Theor Appl Genet (2010) 121:1047–1058
DOI 10.1007/s00122-010-1370-1

ORIGINAL PAPER

Targeted mapping of *Cdu1*, a major locus regulating grain cadmium concentration in durum wheat (*Triticum turgidum* L. var *durum*)

K. Wiebe · N. S. Harris · J. D. Faris · J. M. Clarke · R. E. Knox · G. J. Taylor · C. J. Pozniak

Received: 10 February 2010 / Accepted: 21 May 2010 / Published online: 18 June 2010

Abstract Some durum wheat (*Triticum turgidum* L. var *durum*) cultivars have the genetic propensity to accumulate cadmium (Cd) in the grain. A major gene controlling grain Cd concentration designated as *Cdu1* has been reported on 5B, but the genetic factor(s) conferring the low Cd phenotype are currently unknown. The objectives of this study were to saturate the chromosomal region harboring *Cdu1* with newly developed PCR-based markers and to investigate the colinearity of this wheat chromosomal region with rice (*Oryza sativa* L.) and *Brachypodium distachyon* genomes. Genetic mapping of markers linked to *Cdu1* in a population of recombinant inbred substitution lines revealed that the gene(s) associated with variation in Cd concentration resides in wheat bin 5BL9 between fraction breakpoints 0.76 and 0.79. Genetic mapping and quantitative trait locus (QTL) analysis of grain Cd concentration was performed in 155 doubled haploid lines from the cross W9262-260D3 (low Cd) by Kofa (high Cd) revealed two expressed sequence tag markers (ESMs) and one sequence tagged site (STS) marker that co-segregated with *Cdu1* and explained >80% of the phenotypic variation in grain Cd concentration. A second, minor QTL for grain Cd concentration was also identified on 5B, 67 cM proximal to *Cdu1*. The *Cdu1* interval spans 286 kbp of rice chromosome 3 and 282 kbp of *Brachypodium* chromosome 1. The markers and rice and *Brachypodium* colinearity described here represent tools that will assist in the positional cloning of *Cdu1* and can be used to select for low Cd accumulation in durum wheat breeding programs targeting this trait. The isolation of *Cdu1* will further our knowledge of Cd accumulation in cereals as well as metal accumulation in general.

Communicated by C. Feuillet.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-010-1370-1) contains supplementary material, which is available to authorized users.

K. Wiebe · J. M. Clarke · C. J. Pozniak (✉)
Department of Plant Sciences, Crop Development Center, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N 5A8, Canada
e-mail: curtis.pozniak@usask.ca

N. S. Harris · G. J. Taylor
Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9, Canada

J. D. Faris
Northern Crop Science Laboratory, USDA-ARS, 1307 18th Street North, Fargo, ND 58105-5677, USA

R. E. Knox
Semiarid Prairie Agricultural Research Centre, Agriculture and Agri-Food Canada, P.O. Box 1030, Swift Current, SK S9H 3X2, Canada

Introduction

Cadmium (Cd) is a toxic metal that is naturally present in trace quantities in almost all soils. Anthropogenic activities can elevate Cd concentrations in agricultural soils via atmospheric deposition or direct application of sewage sludge, manure, fertilizer, and irrigation water (Alloway and Steinnes 1999). Cadmium in soil is readily absorbed by roots and transported in plants (Grant et al. 1998). Cadmium-contaminated foods are the dominant source of human exposure to environmental Cd (Satarug and Moore 2004), with cereals and vegetables contributing the majority of dietary Cd (McLaughlin et al. 1999). Recently the Codex Alimentarius Commission of FAO/WHO has set maximum limits for Cd levels in a variety of foods. The

