IDENTIFICATION AND VALIDATION OF A NEW SOURCE OF LOW GRAIN CADMIUM ACCUMULATION IN DURUM WHEAT (*TRITICUM TURGIDUM* L. SUBSP. DURUM (DEFS.))

A Dissertation
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Atena Oladzad Abbasabadi

In Partial Fulfillment of the Requirements
for the Degree of
DOCTOR OF PHILOSOPHY

Major Department:
Plant Sciences

March 2017

Fargo, North Dakota

North Dakota State University
Graduate School

Title

Identification and Validation of A New Source of Low Grain Cadmium Accumulation In Durum wheat (*Triticum turgidum* L. subsp. durum (Defs.))

By

Atena Oladzad Abbasabadi

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

SUPERVISORY COMMITTEE:

Dr. Elias M. Elias
Chair

Dr. Phil McClean

Dr. Justin Faris

Dr. Shiaoman Chao

Dr. Sam Markell

Approved:

March 9, 2017 — Date

Dr. Richard Horsley — Department Chair

ABSTRACT

Cadmium (Cd) is a toxic heavy metal with no known biological function. The maximum level of Cd concentration allowed in the international market for wheat grain is 0.2 mg kg-1. Higher Cd levels in durum wheat (*Triticum turgidum* L. var. *durum* Desf) may threaten its export. To develop new durum wheat cultivars low in Cd uptake and speed up the selection process in breeding programs, this study attempted to identify SNP(s) associated with a low Cd uptake in the durum experimental line D041735. D041735 was developed from a cross between hexaploid (Sumai 3) and durum wheat by NDSU breeding program and has consistently shown low grain Cd levels. Therefore, this study sought 1) to identify SNP marker(s) tightly linked to Cd uptake and genetic dissection of the grain Cd content in a recombinant inbred line mapping population derived from D041735 and Divide (a high Cd accumulator cultivar) using wheat 90k SNP chips and 2) to test for alleles from detected Cd-linked markers among three sources of low Cd accumulators, including Strongfield, Haurani, and D041735. The QTL analysis performed in this study identified only a single major QTL for Cd uptake on chromosome arm 5BL. The QTL was detected in a 0.3 cM interval flanked by SNP markers RAC875_c20785_1219 and Kukri_c66357_357. Validation results using these flanking markers initially suggested the existence of a different gene or allele for low Cd uptake in the D041735 line as a new source for the durum breeding program at NDSU. The BLAST analysis of these flanking markers suggested the Aluminum Induced Protein Like Protein and heavy metal transporter ATPase 3 as candidate genes for the major QTL. Allelism testing revealed that the identified QTL in this study is novel and not the previously mapped QTL *Cdu1*on 5BL. This study therefore confirmed that the D041735 experimental line is a novel source of low Cd uptake in durum wheat germplasms, where the major QTL is most likely introduced from hexaploid wheat.

ACKNOWLEDGEMENTS

A special thanks goes to my advisor, Dr.Elias Elias,whose tremendous and endless support made this dissertation possible. I cannot thank him enough as he was not only my thesis advisor, but also my life guide, helping me through the many difficulties I faced during a journey that made me a much stronger and better person.

I would like to express my deep gratitude to Stan Stancyk, my great teacher in the durum breeding program, whose knowledge and expertise helped sharpen my thinking for a better understanding of plant breeding.

I greatly appreciate all the valuable time and help that Sally Mann and Megan Shawgo devoted to me during my fieldwork.

Many thanks go to Dr. Ajay Kumar and Dr. Seyed Mostafa Pirseyedi, who generously shared their knowledge with me, enabling me to shape and complete my research.

I would like to thank Dr. Phil McClean, Dr. Shiaoman Chao, Dr. Justin Faris. and Dr. Sam Markell for serving on my supervisory committee and sharing their valuable time and knowledge with me. Also, my thanks are extended to Mary Osenga, Dr. Francois Marais, Roshan Sharma, Justin Hegstad, Dr. Jim Hammond, Dr. Edward Deckard, Marina Dobrydina, Wesam Abouhammed, Evan Salsman, Patricia Cabas Luhmann, Rahil Ashtari, and Naa Korkoi Ardayfio for their valuable assistance, support, and encouragement.

Finally, I would like to thank my family for their heart-warming encouragement, and especially Ashkan, for his unconditional support and immense patience every step of the way.

DEDICATION

To my beloved parents, my beautiful sisters, Atefeh and Azarakhsh, and my amazing brothers, Arman and Arian, for their love, support, and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

bp.....Base Pair

CAPs .....Cleaved Amplified Polymorphic Site

Cd.....Cadmium

Cdu1 .....Cadmium Uptake Gene

CIM .....Composite Interval Mapping

CV .....Coefficient of Variation

DArT .....Diversity Arrays Technology

ESM .....Express Site Marker

EST .....Expressed Sequence Tag

KASPar .....Kompetitive Allele Specific PCR

LOD .....Logarithm of the Odds

LSD .....Least Significant Differences

MAS .....Marker Assisted Selection

PCR .....Polymerase Chain Reaction

QTL.....Quantitative Trait Loci

RAPD .....Random Amplification Polymorphism DNA

RILs.....Recombinant Inbred Lines

SNP .....Single Nucleotide Polymorphism

STS.....Sequence Tagged Site

INTRODUCTION

This dissertation is presented in four chapters. In Chapter 1, different characteristics of durum wheat, features of Cadmium (Cd) in the soil and plants, and breeding approaches for improvement of grain Cd content in wheat are reviewed. In Chapter 2, the methods and results for detecting QTL(s) associated with Cd uptake in wheat are explained, leading to the suggestion to perform a diagnostic test of alleles associated with Cd uptake. Chapter 3 explains this step, including a discussion of the methodology employed and the test results. Chapters 2 and 3 both discuss how this study ultimately identified a new source for Cd uptake in wheat. Finally, Chapter 4 provides the general conclusions and recommendations for the application of the findings of this study in durum wheat breeding programs.

CHAPTER 1. LITERATURE REVIEW

1.1. Introduction

This chapter reviews different aspects of durum wheat, from its taxonomy to its molecular characteristics. The studies that detected significant QTLs in durum wheat demonstrate how marker-assistant selection (MAS) is becoming an important approach in durum breeding programs. Since this study focuses on durum wheat with a low level of grain Cd, the review was directed to important features of Cd in soil and plants generally, but with an emphasis on durum wheat. An overview of breeding efforts to improve grain Cd demonstrates part of the approach used in this study.

1.2. Durum Wheat

Durum wheat is a member of the *Poaceae* (grasses) family, which evolved about 70 million years ago, (Kellogg, 2001). Based on the morphological characteristics of grass structures, the physiology, cytology, anatomy, genomics, and proteomics studies, 11,000 species have been classified into this family (Peterson, 2003). The taxonomic studies of *Poaceae* classify 12 subfamilies within the grass family, consisting of 50 tribes and 81 subtribes (Peterson, 2003). The major sub-family of the 12 of which the genus *Triticum* is a member is known as *Pooideae* (Inda et al. 2008). Durum wheat in turn belongs to the genus *Triticum* and the species *turgidum*. About 300,000 to 500,000 year ago, the hybridization and subsequent polyploidization between *Triticum urartu* ($2n = 2x = 14$), and *Aegilops speltoides* ($2n = 2x = 14$) led to the formation of emmer wheat (*T. dicoccoides*, $2n = 4x = 28$, genome A^uA^uBB). The A^uA^u genome ancestor descended from the genus *T. urartu* and the B genome from *Ae. speltoides* (Dvorak and Akhunov, 2005). *Aegilops* is the most closely related genus to *Triticum* and played an important role in the evolution of durum wheat. Kilian et al. (2011) identified *Aegilops* as the "largest part

of the secondary gene pool of wheat." Emmer wheat was planted by humans 10,000 years ago, and unconscious selections over the years resulted in cultivated emmer. Mutations slowly modified cultivated emmer into a free-threshing allotetraploid species with $2n = 4x = 28$, known as *Triticum turgidum* L. subsp. durum (Desf.) Husn (Peng et al., 2011).

A phylogenic relationship study by Petersen et al. (2006), presented strong evidence that *T.urartu* and *Ae. speltoides* are progenitors of the A^u and B genomes in durum wheat, respectively. Different studies have shown that the B genome is more variable than the A genome (Wendel, 2000). This suggests that the B genome has a higher modification rate in polyploidy wheat (Petersen et al.,2006).

1.2.1. The Domestication of Durum

The main qualitative traits of wheat studied in domestication research are brittle rachis (Br/br), tough glume (Tg/tg), and free-threshing (Q/q) (Gill et al., 2006, Peng et al., 2011). Studies show that these characters are the most obvious difference between wild type of wheat and durum wheat which make a distinct difference in seed dispersal mechanism as well (Elbaum et al., 2007, and Tzarfati et al., 2013). Important quantitative traits from domestication point of view are seed size, flowering time and grain yield (Peng et al., 2011).

1.2.2. Dissemination of the Domesticated Lines

Feldman and Kislev (2007) collected evidence that dated the human use of the wild emmer (*T. dicoccoides*, 2n = 4x = 28, genome A^uA^uBB) to 19,000 years ago in the southwestern area by the Sea of Galilee in Israel and in many locations in the Fertile Crescent (a crescent-shaped mountainous area that covers parts of the Zagros Mountains in southwestern Iran, the Tigris-Euphrates delta in northern Iraq, southeastern Turkey, south western Syria near the Mediterranean Sea and Israel, and Jordan). This race was then cultivated 10,000 years before

present. Many natural hybridizations, spontaneous mutations, and human selections resulted in the domesticated emmer race. Dvorak et al. (2011) believe that cultivated emmer (*T. dicoccum*) was possibly domesticated in southeastern Turkey 9,000 to 7,500 years ago, as free-threshing types of seed derived from cultivated emmer had been discovered by that time, resulting in domesticated tetraploids. According to Feldman and Kislev (2007), durum wheat was domesticated in an area that coincided with the distribution area of wild emmer, specifically the southwestern part of the Fertile Crescent (the Levant area of the Near East and parts of the Ethiopian Hilltops). According to the archaeological record, the production of durum wheat centered in parts of the Middle East, North Africa, the European regions bordering the Mediterranean, the former Soviet Union, and the Great Plains of India as durum wheat is better suited to semi-arid climates (Elias and Manthey, 2005). In the United States, western parts of North Dakota and eastern Montana, and, in Canada, southern parts of Saskatchewan, Manitoba, and Alberta are growing good quality of durum wheat.

1.2.3. Durum Wheat in the United States

According to Joppa and Williams (1988), in 1850, four varieties of durum wheat were introduced in the United States: Algerian Flint, Arnautka, Syrian Spring, and Turkish Flint. Twenty years later, durum wheat from Nicaragua was introduced in Texas. Until 1894, durum cultivation was not successful in the United States because of durum wheat's low adaptation to this area. However, from 1910 to 1920, agronomic studies on over 1,000 durum varieties brought from all over the world resulted in the release of new durum varieties adapted for the United States. Although these varieties were well adapted to the North American climate, some constraints existed, including a lack of resistance to stem rust and a general poor seed quality. To address these constraints, many breeding efforts were initiated from 1920 to 1940. Since then,

different durum breeding programs have attempted to improve or develop such traits as disease resistance, semi-dwarfism, and a high gluten content. Molecular genetic techniques also have been integrated into traditional breeding approaches to improve productivity and other traits. Identifying different molecular markers, explained later in this chapter, has played an important role in durum wheat diversity studies. Montana and North Dakota are the leadings producers of durum wheat in the United States. According to the NASS (National Agricultural Statistics Service, 2015), 1.95 million acres of durum wheat production was concentrated in the United States, with approximately 56% of this acreage in North Dakota.

1.2.4. Molecular Characterization of Durum Wheat

Durum wheat is an allotetraploid crop with 28 chromosomes and a genome size of 13,000 Mbp (Kubaláková et al., 2005). Numerous molecular maps have been created, some using multiple classes of molecular markers. The diversity of the markers used indicates that several techniques can be employed to identify the locations of durum wheat genes. The newest and most extensive consensus map of durum wheat was constructed by Maccaferri et al. (2015). This map harbors 26,626 Single Nucleotide Polymorphisms (SNPs) and 791 Simple Sequence Repeats (SSRs) on the 2,631 cM map length of all 14 durum wheat chromosomes and has been used for many quantitative/qualitative trait loci (QTL) studies of durum wheat, including this study. The map shows fewer regions with recombination suppression than that for bread wheat (*T. aestivum* L.), which makes it suitable for mapping on the A and B genomes of durum wheat.

There are several important QTL mapping studies of durum wheat. The association mapping study conducted by Reimer et al. (2008) for yellow pigmentation used consensus map of SSR markers to localize a QTL close to phytoene synthase gene, *Psy1-b1*, on the distal end of chromosome arm 7BL. This gene is a potential candidate gene for yellow pigmentation. In a

study of leaf rust resistance, a major QTL was mapped on the distal region of chromosome 7BL using SSR and Diversity Arrays Technology (DArT) markers (Maccaferri et al., 2008). Major QTLs for pasta color on chromosomes 1B, 4B, 6A, 7A, and 7B were identified by a composite microsatellite and SNP map of durum wheat. The 7B QTL for pasta color was also associated with the Phytoene synthase 1 (*Psy-B1*) locus, which suggested a difference in pigment biosynthesis (Zhang et al., 2008). Different studies have mapped a QTL associated with the low Cd uptake gene (*Cdu1*) on the long arm of chromosome 5B using 9K SNPs, SSRs, RAPD (Random Amplified Polymorphic DNA) and DArT (AbuHammad et al., 2016,; Knox et al., 2009; Penner et al., 1995; Pozniak et al., 2012). Several wheat ESM developed from Expressed Sequences Tags (EST) have allowed a finer mapping of the *Cdu1* region (Wiebe et al., 2010). Studies show that a gene responsible for insensitivity to the toxin *Ptr ToxA* (*Tsn1*) caused by some *Pyrenophora tritici-repentis* races is located on the 5BL in the deletion bin 0.75-0.76 (Faris et al., 1996, and Faris et al., 2010). Lu et al. (2006) identified a SSR marker (*Xfcp2*) tightly linked to *Tsn1*, and Knox et al. (2009) showed that this region is mapped approximately 12 cM proximal from the Cd uptake gene (*Cdu1*). A major QTL for the vernalization locus *Vrn- 53 B1* mapped on the 5BL chromosome also showed a significant linkage with the Cdu1 proximal position (Iwaki et al., 2002, and Wiebe et al., 2010). Several wheat Expressed Sequences Tags (EST) have been localized to the long arm of 5B (Faris et al., 2000, and Qi et al., 2004). These ESTs were used for further marker development to obtain a fine map of chromosome 5B.

The most important point to remember from these studies is that the parents are selected based on the trait(s) under study. For example, Maccaferri et al. (2008) chose cultivars Colosseo and Lloyd as the parents in a stem rust experiment since they differed in susceptibility to a type of rust normally found in Italy. For a study of QTLs associated with protein content, double

haploid lines were developed from a cross between Strongfield and DT 695 because Strongfield had an average 1.4% higher grain protein concentration than the other parent (Suprayogi et al. 2009). In an experiment conducted by Kumar et al. (2013), a segregating population from a cross between high gluten (Maier) and low gluten (Rugby) durum cultivars resulted in identification of a major QTL for gluten strength on chromosome 1B that explained approximately 93% of the variation. The association mapping study on yellow pigmentation mentioned earlier used 93 different accessions collected from all over the world (Reimer et al., 2008). One of the cultivars with several excellent pasta quality characteristics, but a high Cd uptake, is Kofa from the United States. Many different molecular mapping studies have used Kofa as one of the parental lines. In two of the important studies of low Cd uptake genes in durum wheat, a double haploid population from a cross between Kofa and W926226D3 (a low Cd accumulator) was used to study mapping of a major gene for this trait (Knox et al., 2009 and Wiebe et al., 2010). According to Clarke et al. (2005), the primary source of the low Cd phenotype Nile was obtained from the International Centre for Agricultural Research in the Dry Areas, Syria.

The breeding objectives of the NDSU durum breeding program have changed over time, necessitating a continuous introgression of new material. After resolving the main issues related to rust epidemics in the late 1950s, breeders had more freedom to select for other traits of interest in the 1960s and 70s. Recently, the molecular characterization of these traits has been more focused. A recent concern of durum breeders in North America is selecting for low Cd uptake lines. Knox et al. (2009) laid the groundwork for current research in this area by describing the chromosomal location of *Cdu1*using a saturated microsatellite map, resulting in incorporation of this low Cd gene into durum breeding programs in the United States. In a recent study by AbuHammad et al. (2016) at NDSU, Haurani (low Cd source) and Granola (high Cd source)

were used to develop a bi-parental population for genetic mapping of SNPs linked to the *Cdu1* gene. The detected marker (wsnp_Ex_c1343_2570756) has been shown to be as useful as the RAPD marker linked to the low Cd uptake, *Cdu1,* developed by Penner et al. (1995) for the NDSU breeding program (Salsman, 2016).

1.3. Cadmium

Cadmium (Cd) is a toxic heavy metal with an atomic number of 48 that usually presents in an oxidation state of +2 (less exists in the +1 state); it has no known biological function (Cotton et al., 1999). Cd exists extensively in nature, with an average content of 0.2 mg kg−1 in the geosphere (Vig et al., 2003). It is accumulated to comestible parts of plants and moves up the food chain to humans primarily through consumption of cereals and vegetables. Under environmental conditions that cause accumulation of Cd in the soil, the risk of Cd exposure through consuming foods grown in that soil is increasing. High levels of Cd in food crops is a major concern because of the negative effects of Cd consumption on health. This nephrotoxic chemical primarily affects the kidneys in the human body. As Cd accumulates in the skeletal system, it interferes with the metabolism of calcium, resulting in bone demineralization and the formation of kidney stones (Kazantzis, 2004). Beyersmann and Hechtenberg (1997) found that chromosomal aberrations within the cell are caused by Cd binding to and breaking DNA strands. A study by Jin et al. (2003) showed that Cd can cause genome mutability by inhibiting DNA mismatch repair, thereby enhancing the likelihood of developing different types of cancers. Other studies have found an association between Cd concentration in the urine and breast cancer (Gallagher et al., 2010) and modifications of gene expression at the cellular level (Luparello et al., 2011).

The high level of Cd concentration is a food safety issue that affects durum wheat breeding programs around the world. Semolina from durum wheat is the main ingredient used in pasta production. In 2002, durum wheat was found to have high levels of Cd in northern states in the United States and in Canada. The maximum level of Cd concentration for wheat grains allowed to trade in international markets is 0.2 mg kg-1 (0.2 ppm) (Codex Alimentarius Commission, 2011). North Dakota produces 56% of the U.S. durum crop (National Agricultural Statistics Service, 2015). Since high Cd may threaten the export market for durum wheat, it is important to reduce its Cd uptake to meet international trade regulations. Significant cultivar variations for Cd uptake have been detected in various crops, including durum wheat (Beyersmann and Hechtenberg, 1997; Hinsley et al., 1978; and McLaughlin et al., 2000). And, many breeding programs have been established around the world to develop and release cultivars low in Cd accumulation.

1.3.1. Cd Accumulation in Soil and Plants

The main source of Cd uptake for plants is the soil. Mass flaw, diffusion, and interception are three possible systems that transport Cd from the soil to the plant roots (Ingwersen and Streck, 2005). Cd ions can be taken up into the living system of a plant by being sequestered in the root tissue. This Cd later can be translocated to the stem and leaf via the xylem (Benavides et al., 2005; Hart et al., 1998; and Salt et al., 1995) and to the grain, probably via the phloem (Harris and Taylor, 2001; Hart et al., 1998; and Popelka et al., 1996).

1.3.1.1. Cd in the Soil

Cd contamination in soils is affected by several soil properties. For example, faster Cd accumulation occurs in soils with a lower pH (Page et al., 1987). Tillage management and large-scale use of super phosphate fertilizers also affect Cd accumulation in the soil (Grant et al.,

2008). However, Gao et al. (2010) showed that tillage practices did not consistently influence Cd concentration in durum wheat. In contrast, nitrogen fertilizers have been shown to increase Cd accumulation in plants by their effects on soil chemistry (Andersson, 1976).

Some studies indicate a correlation between soil attributes and Cd concentration uptake by plants in the field (Wu et al., 2002). Perrier et al. (2016) showed that the concentration of other minerals in the soil can also affect grain Cd accumulation in French durum wheat cultivars; the Cd content had a positive correlation with the concentration of phosphorous (P) < Manganese (Mn) < zinc (Z). These durum cultivars, however, did not show any significant correlation between Cd content and Iron (Fe) or Copper (Cu) content. Two Canadian cultivars, Kyle and Strongfield, also showed the same relationships with these other minerals in their experiment (Perrier et al.. 2016). However, another study indicated that Cd and Zn persuaded changes in the Cu/Mn accumulation in the roots of rice (Yoshihara, Goto, et al., 2010).

1.3.1.2. Cd in Plants

Cd uptake and accumulation have been shown to differ among plant species (Grant et al., 1998, and Özturket al., 2003) and cultivars (Clarke et al., 2002; Dunbar et al. 2003; and Özturk et al., 2003). The dose of Cd toxicity in plants is affected by mechanisms of root uptake and shoot transport of Cd (Dunbar et al., 2003, and Özturk et al., 2003). Harris and Taylor (2004) clearly proved that differences between a pair of near-isogenic durum lines for grain Cd accumulation in shoots are solely due to differences in root-to-shoot Cd translocation and unrelated to whole plant Cd accumulation. In their study, high and low grain Cd lines had a similar whole Cd accumulation. This result coincides with results of another study, this time with rice, which showed that whole plant Cd was mainly accumulated in the roots in the low Cd rice grains (Ueno et al., 2009). It is possible that the same genetic mechanism is governing the low

Cd uptake in both rice and durum since the phenotypic variation in the Cd level of shoots and grains in different varieties in both studies was caused by the physiological process translocating Cd from the root to the shoot regardless of the amount of Cd accumulated by the root from the soil. The physiological studies suggest that two mechanisms most likely cause a low level of Cd to accumulate in the edible parts of most plants. In the first, plant root cell walls can effectively minimize the Cd uptake into the root cells from the soil (Ovečka and Takáč, 2014). In the second and more likely mechanism, called the Cd-chelating pathway, Cd-binding proteins, such as phytochelatins or metallothionins, in the root cells of some plants detoxify the Cd toxicity so that only a low level of Cd is translocated into the vegetative parts of the plant. This ability to detoxify the Cd toxicity is different between and within plant species (Grant et al., 1998; Hall, 2002; and Sugiyama et al., 2007). This mechanism was reported as a possible method for decreasing Cd levels in durum wheat (Harris and Taylor, 2013), as well in a soybean cultivar, Enrei (Ahsan et al., 2012), and in rice, where, a P1B-ATPase protein coded by the *OsHMA3* gene was found to translocate Cd from the cytosol to the vacuole as Cd is prevented from being translocated to the shoot (Miyadate et al., 2011, and Ueno et al., 2010). Several different studies in Arabidopsis demonstrated that the ATP-binding cassette transporters and P1B-ATPases were related to low Cd uptake (Kim et al., 2007, and Wojas et al., 2009). However, a consistent pathway for Cd accumulation in durum grain has not yet been established. Wiebe et al. (2010) presented a hypothesis regarding the low Cd uptake in durum wheat, which states that in root tissue, a functional transporter or chelator prevents Cd translocation to shoots by sequestration of Cd into chemical complexes or physical compartments, thereby reducing the Cd availability for loading into the xylem and phloem. More studies have discussed the role of ABC transporters, which transport Cd-glutathione or Cd-phytochletain complex into the vacuole and consequently

limit Cd mobilization to the grain (Klein et al., 2006; Stolt et al., 2003; and Wojas et al., 2009). In one study, a gene coding a phytochelatin protein (*PCS2*) was reported on chromosome 5B in wheat (Yan et al., 2003), and it has been shown that this gene is tightly linked to *Xwg644*, which codes for a half-sized ABC transporter (Dubcovsky et al., 2001). Wiebe et al. (2010) have mapped a Cdu1 close to *PCS2*, which suggests *PCS2* could be a candidate gene because of its potential role in chelating Cd and sequestering it into the vacuole.

To conclude, by selecting cultivars highly capable of repressing the root uptake and shoot transport of Cd, the effects of Cd toxicity can be reduced in crop plants (Özturket al., 2003).

1.3.2. Genetic Inheritance of Low Cd Uptake

Genetic variation for grain Cd accumulation was studied in many crops, including durum wheat, rice, oats, sunflower, flax, and soybeans (Andersen and Hansen, 1984; Cieśliński et al., 1996; Ishikawa et al., 2005; Kobori et al., 2011; Penner et al., 1995; and Tanhuanpää et al., 2007). Clarke et al. (1997) have shown that genetic inheritance of the low Cd trait in durum wheat is controlled by a single gene. Hexaploid wheat cultivars commonly accumulate low Cd in their grains compared with durum wheat grains (Zook et al., 1970). However, considering the complexity of Cd accumulation in grain, from absorption to sequestration to translation, it is questionable that only one gene contributes to the genetic differences among grains with a low Cd uptake. Nevertheless, to date, only one to three genes have been reported to govern Cd content in most crops. For example, Ishikawa et al. (2005) identified three QTL in rice for grain Cd content on chromosomes 3, 6, and 8, though more recently, a single QTL on chromosome 7 of rice has been reported for regulation of Cd. Only one major QTL in soybeans has been identified (Benitez, Hajika, et al., 2010, and Jegadeesan, Yu, et al., 2010). In durum wheat, Knox et al. (2009) reported a major QTL for low grain Cd content on chromosome 5B, but as

mentioned earlier, many studies have shown an association among some transporter proteins with the phenotypic variation of this trait, such as P1B-ATPase, *OsNRAMP1*(an iron transporter), *OsZIP8* (a Cadmium/zinc transporter), and ABC transporters (Miyadate et al., 2011; Ueno et al., 2010; Wojas et al., 2009 and Yan et al., 2003); the genes controlling these transporters should be taken into consideration in map-based cloning studies.

1.4. Plant Breeding Approach to Cd Accumulation Reduction

Because a genetic system is the likely mechanism to limit Cd translocation from the roots to the shoots, screening genotypes at the early stages to identify plants carrying the low Cd allele with a lower Cd concentration in the leaves and grain (Harris and Taylor, 2004) might be a good solution for reducing the level of Cd in our diet. Previous research demonstrated that a single dominant gene, Cdu1, confers the low grain Cd phenotype (Clarke et al., 1997), which is localized on chromosome arm 5BL (Knox et al., 2009). Different cultivars in durum wheat show genotypic variation in Cd accumulation in the grain (Van der Vliet et al., 2007). This genetic diversity provides an opportunity to apply plant breeding to develop cultivars with a low Cd uptake that possess excellent agronomic and quality traits and disease resistance. However, for plant breeding programs to find low-Cd phenotypes through chemical analysis of the grains is expensive. The ability to identify and select for genetic differences in Cd concentration at an early growth stage would save time and cost to the breeding program (Grant et al., 2008). One of the most common and practical techniques for selecting genes with Mendelian heritability, such as Cdu1 is MAS, is an indirect selection method where a trait of interest is selected based on markers (morphological, biochemical, or a DNA/RNA variation) that are tightly linked to the gene or quantitative trait locus (QTL) of interest (Bernardo, 1998). In this approach, breeders can select for desirable traits in early segregating generations at the seedling stage. Likewise,

selection of two or more markers in the region where these traits might also be tightly linked with the trait of interest can leads to a fast and easy selection for both loci. As explained earlier in this chapter, the *Tns1* gene that conditions sensitivity to *Ptr ToxA* (Faris et al., 1996) is widely known to be linked with the Cdu1 gene in durum wheat. Therefore, a simultaneous selection for both loci can be obtained by identifying markers in the region of *TN1/Cdu1*. This method will save time, resources, and energy that can be channeled to develop large segregating population for several generations and estimate the parameters appropriate for direct selection.

1.4.1. Use of Marker Assisted Selection (MAS) for Reducing Cd Concentration

MAS may provide an effective selection tool for low Cd uptake genotypes. The main requirement for such a selection strategy is the identification of a suitable marker linked to a gene controlling Cd uptake. The five considerations for evaluating a valuable marker for a breeding program are 1) the recombination between the marker and target loci should preferably be less than 5 cM genetic distance 2) the high DNA quantity and quality of the marker 3) a high-throughput simple and quick procedure 4) the marker should be highly polymorphic in breeding material, and 5) the cost of the marker (Collard and Mackill, 2008). The general steps of a MAS technique are:

(i) Develop a Mapping Population

A mapping population is a population in which recombination of parental alleles can be traced. The type and size of the mapping population depends on the type of marker system, traits to be mapped, the availability of time, and the objective of the mapping project. As explained earlier in this chapter, crossing parents should differ in the traits of interest to facilitate linkage mapping and segregation analysis.

The mapping population can be developed from F_2 and testcross populations derived from crossing parents showing polymorphism for the traits desired for mapping. A major disadvantage of such populations is that they are only temporary and cannot be repeated or grown again. Recombinant Inbred Line (RILs) populations, and double haploid method, can overcome this problem since they can be regrown repeatedly. RILs also can be developed from a Single Seed Descent (SSD) propagation. Individual plants from an F_2 population are harvested and continued through several generations (often up to the F_6) until the lines are homozygous. Thenceforth, each line is harvested in bulk and maintained separately. The research lines from these populations can be scored for various molecular markers and traits. Replicated trials across space and time will allow for accurate characterization of these lines, especially in terms of quantitatively inherited traits such as yield.

(ii) Identification of Molecular Marker(s) for Genotyping the Mapping Population

The most common molecular markers used in genomic mapping programs are restriction fragment length polymorphisms (RFLP), randomly amplified polymorphic DNA (RAPD), simple sequence repeats (SSR) or microsatellites, and amplified fragment length polymorphisms (AFLP). Currently, Single nucleotide polymorphisms (SNPs) markers seem to be more applicable than other markers due to their high frequency throughout a genome. However, many of these markers need to be modified to be applicable in breeding programs seeking greater efficiency and a reduced cost. Modified markers are called “breeder-friendly markers.” RAPD markers generally are converted to the sequence characterized amplified region (SCAR) marker, which is more stable (Dnyaneshwar et al., 2006, and Rajesh et al., 2013), and analysis gets less tedious by using a simple PCR analysis. The PCR primers are designed from the sequence of the amplicon of RAPD (Kumla et al., 2012, and Rajesh et al., 2013). For example, Knox et al.

(2009) developed the SCAR-OPC20 (*ScOPC20*) marker from the RAPD- OPC20 marker identified by Penner et al. (1995) to map their Cd segregated population. Cleaved Amplified Sequences (CAPs) are another user-friendly marker commonly derived from an RFLPs or from markers developed from an Expressed Sequenced Target (EST) or Sequence Site Target (SST). The basic principal is to convert an amplified band that does not show variation into a polymorphic band. Wiebe et al. (2010) localized a CAPs marker (*Xusw47*) for wheat in a 0.14 cM interval of the Cdu1 gene 5 cM apart from the ScOPC20 marker on 5BL. In a collinearity study of this chromosomal region with rice (*Oryza sativa* L.) and Brachypodium, *Xusw47* with some other CAPs derived from ESMs and STS markers plus newly mapped SSRs used to fine map the Cdu1 region on chromosome 5B. The efficiency of *Xusw47* was validated by Zimmerl et al. (2014).

Pozniak et al. (2012) found five DArT (diversity arrays technology) markers linked with phenotypic grain Cd concentration. Three of these markers, including *wPt-1733*, *wPt-2453* and *wPt-9300*, showed similar effects and proportions of the phenotypic variance as the *Xusw47* marker and were physically associated with *Cdu-B1*. They suggested that these markers are either detecting the same locus or are closely linked to *Cdu- B1*.

Likewise, breeder-friendly markers derived from SNPs for low Cd uptake genotypes were developed in the durum breeding program at NDSU. The KASPar (Kompetitive AlelleSpecific PCR) genotyping assay was derived from the SNP marker (Ex_c1343_2570756) associated with the major QTL for low Cd uptake on 5BL (AbuHammad et al., 2016).

(iii) Linkage Mapping of Markers and QTL Analysis

Linkages analyses are statistical methods used to estimate the functionality relation of genes to their location on chromosomes (Gupta et al., 2005). Different methods for Linkages

analyses in plants have been described in various publications (Flint-Garcia et al., 2003, and Gaut and Long, 2003).

It is not possible to determine linkages between large numbers of markers manually, but some computer software programs have this capability. The calculation is based on using odds ratios (the ratio of linkage versus no linkage) defined as the logarithm of the ratio and called a logarithm of odds (LOD) value or LOD score (Risch, 1992). In linkage maps, LOD scores greater than three are usually used for developing the linkage maps. If this value is equal to three between two markers, the probability of linkage will be 1,000 times more likely than no linkage (1,000:1) (Collard and Mackill, 2008). Mapmaker/EXP, MapManager QTX, JoinMap, and Mapdisto are common software programs used for constructing linkage maps.

To detect an association between phenotype data and the genotype of markers in a linkage map, several methods of QTL analysis can be performed (Collard and Mackill, 2008). For examples, single-marker analysis, which analyzes only a single marker, is the simplest method. In this method, different statistical analyses may be used, such as t-tests, analysis of variance (ANOVA), and linear regression. The outcomes from this method of analysis show the linkage group covering the markers, probability values, and the percentage of phenotypic variation. Another method, simple interval mapping (SIM), tests for the existence of QTL between each pair of adjacent markers along chromosomes. SIM is statistically more powerful than single-marker analysis. Finally, composite interval mapping (CIM) combines interval mapping with linear regression. It is more accurate and effective at mapping QTLs. The most common software programs for QTL analysis are QTL Cartographer, QTL MapManager, Qgene, IciMapping, QTX, and PLABQTL.

In the latest study on targeted mapping of *Cdu1*, Weibe et al. (2010), revealed an accurate chromosomal position for the *Cdu1* gene on chromosome 5B in the 0.14 cM interval that explained 80% of phenotypic variation for low Cd uptake. They suggested that the map be used for a map-based cloning of the *Cdu1* gene.

Although the detected QTL was mapped using the accurate fine mapping approach, to date, no coding sequence for the *Cdu1* gene in durum wheat has been reported or published; this means the possibility for detecting tighter markers linked to the *Cd* uptake gene should not be ruled out, especially if a new source of this phenotype in a durum breeding program is introduced. New sources for a phenotype of interest are the most important part of generating diversity and crop improvement in a breeding program as they can reveal the existence of new genes for the same phenotype. Such studies are always helpful for a better understanding of the molecular characteristics of a functional gene.

1.5. References

AbuHammad, W.A., S. Mamidi, A. Kumar, S. Pirseyedi, F.A. Manthey, S.F. Kianian, et al. 2016. Identification and validation of a major cadmium accumulation locus and closely associated SNP markers in North Dakota durum wheat cultivars. Molecular Breeding 36: 112.

Agricultural Statistics Board. 2015. National Agricultural Statistics Service, U.S. Department of Agriculture.

Ahsan, N., T. Nakamura and S. Komatsu. 2012. Differential responses of microsomal proteins and metabolites in two contrasting cadmium (Cd)-accumulating soybean cultivars under Cd stress. Amino Acids 42: 317-327.

Andersen, A. and H.N. Hansen. 1984. High cadmium and nickel contents in sunflower kernels. Zeitschrift für Lebensmittel-Untersuchung und Forschung 179: 399-400.

Andersson, A. 1976. On the influence of manure and fertilizers on the distribution and amounts of plant-available Cd in soils. Swedish Journal of Agricultural Research.

Benavides, M.P., S.M. Gallego, and M.L. Tomaro. 2005. Cadmium toxicity in plants. Brazilian Journal of Plant Physiology 17: 21-34.

Benitez, E.R., M. Hajika, T. Yamada, K. Takahashi, N. Oki, N. Yamada, et al. 2010. A major QTL controlling seed cadmium accumulation in soybeans. Crop Science 50: 1728-1734.

Bernardo, R. 1998. A model for marker-assisted selection among single crosses with multiple genetic markers. Theoretical and Applied Genetics 97: 473-478.

Beyersmann, D. and S. Hechtenberg. 1997. Cadmium, gene regulation, and cellular signalling in mammalian cells. Toxicology and Applied Pharmacology 144: 247-261.

Cieśliński, G., K. Van Rees, P. Huang, L. Kozak, H. Rostad, and D. Knott. 1996. Cadmium uptake and bioaccumulation in selected cultivars of durum wheat and flax as affected by soil type. Plant and Soil 182: 115-124.

Codex Alimentarius Commission. 2011. Working document for information and use in discussions related to contaminants and toxins in the GSCTFF. CODEX: Rome, Italy.

Clarke, J., D. Leisle and G. Kopytko. 1997. Inheritance of cadmium concentration in five durum wheat crosses. Crop Science 37: 1722-1726.

Clarke, J., T. McCaig, R. DePauw, R. Knox, F. Clarke, M. Fernandez, et al. 2005. Strongfield durum wheat. Canadian Journal of Plant Science 85: 651-654.

Clarke, J., W. Norvell, F. Clarke, and W. Buckley. 2002. Concentration of cadmium and other elements in the grain of near-isogenic durum lines. Canadian Journal of Plant Science 82: 27-33.

Collard, B.C. and D.J. Mackill. 2008. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Philosophical Transactions of the Royal Society B: Biological Sciences 363: 557-572.

Cotton, A.F., G. Wilkinson, M. Bochmann, and C.A. Murillo. 1999. Advanced Inorganic Chemistry, Wiley.

Dnyaneshwar, W., C. Preeti, J. Kalpana, and P. Bhushan. 2006. Development and Application of RAPD-SCAR Marker for Identification of Phyllanthus emblica LINN. Biological and Pharmaceutical Bulletin 29: 2313-2316.

Dubcovsky, J., W. Ramakrishna, P.J. SanMiguel, C.S. Busso, L. Yan, B.A. Shiloff, et al. 2001. Comparative sequence analysis of colinear barley and rice bacterial artificial chromosomes. Plant Physiology 125: 1342-1353.

Dunbar, K.R., M.J. McLaughlin and R. Reid. 2003. The uptake and partitioning of cadmium in two cultivars of potato (Solanum tuberosum L.). Journal of Experimental Botany 54: 349-354.

Dvorak, J. and E.D. Akhunov. 2005. Tempos of gene locus deletions and duplications and their relationship to recombination rate during diploid and polyploid evolution in the Aegilops-Triticum alliance. Genetics 171: 323-332.

Dvorak, J., M.-C. Luo and E. Akhunov. 2011. NI Vavilov's theory of centres of diversity in the light of current understanding of wheat diversity, domestication and evolution. Genet Plant Breed 47: S20-S27.

Elbaum, R., L. Zaltzman, I. Burgert, and P. Fratzl. 2007. The role of wheat awns in the seed dispersal unit. Science 316: 884-886.

Elias, E. and F. Manthey. 2005. End products: present and future uses. In 'Durum wheat breeding: current approaches and future strategies. Vol. 1,.(Eds C. Royo, M.M. Nachit, N. Di Fonzo, J.L/ Araus, W.H. Pfeiffer, and G.A. Slafer) pp. 63-85. Food Products Press: New York.

Faris, J., J. Anderson, L. Francl, and J. Jordahl. 1996. Chromosomal location of a gene conditioning insensitivity in wheat to a necrosis-inducing culture filtrate from Pyrenophora tritici-repentis. Phytopathology 86: 459-463.

Faris, J.D., K.M. Haen, and B.S. Gill. 2000. Saturation mapping of a gene-rich recombination hot spot region in wheat. Genetics 154: 823-835.

Faris, J.D., Z. Zhang, H. Lu, S. Lu, L. Reddy, S. Cloutier, et al. 2010. A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. Proceedings of the National Academy of Sciences 107: 13544-13549.

Feldman, M. and M.E. Kislev. 2007. Domestication of emmer wheat and evolution of free-threshing tetraploid wheat. Israel Journal of Plant Sciences 55: 207-221.

Flint-Garcia, S.A., J.M. Thornsberry, and B. IV. 2003. Structure of linkage disequilibrium in plants. Annual Review of Plant Biology 54: 357-374.

Gallagher, C.M., J.J. Chen, and J.S. Kovach. 2010. Environmental cadmium and breast cancer risk. Aging (Albany NY) 2: 804-814.

Gao, X., K.R. Brown, G.J. Racz, and C.A. Grant. 2010. Concentration of cadmium in durum wheat as affected by time, source and placement of nitrogen fertilization under reduced and conventional-tillage management. Plant and Soil 337: 341-354.

Gaut, B.S. and A.D. Long. 2003. The lowdown on linkage disequilibrium. The Plant Cell 15: 1502-1506.

Gill, B.S., B. Friebe, W.J. Raupp, D.L. Wilson, T.S. Cox, R.G. Sears, et al. 2006. Wheat genetics resource center: the first 25 years. Advances in Agronomy 89: 73-136.

Grant, C., W. Buckley, L.D. Bailey and F. Selles. 1998. Cadmium accumulation in crops. Canadian Journal of Plant Science 78: 1-17.

Grant, C., J. Clarke, S. Duguid, and R. Chaney. 2008. Selection and breeding of plant cultivars to minimize cadmium accumulation. Science of the Total Environment 390: 301-310.

Gupta, P.K., S. Rustgi, and P.L. Kulwal. 2005. Linkage disequilibrium and association studies in higher plants: present status and future prospects. Plant molecular biology 57: 461-485.

Hall, J. 2002. Cellular mechanisms for heavy metal detoxification and tolerance. Journal of Experimental Botany 53: 1-11.

Harris, N.S. and G.J. Taylor. 2001. Remobilization of cadmium in maturing shoots of near isogenic lines of durum wheat that differ in grain cadmium accumulation. Journal of Experimental Botany 52: 1473-1481.

Harris, N.S. and G.J. Taylor. 2004. Cadmium uptake and translocation in seedlings of near isogenic lines of durum wheat that differ in grain cadmium accumulation. BMC Plant Biology 4: 1.

Harris, N.S. and G.J. Taylor. 2013. Cadmium uptake and partitioning in durum wheat during grain filling. BMC plant biology 13: 1.

Hart, J.J., R.M. Welch, W.A. Norvell, L.A. Sullivan, and L.V. Kochian. 1998. Characterization of cadmium binding, uptake, and translocation in intact seedlings of bread and durum wheat cultivars. Plant Physiology 116: 1413-1420.

Hinsley, T., D. Alexander, E. Ziegler, and G. Barrett. 1978. Zinc and cadmium accumulation by corn inbreds grown on sludge amended soils.

Inda, L.A., J.G. Segarra-Moragues, J. Müller, P.M. Peterson, and P. Catalán. 2008. Dated historical biogeography of the temperate Loliinae (Poaceae, Pooideae) grasses in the northern and southern hemispheres. Molecular Phylogenetics and Evolution 46: 932-957.

Ingwersen, J. and T. Streck. 2005. A regional-scale study on the crop uptake of cadmium from sandy soils. Journal of Environmental Quality 34: 1026-1035.

Institute, S. 2008. SAS/STAT 9.2 user's guide. SAS Institute Cary, NC.

Ishikawa, S., N. Ae, and M. Yano. 2005. Chromosomal regions with quantitative trait loci controlling cadmium concentration in brown rice (Oryza sativa). New Phytologist 168: 345-350.

Iwaki, K., J. Nishida, T. Yanagisawa, H. Yoshida, and K. Kato. 2002. Genetic analysis of Vrn-B1 for vernalization requirement by using linked dCAPS markers in bread wheat (Triticum aestivum L.). Theoretical and Applied Genetics 104: 571-576.

Jegadeesan, S., K. Yu, V. Poysa, E. Gawalko, M.J. Morrison, C. Shi, et al. 2010. Mapping and validation of simple sequence repeat markers linked to a major gene controlling seed cadmium accumulation in soybean [Glycine max (L.) Merr]. Theoretical and Applied Genetics 121: 283-294.

Jin, Y.H., A.B. Clark, R.J. Slebos, H. Al-Refai, J.A. Taylor, T.A. Kunkel, et al. 2003. Cadmium is a mutagen that acts by inhibiting mismatch repair. Nature Genetics 34: 326-329.

Joppa, L. and N. Williams. 1988. Genetics and breeding of durum wheat in the United States.

Kazantzis, G. 2004. Cadmium, osteoporosis and calcium metabolism. Biometals 17: 493-498.

Kellogg, E.A. 2001. Evolutionary history of the grasses. Plant Physiology 125: 1198-1205.

Kilian, B., K. Mammen, E. Millet, R. Sharma, A. Garner, F. Salamini, et al. 2011. Aegilops. Wild Crop Relatives: Genomics and Breeding Resources, Cereals. Berlin/Heidelberg: Springer-Verlag.

Kim, D.Y., L. Bovet, M. Maeshima, E. Martinoia, and Y. Lee. 2007. The ABC transporter AtPDR8 is a cadmium extrusion pump conferring heavy metal resistance. The Plant Journal 50: 207-218.

Klein, M., B. Burla and E. Martinoia. 2006. The multidrug resistance-associated protein (MRP/ABCC) subfamily of ATP-binding cassette transporters in plants. FEBS Letters 580: 1112-1122.

Knox, R., C. Pozniak, F. Clarke, J. Clarke, S. Houshmand, and A. Singh. 2009. Chromosomal location of the cadmium uptake gene (*Cdu1*) in durum wheat. Genome 52: 741-747.

Kobori, G., M. Okazaki, T. Motobayashi, and K. Matzukawa. 2011. Differences in Cadmium Uptake and Accumulation in Three Soybean Cultivars (Symposium 3.5. 1 Heavy Metal Contaminated Soils,< Special Issue> International Symposium: Soil Degradation Control, Remediation, and Reclamation, Tokyo Metropolitan University Symposium Series No. 2, 2010). 54: 214-221.

Kubaláková, M., P. Kovářová, P. Suchánková, J. Číhalíková, J. Bartoš, S. Lucretti, et al. 2005. Chromosome sorting in tetraploid wheat and its potential for genome analysis. Genetics 170: 823-829.

Kumar, A., E.M. Elias, F. Ghavami, X. Xu, S. Jain, F.A. Manthey, et al. 2013. A major QTL for gluten strength in durum wheat (Triticum turgidum L. var. durum). Journal of Cereal Science 57: 21-29.

Kumla, S., S. Doolgindachbaporn, R. Sudmoon, and N. Sattayasai. 2012. Genetic variation, population structure and identification of yellow catfish, Mystus nemurus (C&V) in Thailand using RAPD, ISSR and SCAR marker. Molecular Biology Reports 39: 5201-5210.

Lu, H.-J., J. Fellers, T. Friesen, S. Meinhardt, and J. Faris. 2006. Genomic analysis and marker development for the Tsn1 locus in wheat using bin-mapped ESTs and flanking BAC contigs. Theoretical and Applied Genetics 112: 1132-1142.

Luparello, C., R. Sirchia, and A. Longo. 2011. Cadmium as a transcriptional modulator in human cells. Critical Reviews in Toxicology 41: 73-80.

Maccaferri, M., P. Mantovani, R. Tuberosa, E. DeAmbrogio, S. Giuliani, A. Demontis, et al. 2008. A major QTL for durable leaf rust resistance widely exploited in durum wheat breeding programs maps on the distal region of chromosome arm 7BL. Theoretical and Applied Genetics 117: 1225-1240.

Maccaferri, M., A. Ricci, S. Salvi, S.G. Milner, E. Noli, P.L. Martelli, et al. 2015. A high-density, SNP-based consensus map of tetraploid wheat as a bridge to integrate durum and bread wheat genomics and breeding. Plant Biotechnology Journal 13: 648-663.

McLaughlin, M.J., M. Bell, G. Wright, and G. Cozens. 2000. Uptake and partitioning of cadmium by cultivars of peanut (Arachis hypogaea L.). Plant and Soil 222: 51-58.

Miyadate, H., S. Adachi, A. Hiraizumi, K. Tezuka, N. Nakazawa, T. Kawamoto, et al. 2011. OsHMA3, a P1B-type of ATPase affects root-to-shoot cadmium translocation in rice by mediating efflux into vacuoles. New Phytologist 189: 190-199.

Ovečka, M. and T. Takáč. 2014. Managing heavy metal toxicity stress in plants: biological and biotechnological tools. Biotechnology Advances 32: 73-86.

Özturk, L., S. Eker, F. Özkutlu, and İ. Çakmak. 2003. Effect of cadmium on growth and concentrations of cadmium, ascorbic acid and sulphydryl groups in durum wheat cultivars. Turkish Journal of Agriculture and Forestry 27: 161-168.

Page, A., A. Chang, and M. El-Amamy. 1987. Cadmium levels in soils and crops in the United States. Lead, Mercury, Cadmium and Arsenic in the Environment: 119-146.

Peng, J.H., D. Sun, and E. Nevo. 2011. Domestication evolution, genetics and genomics in wheat. Molecular Breeding 28: 281-301.

Penner, G.A., L.J. Bezte, D. Leisle, and J. Clarke. 1995. Identification of RAPD markers linked to a gene governing cadmium uptake in durum wheat. Genome 38: 543-547.

Perrier, F., B. Yan, F. Candaudap, O. Pokrovsky, E. Gourdain, B. Meleard, et al. 2016. Variability in grain cadmium concentration among durum wheat cultivars: impact of aboveground biomass partitioning. Plant and Soil: 1-14.

Petersen, G., O. Seberg, M. Yde, and K. Berthelsen. 2006. Phylogenetic relationships of Triticum and Aegilops and evidence for the origin of the A, B, and D genomes of common wheat (Triticum aestivum). Molecular Phylogenetics and Evolution 39: 70-82.

Peterson, P.M. (2003) Poaceae (Gramineae). In *Encyclopedia of Life Sciences*. Wiley, Chichester, U.K. Available at *http://www.els.net/* [doi: 10.1038/npg.els.0006101]

Popelka, J., S. Schubert, R. Schulz, and A. Hansen. 1996. Cadmium uptake and translocation during reproductive development of peanut (Arachis hypogaea L.). Angewandte Botanik 70: 140-143.

Pozniak, C., J. Clarke and F. Clarke. 2012. Potential for detection of marker–trait associations in durum wheat using unbalanced, historical phenotypic datasets. Molecular Breeding 30: 1537-1550.

Qi, L., B. Echalier, S. Chao, G. Lazo, G. Butler, O. Anderson, et al. 2004. A chromosome bin map of 16,000 expressed sequence tag loci and distribution of genes among the three genomes of polyploid wheat. Genetics 168: 701-712.

Rajesh, M., B. Jerard, P. Preethi, R.J. Thomas, T. Fayas, K. Rachana, et al. 2013. Development of a RAPD-derived SCAR marker associated with tall-type palm trait in coconut. Scientia Horticulturae 150: 312-316.

Reimer, S., C. Pozniak, F. Clarke, J. Clarke, D. Somers, R. Knox, et al. 2008. Association mapping of yellow pigment in an elite collection of durum wheat cultivars and breeding lines. Genome 51: 1016-1025.

Risch, N. 1992. Genetic linkage: Interpreting LOD scores. Science 255: 803.

Salsman, E.L. 2016. Validation of molecular markers associated with grain cadmium in durum wheat (Triticum turgidum L. var. Desf.). North Dakota State University.

Salt, D.E., R.C. Prince, I.J. Pickering, and I. Raskin. 1995. Mechanisms of cadmium mobility and accumulation in Indian mustard. Plant Physiology 109: 1427-1433.

Stolt, J., F. Sneller, T. Bryngelsson, T. Lundborg, and H. Schat. 2003. Phytochelatin and cadmium accumulation in wheat. Environmental and Experimental Botany 49: 21-28.

Sugiyama, M., N. Ae, and T. Arao. 2007. Accumulation of large amounts of Cd in the root may limit the accumulation of Cd in edible above-ground portions of the plant. Plant Soil 295: 1-11.

Suprayogi, Y., C.J. Pozniak, F. Clarke, J. Clarke, R. Knox, and A. Singh. 2009. Identification and validation of quantitative trait loci for grain protein concentration in adapted Canadian durum wheat populations. Theoretical and Applied Genetics 119: 437-448.

Tanhuanpää, P., R. Kalendar, A.H. Schulman, and E. Kiviharju. 2007. A major gene for grain cadmium accumulation in oat (Avena sativa L.). Genome 50: 588-594.

Tzarfati, R., Y. Saranga, V. Barak, A. Gopher, A.B. Korol, and S. Abbo. 2013. Threshing efficiency as an incentive for rapid domestication of emmer wheat. Annals of Botany 112: 829-837.

Ueno, D., E. Koyama, I. Kono, T. Ando, M. Yano, and J.F. Ma. 2009. Identification of a novel major quantitative trait locus controlling distribution of Cd between roots and shoots in rice. Plant and Cell Physiology 50: 2223-2233.

Ueno, D., N. Yamaji, I. Kono, C.F. Huang, T. Ando, M. Yano, et al. 2010. Gene limiting cadmium accumulation in rice. Proceedings of the National Academy of Sciences 107: 16500-16505.

Van der Vliet, L., C. Peterson, and B. Hale. 2007. Cd accumulation in roots and shoots of durum wheat: the roles of transpiration rate and apoplastic bypass. Journal of Experimental Botany 58: 2939-2947.

Vig, K., M. Megharaj, N. Sethunathan, and R. Naidu. 2003. Bioavailability and toxicity of cadmium to microorganisms and their activities in soil: a review. Advances in Environmental Research 8: 121-135.

Wendel, J. F. 2000. Genome evolution in polyploids. Plant molecular evolution. Springer. p. 225-249.

Wiebe, K., N. Harris, J. Faris, J. Clarke, R. Knox, G. Taylor, et al. 2010. Targeted mapping of Cdu1, a major locus regulating grain cadmium concentration in durum wheat (Triticum turgidum L. var durum). Theoretical and Applied Genetics 121: 1047-1058.

Wojas, S., J. Hennig, S. Plaza, M. Geisler, O. Siemianowski, A. Skłodowska, et al. 2009. Ectopic expression of Arabidopsis ABC transporter MRP7 modifies cadmium root-to-shoot transport and accumulation. Environmental Pollution 157: 2781-2789.

Wu, J., W. Norvell, D. Hopkins, and R. Welch. 2002. Spatial variability of grain cadmium and soil characteristics in a durum wheat field. Soil Science Society of America Journal 66: 268-275.

Yan, L., A. Loukoianov, G. Tranquilli, M. Helguera, T. Fahima, and J. Dubcovsky. 2003. Positional cloning of the wheat vernalization gene VRN1. Proceedings of the National Academy of Sciences 100: 6263-6268.

Yoshihara, T., F. Goto, K. Shoji, and Y. Kohno. 2010. Cross relationships of Cu, Fe, Zn, Mn, and Cd accumulations in common japonica and indica rice cultivars in Japan. Environmental and Experimental Botany 68: 180-187.

Zhang, W., S. Chao, F. Manthey, O. Chicaiza, J. Brevis, V. Echenique, et al. 2008. QTL analysis of pasta quality using a composite microsatellite and SNP map of durum wheat. Theoretical and Applied Genetics 117: 1361-1377.

Zimmerl, S., J. Lafferty and H. Buerstmayr. 2014. Assessing diversity in Triticum durum cultivars and breeding lines for high versus low cadmium content in seeds using the CAPS marker usw47. Plant Breeding 133: 712-717.

Zook, E.G., F.E. Greene, and E. Morris. 1970. Nutrient composition of selected wheats and wheat products. VI. Distribution of manganese, copper, nickel, zinc, magnesium, lead, tin, cadmium, chromium, and selenium as determined by atomic absorption spectroscopy and colorimetry. Cereal Chem 47, 720-731.

CHAPTER 2. GENETIC MAPPING OF A NEW SINGLE NUCLEOTIDE POLYMORPHISM (SNP) TIGHTLY ASSOCIATED WITH LOW CADMIUM UPTAKE IN DURUM WHEAT (TRITICUM TURGIDUM L.)