Codex maximum level for Cd in wheat grain is 200 ng g^{-1} (CODEX STAN 193-1995 2009). Among cereals, some durum wheat (*Triticum turgidum* L. var *durum*) cultivars have the genetic potential to accumulate Cd in grain to levels that exceed the Codex standard (Grant et al. 2008). In contrast, common wheat (*Triticum aestivum* L.) accumulates very little Cd in grain (Zook et al. 1970). Some cultivars from other crops, including rice (*Oryza sativa* L.), oat (*Avena sativa* L.), flax (*Linum usitatissimum* L.) and sunflower (*Helianthus annuus* L.) can also accumulate high concentrations of Cd in the grain (Grant et al. 2008). Genetic variation for grain Cd accumulation exists in durum (Clarke et al. 1997), and breeding for low grain Cd concentration is a target of durum wheat breeding programs globally (Grant et al. 2008).

Genetically, Cd accumulation in grain can be regulated by multiple genes with combined effects on uptake, translocation and sequestration (Tanhuanpää et al. 2007). Several quantitative trait loci (QTL) associated with cadmium accumulation in rice grain (*Oryza sativa* L.) have been reported (Kashiwagi et al. 2009; Ishikawa et al. 2005, 2010; Xue et al. 2009; Ueno et al. 2009). In contrast, a single QTL has been reported in oat (*Avena sativa* L.) (Tanhuanpää et al. 2007). In durum wheat, Cd accumulation is governed by the major gene *Cdu1* (Clarke et al. 1997), which is localized to chromosome arm 5BL (Knox et al. 2009). Genotypic differences in accumulation of Cd in grain of durum wheat have been associated with restricted root-to-shoot translocation, which limits the pool of available Cd in vegetative tissues for subsequent remobilization during grain filling (Harris and Taylor 2004). Root-to-shoot Cd translocation is also the major physiological process determining Cd accumulation in shoots and grains of rice (Uraguchi et al. 2009). Genes coding for ATP-binding cassette transporters (Wojas et al. 2009; Kim et al. 2007), P_{1B}-ATPases (Morel et al. 2009), and selenium-binding proteins (Dutilleul et al. 2008) have been associated with Cd accumulation and detoxification in *Arabidopsis*, but the genetic factor(s) associated with variable Cd accumulation in durum grain have yet to be identified.

The long-term goal of our research is to clone the gene(s) regulating Cd accumulation in durum wheat grain. However, to accomplish this, a saturated map of the *Cdu1* region is required to initiate fine mapping and map-based cloning experiments. Wheat expressed sequence tags (ESTs) have been used to develop PCR-based molecular markers for saturation and fine mapping of several traits in wheat (Yu et al. 2009; Lu et al. 2006). Over 1 million wheat ESTs are available (http://www.ncbi.nlm.nih.gov/sites/entrez) and 16,000 of these have been localized to wheat deletion bins (Qi et al. 2004). These ESTs are useful for developing PCR-based DNA markers for saturation and fine mapping of chromosomal regions that contain genes of interest. In addition, genomic information from the model species rice and *Brachypodium distachyon* (here after referred to as *Brachypodium*) have been used for marker development for fine mapping and gene isolation in wheat (Lu and Faris 2006). Current information suggests that *Brachypodium* is more closely related to wheat than rice (Bossolini et al. 2007; Huo et al. 2008; Kumar et al. 2009) and the availability of the sequence of the *Brachypodium* genome is an additional resource for marker development for saturation mapping and gene cloning in wheat. The objectives of this study were to use available wheat EST information to accurately map *Cdu1* by increasing the marker density in the *Cdu1* region, identify the orthologous regions in the rice and *Brachypodium* genomes, and evaluate the levels of colinearity. This information will be used in future studies to fine map and sequence *Cdu1* from durum wheat.