2.1. Abstract

Cadmium (Cd) is a toxic heavy metal with no known biological function. The maximum level of Cd concentration allowed in the international market for wheat grain is 0.2 mg kg-1. Higher Cd levels in durum wheat (*Triticum turgidum* L. var. *durum* Desf) may threaten its export. To develop new durum wheat cultivars low in Cd uptake and speed up the selection process in breeding programs, this study attempted to identify SNP(s) associated with a low Cd uptake in the durum experimental line D041735. The QTL analysis of the grain Cd content in a recombinant inbred line mapping population derived from D041735 and Divide (a high Cd accumulator cultivar) using wheat 90k SNP chips identified only a single major QTL for Cd uptake on chromosome arm 5BL. The QTL was detected in a 0.3 cM interval flanked by SNP markers RAC875_c20785_1219 and Kukri_c66357_357. The BLAST analysis of these flanking markers suggested the Aluminum Induced Protein Like Protein and heavy metal transporter ATPase 3 as putative candidate genes for the major QTL

2.2. Introduction

Accumulation of Cd in soils and edible crops and its transfer to the human diet is a well-known problem around the world. Durum wheat (*Triticum durum* L. var. durum Desf.), in particular, accumulates high Cd concentrations relatively easily. Grain harvested in North Dakota has been reported to have Cd levels ranging from 0.025 to 0.359 mg kg^{-1} (Knox et al., 2009, and Wu et al., 2002). Developing varieties with a low Cd level is always an important objective of durum wheat breeding programs not only for the domestic market, but also for the

export market, as high Cd levels may threaten the wheat's exportability. Unfortunately, phenotyping the Cd content in seeds is costly and time consuming. After Penner et al. (1995) and Knox et al. (2009) identified molecular markers associated with this trait on chromosome 5BL, the use of genomic tools became a practical and efficient approach for developing new cultivars with a low Cd content in durum wheat. Durum cultivars containing the low Cd uptake gene on this chromosome concentrate 50% less Cd in their seeds (Randhawa et al., 2013). Recently, the use of SNP array became a powerful genetic tool for identifying marker-trait association in mapping studies. According to Pootakham et al. (2015), depending on the species, the frequency of SNPs that occur in the plant genome is one per~100-500 bp. Advances in high throughput sequencing led to the development of 90K SNP wheat chip, which provides a large database of marker information (Wang et al., 2014). Fortunately, Maccaferri et al. (2015) have published the consensus map of the 90K SNP marker in tetraploid wheat. Tightly linked user-friendly markers developed from these SNPs are more effective in molecular wheat breeding due to their speed and cost effectiveness. According to NASS (2015), the economic value of durum wheat production in North Dakota is more than $329 million per year, making it an especially economically important crop.

As is clear from above, detecting SNP markers for important traits such as Cd uptake and subsequently develop user-friendly markers from these SNPs can ease and speed up the selection progress in durum breeding programs. The recent discovery of linked SNP markers to the Cd accumulation locus has been reported in some North Dakota durum cultivars (AbuHammad et al., 2016). In this study, six SNPs were discovered associated with Cd accumulation on chromosome 5BL using a bi-parental and an association mapping population. The major QTL from the bi-parental population derived from a cross between high CD Grenora (Elias and

Manthey, 2007) and low Cd Haurani explained 54.3% of the phenotypic variation for Cd uptake in this population. The KASP marker developed from the Cd associated SNP (Ex_c1343_2570756) was validated in another population from a cross between low Cd Strongfield (Clarke et al., 2005) and high Cd Alkabo (Elias and Manthey, 2007). In this population, it explained 64% of the phenotypic variation. Further validation of the associated markers showed that Ex_c1343_2570756 was the most useful marker for tracking the introgression of major CD locus in NDSU's germplasm (Salsman, 2016).

Recently, an experimental line called D041735 was developed in the durum wheat breeding program at NDSU for Fusarium head blight (FHB) caused by *Fusarium graminearium Schwabe* resistance. The line displays a very low Cd uptake phenotype. Because the genetic basis of Cd accumulation in durum wheat is still unclear and has not been well-defined, a phenotype and genotype dissection study on any new sources of diversity in the phenotype of this trait, either from un-adapted or bred sources, can lead to a better understanding of Cd accumulation in durum wheat.

That D041735 is a well-adapted line in North Dakota is of great significance for breeding low Cd genotypes. At present, two sources of low Cd uptake are being used in the durum wheat breeding program at NDSU: the Canadian cultivar Strongfield (Clarke et al., 2005), and the Syrian cultivar Hurrani. Strongfield is adapted to the western part of the North Dakota durum growing region, while Hurrani is un-adapted for this region. Therefore, generating widely adaptive durum wheat with a solid low Cd uptake for the state would mark a great success for the program. Any new source for low Cd uptake could be used to produce high quality durum wheat that meets food safety and exportability requirements.

This chapter covers the first step of dissecting phenotype/genotype association in D041735 to gain an understanding of the genetic basis for low Cd uptake. The objective was to identify the major QTL associated with low Cd uptake in a population derived from a cross between a high Cd cultivar and the low Cd durum experimental line D041735 using a high-density genotyping assay. This genotyping assay enabled the analysis of data across 90K of markers and provides a robust size of genetic variation. Finally, this chapter tests the null hypothesis that a major QTL associated with low Cd uptake exists on chromosome 5B in the population derived from D041735 line.

2.3. Material and Methods

2.3.1. Mapping Population

In this study, a RIL mapping population was developed from a cross between the low Cd uptake line D041735 and the high Cd uptake cultivar Divide. NDSU released Divide was (Elias and Manthey, 2007) as a high yielding cultivar of excellent quality and a moderate level of FHB resistance.

All RIL populations in this study were developed by the single seed descent method. A cross between D041735 and Divide was made in the spring of 2012 in the Lord and Burnham greenhouse at NDSU. F_1 seeds were planted in the fall of 2012, and F_2 seeds were harvested by the end of December 2012. In the spring of 2013, 300 F_2 seeds were planted in the greenhouse. One spike per plant was harvested and planted as F_3 head rows in the summer of 2013 in Langdon, North Dakota. One F_3 spike was harvested from each row. Three F_4 seeds from each spike were planted in pots in the greenhouse in the fall of 2013. These pots were then thinned to one plant per pot after germination and seedling establishment. F_4 spikes were harvested from each plant. F_5 seeds from the harvested F_4 spikes were planted in the spring of 2014 in the

greenhouse in 6 to 8 pots for seed increase. $F_{4:6}$ RIL was planted in two replications in preliminary yield trials at two locations (Prosper and Langdon, North Dakota).

A total of 196 individuals, including parents and checks with a high phenotype variation in Cd level were evaluated for Cd and other macro- and micro-elements in seeds. The checks used in this study were low Cd level Strongfield and CDC-Verona (Pozniak, Fox, et al., 2009), and high Cd uptake Carpio (Elias, Manthey, et al., 2015) and Joppa (Elias and Manthey, 2016). A simple lattice design was used to plant the preliminary trials at the two locations. In addition to the Cd phenotyping, data were collected on yield, test weight, kernel weight, plant height, heading date, and lodging for breeding purposes.

2.3.2. Phenotyping

To phenotype the grain Cd content, eight spikes of each genotype were randomly harvested using chromium knives and were threshed using an uncolored metal thresher to prevent any risk of Cd contamination in the samples. Seeds from one spike were kept as a remnant, some of which were later grown in the greenhouse for DNA extraction. The seeds from seven spikes for each genotype were sent to the College of Agriculture and Life Science, Nutrient Analysis Laboratory at Cornell University in Ithaca, New York, to estimate their concentration of Cd and other elements. For this purpose, seeds from each genotype were milled and dried in an oven. Then, 0.5 g of the flour of each genotype was dissolved into a solution consisting of Nitric and Perchloric acids. To break down the flour into its components, the mixture of each sample was placed in a fluorocarbon container and heated. After the containers were sufficiently cooled, each sample was diluted up to 20 ml in volume. Finally, a SW-846 method defined by the Environmental Protection Agency was used to analyze the concentration of Cd and other elements for each sample.

The phenotypic data were analyzed for each location separately using the Statistical Analysis System (2008). Homogeneity of error variances between the two locations were tested by Levene's as well as Brown and Forsythe's homogeneity tests. Analysis of the variance across locations was also performed to determine if there was a significant genotype by location interaction. In the statistical analysis, RILs (genotypes) were considered fixed effects, while locations, replication within locations, block per replication per RIL, and location per RIL were treated as random effects. Means were scored and separated by Fisher's Protected LSD at the 5% level of significance.

2.3.3. Genotyping

2.3.3.1. Molecular Marker Analysis

Seeds from each genotype were grown in the greenhouse. Two inches of leaf tissue were collected for each genotype and used for DNA extraction. To avoid DNA contamination between individuals, collection tools were cleaned with ethanol after harvesting each genotype. The leaf tissues were collected in 96-well blocks containing 2.5 mg of silica gel. Samples were then sent to the USDA-ARS Cereal Crop Genotyping Laboratory in Fargo, North Dakota, for molecular analysis. The DNA extraction protocol can be found on the USDA website: *http://wheat.pw.usda.gov/GenotypingLabs/fargo.html.* This protocol is a modification of the original protocol developed by Pallota et al. (2003).

The DNA samples were genotyped using wheat 90k SNP chips (Wang, Wong, et al., 2014) at the same laboratory.

The genotyping data was analyzed using Illumina's GenomStudio Software. When the assay data from several individuals are plotted, distinct patterns emerge. Samples with identical genotypes for an assay locus exhibit similar signal profiles. For diploid organisms, bi-allelic loci

are expected to exhibit three clusters. Each cluster represents one of the three genotypic classes AA, AB, and BB. In an $_{F6}$ RIL population, two clusters (AA and BB) are expected for each segregating marker assay. The necessary corrections for each genotype were done manually to make sure all possible errors related to cluster assessment were edited. Moreover, SNPs with a high missing data rate (> 20 %) and low allele frequency (< 0.4) for any of the parental genotypes were removed. The final genotyping data were exported to an Excel file for constructing the genetic map.

2.3.3.2. Genetic Linkage Map Construction

To construct the SNPs linkage map for each chromosome, genotyping data from the GenomeStudio software were exported to an Excel file. Markers were sorted according to parental genotypes. Initially, all monomorphic markers were deleted. The linkage maps were developed using MapMaker 3.0 (Lander and Botstein, 1989) and CartaGene V.1.2.3R (De Givry et al., 2005)). From the final list of polymorphic markers, a total of 5 to 10 markers per chromosome were selected as anchors for developing linkage maps. The markers were selected to represent the length of the chromosome based on a published durum wheat consensus map (Maccaferri et al., 2015). MapMaker was used to generate linkage groups based on a minimum LOD score of 3.0 and a maximum recombination frequency of 50%. The markers in each linkage group were then individually used in CarthaGene to estimate the marker order and genetic distances as described elsewhere (Kumar et al., 2012a, 2012b, and 2016). Kosambi function (Kosambi, 1943) was used to convert recombination frequency into map distances (CentiMorgans).

2.3.4. QTL Analysis

QTL analysis was performed on individual environmental data as well as on the mean data across environments using the Inclusive Composite Interval Mapping (ICIM) method available in software QTL IciMapping V 4.1 (Meng et al., 2015). The statistical method for ICIM is based on an initial step-wise regression followed by a one-dimensional scanning for mapping additive effect and completed by a two-dimensional scanning for mapping epistasis effects. In ICIMAPPING, the markers' unique loci were used for each linkage group. Markers for a linkage group were defined by the cumulative distance. A permutation test was used to determine the threshold LOD value for asserting significant additive QTL or epistatic QTL.

2.3.5. KASPar Assay Development for Validation Testing

To successfully apply the MAS approach for breeding programs, it is essential to convert trait-associated markers to the breeder-friendly markers. In this study, the sequences of two flanking SNPs associated with a major QTL for Cd were used to develop KASPar primers (Kompetitive Alelle Specific PCR; LGC Ltd., Teddington, United Kingdom). KASPar is a user-friendly PCR-based assay that can easily separate two different SNPs at a specific locus and is used for genotyping at the USDA-ARS Cereal Crop Genotyping Laboratory in Fargo, North Dakota. In this laboratory, first DNA samples are added to 96-well plates using a matrix 2×2 robot. The samples are then dried at 65˚C. A PCR reaction is prepared from 2 µl water, 2 µl master mix (specific for the Roche Light Cycler ordered from LGC Ltd., Teddington, United Kingdom), and 0.055 µl allele specific primer. The reactions are placed on an ABI GeneAmp PCR machine for 32 cycles, and the plates are read on a Roch Light Cycler 480 (Roche Life Sciences, United States).

2.4. Results

2.4.1. Phenotypic Analysis

The analysis for phenotypic performance of parents showed that D041735 and Divide are significantly different for grain Cd level. The range of the Cd level among progenies varied from 0.021 to 0.242 mg/kg in Langdon, and from 0.092 to 0.524 mg/kg in Prosper (Table 2-1). The ANOVA showed that genotypes were highly variable for Cd content in both locations. Figure 2-1. shows the distribution of phenotypic performance of RILs in the two locations. Nineteen lines in Langdon and 14 lines in Prosper showed a lower Cd level than D041735, the low Cd parent. Moreover, 50 lines in Langdon and 37 lines in Prosper showed Cd levels higher than Divide. The genotype × environment interaction was therefore due to magnitude rather than rank. In general, the Cd content of genotypes in Langdon was always lower than in Prosper. This is probably because of the different soil type in these two locations even though both are considered to be high Cd possessors. The soil type in Langdon is Svea and Barns, while it is Perella and Bearden in Prosper.

Although the continuous variability among the data might suggest that Cd accumulation can be regulated by multiple genes, the high heritability value (87% in Langdon and 82% in Prosper) indicates the genetic components have more of an effect on grain Cd content than environment (Table 2-2). Because Levene's homogeneity test showed a different result from Brown and Forsythe's homogeneity test, the data were not pooled across locations, instead the results for the single locations are reported in Table 2-1.

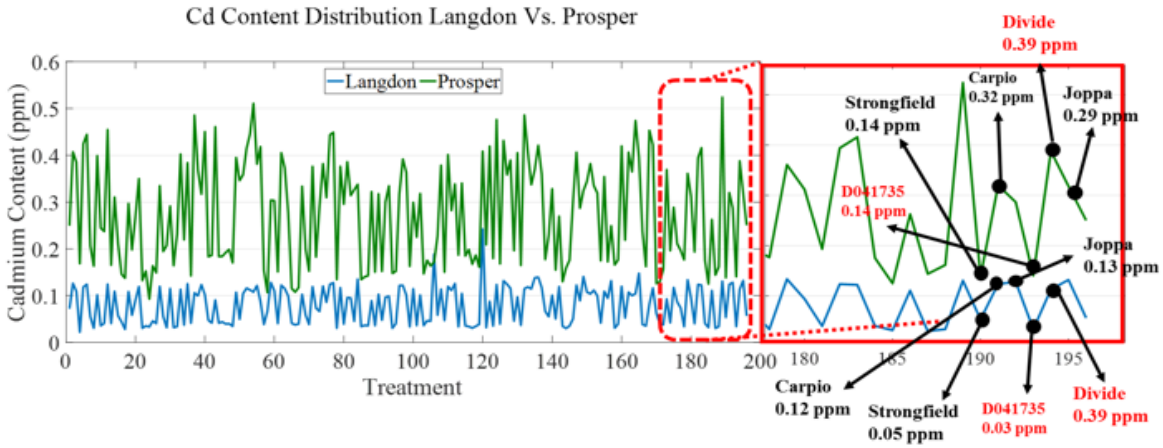


Figure 2-1. Comparative phenotypic performance distribution of RILs in two locations (Langdon and Prosper, North Dakota)

Table 2-1. Mean Cd uptake of parents and checks and the range of the mapping population

D041735 × Divide (RIL population)		
Parents	Cd Content (mg/kg)	
	Langdon	Prosper
D041735	0.034	0.140
Divide	0.113	0.387
Checks		
Strongfield	0.053	0.138
CD-Veronica	0.056	0.250
Joppa	0.128	0.286
Carpio	0.124	0.320
The rang of the data		
Minimum	0.021	0.092
Maximum	0.242	0.524
LSD	0.047	0.142

Table 2-2. Descriptive statistical parameters in RILs derived from D041735 × Divide

Location	Langdon	Prosper
No.of lines	392	392
Mean	0.079	0.27
Sd	0.043	0.120
CV%	30.3	26.3
MS (Lines)	0.003	0.023
MS (Error)	0.00058	0.0052
F	5.50**	4.58**
Pr>F	<.0001	<.0001
Heritability	87 %	82 %

Sd= Standard Deviation, CV%= Coefficient of Variation, MS= Mean Square, F= Fisher's test, LSD = Least Significant Difference comparing individuals

2.4.2. Framework Linkage Map

After filtering genotypic data, a total of 3,973 polymorphic SNP markers were selected for constructing a linkage map. Out of those markers, a total of 3,923 were successfully mapped onto 28 linkage groups, representing all 14 durum wheat chromosomes (Apendix). One linkage group was observed for chromosomes 1A, 3B, 4B, and 6B. Two linkage groups each were observed for chromosomes 1B, 2B, 3A, 5B, 7A, and 7B. Finally, three linkage groups each were observed for chromosomes 2A, 4A, 5B, and 6A. Linkage groups 4A1 and 7A1 had the minimum number of markers (two), and linkage groups 3A1 and 3B had the maximum number of markers (74). The total map length was 2,137.5 cM. The 3,923 markers represented 849 unique loci, with an average distance of 2.51 cM between two loci. A total of 1,856 markers representing 426 unique loci were assigned onto the A-genome. These loci markers cover a map length of 953.5 cM, with an average distance of 2.2 cM per locus. A total of 2,067 markers representing 423 loci markers were mapped onto the B-genome. These loci cover a map length of 1,184.0 cM, with an average distance of 2.8 cM per locus (Table 2-3).

Table 2-3. Summary of the SNPs linkage map of the durum wheat population derived from D041735 × Divide

Linkage Groups	No. of Markers	No. of unique loci	Map length (cM)	Average map density (cM/locus)
1A	238	54	117	2.1
2A-1	69	20	37.6	1.8
2A-2	8	3	1.1	0.3
2A-3	48	13	14.8	1.1
3A-1	328	74	238.3	3.2
3A-2	37	10	30.5	3.0
4A-1	3	2	2.7	1.3
4A-2	30	14	48.4	3.4
4A-3	312	58	37.5	0.6
5A-1	12	3	1.1	0.3
5A-2	263	71	147.9	2.0
6A-1	76	15	94.8	6.3
6A-2	131	19	10.4	0.5
6A-3	67	17	8.2	0.4
7A-1	3	2	1.7	0.8
7A-2	231	51	161.5	3.1
1B-1	310	58	99.4	1.7
1B-2	228	36	53.4	1.4
2B-1	374	65	131.2	2.0
2B-2	184	30	67.3	2.2
3B	338	74	226.4	3.0
4B	99	25	158.8	6.3
5B-1	28	11	6.1	0.5
5B-2	164	41	61.4	1.4
5B-3	130	29	86.3	2.9
6B	95	27	162.4	6.0
7B-1	21	8	6.1	0.7
7B-2	96	19	125.2	6.5
A genome	1,856	426	953.5	2.2
B genome	2,067	423	1,184	2.8
Whole genome	3,923	849	2,137.5	2.5

2.4.3. QTL Analysis

Quantitative trait loci mapping detected one major stable QTL associated with Cd uptake in both locations. The SNPs IAAV1448 (left marker) and Kukri_c66357_357 (right marker) tightly flank this QTL within a 0.3 cM region (0.8 to1.1 cM on 5B), with a LOD score of about 50. The left flanking marker also co-segregated with RAC875_c20785_1219, but the right-flanked marker is a unique locus (Table 2-4 Figure 2-2). The Divide allele at the major locus

adds an average of 0.06 mg/kg of Cd into the seeds (0.031 mg/kg in Langdon, and 0.086 mg/kg in Prosper). This QTL explains about 70.6% of the phenotypic variation in seed Cd uptake. In addition to the major QTL, two QTL with a minor effect were also identified, one each on chromosome 4B and 4A2 (Table 2-4, Figure 2-3). These minor QTL were specified to each location. 4B QTL showed an additive effect of only 0.0047 mg/kg Cd content in the Langdon trial, whereas 4A2 QTL had an additive effect of 0.015 mg/kg Cd content in the Prosper trial.

Table 2-4. Summary of the detected major and minor QTLs for grain Cd uptake in the RILs population derived from D041735 × Divide at two locations (Langdon and Prosper, North Dakota) and mean

	Major QTL in Langdon & Prosper			*Minor QTL in Langdon & Prosper*	
Location	Cd-Lnd	Cd-Prs	Cd-Mean	Cd-Lnd	Cd-Prs
Chromosome	5B-3	5B-3	5B-3	4B	4A-2
Position (cM)	1	1	1	86	1
Left Marker*	IAAV1148 RAC875_c20785_1219	IAAV1148 RAC875_c20785_1219	IAAV1148 RAC875_c20785_1219	RAC875_c35152_372	BS00011273_51
Right Marker	Kukri_c66357_357	Kukri_c66357_357	Kukri_c66357_357	Excalibur_c8845_1531	Excalibur_rep_c102565_399
LOD	49.46	41.15	47.96	2.29	2.12
R^2	70.6	64.0	69.5	1.6213	1.8507
Add	0.0313	0.0868	0.059	0.0047	0.0149

*Two co-segregated markers were detected in this position.

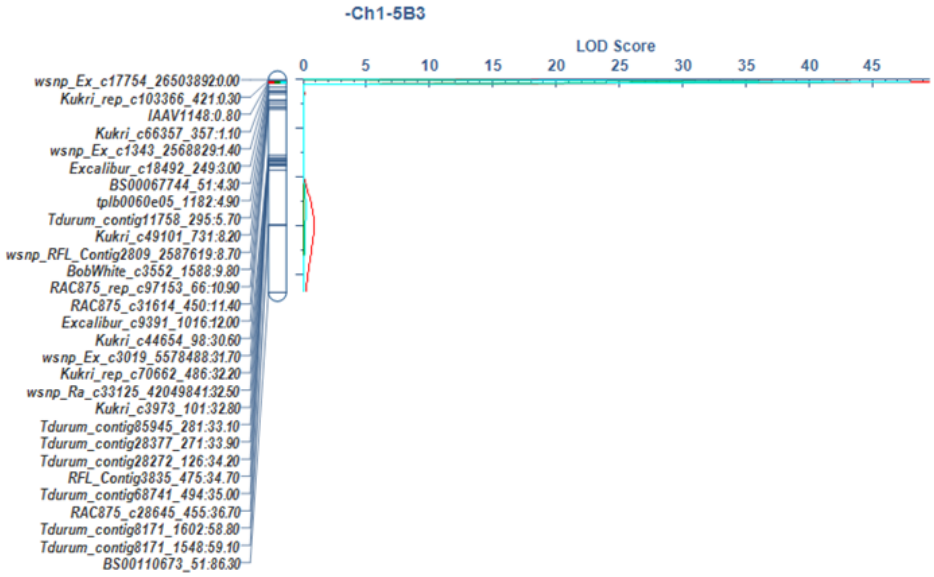


Figure 2-2. A major QTL for Cd grain uptake detected in the D041735 × Divide durum wheat population across two locations (Langdon and Prosper, North Dakota, indicated with the green and red curves, respectively) and mean (the blue curve)

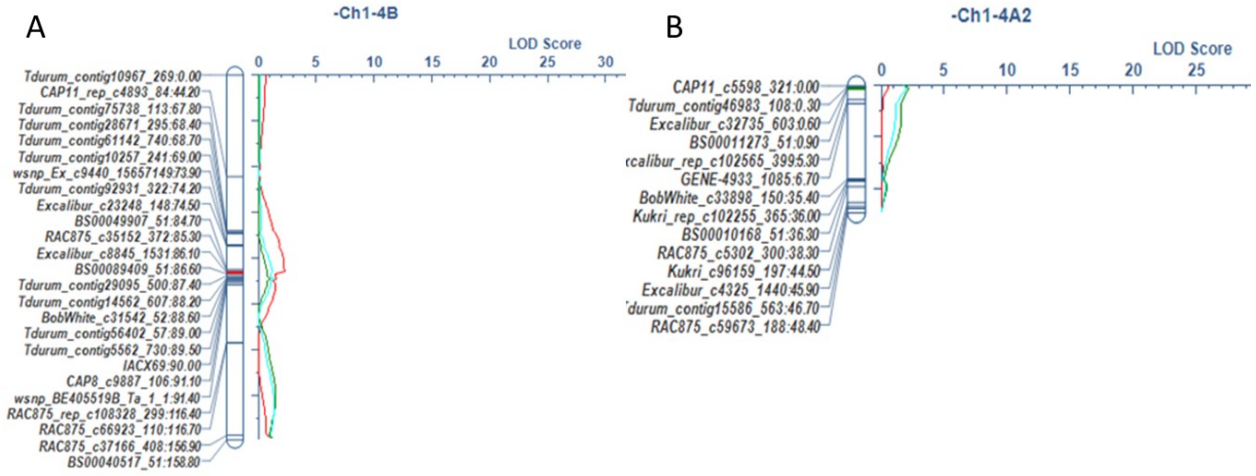


Figure 2-3. Minor QTLs detected in the D041735 × Divide population for grain Cd Content A) The red curve indicates the minor QTL detected in Langdon B) The green curve indicates the minor QTL detected in Prosper. The blue curve indicates the mean.

2.4.4. Epistasis Analysis

IciMapping detected two QTL involved in digenic interactions in the Langdon trial (Figure 2-4, Table 2-5). The first epistatic interaction was placed on linkage group 2B1 (between positions 75 and 85 cM), and the second epistatic interaction was placed on linkage group 4B (between positions 90 and 95 cM). Epistatic interaction 1 explained 4.1% and epistatic interaction 2 explained 2.1% of the phenotypic variation for Cd uptake in seeds. For both epistatic interactions, the D041735 allele at the first locus and the Divide allele at the second locus resulted in a lower Cd level in the Langdon location.

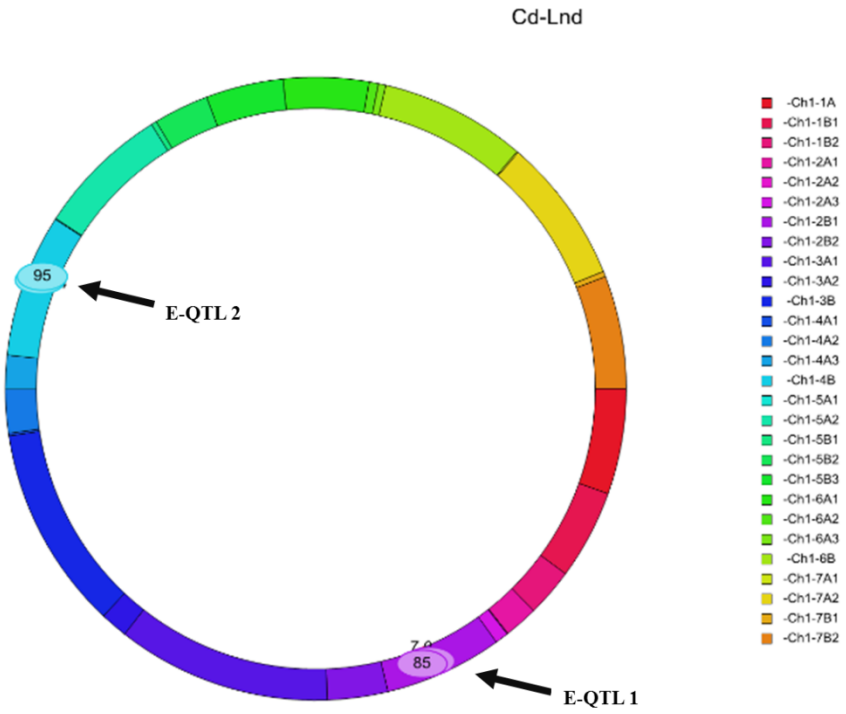


Figure 2-4. The epistasis interaction detected for grain Cd content in the durum wheat population

Table 2-5. Summary of detected epistatic interactions in the RILs population from Langdon

Epistatic Interaction 1				
Location	Chromosome	Position	Left-Marker	Right-Marker
Lnd	2B1	Position 1: 75 Position 2: 85	Tdurum_contig47816_258 Tdurum_contig71139_134	Tdurum_contig71139_134 CAP7_c4304_207
LOD	PVE (%)	Add 1	Add 2	Add by Add
6.9894	4.3804	0.0262	- 0.0277	- 0.0005
Epistatic Interaction 2				
Location	Chromosome	Position	Left-Marker	Right-Marker
Lnd	4B	Position 1: 90 Position 2: 95	IACX69 wsnp_BE405519B_Ta_1_1	CAP8_c9887_106 RAC875_rep_c108328_299
LOD	PVE (%)	Add 1	Add 2	Add by Add
5.3553	2.152	0.0166	- 0.0344	- 0.0204

2.4.5. Candidate Genes or Sequence Analysis of the QTL Region Associated with Cd Uptake

To characterize the major QTL region linked to Cd uptake, sequences of two flanking SNPs were analyzed using EnsemblePlant BLAST (Kersey et al., 2010) against *T. aestivum.* The coding sequence TRIAE_CS42_5BL_TGACv1_405827_AA1335930.1 overlapping with the sequence of Kukri_c66357_357 SNP located in the right side of our QTL was identified (Figure 2-5). The peptide sequence of this query used NCBI BLASTp, which resulted in 100% alignment with a protein in barley. The peptide sequence in the FASTA format was taken and an attempt was made to map it in the IPK-BARLEY database using BLASTp with High-Confidence genes. The analysis showed that the protein translated from this query is MLOC_51340.2, which is an Aluminum Induced Protein-like-Protein. Additionally, three more genes were detected at close distances from Kukri_c66357_357, and the results from BLASTp showed that they are not as important as the detected candidate gene inside the Kukri_c66357_357 SNP (Table 2-6). As explained earlier, Kukri_c66357_357 was a unique locus, and no co-segregated markers were identified in this region. For the other flanking marker, two co-segregated markers were identified (Figure 2-6). Because ensemble blast was not able to find a significant hit for IAAV1148, the blast analysis was performed on the co-segregating SNP RAC875_c20785_1219.