Materials and methods

Plant materials

Genetic mapping was performed using 155 doubled haploid (DH) lines from the W9262-260D3/Kofa mapping population used previously to localize *Cdu1* (Knox et al. 2009). Grain Cd concentration data was available from two environments for the DH population (Knox et al. 2009), and these data were used in the present study. A hexaploid wheat (*Triticum aestivum* L.) population consisting of 115 recombinant substitution lines (RSLs) derived from a cross between Chinese Spring (CS) and a CS-*Triticum dicoccoides* 5B (CS-DIC 5B) described in Gill et al. (1996) was also used for genetic mapping. TA106, the *T. dicoccoides* source of 5B was also included as control in molecular studies.

Cadmium accumulation in CS and CS-DIC 5B

CS and CS-DIC 5B were assessed for Cd uptake in seedlings and Cd concentration in grain at maturity. Seeds were surface sterilized in 1.2% NaOCl for 20 min, rinsed in reverse osmosis (RO) water (<3 μS cm^{-1}), and imbibed for 24 h in an aerated solution of 1 mM $CaCl_2$ and 5 mg L^{-1} Vitavax fungicide (Uniroyal Chemical Ltd, Calgary, AB, Canada). The germinated seeds were placed on nylon mesh suspended over 10 L of aerated chelator-buffered nutrient solution. The nutrient solution was prepared in RO water and contained 1.0 mM $Ca(NO_3)_2$, 0.3 mM $Mg(NO_3)_2$, 0.3 mM NH_4NO_3, 0.25 mM KNO_3, 0.1 mM K_2HPO_4, 0.1 mM K_2SO_4, 50 μM KCl, 100 μM $Fe(NO_3)_3$, 10 μM H_3BO_3, 0.2 μM Na_2MoO_4, 10 μM

$ZnSO_4$, 2 μM $CuSO_4$, 1 μM $MnSO_4$, 0.5 μM $CdCl_2$, 0.1 μM $NiCl_2$, 138 μM *N*-(2-hydroxyethyl)ethylenediaminetriacetic acid (HEDTA), 1.42 mM KOH, and 2 mM 2-(*N*-morpholino)ethanesulfonic (MES) acid buffer (pH 6.0). HEDTA was added at a 25 μM excess over the total concentration of transition metal cations to chelate Cd and micronutrient metals in solution, thereby buffering the free metal activities at environmentally relevant levels (Parker and Norvell 1999). The free ion activity of Cd^{2+} was 14.4 pM as calculated by GEOCHEM-PC (Parker et al. 1995). Seedlings were grown for 3 days in the dark, and then a further 4 days in a controlled environment growth chamber (16 h day, 21/16°C day/night temperature, and 450 μmol m^{-2} s^{-1} photosynthetically active radiation). The seedlings were removed after 7 days and were transferred to 10-L polyethylene buckets (under the same growth conditions) containing aerated, chelator-buffered nutrient solution as described above. Each bucket held two seedlings, supported independently by polyethylene mesh baskets mounted in opaque polycarbonate lids. The buckets were suspended in a common water bath to limit temperature fluctuations and maintain a consistent root temperature in all experimental containers. During the growth period, RO water was added to maintain a constant solution volume, and the solution pH was monitored daily. The pH was adjusted with 1.25 N HNO_3 or KOH when it deviated from 6.0 ± 0.1. The plants were harvested after 14 days. The two plants per bucket were combined at harvest. The shoot tissues were washed in running RO water for 30 s, while the roots were triple rinsed (RO water, 1 min; 1 mM $CaCl_2$, 5 min; RO water, 1 min) and blotted dry.

The Cd concentration in grain of CS and CS-DIC 5B was determined in plants grown in potted soil. A black chernozemic topsoil collected near Edmonton, AB, was air-dried, passed through a 1-cm sieve, and thoroughly mixed. The DTPA-extractable (Lindsay and Norvell 1978) and total Cd concentrations of the soil were 126 and 248 μg kg^{-1}, respectively. The soil was fertilized with 74 mg kg^{-1} $NH_4H_2PO_4$, 403 mg kg^{-1} NH_4NO_3, and 109 mg kg^{-1} K_2SO_4. Each pot (20 cm diameter) was filled with 2.4 kg of air-dried soil and watered to and maintained at 80% of field capacity. For both genotypes, ten replicate pots were planted with two germinated seeds per pot. The plants were grown under the same conditions as described above. The first four heads to begin anthesis on each plant were tagged and these were harvested at 42 days post-anthesis. The heads from both plants in a pot were combined, and the grain was manually separated.