The analysis identified the coding sequence TRIAE_CS42_5BL_TGACv1_404346_AA1296530 at a distance of 41,424 bp from this SNP. This coding sequence included five splices. The same procedure just explained was done for this query. All splices of this coding sequence were aligned with heavy metal transporting P1B-ATPase 3 in *T.aestivum* (100%), durum wheat (99%) and rice (71%). (Table 2-6). The same approach was performed for markers associated with minor QTLs. Blast and annotation analysis for SNPs flanking minor QTL in the Prosper population detected a coding sequence overlapping SNP Excalibur_rep_c102565_399, which was aligned 88% with Peptide Transporter PTR2 in *T. urartu* (Table 2-7).

Table 2-6. Summary of BLAST and annotation analysis for SNP markers flanking the major QTL for Cd uptake detected in the D041735 × Divide population

Flanked Marker Name	Scaffold Name and Position			
Kukri-c66357-357	TGACv1_scaffold_405827_5BL:59771-59864 SNP position in 59813 bp position			
Number	Gene	Distance From SNP (bp)	Function	ID % with
1	**TRIAE_CS42_5BL_TGACv1_405827_AA1335930.1**	0	**AIP-like-Protein**	**100%-Barley**
2	TRIAE_CS42_5BL_TGACv1_405827_AA1335940	974	Transcription factor 3	91 % *T.urartu*
3	TRIAE_CS42_5BL_TGACv1_405827_AA1335950 Splice1-AA1335950.1	63904	Putative/TPK	98% *Ae.tauschii*
	Splice-2AA1335950.2	6520	Putative/TPK	94% *Ae.tauschii*
RAC875_c20785_1219	TGACv1_scaffold_404346_5BL: 180582:181282:1 SNP position in 181231bp position			
1	**TRIAE_CS42_5BL_TGACv1_404346_AA1296530**			
	Splice form-1 AA1296530.3	41424	Heavy metal transporting P1B-ATPase 3	100 % *T.aestivum* 99 % Durum 71 % Rice
	Splice form-2 AA1296530.4	41424	Heavy metal transporting P1B-ATPase 3	
	Splice form-3 AA1296530.1	41388	Heavy metal transporting P1B-ATPase 3	
	Splice form-4 AA1296530.2	41424	Heavy metal transporting P1B-ATPase 3	
	Splice form-5 AA1296530.5	41433	Heavy metal transporting P1B-ATPase 3	

Table 2-7. Summary of BLAST and annotation analysis for SNPs associated with the minor QTL for Cd uptake

Flanked Marker Name	Scaffold Name and Position			
Excalibur_rep_c102565_399	Scaffold TGACv1_scaffold_295511_4AL: 2,019-2,129			
Number	Gene	Distance From SNP (bp)	Function	ID % with
1	TRIAE_CS42_4AL_TGACV1_295511_AA1001980	0	Peptide Transporter PTR2	88% *T urartu*

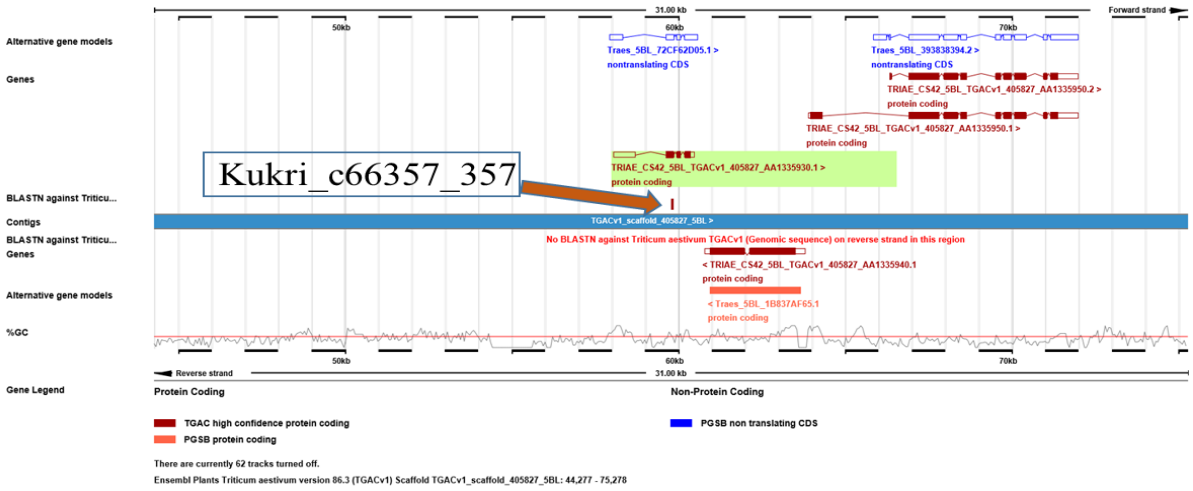


Figure 2-5. Candidate gene showing sequence similarity with flanking marker Kukri_c66357_357, the SNP on the left side of the major QTL (gene region in green)

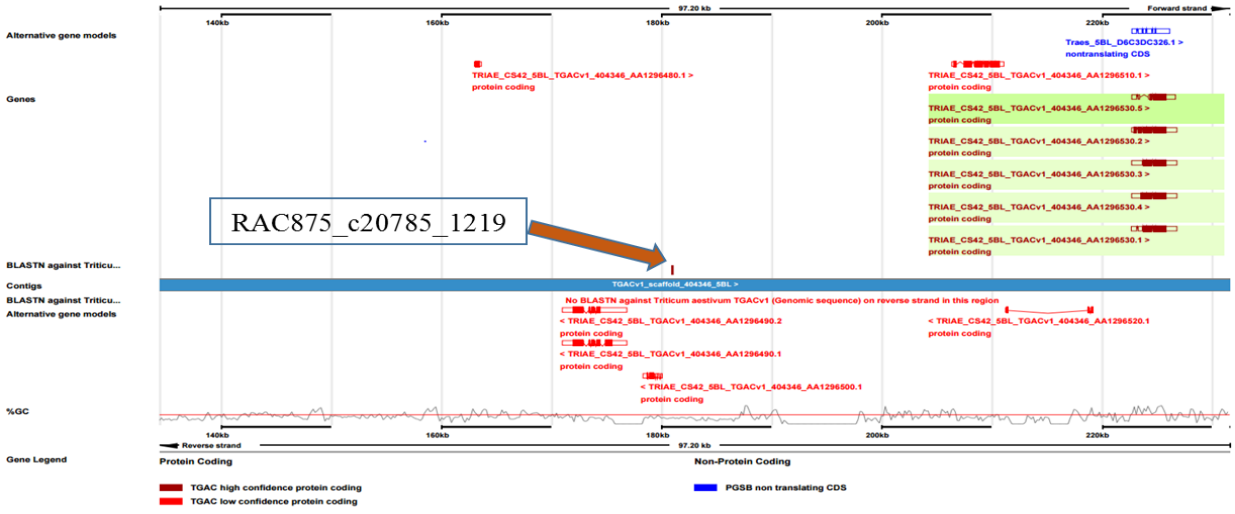


Figure 2-6. Candidate gene identified in the region of RAC875_c20785_1219, the SNP on the right side of the major QTL for grain Cd (gene region in green)

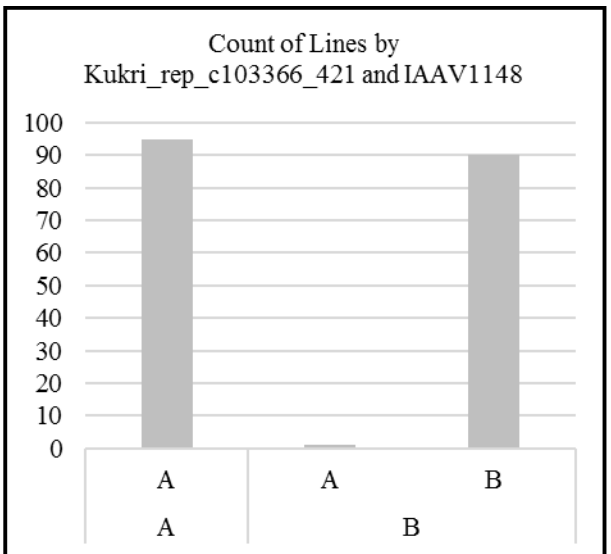
The overlapping genes and related proteins were identified for most of E-QTLs using ensemble *T. aestivum* and NCBI-BLASTp tools, respectively, and are summarized in (Table 2-8).

Table 2-8. Summary of BLASTp results for E-QTLs associated with the grain Cd level

E-QTL SNPs	Overlapping Gene	Function	ID %
Tdurum_contig 71139_134	TRIAE_CS42_2BS_TGACv1 _146086_AA0455010	Ribulose bisphosphate carboxylase small chain	99% *T.aestivum* 100% Barley
	TRIAE_CS42_2BS_TGACv1 _146086_AA0454980	Glyoxylate/hydroxypyruvate reductase B	81% *T.aestivum* 80% Barley
CAP7_c4304_207	TRIAE _2BS_04089466E	Protein kinase	98% Barley
IACX69	TRIAE_CS42_4BL_TGACv1 _320837_AA1049900	Ubiquitin-conjugating enzyme 31	95% Barley
Wsnp_BE405519B Ta_1_1	TRIAE_CS42_4BL_TGACv1 _321115_AA1055680	Ubiquitin carrier protein enzyme 32	100% *Ae.tauschii* 100% rice 100% Brachypodium
RAC875_rep c108328_299	TRIAE_CS42_4BL_TGACv1 _321807_AA1065680	Protein kinase	98% barley 95% *Ae.tauschii*

2.4.6. Effectiveness of Selection for Detected QTLs

Two Cd flanking markers, Kukri_c66357_357 and IAAV1148/ RAC875_c20785_1219, segregated the parental alleles in the progenies in a 1:1 ratio (Figure 2-7). To estimate the efficiency of selection for the *Cdu1* gene, a radar chart was generated based on the genotypic data of these two flanking markers for the RIL population. As shown in Figure 2-8, only 13 out of 184 lines fell into the overlapping area, meaning that 93% of the RILs were successfully divided into two groups of low and high Cd based on the different alleles for these two markers. More importantly, only six individuals with a high Cd level fell into the low Cd category, which means that 96.5% of the lines were accurately selected based on desirable marker alleles.



Kukri_rep_c10 3366_421	IAAV1148/ RAC875_c20785_1219	Count of Lines
A	**A**	**95**
B	**B**	**90**
B	**A**	**1**

Figure 2-7. Segregation ratio of high Cd individuals to low Cd individuals based on parental alleles for flanking markers (Kukri_c66357_357 and IAAV1148/ RAC875_c20785_1219) of the major QTL. (Allele A presents the parent Divide with the high Cd uptake, and allele B presents the parent D041735 with the low Cd uptake)

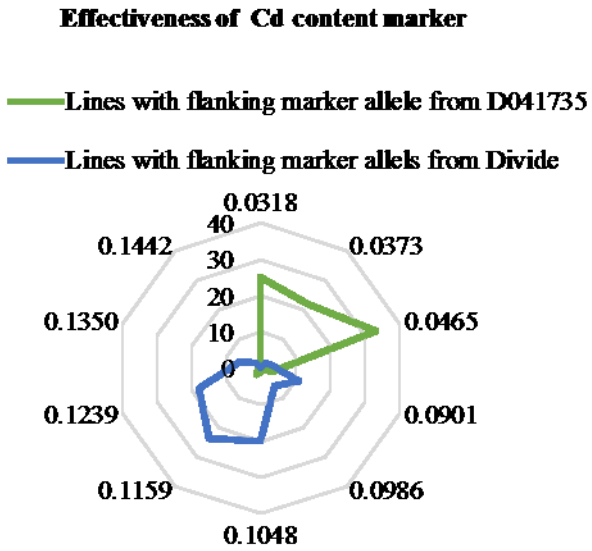


Figure 2-8. Effectiveness of Kukri_c66357_357 and IAAV1148/ RAC875_c20785_1219 in separating low Cd individuals from high Cd individuals in the RILs population derived from D041735 × Divide

2.4.7. KASPar Developed from Flanking Markers

As shown in Table 2-9, the designed KASPar assays separated the high accumulator parent (Divide) from the low accumulator parent (D041735) in the initial test by assigning allele T to the low Cd parent and allele C to the high Cd parent for Kukri_c66357_357. For the second flanking marker, KASPar assays for IAAV1148, which assigned allele C for the low Cd parent and allele T for the high Cd allele, were used.

Table 2-9. Allele assignment to the genotypes of the high and low Cd grain level parents

Marker Name	High Cd Allele from Divide	Low Cd Allele from D041735
Kukri_c66357_357	C	T
IAAV1148	T	C

2.4.8. Validation Test

The KASPar markers designed from the SNPs flanking the major QTL were used for the validation test of 100 inbred lines from preliminary trials (PYT) grown in two locations (Langdon and Williston, North Dakota) in 2014. To further test if these markers could distinguish the integrated low Cd uptake gene into the high Cd uptake cultivars, an additional 212 individuals derived from Transcend were tested. These 212 lines were developed from crosses between North Dakota germplasm and the low Cd uptake Canadian cultivar Transcend (Singh et al., 2012). Surprisingly, all lines, regardless of their phenotype, displayed only one genotype (the same as the high Cd genotype). It was expected that the 50 PYT lines and all the Transcend lines with a low Cd phenotype would show a different genotype at the major Cd locus than the lines with a high Cd phenotype. Two of the low Cd sources, Strongfield and Huarani, also showed the same allele as in the high Cd checks of Divide and Joppa. This result might be because the major QTL was not linked to the Cd uptake gene in these two sources. Therefore, to test the specificity of these markers for lines with the D041735 background, the KASPar markers

were tested on 50 lines derived from a cross between Joppa (a high Cd uptake cultivar) and D041735 grown in 2013 in two locations (Langdon and Williston). In this set of lines, the KASPar markers were able to perfectly separate low and high Cd uptake phenotypes into separate groups. This means that these two flanking markers are effective in selecting low Cd lines in populations where D041735 is one of the parents. Although these markers showed high Cd alleles for Strongfield and Haurani, they were low for Cd uptake.

The SNPs associated with the major QTL identified in this study were located very close to the major Cd uptake gene on chromosome 5B. The validation test suggests that the markers identified in this study are only effective in lines with the D041735 parental background. This encouraged a test to see if this identified QTL is the same as *Cdu1* reported earlier in other low Cd sources. This hypothesis in examined in the next chapter.

2.5. Discussion

This study was conducted to map the genetic region associated with the Cd content phenotype in a RIL population derived from a cross between DO14735 and Divide. The DO14735 line potentially could be considered as a new source for a low Cd content phenotype because it is better adapted to the Upper Midwest than the other two sources (Haurani from Syria and Strongfield from Canada) of low Cd used by the NDSU breeding program.

The RILs in this study's population were significantly different in seed Cd content in two locations in North Dakota. The range of Cd in each environment was adequate to compare different Cd content phenotypes of RILs, which in turn made an excellent population for high density genetic mapping. The RILs showed continuous variation for Cd content in the two environments, suggesting a quantitative nature for the trait. However, high heritability in both locations coincided with results of a previous study (Clarck et al., 1997), suggesting that the

genetic components play a major role in grain Cd content than environmental factors. Moreover, observing transgressive phenotypes in both locations for the mapping population indicates the effect of some minor QTLs and probably epistatic effects on Cd accumulation in grains, which will be discussed below.

This study is the first to report using a 90K SNPs assay for dissection of Cd uptake in wheat, suggesting a more robust QTL detection approach compared to previous QTL mapping studies. A recent study on the genetic dissection of Cd in durum wheat used 9K SNPs and 255 SSRs to assign a total of 330 markers on the whole durum genome (AbuHammad et al., 2016). In comparison, this study mapped 849 unique loci displaying almost three times better coverage of the genome. Further, Shavruvkov (2016) showed that the KASPar markers developed fom 90K SNPs are more useful than CAPs markers developed from EST-derived markers for large scale-wheat experiments. Therefore, compared to the study by Wiebe et al. (2010), which developed CAPs markers from ESTs associated with *Cdu1*, the linkage map in this study may be the most suitable approach for genetic mapping or for detecting the closest marker for Cd grain content. The framework linkage map in this study assigned more SNPs onto the B-genome compared to the A-genome. This coincides with the findings of previous studies, which found more diversity in the B-genome of wheat than the A-genome (Petersen et al., 2006; Feldman et al., 2012; and Panchy et al., 2006).

Since DO14735 is a new source of low Cd with no parental background from other low Cd sources in NDSU's germplasm, an attempt was made to understand the genetics behind the low Cd in this line. This study identified a major QTL tightly linked to the grain Cd content on the long arm of the chromosome 5B. Previous studies also indicated a major QTL for Cd uptake on chromosome 5B (Knox et al., 2009; Wiebe et al., 2010; and Abouhammad et al., 2016). In the

current study, this QTL was placed in a 0.3 cM interval. Comparatively, Abouhammad et al. (2016) placed the major Cd QTL within the region 76-85.5 cM, meaning that this study was able to narrow down the 5B locus 30 times using the 90K SNP assay. The stronger association in this study also resulted in a higher PV and LOD score for the major QTL compared to Abouhammad et al. (2016). The availability of a large amount of wheat sequence data (Maccaferri et al., 2015) made it possible to identify important candidate genes for the major Cd uptake locus that have not been reported previously. Moreover, as expected from phenotypic variation, two minor QTLs as well as some epistasis effects associated with grain Cd content were also detected in this study. Minor QTLs for Cd have been reported previously on chromosomes 2B and 5B (Abouhammad et al., 2016 and Wiebe et al., 2010). As Uemoto et al. (2016) stated, detecting minor QTLs and digenic interaction effects are important for estimating heritability and increasing predictive accuracy in breeding programs. Knowledge about the existence of such effects might open a new window towards a better understanding the relative differences in the range of Cd content phenotypes in different environments. For example, individuals in the Prosper location, constantly showed a higher Cd uptake compared to those in Langdon location. Some possible environmental conditions in these two locations might have an effect on up or down regulation of some minor QTLs associated with Cd uptake. Another reason for the difference could be the interactions between some E-QTLs identified in the Langdon location with a relatively lower Cd uptake compared to those in the Prosper location. Several studies have reported the effect of epistatic interactions in their populations (Kulwal et al., 2005; Zhao et al., 2010; and Talukder et al., 2014). Lower heritability in the Prosper location also indicates a slight genotype by environment (G×E) interaction. Therefore, using statistical tools like QTLNetwork

can be useful to detect the possible gene network and its interactions with the environment for any trait.

The sequence analysis of the markers flanking the major QTL identified two important candidate genes for Cd uptake not reported in durum wheat. One of the candidate genes is Aluminum-induced protein like protein (AIP). To date, the mechanism of AIP families in plants have not been clearly described. However, in hexaploid wheat, a domain present in AIP called Wali 7 (Wheat aluminum induced 7) was isolated from the root with no known function for the encoded protein (Snowden and Gardner, 1993). But recently, it was shown that the transcription of AIP containing the Wali 7 domain increases when a plant is exposed to aluminum, Cd, and copper compared to a control treatment (Jang, et al., 2014). The expression occurs in the plasma membrane, which was expected because it is where the plant is able to protect itself against heavy metal transportation into the vital organs (Singh, Parihar, et al., 2015). Moreover, a proteomics profiling analysis showed that the Wali 7 domain is an important osmotic resistance protein in hexaploid wheat (Ma, Dong, et al., 2016). These observations suggest a possible involvement of an annotated gene in the common mechanism of plant response to the heavy metal stress condition in this study. This is the first study to report finding this candidate gene linked to the Cd uptake locus. Another candidate gene identified in this region is heavy metal transporting PIB-ATPase 3. PIB-ATPase are membrane proteins that use energy from ATP hydrolysis to transport heavy metal ions (Co^{2+}, Cu^{2+}, Cu^{+1}, Zn^{2+}, Co^{2+}, Ag^{+1}, and Cd^{2+}) across the membrane against their concentration gradient (Gourdon, Liu, et al., 2011). Therefore, they play a key role in maintaining ion homeostasis and lipid asymmetry in cells (Kühlbrandt, 2004). In the past, several studies in other crops have reported an association of heavy metal transporting PIB-ATPase with Cd uptake, but none have reported a close linkage between these transporters

and Cd accumulation in durum wheat. In a recent study, AbuHammad et al. (2016) observed a high-sequence similarity of markers linked to the Cd uptake QTL with Leucine-rich repeat protein kinase family protein (in a bi-parental mapping population) and mitochondrial ATP-dependent RNA helicase (in an association mapping panel).

The physiological and molecular results in a study on Cd accumulation in Arabidopsis, showed that a P-type ATPase called AtHMA4 has an important role in the xylem transporting of Cd (Verret, Gravot, et al., 2004). However, it is less likely to find an encoding transporter gene for xylem-loading Cd in wheat, as several studies have shown that Cd in wheat, barley, and rice is loaded into the grains through the phloem, not the xylem (Chen et al., 2007; Harris and Taylor, 2004; and Tanaka et al., 2003).

Also, the association of some genes with the minor and E-QTLs in this study might be a clue for better understanding the genetics of Cd uptake in durum wheat for future physiological studies. For example, several studies showed that crops accumulate more Cd in soils with a higher level of nitrate (Guan, Fan, et al., 2015; Hu, Yin, et al., 2013; and Luo, Du, et al., 2012). This study identified a possible association between an encoding gene for a sub family of nitrate/peptide ABC-transporters with the minor QTL on chromosome 4A with a 0.015 mg/kg additive effect on Cd content in the Prosper location. Moreover, a putative association between Cd accumulation and encoding genes for Protein kinase, Ubiquitin-conjugating enzyme 31, and Ubiquitin carrier protein enzyme 32 was found in our digenic interaction analysis. The activity of Kinase protein families, which are well known as stress-related proteins in plants, will be increased to regulate Cd^{+2} homeostasis (Dias, Monteiro, et al., 2013; Guo, Hong, et al., 2016; Jonak, Nakagami, et al., 2004; and Viehweger, 2014). Moreover, additional studies suggest the interaction of the Ubiquitin-conjugating enzyme with Kinase protein can regulate resistance to

heavy metal accumulation in plants (Chen, Song, et al., 2015, and Feussner, Feussner, et al., 1997).

The only two sources for low Cd uptake available in the NDSU breeding program are a Canadian cultivar and a Syrian cultivar. Therefore, the need for a novel source belonging to the program and well-adapted to this area has been strong. This study's results suggest that selecting for grain Cd content based on two markers flanking the major QTL will be highly effective for successfully separating low Cd from high Cd uptake lines in the population. The durum breeding program at NDSU is the largest in the United States. In this program, low grain Cd content in the advanced lines is always a desirable trait. However, the estimation of Cd in grain samples is very expensive ($17 per sample). The highly effective markers identified in D041735 can be used in earlier generations for screening lines with low Cd content to reduce the cost of Cd measurement in more advanced lines. The validation results using these flanking markers suggest the existence of a different gene or allele for low Cd uptake in the D041735 line as a new source for NDSU's durum breeding program. The lines were monomorphic in the validation tests and had either a Huarani or Strongfield parental background as the source of low Cd uptakes. Therefore, Chapter 3 reports the results of an allelism test of the DO14735 line and the other two sources of low Cd uptake.

2.6. References

AbuHammad, W.A., S. Mamidi, A. Kumar, S. Pirseyedi, F.A. Manthey, S.F. Kianian, et al. 2016. Identification and validation of a major cadmium accumulation locus and closely associated SNP markers in North Dakota durum wheat cultivars. Molecular Breeding 36: 112.

Agricultural Statistics Board. 2015. National Agricultural Statistics Service, U.S. Department of Agriculture.

Chakraborty, N., J. Curley, R.D. Frederick, D.L. Hyten, R.L. Nelson, G.L. Hartman, et al. 2009. Mapping and confirmation of a new allele from soybean PI 594538A conferring RB Lesion–Type resistance to soybean rust. Crop Science 49: 783-790.

Chen, C., Y. Song, K. Zhuang, L. Li, Y. Xia, and Z. Shen. 2015. Proteomic analysis of copper-binding proteins in excess copper-stressed roots of two rice (Oryza sativa L.) varieties with different Cu tolerances. PloS one 10: e0125367.

Chen, F., F. Wu, J. Dong, E. Vincze, G. Zhang, F. Wang, et al. 2007. Cadmium translocation and accumulation in developing barley grains. Planta 227: 223-232.

Clarke, J., T. McCaig, R. DePauw, R. Knox, F. Clarke, M. Fernandez, et al. 2005. Strongfield durum wheat. Canadian Journal of Plant Science 85: 651-654.

Cotton, A.F., G. Wilkinson, M. Bochmann, and C.A. Murillo. 1999. Advanced Inorganic Chemistry. Wiley.

Dias, M.C., C. Monteiro, J. Moutinho-Pereira, C. Correia, B. Gonçalves, and C. Santos. 2013. Cadmium toxicity affects photosynthesis and plant growth at different levels. Acta Physiologiae Plantarum 35: 1281-1289.

De Givry, S., Bouchez, M, Chabrier, P., Milan, D., & Schiex, T. 2005. Carh ta Gene: multipopulation integrated genetic and radiation hybrid mapping. Bioinformatics, 21(8), 1703-1704.

Elias, E. and F. Manthey. 2007. Registration of ‘Divide’durum wheat. Journal of Plant Registrations 1: 7-8.

Elias, E. and F. Manthey. 2007. Registration of “Alkabo” durum wheat. Journal of Crop Registrations 1:10-11.

Elias, E.M. and F. Manthey. 2016. Registration of ‘Joppa’ durum wheat. Journal of Plant Registrations 10: 139-144.

Elias, E.M., F. Manthey, and W. AbuHammad. 2015. Registration of 'Carpio'durum wheat. Journal of Plant Registrations 9: 78-82.

Feldman, M., & Levy, A. A. (2012). Genome evolution due to allopolyploidization in wheat. Genetics 192: 763-774.

Feussner, K., I. Feussner, I. Leopold, and C. Wasternack. 1997. Isolation of a cDNA coding for an ubiquitin-conjugating enzyme UBC1 of tomato—the first stress-induced UBC of higher plants. FEBS Letters 409: 211-215.

Gourdon, P., X.-Y. Liu, T. Skjørringe, J.P. Morth, L.B. Møller, B.P. Pedersen, et al. 2011. Crystal structure of a copper-transporting PIB-type ATPase. Nature 475: 59-64.

Guan, M.Y., S.K. Fan, X.Z. Fang, and C.W. Jin. 2015. Modification of nitrate uptake pathway in plants affects the cadmium uptake by roots. Plant sSgnaling & Behavior 10: e990794.

Guo, H., C. Hong, X. Chen, Y. Xu, Y. Liu, D. Jiang, et al. 2016. Different Growth and Physiological Responses to Cadmium of the Three Miscanthus Species. PloS one 11: e0153475.

Harris, N.S. and G.J. Taylor. 2004. Cadmium uptake and translocation in seedlings of near isogenic lines of durum wheat that differ in grain cadmium accumulation. BMC Plant Biology 4: 1.

Hu, P., Y.-G. Yin, S. Ishikawa, N. Suzui, N. Kawachi, S. Fujimaki, et al. 2013. Nitrate facilitates cadmium uptake, transport and accumulation in the hyperaccumulator Sedum plumbizincicola. Environmental Science and Pollution Research 20: 6306-6316.

Institute, S. 2008. SAS/STAT 9.2 user's guide. SAS Institute Cary, NC.

Ishikawa, S., T. Abe, M. Kuramata, M. Yamaguchi, T. Ando, T. Yamamoto, et al. 2009. A major quantitative trait locus for increasing cadmium-specific concentration in rice grain is located on the short arm of chromosome 7. Journal of Experimental Botany: erp360.

Jang, M.-G., Y.-J. Kim, G.-H. Jang, J. Sukweenadhi, W.-S. Kwon, and D.-C. Yang. 2014. Ectopic overexpression of the aluminum-induced protein gene from Panax ginseng enhances heavy metal tolerance in transgenic Arabidopsis. Plant Cell, Tissue and Organ Culture (PCTOC) 119: 95-106.

Jonak, C., H. Nakagami, and H. Hirt. 2004. Heavy metal stress. Activation of distinct mitogen-activated protein kinase pathways by copper and cadmium. Plant Physiology 136: 3276-3283.

Kersey, P.J., D. Lawson, E. Birney, P.S. Derwent, M. Haimel, J. Herrero, et al. 2010. Ensembl Genomes: extending Ensembl across the taxonomic space. Nucleic Acids Research 38: D563-D569.

Knox, R., C. Pozniak, F. Clarke, J. Clarke, S. Houshmand, and A. Singh. 2009. Chromosomal location of the cadmium uptake gene (Cdu1) in durum wheat. Genome 52: 741-747.

Kosambi, D.D. 1943. The estimation of map distances from recombination values. Annals of Eugenics 12: 172-175.

Kulwal, P., Kumar, N., Kumar, A., Gupta, R. K., Balyan, H. S., & Gupta, P. K. 2005. Gene networks in hexaploid wheat: interacting quantitative trait loci for grain protein content. Functional & integrative genomics 5: 254-259.

Kumar, A., F.M. Bassi, E. Paux, O. Al-Azzam, M. Michalak de Jimenez, A.M. Denton, et al. 2012a. DNA repair and crossing over favor similar chromosome regions as discovered in radiation hybrid of Tr it icum. BMC Genomics 13:339. doi:10.1186/1471-2164-13-339

Kumar, A., K. Simons, M.J. Iqbal, et al. 2012b. Physical mapping resources for large plant genomes: Radiation hybrids for wheat D-genome progenitor Aegilops tauschiiaccession AL8/78. BMC Genomics 13:597. doi:10.1186/1471-2164-13-597

Kumar, A., E. Mantovani, R. Seetan, A. Soltani, M. Echeverry-Solarte, S. Jain, S. Simsek, D, Doehlert, M.S. Alamri, E.M. Elias, S.F. Kianian, and M. Mergoum. 2016. Dissection of genetic factors underlying wheat kernel characteristics in an elite × non-adapted cross using 90k SNP iSelect assay. The Plant Genome 9, doi:10.3835/plantgenome2015.09.0081

Kühlbrandt, W. 2004. Biology, structure and mechanism of P-type ATPases. Nature Reviews Molecular Cell Biology 5: 282-295.

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

Luo, B.F., S.T. Du, K.X. Lu, W.J. Liu, X.Y. Lin, and C.W. Jin. 2012. Iron uptake system mediates nitrate-facilitated cadmium accumulation in tomato (Solanum lycopersicum) plants. Journal of Experimental Botany 63: 3127-3136.

Ma, J., W. Dong, D. Zhang, X. Gao, L. Jiang, Y. Shao, et al. 2016. Proteomic profiling analysis reveals that glutathione system plays important roles responding to osmotic stress in wheat (Triticum aestivum L.) roots. PeerJ 4: e2334.

Maccaferri, M., A. Ricci, S. Salvi, S.G. Milner, E. Noli, P.L. Martelli, et al. 2015. A high-density, SNP-based consensus map of tetraploid wheat as a bridge to integrate durum and bread wheat genomics and breeding. Plant Biotechnology Journal 13: 648-663.

Meng, L., H. Li, L. Zhang, and J. Wang. 2015. QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. The Crop Journal 3: 269-283.

Penner, G.A., L.J. Bezte, D. Leisle, and J. Clarke. 1995. Identification of RAPD markers linked to a gene governing cadmium uptake in durum wheat. Genome 38: 543-547.

Petersen, G., Seberg, O, Yde, M, and K. Berthelsen. 2006. Phylogenetic relationships of Triticum and Aegilops and evidence for the origin of the A, B, and D genomes of common wheat (Triticum aestivum). Molecular phylogenetics and evolution 39(1), 70-82.

Pootakham, W., N. Jomchai, P. Ruang-areerate, J.R. Shearman, C. Sonthirod, D. Sangsrakru, et al. 2015. Genome-wide SNP discovery and identification of QTL associated withagronomic traits in oil palm using genotyping-by-sequencing (GBS). Genomics 105: 288-295.

Pozniak, C., S. Fox and D. Knott. 2009. CDC Verona durum wheat. Canadian Journal of Plant Science 89: 321-324.

Randhawa, H.S., M. Asif, C. Pozniak, J.M. Clarke, R.J. Graf, S.L. Fox, et al. 2013. Application of molecular markers to wheat breeding in Canada. Plant Breeding 132: 458-471.

Salsman, E.L. 2016. Validation of molecular markers associated with grain cadmium in durum wheat (Triticum turgidum L. var. Desf.). North Dakota State University.

Shavrukov Y. 2016. Comparison of SNP and CAPS markers application in genetic research in wheat and barley. *BMC Plant Biol.* 16:11

Singh, S., P. Parihar, R. Singh, V.P. Singh, and S.M. Prasad. 2015. Heavy Metal Tolerance in Plants: Role of Transcriptomics, Proteomics, Metabolomics, and Ionomics. Frontiers in Plant Science. 6.

Snowden, K.C. and R.C. Gardner. 1993. Five genes induced by aluminum in wheat (Triticum aestivum L.) roots. Plant Physiology 103: 855-861.

Stacey, G., S. Koh, C. Granger, and J.M. Becker. 2002. Peptide transport in plants. Trends in Plant Science 7: 257-263.

Tanaka, K., S. Fujimaki, T. Fujiwara, T. Yoneyama, and H. Hayashi. 2003. Cadmium concentrations in the phloem sap of rice plants (Oryza sativa L.) treated with a nutrient solution containing cadmium. Soil Science and Plant Nutrition 49: 311-313.

Talukder, S. K., Babar, M. A, Vijayalakshmi, K, Poland, J, Prasad, P. V. V, Bowden, R, & A. Fritz. 2014. Mapping QTL for the traits associated with heat tolerance in wheat (Triticum aestivum L.). BMC genetics 15: 97.

Tsay, Y.-F., C.-C. Chiu, C.-B. Tsai, C.-H. Ho, and P.-K. Hsu. 2007. Nitrate transporters and peptide transporters. FEBS Letters 581: 2290-2300.

Uemoto, Y., Sasaki. S, Kojima. T. Sugimoto, and T.Watanabe. 2015. Impact of QTL minor allele frequency on genomic evaluation using real genotype data and simulated phenotypes in Japanese Black cattle. BMC genetics 16: 134.

Verret, F., A. Gravot, P. Auroy, N. Leonhardt, P. David, L. Nussaume, et al. 2004. Overexpression of AtHMA4 enhances root-to-shoot translocation of zinc and cadmium and plant metal tolerance. FEBS Letters 576: 306-312.

Viehweger, K. 2014. How plants cope with heavy metals. Botanical Studies 55: 1-12.

Wang, S., D. Wong, K. Forrest, A. Allen, S. Chao, B.E. Huang, et al. 2014. Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. Plant Biotechnology Journal 12: 787-796.

Wiebe, K., N. Harris, J. Faris, J. Clarke, R. Knox, G. Taylor, et al. 2010. Targeted mapping of Cdu1, a major locus regulating grain cadmium concentration in durum wheat (Triticum turgidum L. var durum). Theoretical and Applied Genetics 121: 1047-1058.

Wu, J., W. Norvell, D. Hopkins, and R. Welch. 2002. Spatial variability of grain cadmium and soil characteristics in a durum wheat field. Soil Science Society of America Journal 66: 268-275.

Zhao, X., Qin, Y., & Sohn, J. K. 2010. Identification of main effects, epistatic effects and their environmental interactions of QTLs for yield traits in rice.Genes & Genomics32: 37-45.

CHAPTER 3. A DIAGNOSTIC ALLELISM TEST FOR TWO LOW CADMIUM LOCI IDENTIFIED IN CANADIAN DURUM VS NDSU DURUM

3.1. Abstract

D041735 experimental line was developed from a cross between hexaploid (Sumai 3) and durum wheat by NDSU breeding program. The line is a well-adapted line in North Dakota is of great significance for breeding low Cd genotypes. At present, two sources of low Cd uptake are being used in the durum wheat breeding program at NDSU: the Canadian cultivar Strongfield, and the Syrian cultivar Hurrani. Strongfield is adapted to the western part of the North Dakota durum growing region, while Hurrani is un-adapted for this region. Therefore, generating widely adaptive durum wheat with a solid low Cd uptake for the state would mark a great success for the program. Validation results using the flanking markers tightly linked to the low Cd uptake in DO41735 line, initially suggested the existence of a different gene or allele for low Cd uptake. To test for alleles from detected Cd-linked markers among these three sources of low Cd accumulators, Haurani Strongfield, and D041735 were intercrossed. These markers have been tested on 169 RILs. Allelism testing revealed that the QTL in D041735 line is novel and not the previously mapped QTL *Cdu1*on 5BL. This study therefore confirmed that the D041735 experimental line is a novel source of low Cd uptake in durum wheat germplasms, where the major QTL is most likely introduced from hexaploid wheat

3.2. Introduction

The durum wheat breeding program at NDSU has been using two main sources of durum wheat for introducing low Cd uptake into the breeding lines. One of these sources is Haurani, a landrace from Syria that is non-adapted to the North Dakota region. The other source is Strongfield, a Canadian cultivar (Clarke et al., 2005). Recently, an experimental line was

developed at the NDSU durum wheat breeding program with a Hexaploeid wheat, Sumai3, parental background. The purpose was the introgression of Fusarium Head Blight resistance to durum wheat. The line is designated as D041735. D041735 was found to have a low Cd uptake. Therefore, the line has the potential to be used as a new source of low Cd uptake in durum breeding programs. Because of the expensive chemical analysis for phenotyping Cd content of seeds in durum wheat, molecular markers assisted selection can be a much cheaper and faster tool for identifying low Cd *vs* high Cd content lines. In two separate studies by the University of Saskatchewan in Canada and one study by NDSU, three markers linked to a major Cd uptake gene were mapped using RAPD, CAPs, and a SNP assay, respectively (AbuHammad et al., 2016; Penner et al., 1995; and Wiebe et al., 2010). The RAPD marker (OPC 20) was mapped at about a 5 cM distance from the Cd uptake gene, while the CAPs marker *Xusw*47, developed from an EST in the fine mapping approach, was placed in a 0.14 cM interval containing the putative Cd uptake gene (TdHMA3-B1). Finally, the SNP marker Ex_c1343_2570756, which was identified in an association mapping study, was located in a 4.95 cM interval harboring Cd uptake QTL. All three markers are able to separate low Cd content lines from high Cd content lines in the populations with either the Canadian durum wheat or Syrian durum wheat parental background. However, in one NDSU study, it was observed that the KASPar developed from RAPD (OPC-20) was monomorphic in populations with a D014735 parental background (AbuHammad et al., 2016) and the SNP (Ex_c17754_26503892) markers were monomorphic in the other populations with Haurani and Strongfield backgrounds (Salsman, 2016). This showed the limited use of those markers for selecting low Cd uptake lines in different backgrounds and a need to develop tightly linked more suitable markers for Cd uptake.

In the experiment in Chapter 2, the Cd uptake QTL was mapped to a 0.3 cM interval, flanked by two SNPs, in populations with the D041735 parental background. Surprisingly, Strongfield and Haurani possessed high Cd alleles of those flanking markers. Consequently, in a validation test, these markers were monomorphic for the populations with either the Canadian or Syrian parental background. The closest SNP marker (Ex_c1343_2570756) identified by AbuHammad et al. (2016) was not mapped in the population with the D041735 parental background in this study. Further, the pedigree of D041735 did not involve any of the low Cd durum sources. This suggested that the Low Cd gene identified in this study was probably contributed by the hexaploid parent of D041735. All the above-mentioned observations suggested that the major Cd gene identified in this study could be different than the gene *Cdu1*, identified earlier in durum wheat on chromosome 5BL. Thus, in this chapter our objective is to test the hypothesis that two different genes/alleles are governing low Cd uptake in durum breeding lines in the NDSU durum wheat breeding program.

3.3. Materials and Methods

3.3.1. Plant Material and Phenotypic Data

The three sources for low Cd uptake in the NDSU durum wheat breeding program, D041735, Haurani (a landrace collected by the International Center of Agricultural Research in Dry Area, Syria), and Strongfield (Canadian cultivar, Clark et al 2005) were intercrossed in the fall of 2012 to develop segregating populations for allelism testing and breeding purposes. The single seed descent method, explained in Chapter 2, was used to develop a RIL population for each cross. In 2014, a 13 × 13 lattice experiment of F_6 RILs was designed in two replications and two locations (Prosper and Langdon). Three populations, each with a total of 169 individuals consisting of the three low Cd uptake parents and the checks with a high phenotype variation

(CD-Verona (low Cd uptake), Divide Carpio, and Joppa (high Cd-uptake)), were evaluated for grain Cd content. Data collection and analysis were done as described for the previous experiment in Chapter 2. However, the population from the cross between Haurrani and Strongfield was not used for genotyping because of the monomorphism patterns of both parents for the low Cd uptake gene.

After extracting DNA using the method explained in Chapter 2, parents and checks were genotyped using the Cd-associated co-dominant CAPs marker, *Xusw47*. The above-mentioned lines were also genotyped with another CAPs marker, *Xusw14*, which was mapped 0.2 cm away from *Xusw47*.

Table 3-1. PCR reaction components

PCR reaction components	Volume (µl)
5×Buffer	4
$MgCl_2$	1.6
dNTP	2
Primer	1
Taq polymerase	0.2
DNA	4
Double distilled water	7.2

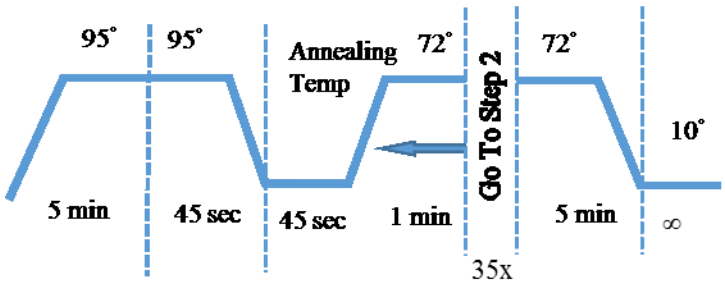


Figure 3-1. PCR Amplification process graph

Table 3-2 shows the details about the CAPs markers used in this study. To date, *Xusw47* is the closest reported marker for *Cdu1*. Therefore, identifying the location of this marker in the

genetic map with reference to the major QTL and SNP for Cd, identified in this study was of interest.

Table 3-2. CAPs markers were subjected to PCR on checks and low Cd parents

CAPs Marker	*Xusw47*	*Xusw14*
Forward primer sequence	GCTAGGACTTGATTCATTGAT	TACAGCCGCTCAGTTGCTC
Reverse primer sequence	AGTGATCTAAACGTTCTTATA	CAACATATGTCTGGCCTACTACTCT
Restriction enzyme	Hpy188I	BsoB1
ESM or STS marker developed from	XBF474090	XBF474164
Annealing Temperature (˚C)	55	55
Digestion incubator (˚C)	37	37

After the polymorphism between the D041735 parent and the other two parents was confirmed by the CAPs marker, a 20µl PCR reaction for each genotype of the RILs population was prepared and amplified. (Table 3-1, Figure 3-1). However, the DNA for 14 individuals from each population was not available because of germination problems. Therefore, a total of 155 individuals from the D041735 × Strongfield population and 155 individuals from the Haurrani × D041735 population plus Sumai 3 were subjected to PCR. The PCR products later were digested by the restriction enzymes mentioned in Table 3-2 and were incubated for three hours at 37˚C. The final digested product was separated and visualized after electrophoresis using a 3.5% agarose gel and 14 µl of ethidium bromide.

3.4. Results

3.4.1. Genotype-phenotype Association

The CAPs markers *Xusw47*and *Xusw14* was polymorphic at the expected alleles among the high Cd uptake genotypes Divide, Carpio, and Joppa and low Cd uptake genotypes Haurani and Strongfield (Figure 3-2. A, B). However, for both *Xusw47* and *Xusw14*, the low Cd uptake genotype D041735 showed the same banding pattern as the high Cd uptake genotypes Divide, Carpio, and Joppa, not the other low Cd genotypes Strongfield and Haurani.

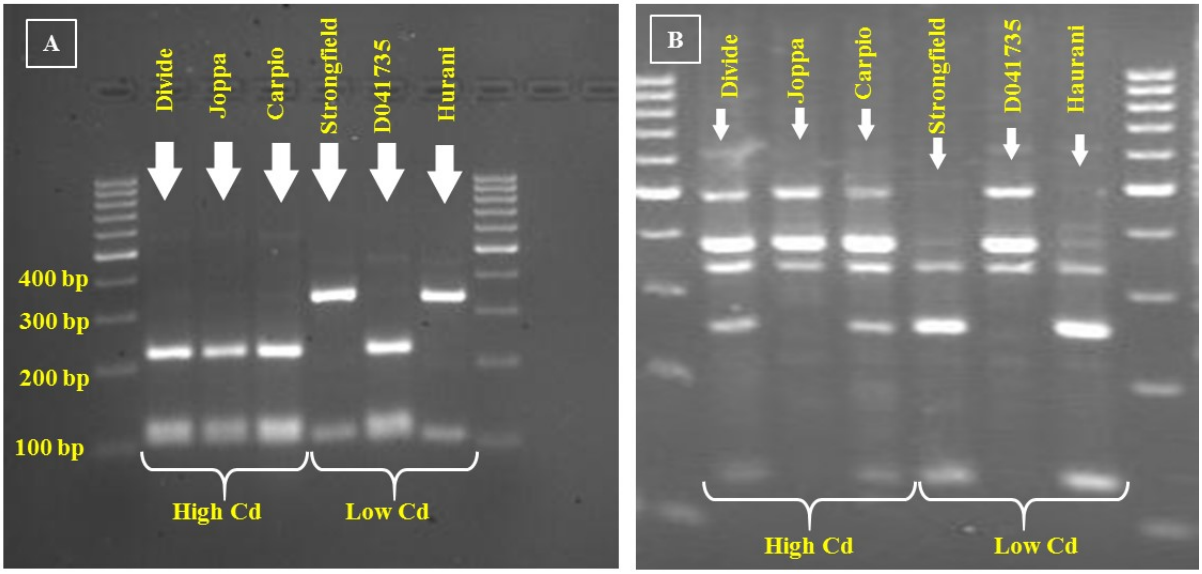


Figure 3-2. Amplification results of *Xusw47* (A) and *Xusw14* (B) on high and low Cd checks. Strongfield, D041735, and Haurani are the low Cd uptake parents in populations derived from intercrossing these three genotypes.

As *Xusw47*showed polymorphism between D041735 (low Cd uptake) and Strongfield (low Cd uptake), it was used to genotype the whole RIL population developed from these two low Cd uptake parents. The results showed that 73 RILs individuals had Strongfield alleles, while 75 RILs had D041735 alleles (Figure 3-3, Figure 3-4, Figure 3-5 and Figure 3-6). Few RILs were heterozygous as expected in the F_6 generation of the population.

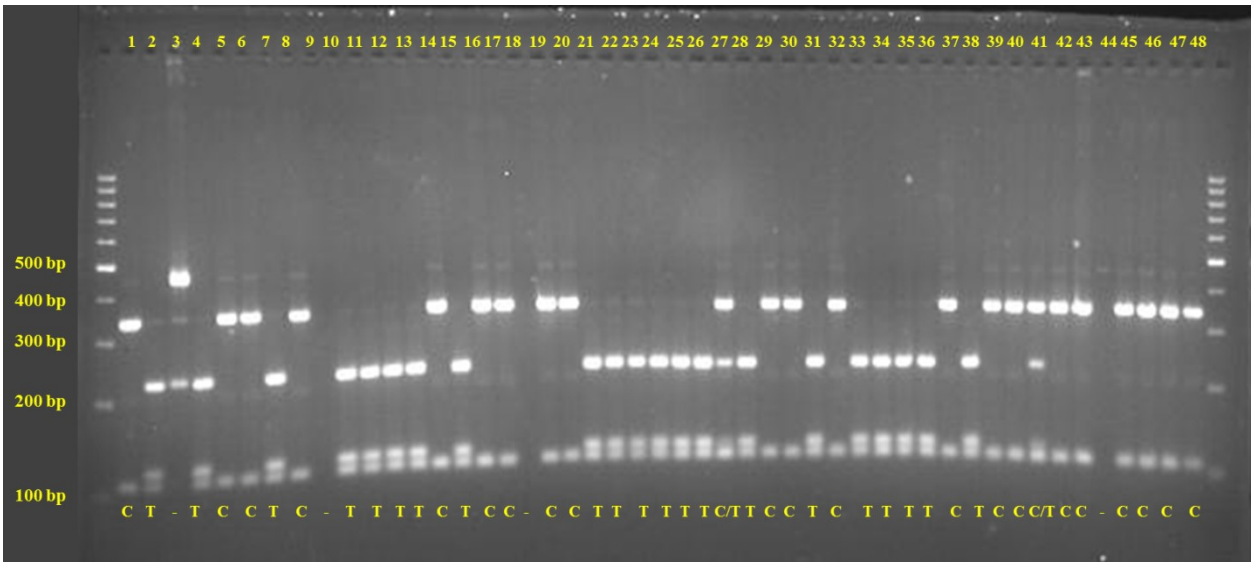


Figure 3-3. Genotyping results of *Xusw47* (banding patterns; 220bp: High Cd, 350 bp low Cd) and designed KASPar of Kukri_c66357_357 (letters at the bottom; T: low Cd, C: High Cd) for lines 1 to 48 of RILs derived from cross D041735 and Strongfield

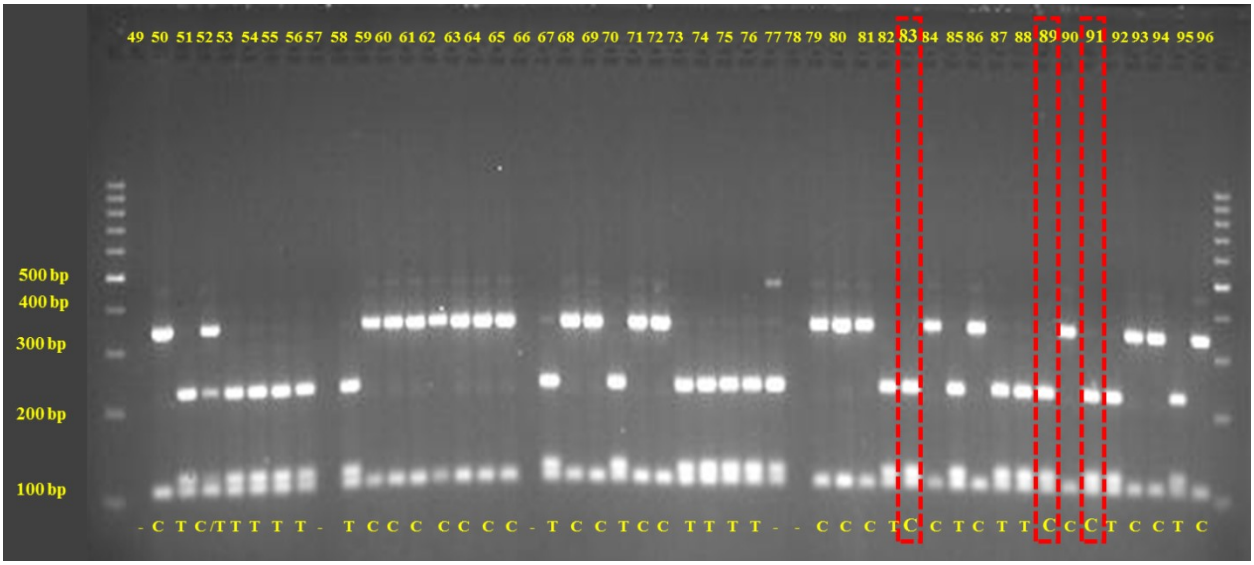


Figure 3-4. Genotyping results of *Xusw47* (banding patterns; 220bp: High Cd, 350 bp low Cd) and designed KASPar of Kukri_c66357_357 (letters at the bottom; T: low Cd, C: High Cd) for lines 49 to 96 of RILs derived from cross D041735 and Strongfield. Red rectangles indicate lines with high Cd content both phenotypically and genotypically.

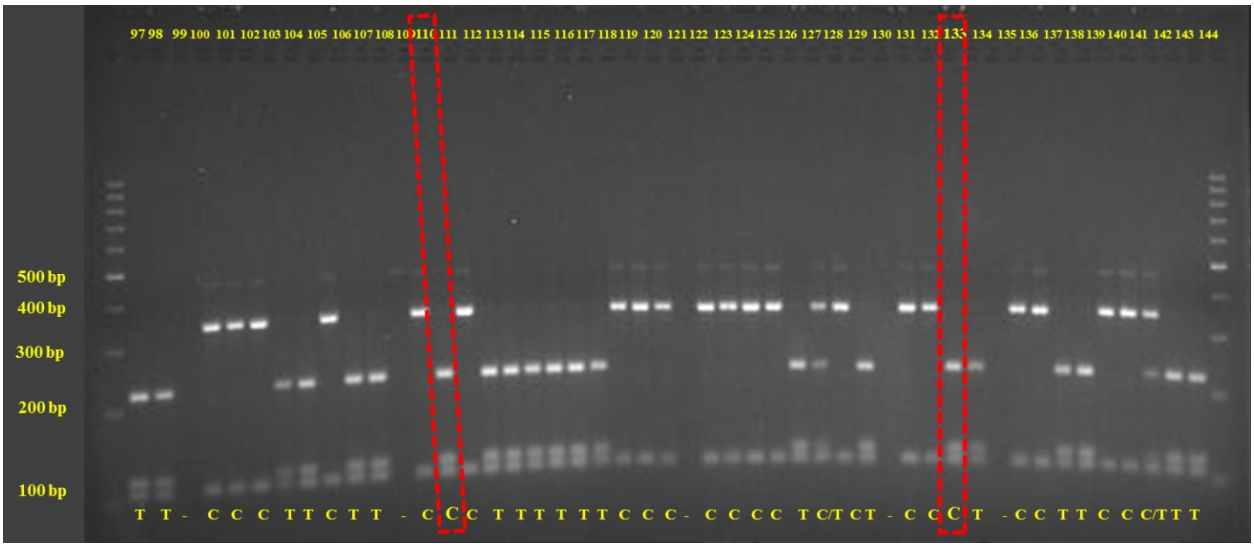


Figure 3-5. Genotyping results of *Xusw47* (banding patterns; 220bp: High Cd, 350 bp low Cd) and designed KASPar of Kukri_c66357_357 (letters at the bottom; T: low Cd, C: High Cd) for lines 97 to 144 of RILs derived from cross D041735 and Strongfield. Red rectangles indicate lines with high Cd content both phenotypically and genotypically.

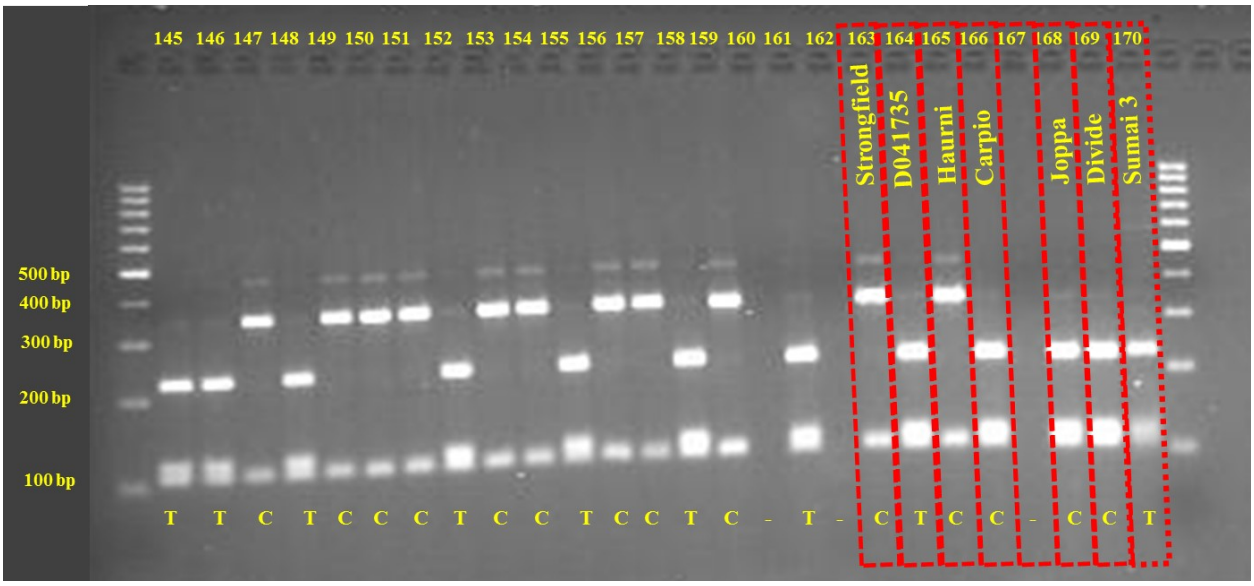


Figure 3-6. Genotyping results of Xusw47 (banding patterns; 220bp: High Cd, 350 bp low Cd) and designed KASPar of Kukri_c66357_357 (letters at the bottom; T: low Cd, C: High Cd) for lines 145 to 170 of RILs derived from cross D041735 and Strongfield. Red rectangles indicate checks.

The RIL population had a mean of 0.35 and 0.084 mg/kg grain Cd level in Prosper and Langdon, respectively. The range was from 0.014 to 0.044 mg/kg grain Cd level in Langdon and from 0.14 to 0.51 mg/kg grain Cd level in Prosper (Figure 3-7). Phenotypic analysis of the

Prosper data using Least Significant Differences (LSD) between the means of progenies and the parents showed that five individuals were significantly different from our low Cd parents and displayed a high Cd phenotype (Table 3-3 and Table 3-4). Therefore, the phenotypic data categorized 143 individuals with a low Cd uptake level and five individuals with a high Cd uptake level (without checks). The phenotypic analysis of the Langdon data similarly categorized these five lines with a high Cd uptake level (Table 3-3 and Table 3-4).

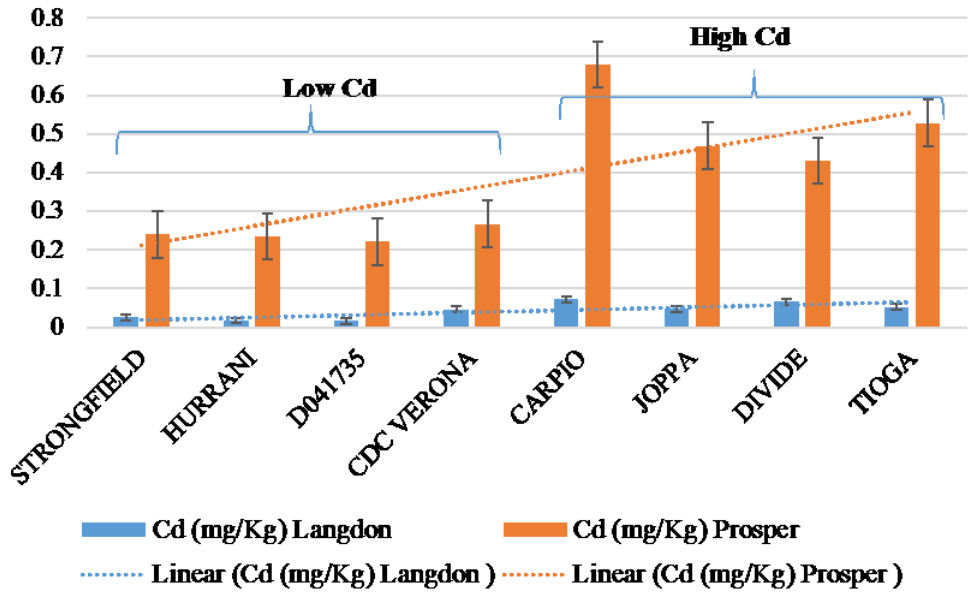


Figure 3-7. Comparing variability of grain Cd level among high accumulators and low accumulators in Langdon (blue bars) and in Prosper (orange bars)

To test if these five individuals also had alleles similar to high Cd checks and the rest of 70 individuals which did not show low Cd band with marker *Xusw47* were inheriting their low Cd uptake gene from D041735, we also tested a KASPar assay for *Kukri_c66357_357*, the marker associated with major Cd QTL in the D041735 × Divide population (Chapter 2). This marker showed different alleles for D041735 (T) and Strongfield (C), and Haurani (C) (Table 3-5). The genotyping of the whole D041735 × Strongfield RIL population with

Kukri_c66357_357, showed that those 70 individuals have the same allele as D041735 (T). As expected, 73 individuals have the same banding pattern as Strongfield and Haurrani, with *Xusw47* marker, and showed allele C with *Kukri_c66357_357*(Figure 3-8). But more importantly, the five individuals with the high Cd uptake phenotype showed the same banding pattern as the high Cd uptake checks with the *Xusw47* marker (220 bp) and the same alleles as the high Cd uptake checks with *Kukri_c66357_357*(C) (Figure 3-4, Figure 3-5 and Figure 3-8). This explains the segregation between two genes governing Cd uptake in the durum wheat parents in this study (Table 3-5).

Table 3-3. Phenotypic performance of parents and checks in the D041735 × Strongfield RIL population

D041735 × Strongfield (RIL population)		
Parents	Cd Content (mg/kg)	
	Langdon	Prosper
D041735	0.016	0.221
Strongfield	0.025	0.240
Checks		
Haurani	0.017	0.235
CD-Veronica	0.046	0.267
Carpio	0.072	0.679
Joppa	0.047	0.469
Divide	0.066	0.430
Tioga	0.052	0.528
Parameter for Normal Distribution		
Mean	0.027	0.248
Minimum	0.014	0.144
Maximum	0.044	0.518
Standard Deviation	0.006	0.051
LSD (0.05)	0.011	0.101

Table 3-4. Lines with a significantly high Cd phenotype derived from the cross D041735 × Strongfield

D041735 × Strongfield (RIL population)		
Significantly high Cd lines	Cd Content (mg/kg)	
	Langdon	Prosper
83	0.032	0.391
89	0.031	0.518
91	0.042	0.423
110	0.028	0.360
133	0.036	0.362

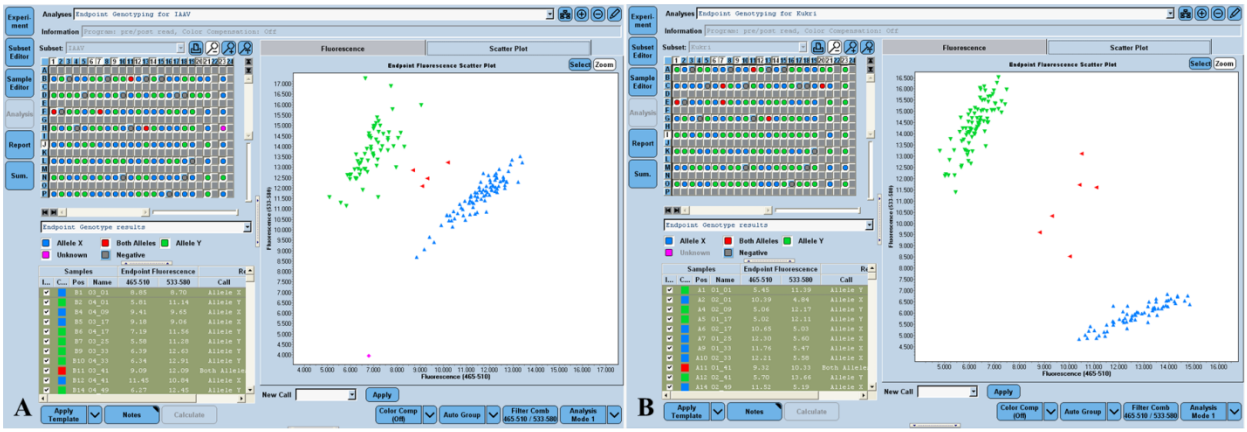


Figure 3-8. Polymorphism results of running KASPar assay *Kukri_c66357_357* (A) and *IAAV1448* (B) on RILs population derived from D041735 × Strongfield

Table 3-5. Summary of the comparison of the genotypic results of CAPs Cd-linked marker (*Xusw47*) with Kasspar assay Cd-linked flanking markers (*Kukri_c66357_357 and IAAV1448*) among parents and high Cd phenotype lines.

High Cd Phenotype Lines	Phenotype	Genotype		
		Cd uptake marker source(Strongfield/Haurani)	Cd uptake marker source(D041735)	
		Xusw47	*Kukri_c66357_357*	*IAAV1448*
		350bp Low Cd 220bp High Cd	T Low Cd C High Cd	C Low Cd T High Cd
83	High Cd	220	C	T
89	High Cd	220	C	T
91	High Cd	220	C	T
110	High Cd	220	C	T
133	High Cd	220	C	T
Parents	**Phenotype**			
D041735	Low Cd	220	T	C
Strongfield	Low Cd	350	C	T

Because Haurani and Strongfield (both low Cd genotypes) showed the same allelic pattern for Cd associated markers (*Xusw47* and *Kukri_c66357_357*), another population developed from a cross between D041735 and Haurani was also used for allelism testing and

validation of the results of the earlier Strongfield and D041735 cross. The summary of descriptive phenotypic performance of this population is shown in Table 3-6.

Table 3-6. Phenotypic performance of parents and checks in the mapping population D041735 × Haurani

D041735 × Haurrani (RIL population)		
Parents	Cd Content (mg/kg)	
	Langdon	Prosper
D041735	0.020	0.196
Haurani	0.022	0.258
Checks		
Strongfield	0.022	0.242
CD-Veronica	0.028	0.262
Carpio	0.127	0.778
Joppa	0.088	0.639
Divide	0.046	0.534
Carpio	0.121	0.786
Parameter for Normal Distribution		
Mean	0.028	0.260
Minimum (no checks)	0.005	0.117
Maximum (no checks)	0.080	0.592
Standard Deviation	0.010	0.069
LSD (0.05)	0.021	0.137

Table 3-7. Lines with a significantly high Cd phenotype derived from the cross D041735 × Haurani

D041735 × Haurrani (RIL population)		
Significantly high Cd lines	Cd Content (mg/kg)	
	Langdon	Prosper
48	0.080	0.541
63	0.066	0.592
126	0.055	0.405
127	0.053	0.527
135	0.046	0.408
139	0.043	0.402

The D041735 × Haurani RIL population showed the same allelic patterns for the *Xusw47* marker as was observed for the D041735 × Strongfield population (Figure 3-9, Figure 3-10, Figure 3-11 and, Figure 3-12). Sixty-one individuals showed the same banding pattern as Haurrani, and 82 individuals showed the same banding pattern as the high Cd uptake checks.

Phenotypic analysis of these 82 individuals explained that 76 of them are categorized as low Cd uptake and only six of them are categorized as high Cd uptake (Table. 2-7).

The DNA from three of these six individuals was not available because of a germination problem in the greenhouse). However, the other three individuals did not show low Cd alleles like in D041735 or a low Cd banding pattern like Haurani, which confirms the high Cd phenotype for these individuals. The 76 individuals inherited their low Cd uptake gene from D041535, and that is why they did not show a low Cd uptake banding pattern like Haurani (Table 3-8).

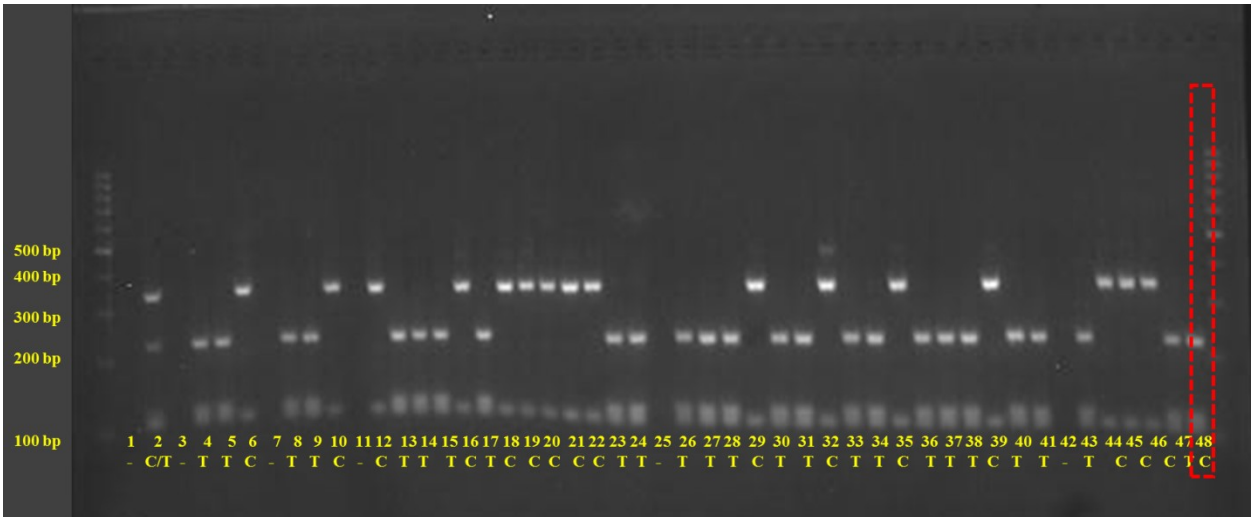


Figure 3-9. Genotyping results of *Xusw47* (banding patterns; 220bp: High Cd, 350 bp low Cd) and designed KASPar of Kukri_c66357_357 (letters at the bottom; T: low Cd, C: High Cd) for lines 1 to 48 of RILs derived from the cross D041735 and Haurani. Red rectangles indicate lines with a high Cd content both phenotypically and genotypically.

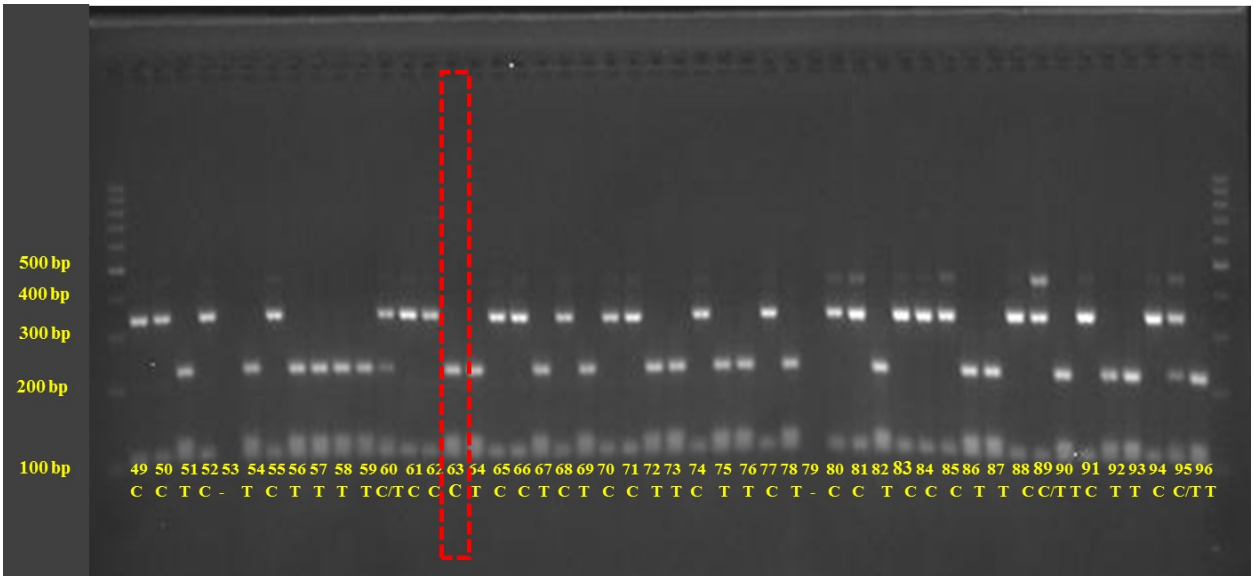


Figure 3-10. Genotyping results of *Xusw47* (banding patterns; 220bp: High Cd, 350 bp low Cd) and designed KASPar of Kukri_c66357_357 (letters at the bottom; T: low Cd, C: High Cd) for lines 49 to 96 of RILs derived from the cross D041735 and Hauraani. Red rectangles indicate lines with a high Cd content both phenotypically and genotypically.

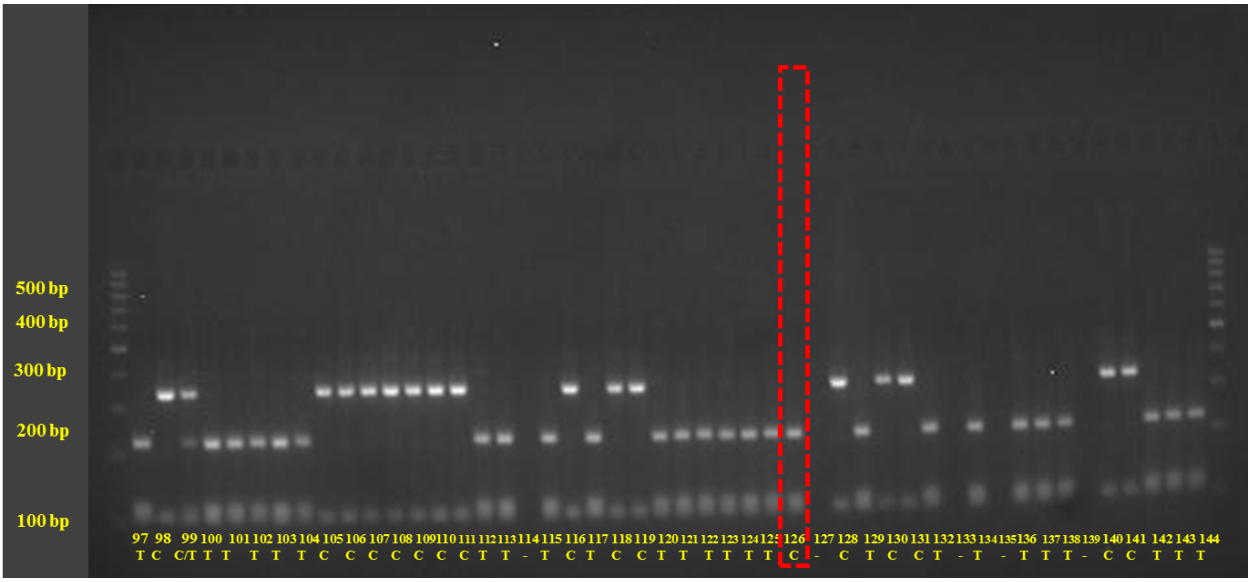


Figure 3-11. Genotyping results of Xusw47 (banding patterns; 220bp: High Cd, 350 bp low Cd) and designed KASPar of Kukri_c66357_357 (letters at the bottom; T: low Cd, C: High Cd) for lines 97 to 144 of RILs derived from the cross D041735 and Haurani. Red rectangles indicate lines with a high Cd content both phenotypically and genotypically.

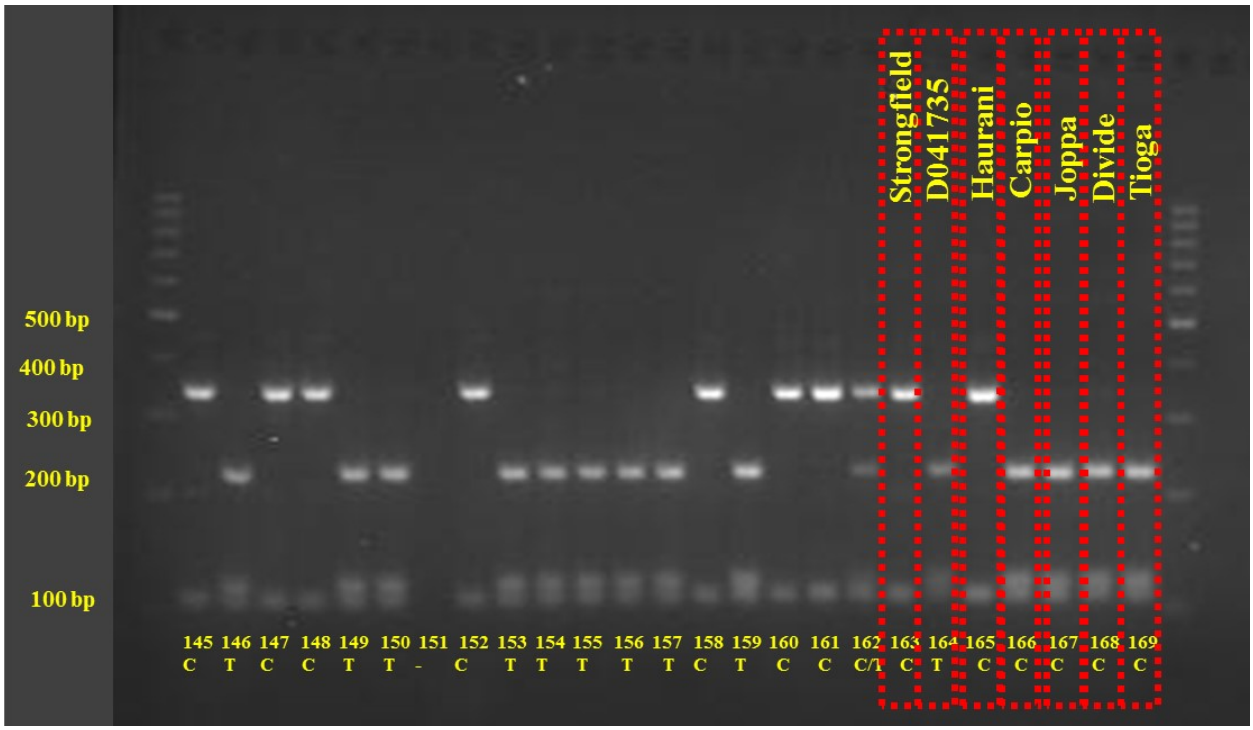


Figure 3-12. Genotyping results of *Xusw47* (banding patterns; 220bp: High Cd, 350 bp low Cd) and designed KASPar of *Kukri_c66357_357* (letters at the bottom; T: low Cd, C: High Cd) for lines 145 to 169 of RILs derived from the cross D041735 and Haurani. Red rectangles indicate checks.

Table 3-8. Summary of the comparison of genotypic pattern of CAPs marker *Xusw47* and with KASPar markers *Kukri_c66357_357* and *IAAV1448* among parents and high Cd RILs

High Cd Phenotype Lines	Phenotype	Genotype		
		Cd uptake marker source (Strongfield/Haurani)	Cd uptake marker source (D041735)	
		Xusw47	*Kukri_c66357_357*	*IAAV1448*
		350 Low Cd 200bp High Cd	T Low Cd C High Cd	C Low Cd T High Cd
48	High Cd	220	C	T
63	High Cd	220	C	T
126	High Cd	220	C	T
127	High Cd	-	-	-
135	High Cd	-	-	-
139	High Cd	-	-	-
Parents	**Phenotype**			
D041735	Low Cd	220	T	C
Haurani	Low Cd	350	C	T

Overall, this study identified about five to six lines with a high Cd uptake in populations derived from the crosses D041735/Strongfield and D041735/Haurani. These results clearly

indicate that two different genes or alleles are controlling Cd uptake in D041735 and Strongfield or Haurani. This also means that D041735 is a new source for low Cd uptake in durum wheat, the gene for which can be introgressed from hexaploid wheat.

3.5. Discussion

This study investigated whether the major low Cd uptake QTLs identified on 5BL in D041735 is same which was identified earlier in populations derived from other low Cd uptake sources Strongfield and Haurani or if they are different. This is because the newly developed low Cd genotype D041735 did not involve any low Cd uptake durum wheat source (Strongfield and Haurani) in its pedigree; rather it was developed from a hexaploid wheat as one of the parents. Hexaploid wheat possesses a low Cd uptake phenotype.

A major QTL for Cd uptake in the Canadian cultivar Strongfield was associated with marker *Xusw47* (Wiebe et al., 2010). This EST-derived CAPs marker successfully separates low Cd uptake from high Cd uptake durum lines by different banding patterns on electrophoresis gel. The same research team also designed other CAPs markers, including *Xusw14*, *Xusw15* and *Xusw17,* from EST-derived markers flanking *Xusw47,* all of which were polymorphic in durum wheat populations (Wiebe et al., 2010). The marker *Xusw47* was found to be highly efficient for the MAS approach for Cd uptake in durum wheat (Zimmerl et al., 2014). However, it has been reported that, surprisingly, none of these markers were polymorphic in a hexaploid wheat population derived from a cross between Chinese Spring (CS-low Cd) and CS-*Triticum dicoccoides* (High Cd), (Wiebe et al., 2010). Wiebe et al. hypothesized that it could be due to existence of another gene on the D-genome of hexaploid wheat controlling low Cd uptake in CS-low Cd. In this study, the markers *Xusw47* and *Xusw14* associated with Cd uptake in Strongfield did not show any polymorphism between D041735 and high Cd uptake durum lines such as

Divide, Joppa, and Carpio. Moreover, the SNPs associated with a low Cd from D041735 did not show polymorphism between low Cd sources (Haurani and Strongfield) and high Cd uptake genotypes (Divide, Joppa, and Carpio), which could confirm the existence of another allele/gene for Cd uptake in wheat.

Table 3-9. Summary of the genotyping results of previously and current markers linked to Cd uptake among three sources of grain low Cd level

Identified markers for Cd accumulation in durum	Allele for Strongfield (Canadian Durum)	Allele for Haurrani (Land Race-Syria)	Allele for D041737 (NDSU experimental line)
Xusw47 (Wiebe et al., 2010)	Low allele	Low allele	High allele
wsnp_Ex_c1343_2570756 (AbuHammad, et al., 2016)	Low allele	Low allele	not been mapped in our population
Kukri_c66357_357current research, 2017	High allele	High allele	Low allele

Strongfield is a Canadian cultivar with a pedigree of /Kyle/Niel/2/Ac Avonlea/DT665. (Clarke, McCaig, et al., 2005). The low Cd characteristic in Strongfield was obtained from Niel, which like Haurani, is a landrace collected by the International Center of Agricultural Research in Dry Area, Syria. In contrast, D041735 is an experimental line developed from a cross between D011543 and Lebsock. The experimental line D011543 was developed from a cross between a hexaploid wheat Sumai 3 and Lebsock. Sumai 3 is a Chinese wheat cultivar (CS). CS is a land race collected from china that has been used in many wheat breeding programs as a source of Fusarium head blight resistance. Since Sumai 3, like most of the hexaploid wheat cultivars, possesses the low Cd uptake characteristic, this study assumed that D041735 inherited the low Cd uptake phenotype from the hexaploid wheat Sumai 3. This also means that D041735 is a new source for low Cd uptake now available in durum wheat. This was confirmed by analysis of markers associated with Cd uptake in D041735 and other sources of low Cd Strongfield and Haurani.

Based on the results collectively from Chapter 2 and Chapter 3, the following discusses three results that suggest that D041735 is a new source for Cd uptake originating in hexaploid wheat and that the major QTL identified in the D041735 × Divide population is different than the one identified in populations derived from the other low Cd sources Strongfield and Haurani.

1- Differences in amplification patterns detected by molecular markers linked to Cd in the populations developed from D041735 and populations developed from Strongfield and Haurani; all three sources have low Cd content in grains

In the Chapter 2, the Cd-linked SNPs identified in this study were found to be insignificant in any population with a background other than D041735. Similarly, none of the previously identified Cd-linked CAPs markers were significant in the population developed from D041735. Also, a validation test study by Salsman (2016) on NDSU durum wheat germplasm showed that the Cd-associated RAPD marker OPC20 (Penner et al., 1995), is insignificant in populations derived from D041735. The same study showed that the SNP for Cd identified by AbuHammad et al. (2016) (wsnp_Ex_c17754_26503892) was a less useful marker, and the SNP wsnp_Ex_c1343_2570756 was the most useful for the NDSU durum wheat breeding program. In this study, wsnp_Ex_c17754_26503892 was mapped tightly linked to the markers flanking the major QTL, while wsnp_Ex_c1343_ 2570756 did not show polymorphism when mapped in this population. From the above, it can be concluded that the detected QTL in this study is not linked to the previously detected QTLs for Cd uptake. Several studies that have introduced new sources for specific traits also reported the findings of different amplification patterns in the new sources (Burnham et al., 2003; Garcia et al., 2008; Lin et al., 1996; McCabe 2015; and Sun et al., 2011).

2- ***Progenies segregation in allelism testing of intercrossing low Cd uptake sources and detecting genotypically and phenotypically high Cd grain content among progenies of two population in two locations***

The alleism test in this study showed genetic segregation among progenies of the populations derived from the Strongfield/D041735 and Haurrani/D041735 for Cd uptake associated identified markers in different plant sources (*Xusw47* or *Kukri_c66357_357*). If the low Cd uptake gene in Strongfield and Haurani is the same as that in D041735, then polymorphism should not have been seen among progenies. Further, if low Cd uptake in D041735 is controlled by the same gene as was found in Strongfield and Haurani but different allele, then progenies with a high level of Cd (genotypically and phenotypically in both populations and both locations) should not be seen. In many studies, allelism testing was used to confirm the existence of a new gene/allele for a specific trait. For example, a new allele for Powdery mildew resistance was discovered in a wheat experimental line (FG-1) using SNPs and SSR markers in the $F_{2:3}$ population (Ma et al., 2016). In soybeans, a new allele for rust resistance was mapped and confirmed in the 98 $F_{3:4}$ population derived from a new source of resistance line called pi 594538A using SNP and SSR markers (Chakraborty et al., 2009). A novel gene for bacterial blight resistance was identified in the Zhachanglong cultivar of rice. The RFLP markers and F_2 segregation population confirmed that this gene is none allelic to other resistance genes previously reported (Lin et al., 1996). More studies in different species have used the same strategy for finding new sources for improving germplasm either with a gene pyramiding approach or releasing more suitable cultivars into the breeding program (Chen et al., 2001; Himabindu et al., 2007; Pan et al., 1999; Randhawa et al., 2015; Sallaud et al., 2003; and Soria et al., 2003)

3- Same amplification pattern of D041035 DNA for low Cd uptake with Sumai 3 and the origin of three low Cd uptake sources

From the above, it can be concluded that hexaploid wheat has a different gene for low Cd uptake than durum wheat. This study tested this hypothesis using reciprocal markers. Results showed the same amplification pattern of Sumai 3 as with the D041735 line. This result was exactly what was expected because D0417735 was developed from Sumai 3. Therefore, this study contends that another gene for low Cd uptake in hexaploid wheat prevents it from accumulating high grain Cd, but rejects that the gene would be located on genome D (Wiebe et al., 2010). This study's findings show that the low Cd uptake gene in hexaploid wheat is located on the long arm of chromosome 5B.

Several studies show that grain Cd variation is significant among modern durum wheat lines compared with hexaploid wheat, (Cakmak et al., 2000; Greger and Löfstedt, 2004;Harris and Taylor, 2001; Harris and Taylor, 2004; and Hart et al., 1998). It has been reported that some durum genotypes accumulate eight times and even in one case up to 20 times more Cd compared to some low Cd durum genotypes. Based on the findings of these studies, the Cd concentration in the root surface in durum wheat and hexaploid wheat is not significantly different. Therefore, one suggested possibility is that hexaploid wheat has a possible redistribution mechanism that rectifies Cd from shoot to root. Looking at the evolutionary history of hexaploid and durum wheat may lead to a better understanding of the existence of two different genes in these two species. Hexaploid wheat evolved from a hybridization between tetraploid wheat (A and B genome) with a diploid *Ae. tauschii* (D genome) (Faris, 2014). However, it does not mean that gene expression in hexaploid wheat is the same as its ancestors. A dynamic genome of hexaploid wheat makes it capable of creating some level of its own variety via experiencing forms of

mutations affecting genes and regulatory elements (Faris, 2014). Hexaploid wheat has taken excessive gene diversity from the tetraploid progenitor (Dubcovsky and Dvorak, 2007). Therefore, the changes during the long-term of evolution of wheat at the micro level, followed by genetic screening at the macro level, might have influenced the resistance of hexaploid wheat to Cd uptake with precipitating new gene. However, identifying two genes for low Cd uptake in wheat suggests a possible involvement of the gene family in Cd uptake mechanism. Yet, future studies may focus on the map base cloning and sequencing of these genes to discover the origin and development of low Cd uptake family gene in durum and hexaploidy wheat.

3.6. References

AbuHammad, W.A., S. Mamidi, A. Kumar, S. Pirseyedi, F.A. Manthey, S.F. Kianian, et al. 2016. Identification and validation of a major cadmium accumulation locus and closely associated SNP markers in North Dakota durum wheat cultivars. Molecular Breeding 36: 112.

Burnham, K., A. Dorrance, D. Francis, R. Fioritto, and S. St Martin. 2003. A New Locus in Soybean for Resistance to . Crop Science 43: 101-105.

Cakmak, I., R. Welch, J. Hart, W. Norvell, L. Oztürk, and L. Kochian. 2000. Uptake and retranslocation of leaf-applied cadmium (109Cd) in diploid, tetraploid and hexaploid wheats. Journal of Experimental Botany 51: 221-226.

Chakraborty, N., J. Curley, R.D. Frederick, D.L. Hyten, R.L. Nelson, G.L. Hartman, et al. 2009. Mapping and Confirmation of a New Allele at from Soybean PI 594538A Conferring RB Lesion–Type Resistance to Soybean Rust. Crop Science 49: 783-790.

Chao, S., W. Zhang, E. Akhunov, J. Sherman, Y. Ma, M.-C. Luo, et al. 2009. Analysis of gene-derived SNP marker polymorphism in US wheat (Triticum aestivum L.) cultivars. Molecular Breeding 23: 23-33.

Chen, P., G. Ma, G. Buss, I. Gunduz, C. Roane, and S. Tolin. 2001. Inheritance and allelism tests of Raiden soybean for resistance to soybean mosaic virus. Journal of Heredity 92: 51-55.

Clarke, J., T. McCaig, R. DePauw, R. Knox, F. Clarke, M. Fernandez, et al. 2005. Strongfield durum wheat. Canadian Journal of Plant Science 85: 651-654.

Dubcovsky, J. and J. Dvorak. 2007. Genome plasticity a key factor in the success of polyploid wheat under domestication. Science 316: 1862-1866.

Faris, J.D. 2014. Wheat domestication: key to agricultural revolutions past and future. Genomics of plant genetic resources. Springer. p. 439-464.

Garcia, A., É.S. Calvo, R.A. de Souza Kiihl, A. Harada, D.M. Hiromoto, and L.G.E. Vieira. 2008. Molecular mapping of soybean rust (Phakopsora pachyrhizi) resistance genes: discovery of a novel locus and alleles. Theoretical and Applied Genetics 117: 545-553.

Greger, M. and M. Löfstedt. 2004. Comparison of uptake and distribution of cadmium in different cultivars of bread and durum wheat. Crop Science 44: 501-507.

Harris, N.S. and G.J. Taylor. 2001. Remobilization of cadmium in maturing shoots of near isogenic lines of durum wheat that differ in grain cadmium accumulation. Journal of Experimental Botany 52: 1473-1481.

Harris, N.S. and G.J. Taylor. 2004. Cadmium uptake and translocation in seedlings of near isogenic lines of durum wheat that differ in grain cadmium accumulation. BMC Plant Biology 4: 1.

Hart, J.J., R.M. Welch, W.A. Norvell, L.A. Sullivan, and L.V. Kochian. 1998. Characterization of cadmium binding, uptake, and translocation in intact seedlings of bread and durum wheat cultivars. Plant Physiology 116: 1413-1420.

Himabindu, K., R. Sundaram, C. Neeraja, B. Mishra, and J. Bentur. 2007. Flanking SSR markers for allelism test for the Asian rice gall midge (Orseolia oryzae) resistance genes. Euphytica 157: 267-279.

Jang, M.-G., Y.-J. Kim, G.-H. Jang, J. Sukweenadhi, W.-S. Kwon, and D.-C. Yang. 2014. Ectopic overexpression of the aluminum-induced protein gene from Panax ginseng enhances heavy metal tolerance in transgenic Arabidopsis. Plant Cell, Tissue and Organ Culture (PCTOC) 119: 95-106.

Kerber, E. and V. Bendelow. 1977. The role of Triticum carthlicum in the origin of bread wheat based on comparative milling and baking properties. Canadian Journal of Plant Science 57: 367-373.

Kihara, H. and F. Lilienfeld. 1949. A new sunthesized 6x-wheat. Hereditas 35: 307-319.

Kihara, H., K. Yamashita and M. Tanaka. 1955. Morphological, physiological, genetical and cytological studies in Aegilops and Triticum collected from Pakistan, Afghanistan and Iran. In "Results of the Kyoto University Scientific Expedition to the Karakoram and Hindukush, 1955," Vol. 1, p. 118. Kyoto University, Japan.

Kuckuck, H. 1979. On the origin of Triticum carthlicum Neyski (= Triticum persicum Vav.). Wheat information service. Wheat Inf Serv 50:1–5

Laidò, G., G. Mangini, F. Taranto, A. Gadaleta, A. Blanco, L. Cattivelli, et al. 2013. Genetic diversity and population structure of tetraploid wheats (Triticum turgidum L.) estimated by SSR, DArT and pedigree data. PLoS One 8: e67280.

Lin, X., D. Zhang, Y. Xie, H. Gao, and Q. Zhang. 1996. Identifying and mapping a new gene for bacterial blight resistance in rice based on RFLP markers. Phytopathology 86: 1156-1159.

Linde-Laursen, I., J. Heslop-Harrison, K. Shepherd, and S. Taketa. 1997. The barley genome and its relationship with the wheat genomes. A survey with an internationally agreed recommendation for barley chromosome nomenclature. Hereditas 126: 1-16.

Ma, P., H. Xu, L. Li, H. Zhang, G. Han, Y. Xu, et al. 2016. Characterization of a new Pm2 allele conferring powdery mildew resistance in the wheat germplasm line FG-1. Frontiers in Plant Science 7.

Matsuoka, Y. and S. Nasuda. 2004. Durum wheat as a candidate for the unknown female progenitor of bread wheat: an empirical study with a highly fertile F1 hybrid with Aegilops tauschii Coss. Theoretical and Applied Genetics 109: 1710-1717.

Mayer, K.F., M. Martis, P.E. Hedley, H. Šimková, H. Liu, J.A. Morris, et al. 2011. Unlocking the barley genome by chromosomal and comparative genomics. The Plant Cell 23: 1249-1263.

Monneveux, P., M. Zaharieva, and D. Rekika. 2000. The utilization of Triticum and Aegilops species for the improvement of durum wheat. Génétique et Amélioration des Plantes INRA: 71-81.

Morris, R. and E. Sears. 1967. The cytogenetics of wheat and its relatives. Wheat and Wheat Improvement 19.

Pan, Q., L. Wang, and T. Tanisaka. 1999. A new blast resistance gene identified in the Indian native rice cultivar Aus373 through allelism and linkage tests. Plant Pathology 48: 288-293.

Penner, G.A., L.J. Bezte, D. Leisle, and J. Clarke. 1995. Identification of RAPD markers linked to a gene governing cadmium uptake in durum wheat. Genome 38: 543-547.

Randhawa, M.S., H.S. Bariana, R. Mago, and U.K. Bansal. 2015. Mapping of a new stripe rust resistance locus Yr57 on chromosome 3BS of wheat. Molecular Breeding 35: 1-8.

Sallaud, C., M. Lorieux, E. Roumen, D. Tharreau, R. Berruyer, P. Svestasrani, et al. 2003. Identification of five new blast resistance genes in the highly blast-resistant rice variety IR64 using a QTL mapping strategy. Theoretical and Applied Genetics 106: 794-803.

Salsman, E.L. 2016. Validation of molecular markers associated with grain cadmium in durum wheat (Triticum turgidum L. var. Desf.). NORTH DAKOTA STATE UNIVERSITY.

Snowden, K.C. and R.C. Gardner. 1993. Five genes induced by aluminum in wheat (Triticum aestivum L.) roots. Plant Physiology 103: 855-861.

Soria, C., E. Moriones, A. Fereres, E. Garzo, and M. Gómez-Guillamón. 2003. New source of resistance to mosaic virus transmission by Aphis gossypii in melon. Euphytica 133: 313-318.

Sun, S., X. Wu, J. Zhao, Y. Wang, Q. Tang, D. Yu, et al. 2011. Characterization and mapping of RpsYu25, a novel resistance gene to Phytophthora sojae. Plant breeding 130: 139-143.

Wiebe, K. 2014. Molecular characterization of Cdu-B1, a major locus controlling cadmium accumulation in durum wheat (Triticum turgidum L. var durum) grain. University of Saskatchewan

Wiebe, K., N. Harris, J. Faris, J. Clarke, R. Knox, G. Taylor, et al. 2010. Targeted mapping of Cdu1, a major locus regulating grain cadmium concentration in durum wheat (Triticum turgidum L. var durum). Theoretical and Applied Genetics 121: 1047-1058.

Zhao, L., Q. Zhao, G. Ao, and J. Yu. 2009. The foxtail millet Si69 gene is a Wali7 (wheat aluminum-induced protein 7) homologue and may function in aluminum tolerance. Chinese Science Bulletin 54: 1697-1706.

Zimmerl, S., J. Lafferty and H. Buerstmayr. 2014. Assessing diversity in Triticum durum cultivars and breeding lines for high versus low cadmium content in seeds using the CAPS marker usw47. Plant Breeding 133: 712-717.

CHAPTER 4. GENERAL CONCLUSIONS AND SUGGESTIONS

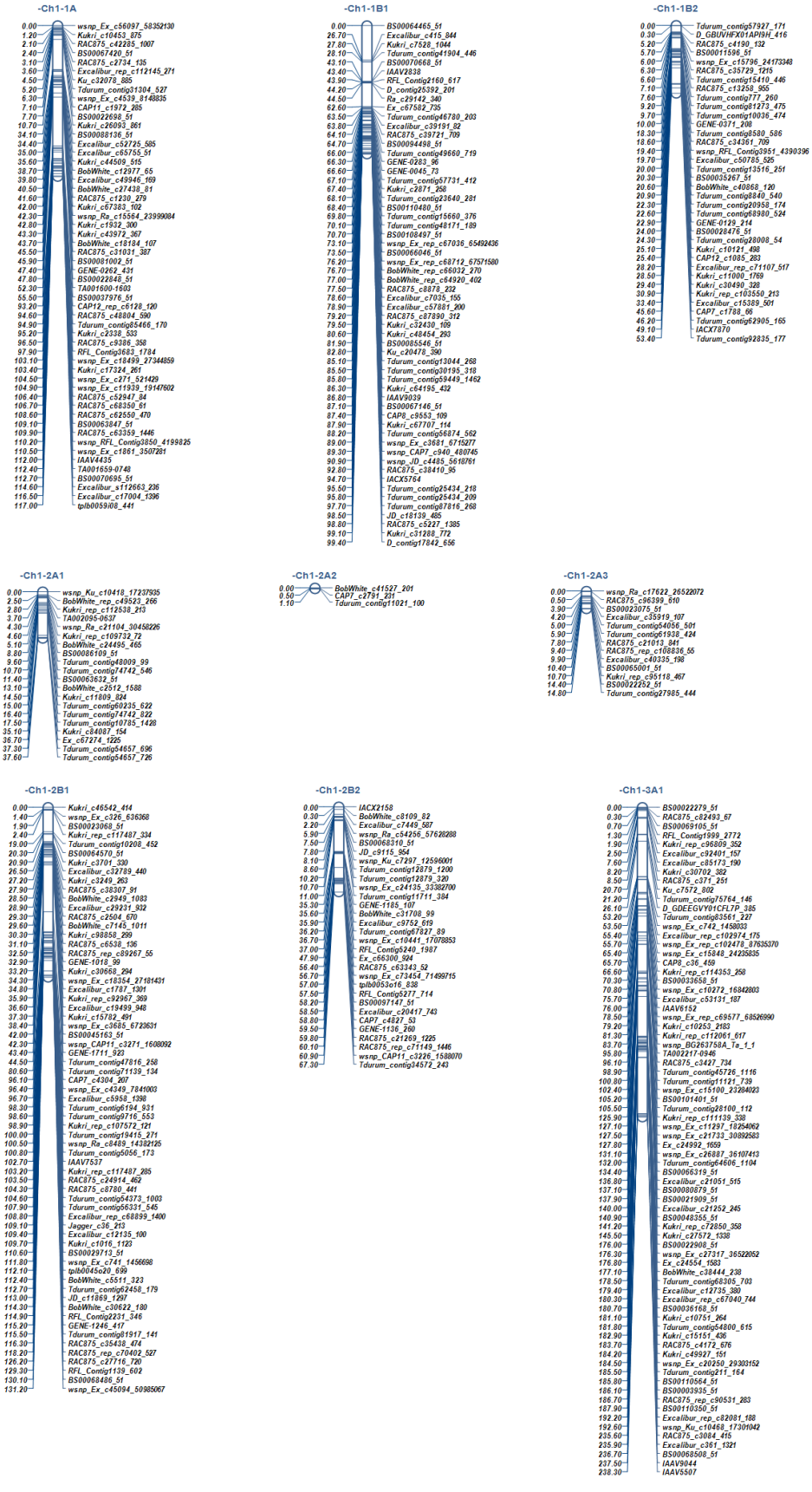
This study suggests that Cd uptake resistance is controlled by different genes in hexaploid and tetraploid wheat. The durum wheat line D041735 developed by the NDSU durum wheat breeding program is a novel source of low Cd as it carries the major gene for Cd uptake resistance from hexaploid wheat. This gene from hexaploid wheat along with the major gene identified in the durum wheat cultivars Strongfield and Haurani could function in the same process. But since the NDSU durum wheat breeding program constantly found a lower level of Cd uptake in D041735 compared to Strongfield and Haurrani, this study suggests that this gene from hexaploid wheat has a slightly different mechanism for redistribution of Cd from shoot to root, which also coincides with previously mentioned studies that found the low Cd accumulation in low durum accumulators was 1.5 times higher than that found in hexaploid wheat. However, mapping these two genes close to each other might suggest that is the presence of a hot spot on this region on the long arm of chromosome 5B in wheat that regulates heavy metal transporters. This study suggests the introgression of this new gene from the D041735 durum line into other lines of the NDSU durum wheat breeding program. This line is better adapted to this durum wheat growing region and best suited for the NDSU durum wheat breeding program's focus on developing both Cd uptake and Fusarium Head Blight resistance. This study suggests that markers, *RAC875_c20785_1219* and *Kukri_c66357_357* will be useful in the MAS approach for speeding up the breeding progress for developing low Cd cultivars derived from D041735 and hexaploid wheat. In the future, a fine mapping study using available genomic resources could lead to map-based gene cloning of this low Cd uptake gene and a more efficient resource for selecting low Cd uptake durum wheat using genomic tools. However, to take advantages of both low Cd uptake genes and additive genetic variation, some breeding

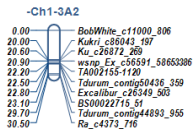
attempts might be taken in future to convert the repulsion phase of these two genes to coupling phase.

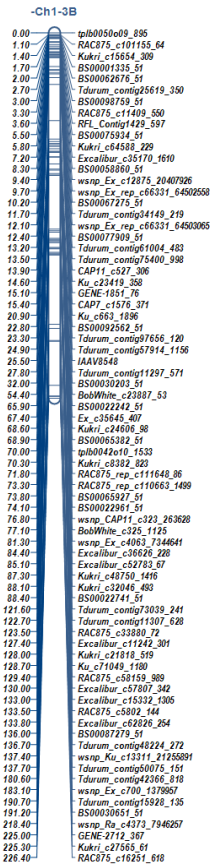
Based on this study's evidence that the Cd uptake gene in D041735 is clearly a new source at the hot spot of heavy transporter regulation of chromosome 5B, assignment of a new gene symbol, Cdu2, would seem appropriate.

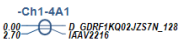
APPENDIX. GENETIC LINKAGE MAP CONSTRUCTED USING D041735×DIVIDE

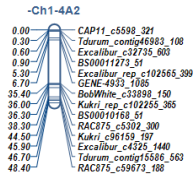
3,923 SNPs were assigned to 14 chromosomes of durum wheat in 28 linkage groups from a RILs mapping population derived from a cross between D041735 and Divide. The position of each marker locus is shown on the left side of each linkage group, and the corresponding marker's name is shown on the right side of each linkage group.

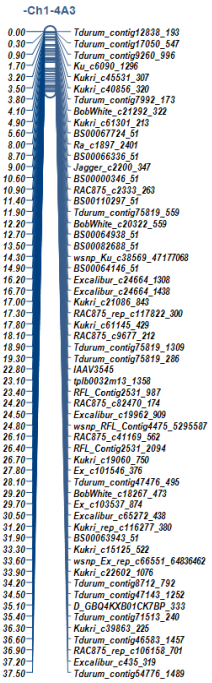

-Ch1-1A
0.00 wsnp_Ex_c56097_58352130
1.20 Kukri_c10453_875
2.10 RAC875_c42285_1007
2.40 BS00067420_51
3.10 RAC875_c2734_135
3.60 Excalibur_rep_c112145_271
4.50 Ku_c32078_885
5.20 Tdurum_contig31304_527
6.30 wsnp_Ex_c4539_8148835
7.10 CAP11_c1972_285
7.70 BS00022698_51
10.70 Kukri_c26093_861
34.10 BS00088136_51
34.40 Excalibur_c52725_585
35.00 Excalibur_c65755_51
35.60 Kukri_c44509_515
38.70 BobWhite_c12977_65
39.80 Excalibur_c49946_169
40.50 BobWhite_c27438_81
41.60 RAC875_c1230_279
42.00 Kukri_c67383_102
42.30 wsnp_Ra_c15564_23999084
42.80 Kukri_c1932_300
43.30 Kukri_c43972_367
43.70 BobWhite_c18184_107
45.50 RAC875_c31031_387
45.90 BS00081002_51
47.40 GENE-0262_431
47.80 BS00022848_51
52.30 TA001600-1603
55.50 BS00037976_51
93.20 CAP12_rep_c6128_120
94.60 RAC875_c48804_590
94.90 Tdurum_contig85466_170
95.20 Kukri_c2338_533
96.50 RAC875_c9386_358
97.90 RFL_Contig3683_1784
103.10 wsnp_Ex_c18499_27344859
103.40 Kukri_c17324_261
104.50 wsnp_Ex_c271_521429
104.90 wsnp_Ex_c11939_19147602
106.40 RAC875_c52947_84
106.70 RAC875_c68350_61
108.60 RAC875_c62550_470
109.10 BS00063847_51
109.90 RAC875_c63359_1446
110.20 wsnp_RFL_Contig3850_4199825
110.50 wsnp_Ex_c1861_3507281
112.00 IAAV4435
112.40 TA001659-0748
112.70 BS00070695_51
114.60 Excalibur_s112663_236
116.50 Excalibur_c17004_1396
117.00 tplb0059i08_441
-Ch1-1B1
0.00 BS00064465_51
26.70 Excalibur_c415_844
27.80 Kukri_c7528_1044
28.10 Tdurum_contig41904_446
43.10 BS00070668_51
43.40 IAAV2838
43.90 RFL_Contig2160_617
44.20 D_contig25392_201
44.50 Ra_c29142_340
62.60 Ex_c67582_735
63.50 Tdurum_contig46780_203
63.80 Excalibur_c39191_82
64.10 RAC875_c39721_709
64.70 BS00094498_51
66.00 Tdurum_contig49660_719
66.30 GENE-0283_96
66.60 GENE-0045_73
67.10 Tdurum_contig57731_412
67.40 Kukri_c2871_258
68.10 Tdurum_contig23640_281
68.40 BS00110480_51
69.80 Tdurum_contig15660_376
70.10 Tdurum_contig48171_189
70.70 BS00108497_51
73.10 wsnp_Ex_rep_c67036_65492436
73.50 BS00066046_51
76.20 wsnp_Ex_rep_c68712_67571580
76.70 BobWhite_rep_c66032_270
77.00 BobWhite_rep_c64920_402
77.50 RAC875_c8878_232
78.60 Excalibur_c7035_155
78.90 Excalibur_c57881_200
79.20 RAC875_c87890_312
79.50 Kukri_c32430_109
80.60 Kukri_c48454_293
81.90 BS00085546_51
82.80 Ku_c20478_390
85.10 Tdurum_contig13044_268
85.50 Tdurum_contig30195_318
85.80 Tdurum_contig59449_1462
86.30 Kukri_c64196_432
86.80 IAAV9039
87.10 BS00067146_51
87.40 CAP8_c8553_109
87.90 Kukri_c67707_114
88.20 Tdurum_contig56874_562
89.00 wsnp_Ex_c3681_6715277
89.30 wsnp_CAP7_c940_480745
90.90 wsnp_JD_c4485_5618761
92.80 RAC875_c38410_95
94.70 IACX5764
95.50 Tdurum_contig25434_218
95.80 Tdurum_contig25434_209
97.70 Tdurum_contig87816_268
98.50 JD_c18139_465
98.80 RAC875_c5227_1385
99.10 Kukri_c31288_772
99.40 D_contig17842_656
-Ch1-1B2
0.00 Tdurum_contig57927_171
0.30 D_GBUVHFX01API9H_416
5.20 RAC875_c4190_132
5.70 BS00011596_51
6.00 wsnp_Ex_c15796_24173348
6.30 RAC875_c35729_1215
6.60 Tdurum_contig15410_446
7.10 RAC875_c13258_955
7.60 Tdurum_contig9777_260
9.20 Tdurum_contig81273_475
9.70 Tdurum_contig10036_474
10.00 GENE-0371_308
18.30 Tdurum_contig8580_586
18.60 RAC875_c34361_709
19.40 wsnp_RFL_Contig3951_4390396
19.70 Excalibur_c50785_526
20.00 Tdurum_contig13516_251
20.30 BS00035267_51
20.60 BobWhite_c40868_120
20.90 Tdurum_contig8840_540
22.30 Tdurum_contig20958_174
22.60 Tdurum_contig68980_524
22.90 GENE-0129_214
24.00 BS00028476_51
24.30 Tdurum_contig28008_54
25.10 Kukri_c10121_498
25.40 CAP12_c1085_283
28.20 Excalibur_rep_c71107_517
28.50 Kukri_c11000_1769
29.40 Kukri_c30490_328
30.90 Kukri_rep_c103550_213
33.40 Excalibur_c15389_501
45.60 CAP7_c1788_66
46.20 Tdurum_contig62905_165
49.10 IACX7870
53.40 Tdurum_contig92835_177
-Ch1-2A1
0.00 wsnp_Ku_c10418_17237935
2.50 BobWhite_rep_c49523_266
2.80 Kukri_rep_c112538_213
3.70 TA002095-0637
4.30 wsnp_Ra_c21104_30458226
4.60 Kukri_rep_c109732_72
5.10 BobWhite_c24495_465
8.80 BS00086109_51
9.60 Tdurum_contig48009_99
10.70 Tdurum_contig74742_546
11.40 BS00063632_51
13.10 BobWhite_c2512_1588
14.50 Kukri_c11809_824
15.00 Tdurum_contig60235_622
16.40 Tdurum_contig74742_822
17.50 Tdurum_contig10785_1428
35.10 Kukri_c34087_154
36.70 Ex_c67274_1225
37.30 Tdurum_contig54657_696
37.60 Tdurum_contig54657_726
-Ch1-2A2
0.00 BobWhite_c41527_201
0.50 CAP7_c2791_201
1.10 Tdurum_contig11021_100
-Ch1-2A3
0.00 wsnp_Ra_c17622_26522072
0.50 RAC875_c96399_610
3.90 BS00023075_51
4.20 Excalibur_c35919_107
5.00 Tdurum_contig54056_501
5.90 Tdurum_contig61938_424
7.80 RAC875_c21013_841
9.40 RAC875_rep_c108836_55
9.90 Excalibur_c40335_198
10.40 BS00065001_51
10.70 Kukri_rep_c95118_467
14.40 BS00022252_51
14.80 Tdurum_contig27985_444
-Ch1-2B1
0.00 Kukri_c46542_414
1.40 wsnp_Ex_c326_636368
1.90 BS00023351_51
2.40 Kukri_rep_c117487_334
19.00 Tdurum_contig10208_452
20.30 BS00064570_51
20.90 Kukri_c3701_330
26.50 Excalibur_c32789_440
27.20 Kukri_c3249_263
27.90 RAC875_c38307_91
28.50 BobWhite_c2949_1083
28.90 Excalibur_c29231_932
29.30 RAC875_c2504_670
29.60 BobWhite_c7145_1011
30.30 Kukri_c9858_299
31.10 RAC875_c6538_136
32.50 RAC875_rep_c89267_55
32.90 GENE-1018_90
33.20 Kukri_c30668_294
34.30 wsnp_Ex_c18354_27181431
34.80 Excalibur_c1787_1301
35.90 Kukri_rep_c92967_369
36.60 Excalibur_c19499_948
37.30 Kukri_c15782_491
38.40 wsnp_Ex_c3685_6723631
42.00 BS00045163_51
42.30 wsnp_CAP11_c3271_1608092
43.40 GENE-1711_923
44.50 Tdurum_contig47816_258
80.60 Tdurum_contig71139_134
96.10 CAP7_c4304_300
96.40 wsnp_Ex_c4349_7841003
96.70 Excalibur_c5958_1398
98.30 Tdurum_contig6194_931
98.60 Tdurum_contig5716_553
98.90 Kukri_rep_c107572_121
100.00 Tdurum_contig19415_371
100.50 wsnp_Ra_c6489_14389125
100.80 Tdurum_contig5056_173
102.70 IAAV7537
103.20 Kukri_rep_c117487_285
103.50 RAC875_c24914_462
104.30 RAC875_c8780_441
104.60 Tdurum_contig54373_1003
107.90 Tdurum_contig56331_545
108.80 Excalibur_rep_c68899_1400
109.10 Jagger_c36_213
109.40 Excalibur_c12135_100
109.70 Kukri_c1016_1123
110.60 BS00029713_51
111.80 wsnp_Ex_c741_1456698
112.10 tplb0045o20_699
112.40 BobWhite_c5511_323
112.70 Tdurum_contig62458_179
113.00 JD_c11869_1297
114.30 BobWhite_c30622_180
114.90 RFL_Contig2231_346
115.20 GENE-1246_417
115.50 Tdurum_contig81917_141
116.30 RAC875_c35438_474
116.20 RAC875_rep_c70402_527
126.20 RAC875_c27716_720
129.30 RFL_Contig1139_602
130.10 BS00068486_51
131.20 wsnp_Ex_c45094_50985067
-Ch1-2B2
0.00 IACX2158
0.30 BobWhite_c3109_82
2.20 Excalibur_c7449_587
5.90 wsnp_Ra_c54256_57628208
7.50 BS00068310_51
7.80 JD_c9115_954
8.10 wsnp_Ku_c7297_12596001
8.60 Tdurum_contig12879_1200
10.20 Tdurum_contig12879_320
10.70 wsnp_Ex_c24135_33392700
11.00 Tdurum_contig11711_384
35.30 GENE-1185_107
35.60 BobWhite_c31708_99
35.90 Excalibur_c9752_619
36.20 Tdurum_contig67827_89
36.70 wsnp_Ex_c10441_17078853
37.00 RFL_Contig5340_1987
47.90 Ex_c66300_924
56.40 RAC875_c63343_52
56.70 wsnp_Ex_c73454_71499715
57.00 tplb0053o16_838
57.50 RFL_Contig5277_714
58.20 BS00097147_51
58.50 Excalibur_c20417_743
58.80 CAP7_c4827_53
59.50 GENE-1136_260
59.80 RAC875_c21269_1225
60.10 RAC875_rep_c71149_1446
60.90 wsnp_CAP11_c3226_1588070
67.30 Tdurum_contig34572_243
-Ch1-3A1
0.00 BS00022279_51
0.30 RAC875_c82493_67
0.70 BS00069105_51
1.30 RFL_Contig1999_2772
1.90 Kukri_rep_c96809_352
2.50 Excalibur_c92401_157
7.60 Excalibur_c85173_190
8.20 Kukri_c30702_382
8.50 RAC875_c371_251
20.70 Ku_c5752_802
21.20 Tdurum_contig75764_146
26.10 D_GDEEGVY01CFL7P_385
53.20 Tdurum_contig83561_227
53.50 wsnp_Ex_c742_1458003
54.40 Excalibur_rep_c102974_175
55.70 wsnp_Ex_rep_c102478_87635370
65.40 wsnp_Ex_c15848_24235835
66.70 CAP8_c36_459
66.60 Kukri_rep_c114353_258
70.30 BS00033658_51
70.80 wsnp_Ex_c10272_16842803
75.70 Excalibur_c53131_187
76.00 IAAV6152
78.50 wsnp_Ex_rep_c69577_68526990
79.20 Kukri_c10253_2183
81.30 Kukri_rep_c112061_617
83.70 wsnp_BG262711A_Ta_1_1
95.80 TA002217-0946
96.10 RAC875_c3427_734
98.90 Tdurum_contig45726_1116
100.80 Tdurum_contig31121_739
102.40 wsnp_Ex_c15100_23284023
105.20 BS00101401_51
105.50 Tdurum_contig28100_112
125.90 Kukri_rep_c111139_338
127.10 wsnp_Ex_c11297_18254062
127.50 wsnp_Ex_c21733_30892583
127.80 Ex_c24992_1659
131.10 wsnp_Ex_c26887_36107413
132.00 Tdurum_contig64606_1104
134.40 BS00066319_51
136.80 Excalibur_c21051_515
137.10 BS00080875_51
137.90 BS00021909_51
140.00 Excalibur_c21252_245
140.90 BS00048355_51
141.20 Kukri_rep_c72850_358
145.50 Kukri_c27572_1338
176.00 BS00022908_51
176.30 wsnp_Ex_c27317_36522052
176.80 Ex_c24554_1503
177.10 BobWhite_c38444_238
178.50 Tdurum_contig68305_703
179.40 Excalibur_c12735_380
180.30 Excalibur_c21040_744
180.70 BS00036168_51
181.10 Kukri_c10751_264
181.80 Tdurum_contig54800_615
182.90 Kukri_c15151_436
183.70 RAC875_c4172_676
184.20 Kukri_c49927_151
184.50 wsnp_Ex_c20250_29303152
185.50 Tdurum_contig211_164
185.80 BS00110564_51
186.10 BS00003935_51
186.70 RAC875_rep_c90531_283
187.90 BS00110350_51
192.20 Excalibur_rep_c82081_188
192.60 wsnp_Ku_c10468_17301042
235.60 RAC875_c3084_415
235.90 Excalibur_c361_1321
236.70 BS00068508_51
237.50 IAAV9044
238.30 IAAV5507

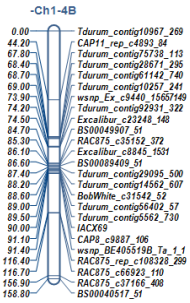

-Ch1-3A2
0.00 BobWhite_c11000_806
20.00 Kukri_c86043_197
20.60 Ku_c26872_269
20.90 wsnp_Ex_c56591_58653386
22.20 TA002155-1120
22.50 Tdurum_contig50436_359
22.80 Excalibur_c26349_503
23.10 BS00022715_51
29.70 Tdurum_contig44893_955
30.50 Ra_c4373_716

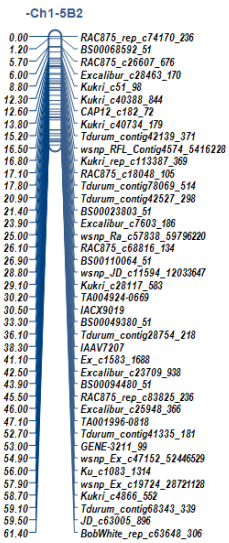

-Ch1-3B
0.00 tplb0050o09_895
1.10 RAC875_c101155_64
1.40 Kukri_c15654_309
1.70 BS00001335_51
2.00 BS00062676_51
2.70 Tdurum_contig25619_350
3.00 BS00098759_51
3.30 RAC875_c11409_550
3.60 RFL_Contig1429_597
5.50 BS00075934_51
5.80 Kukri_c64588_229
7.20 Excalibur_c35170_1610
8.30 BS00058860_51
9.40 wsnp_Ex_c12875_20407926
9.70 wsnp_Ex_rep_c66331_64502558
10.20 BS00067275_51
11.70 Tdurum_contig34149_219
12.10 wsnp_Ex_rep_c66331_64503065
12.40 BS00077909_51
13.20 Tdurum_contig61004_483
13.50 Tdurum_contig75400_998
13.90 CAP11_c527_306
14.60 Ku_c23419_358
15.10 GENE-1851_76
15.40 CAP7_c1576_371
20.90 Ku_c663_1896
22.80 BS00092562_51
23.30 Tdurum_contig97656_120
24.90 Tdurum_contig57914_1156
25.50 IAAV8548
27.80 Tdurum_contig11297_571
32.00 BS00030203_51
54.40 BobWhite_c23887_53
65.90 BS00022242_51
67.40 Ex_c35645_407
68.60 Kukri_c24606_98
68.90 BS00065382_51
70.00 tplb0042o10_1533
70.30 Kukri_c8382_823
71.80 RAC875_rep_c111648_86
73.30 RAC875_rep_c110663_1499
73.80 BS00065927_51
74.10 BS00022961_51
76.80 wsnp_CAP11_c323_263628
77.10 BobWhite_c325_1125
81.30 wsnp_Ex_c4063_7344641
84.40 Excalibur_c36626_228
85.10 Excalibur_c52783_67
87.30 Kukri_c48750_1416
88.10 Kukri_c32046_493
88.40 BS00022744_51
121.60 Tdurum_contig73039_241
122.70 Tdurum_contig11307_628
123.50 RAC875_c33880_72
127.40 Excalibur_c11242_301
128.00 Kukri_c21818_519
128.70 Ku_c71049_1180
129.40 RAC875_c58159_989
130.00 Excalibur_c57807_342
133.00 Excalibur_c15332_1305
133.50 RAC875_c5802_144
133.80 Excalibur_c62826_264
136.00 BS00087279_51
136.70 Tdurum_contig48224_272
137.40 wsnp_Ku_c13311_21255891
137.70 Tdurum_contig50075_151
180.60 Tdurum_contig42366_818
183.10 wsnp_Ex_c700_1379957
190.70 Tdurum_contig15928_135
191.20 BS00030651_51
218.40 wsnp_Ra_c4373_7946257
225.00 GENE-2712_367
225.30 Kukri_c27565_61
226.40 RAC875_c16251_618

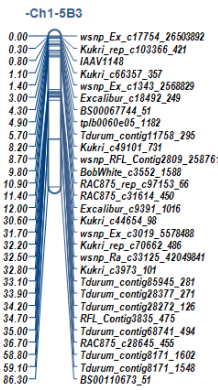

-Ch1-4A1
0.00 D_GDRF1KQ02JZS7N_128
2.70 IAAV2216

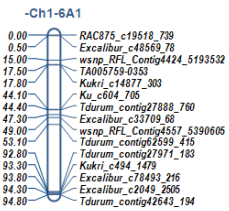

-Ch1-4A2
0.00 CAP11_c5598_321
0.30 Tdurum_contig46983_108
0.60 Excalibur_c32735_603
0.90 BS00011273_51
5.30 Excalibur_rep_c102565_399
6.70 GENE-4933_1085
35.40 BobWhite_c33898_150
36.00 Kukri_rep_c102255_365
36.30 BS00010168_51
38.30 RAC875_c5302_300
44.50 Kukri_c96159_197
45.90 Excalibur_c4525_1440
46.70 Tdurum_contig15586_563
48.40 RAC875_c59673_188

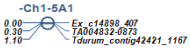

-Ch1-4A3
0.00 Tdurum_contig12838_193
0.30 Tdurum_contig17050_547
0.90 Tdurum_contig9260_996
1.70 Ku_c6090_1296
3.20 Kukri_c45531_307
3.50 Kukri_c40856_320
3.80 Tdurum_contig7992_173
4.10 BobWhite_c21292_322
4.90 Kukri_c61301_213
5.60 BS00067724_51
8.00 Ra_c1897_2401
8.70 BS00066336_51
9.00 Jagger_c2200_347
10.60 BS00000346_51
10.90 RAC875_c2333_263
11.40 BS00110297_51
11.90 Tdurum_contig75819_559
12.20 BobWhite_c20322_559
12.70 BS00064938_51
13.50 BS00082688_51
14.30 wsnp_Ku_c38569_47177068
14.90 BS00064146_51
16.20 Excalibur_c24664_1308
16.70 Excalibur_c24664_1438
17.00 Kukri_c21086_843
17.30 RAC875_rep_c117822_300
17.80 Kukri_c61145_429
18.10 RAC875_c9677_212
18.90 Tdurum_contig75819_1309
19.30 Tdurum_contig75819_286
22.80 IAAV3545
23.10 tplb0032m13_1358
23.40 RFL_Contig2531_987
24.20 RAC875_c82470_174
24.50 Excalibur_c19962_909
24.80 wsnp_RFL_Contig4475_5295587
26.10 RAC875_c41169_562
26.40 RFL_Contig2531_2094
26.70 Kukri_c19060_750
27.80 Ex_c101546_376
28.10 Tdurum_contig47476_495
29.20 BobWhite_c18267_473
29.70 Ex_c103537_874
30.50 Excalibur_c65272_438
31.20 Kukri_rep_c116277_380
31.90 BS00063943_51
33.30 Kukri_c15125_522
33.60 wsnp_Ex_rep_c66551_64836462
33.90 Kukri_c22602_1076
34.20 Tdurum_contig8712_792
34.50 Tdurum_contig47143_1252
35.10 D_GBQHKXB01CK7BP_333
35.40 Tdurum_contig71513_240
36.30 Kukri_c39863_225
36.60 Tdurum_contig46583_1457
36.90 RAC875_rep_c106158_701
37.20 Excalibur_c435_319
37.50 Tdurum_contig54776_1489

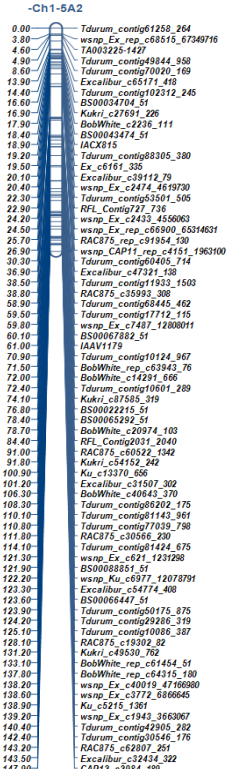

-Ch1-4B
0.00 Tdurum_contig10967_269
44.20 CAP11_rep_c4893_84
67.80 Tdurum_contig75738_113
68.40 Tdurum_contig28671_295
68.70 Tdurum_contig61142_740
69.00 Tdurum_contig10257_241
73.90 wsnp_Ex_c9440_15557149
74.20 Tdurum_contig92931_322
74.50 Excalibur_c23248_148
84.70 BS00049907_51
85.30 RAC875_c35152_372
86.10 Excalibur_c8845_1531
86.60 BS00089409_51
87.40 Tdurum_contig29095_500
88.20 Tdurum_contig14562_607
88.60 BobWhite_c31542_52
89.00 Tdurum_contig56402_57
89.50 Tdurum_contig5562_730
90.00 IACX69
91.10 CAP8_c9887_106
91.40 wsnp_BE405519B_Ta_1_1
116.40 RAC875_rep_c108328_299
116.70 RAC875_c66922_110
156.90 RAC875_c37166_408
158.80 BS00040517_51

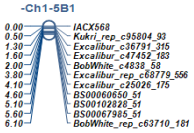

-Ch1-5B2
0.00 RAC875_rep_c74170_236
1.20 BS00068592_51
5.70 RAC875_c26607_676
6.00 Excalibur_c28463_170
8.80 Kukri_c51_98
12.30 Kukri_c40388_844
12.60 CAP12_c182_72
13.80 Kukri_c40734_179
15.20 Tdurum_contig42139_371
16.50 wsnp_RFL_Contig4574_5416228
16.80 Kukri_rep_c113387_369
17.10 RAC875_c18048_105
17.80 Tdurum_contig78069_514
20.90 Tdurum_contig42527_298
21.40 BS00023803_51
23.90 Excalibur_c7603_186
25.00 wsnp_Ra_c57838_59796220
26.10 RAC875_c68816_134
26.90 BS00110064_51
28.80 wsnp_JD_c11594_12033647
29.10 Kukri_c28117_583
30.20 TA004924-0669
30.50 IACX9019
33.30 BS00049380_51
36.10 Tdurum_contig28754_218
38.30 IAAV7207
41.10 Ex_c1583_1688
42.50 Excalibur_c23709_938
43.90 BS00094480_51
45.50 RAC875_rep_c83825_236
46.00 Excalibur_c25948_366
47.10 TA001996-0818
52.70 Tdurum_contig41335_181
53.00 GENE-3211_99
54.90 wsnp_Ex_c47152_52446529
56.00 Ku_c1083_1314
57.90 wsnp_Ex_c19724_28721128
58.70 Kukri_c4866_552
59.10 Tdurum_contig68343_339
59.50 JD_c63005_896
61.40 BobWhite_rep_c63648_306

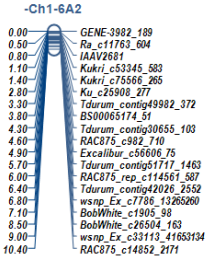

-Ch1-5B3
0.00 wsnp_Ex_c17754_26503892
0.30 Kukri_rep_c103366_421
0.80 IAAV1148
1.10 Kukri_c66357_357
1.40 wsnp_Ex_c1343_2568829
3.00 Excalibur_c18492_249
4.30 BS00067744_51
4.90 tplb0060e05_1182
5.70 Tdurum_contig11758_295
8.20 Kukri_c49101_731
8.70 wsnp_RFL_Contig2809_2587619
9.80 BobWhite_c3552_1588
10.90 RAC875_rep_c97153_66
11.40 RAC875_c31614_450
12.00 Excalibur_c9391_1016
30.60 Kukri_c44654_98
31.70 wsnp_Ex_c3019_5578488
32.20 Kukri_rep_c70662_486
32.50 wsnp_Ra_c33125_42049841
32.80 Kukri_c3973_101
33.10 Tdurum_contig85945_281
33.90 Tdurum_contig28377_271
34.20 Tdurum_contig28272_126
34.70 RFL_Contig3835_475
35.00 Tdurum_contig68741_494
36.70 RAC875_c28645_455
58.80 Tdurum_contig8171_1602
59.10 Tdurum_contig8171_1548
86.30 BS00110673_51

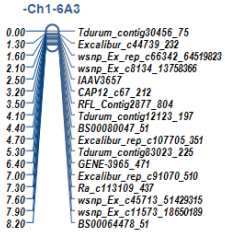

-Ch1-6A1
0.00 RAC875_c19518_739
0.50 Excalibur_c48569_78
15.00 wsnp_RFL_Contig4424_5193532
17.50 TA005759-0353
17.80 Kukri_c14877_303
44.10 Ku_c604_705
44.40 Tdurum_contig27888_760
47.30 Excalibur_c33709_66
49.00 wsnp_RFL_Contig4557_5390605
53.10 Tdurum_contig62599_415
92.80 Tdurum_contig27971_183
93.30 Kukri_c494_1479
93.80 Excalibur_c78493_216
94.30 Excalibur_c2049_2505
94.80 Tdurum_contig42643_194

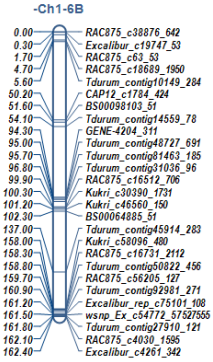

-Ch1-5A1
0.00 Ex_c14898_407
0.30 TA004332-0873
1.10 Tdurum_contig42421_1167

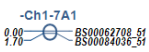

-Ch1-5A2
0.00 Tdurum_contig61258_264
3.80 wsnp_Ex_rep_c68515_67349716
4.60 TA003225-1427
4.90 Tdurum_contig49844_958
8.60 Tdurum_contig70020_169
13.90 Excalibur_c65171_418
14.40 Tdurum_contig102312_245
16.60 BS00034104_51
16.90 Kukri_c27691_226
17.90 BobWhite_c2236_111
18.40 BS00043474_51
18.90 IACX815
19.20 Tdurum_contig88305_380
19.50 Ex_c6161_335
20.10 Excalibur_c39112_79
20.40 wsnp_Ex_c2474_4679730
22.30 Tdurum_contig53501_505
22.90 RFL_Contig727_736
24.20 wsnp_Ex_c2433_4556063
24.50 wsnp_Ex_rep_c66900_65314631
25.70 RAC875_rep_c91954_130
26.90 wsnp_CAP11_rep_c4151_1963100
30.30 Tdurum_contig60405_714
36.90 Excalibur_c47321_158
38.50 Tdurum_contig11933_1503
38.80 RAC875_c35993_308
58.90 Tdurum_contig68445_462
59.50 Tdurum_contig17712_115
59.80 wsnp_Ex_c7487_12008011
60.10 BS00067882_51
61.00 IAAV1179
70.90 Tdurum_contig10124_967
71.50 BobWhite_rep_c63943_76
72.00 BobWhite_c14291_666
72.40 Tdurum_contig10601_289
74.10 Kukri_c87585_319
76.80 BS00022215_51
78.40 BS00065292_51
78.70 BobWhite_c20974_103
84.40 RFL_Contig2031_2040
91.00 RAC875_c60522_1342
91.80 Kukri_c54152_242
100.90 Ku_c13370_656
101.20 Excalibur_c31507_302
106.30 BobWhite_c40643_370
108.30 Tdurum_contig86202_175
110.10 Tdurum_contig81143_961
110.80 Tdurum_contig77039_798
111.80 RAC875_c30566_230
114.10 Tdurum_contig81424_675
121.30 wsnp_Ex_c621_1231298
121.90 BS00088851_51
122.20 wsnp_Ku_c6977_12078791
123.30 Excalibur_c54774_408
123.60 BS00066447_51
123.90 Tdurum_contig50175_875
124.20 Tdurum_contig29286_319
125.10 Tdurum_contig10086_387
128.10 RAC875_c19302_82
131.20 Kukri_c49530_762
133.10 BobWhite_rep_c61454_51
137.80 BobWhite_rep_c64315_180
138.20 wsnp_Ex_c40019_47166980
138.60 wsnp_Ex_c3772_6866645
138.90 Ku_c5215_1361
139.20 wsnp_Ex_c1943_3663067
140.40 Tdurum_contig42905_282
142.40 Tdurum_contig30546_176
143.20 RAC875_c62807_251
143.50 Excalibur_c32434_322

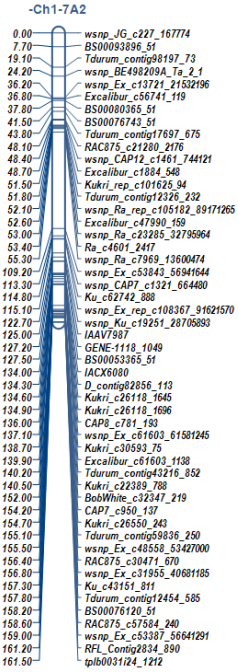

-Ch1-5B1
0.00 IACX568
0.50 Kukri_rep_c95804_93
1.30 Excalibur_c36791_315
1.60 Excalibur_c47452_183
2.00 BobWhite_c4838_58
3.80 Excalibur_rep_c68779_556
4.10 Excalibur_c25026_175
4.60 BS00060650_51
5.10 BS00102936_51
5.60 BS00067985_51
6.10 BobWhite_rep_c63710_181

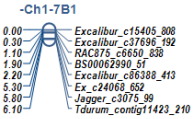

-Ch1-6A2
0.00 GENE-3982_189
0.50 Ra_c11763_604
0.80 IAAV2681
1.10 Kukri_c53345_583
1.40 Kukri_c75566_265
2.80 Ku_c25908_277
3.30 Tdurum_contig49982_372
3.80 BS00065174_51
4.30 Tdurum_contig30655_103
4.60 RAC875_c982_710
4.90 Excalibur_c56606_75
5.70 Tdurum_contig51717_1463
6.00 RAC875_rep_c114561_587
6.40 Tdurum_contig42026_2552
6.80 wsnp_Ex_c7786_13265260
7.10 BobWhite_c1905_98
8.50 BobWhite_c26504_183
9.00 wsnp_Ex_c33113_41653134
10.40 RAC875_c14852_2171

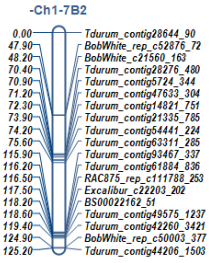

-Ch1-6A3
0.00 Tdurum_contig30456_75
1.30 Excalibur_c44739_202
1.60 wsnp_Ex_rep_c66342_64519823
2.10 wsnp_Ex_c6134_13758366
2.50 IAAV3657
3.20 CAP12_c67_212
3.50 RFL_Contig2877_804
4.10 Tdurum_contig12123_197
4.40 BS00080047_51
4.70 Excalibur_rep_c107705_351
5.30 Tdurum_contig63023_226
6.40 GENE-3965_471
7.00 Excalibur_rep_c91070_510
7.30 Ra_c113109_437
7.60 wsnp_Ex_c45713_51429315
7.90 wsnp_Ex_c11573_18650189
8.20 BS00064478_51


-Ch1-6B
0.00 RAC875_c38876_642
0.30 Excalibur_c19747_53
1.70 RAC875_c63_53
4.70 RAC875_c18589_1950
5.60 Tdurum_contig10149_284
50.20 CAP12_c1784_424
51.60 BS00098103_51
54.10 Tdurum_contig14559_78
94.30 GENE-4204_311
95.00 Tdurum_contig48727_691
95.70 Tdurum_contig81463_185
96.80 Tdurum_contig31036_96
99.90 RAC875_c16512_706
100.30 Kukri_c30390_1731
101.20 Kukri_c46560_150
102.30 BS00064885_51
137.00 Tdurum_contig45914_283
158.00 Kukri_c58096_480
158.30 RAC875_c16731_2112
158.80 Tdurum_contig50822_456
159.70 RAC875_c56205_127
160.90 Tdurum_contig92981_271
161.20 Excalibur_rep_c75101_108
161.50 wsnp_Ex_c54772_57527555
161.80 Tdurum_contig27910_121
162.10 RAC875_c4030_1595
162.40 Excalibur_c4261_342


-Ch1-7A1
0.00 BS00062708_51
1.70 BS00084036_51


-Ch1-7A2
0.00 wsnp_JG_c227_167774
7.70 BS00093896_51
19.10 Tdurum_contig98197_73
24.20 wsnp_BE498209A_Ta_2_1
36.20 wsnp_Ex_c13721_21532196
36.80 Excalibur_c56741_119
37.80 BS00080365_51
41.50 BS00076743_51
43.80 Tdurum_contig17697_675
48.10 RAC875_c21280_2176
48.40 wsnp_CAP12_c1461_744121
48.70 Excalibur_c1884_548
51.50 Kukri_rep_c101625_94
51.80 Tdurum_contig12326_232
52.10 wsnp_Ra_rep_c105182_89171265
52.60 Excalibur_c47990_159
53.00 wsnp_Ra_c23285_32795964
53.40 Ra_c4601_2417
55.30 wsnp_Ra_c7969_13600474
109.20 wsnp_Ex_c53843_56941644
113.30 wsnp_CAP7_c1321_664480
114.80 Ku_c62742_888
115.10 wsnp_Ex_rep_c108367_91621570
122.70 wsnp_Ku_c19251_28705893
125.00 IAAV7987
127.20 GENE-1118_1049
127.50 BS00053365_51
134.00 IACX6080
134.30 D_contig82856_113
134.60 Kukri_c26118_1645
134.90 Kukri_c26118_1696
136.00 CAP8_c781_193
137.10 wsnp_Ex_c61603_61581245
138.70 Kukri_c30593_75
139.90 Excalibur_c61603_1138
140.20 Tdurum_contig43216_852
140.50 Kukri_c22389_788
152.00 BobWhite_c32347_219
154.20 CAP7_c950_137
154.70 Kukri_c26550_243
155.10 Tdurum_contig59836_250
155.50 wsnp_Ex_c48558_53427000
156.40 RAC875_c30471_670
156.80 wsnp_Ex_c31955_40681185
157.30 Ku_c43151_811
157.80 Tdurum_contig12454_585
158.20 BS00076120_51
158.60 RAC875_c57584_240
159.00 wsnp_Ex_c53387_56641291
161.20 RFL_Contig2834_890
161.50 tplb0031i24_1212


-Ch1-7B1
0.00 Excalibur_c15405_808
0.30 Excalibur_c37696_192
1.10 RAC875_c6650_838
1.90 BS00062990_51
2.20 Excalibur_c86388_413
5.30 Ex_c24068_652
5.80 Jagger_c3075_99
6.10 Tdurum_contig11423_210


-Ch1-7B2
0.00 Tdurum_contig28644_90
47.90 BobWhite_rep_c52876_72
48.20 BobWhite_c21560_163
70.40 Tdurum_contig28276_480
70.90 Tdurum_contig5724_344
71.20 Tdurum_contig47633_304
72.30 Tdurum_contig14821_751
73.90 Tdurum_contig21335_785
74.20 Tdurum_contig54441_224
75.60 Tdurum_contig63311_285
115.90 Tdurum_contig93467_337
116.20 Tdurum_contig61884_836
116.50 RAC875_rep_c111788_253
117.50 Excalibur_c22203_202
118.20 BS00022162_51
118.60 Tdurum_contig49575_1237
119.40 Tdurum_contig42260_3421
124.90 BobWhite_rep_c50003_377
125.20 Tdurum_contig44206_1503