Plant samples were oven-dried at 65°C for 3 days, weighed, and finely ground in a stainless steel mill. Ground samples (0.5 g) were digested in a 5 mL:2 mL mixture of trace-metal grade concentrated HNO_3:30% H_2O_2 and diluted to 50 mL with deionized water (>18 MΩ purity). Cadmium concentration was determined by graphite furnace atomic absorption spectroscopy (AAnalyst 700; PerkinElmer, Waltham, MA). Reagent blanks and a NIST Standard Reference Material (NIST No. 8436 durum wheat flour) were included in each batch of samples for quality control. Recovery of the reference Cd concentration was 99 ± 4% (±SD). The nutrient solution and soil culture experiments were arranged in completely randomized designs. Differences between genotypes in biomass and Cd accumulation were determined by Student's *t* test.

Marker development

Markers were developed for ESTs previously localized to bin 5BL9 0.76–0.79. EST sequences were blasted against MSU rice genome annotation release 6.1 (Ouyang et al. 2007) using BLASTn (http://rice.plantbiology.msu.edu/blast.shtml). Rice genes with the best hit (e-values $<10^{-7}$ and ≥80% nucleotide identity for at least 60 bases) were then used as queries in BLASTn searches of *Triticum* sequences (NCBI Blast http://blast.ncbi.nlm.nih.gov/Blast.cgi). The *Triticum* coding (CDS) and EST sequences were aligned with rice CDS and genomic sequences using AlignX (Vector NTI Advance 10.3; Invitrogen, Carlsbad, CA). Primer pairs were designed from the wheat CDS sequences, and the target products included at least one intronic region. Overlapping primers were designed to ensure coverage of the majority of the CDS of each gene. Markers developed were designated expressed sequence markers (ESM) (Lu and Faris 2006). A total of 120 ESM primer pairs were designed from 54 ESTs. In addition, an earlier study (Knox et al. 2009) suggested that *Cdu1* resides in an area close to the major vernalization locus *vrn-B1*. The homoeologous locus *Vrn-A^{m}1* has been sequenced, so primers were designed for six genes physically linked to that locus (Table 1) using the same procedures as described above.

Marker analysis

A saturated map of 5BL has been reported previously for the CS/CS-DIC 5B population (Lu et al. 2006). As such, a dominant marker most closely associated with *Cdu1*, previously designated as *ScOpc20* (Knox et al. 2009), was amplified in this population using primers and PCR reaction conditions reported previously (Knox et al. 2009).

All ESM primers and primers for the six *Vrn-B1* associated genes were evaluated for polymorphisms first on genomic DNA from Kofa and W9262-260D3, and only those primers that produced polymorphic amplicons were assessed on the DH population and are reported here (Table 1). The PCRs consisted of 50 mM KCl, 10 mM

Table 1 ESM and STS markers polymorphic between W9262-260D3 and Kofa

Marker name	Detection method	Map location	Primer sequence (3′–5′)[a]		Putative rice ortholog
			Forward	Reverse	
XBE425993	SSCP	5A	AAGACATCCTGAACCTGGTGTA	GTCCCAGTCGAACTTGTTCAT	Os03g55070
XBE426348	SSCP	5A	CTATAAGATGAACCGGGGTTTT	TACGCTACCTATGAAGTACTTGGAC	Os03g53800
XBE604920	Agarose	5A	TCCCCTACATGCTGCTCTAC	CAACATCGACTTCATTATTGGAC	Os03g52860
XBF474090	SSCP	5A	GTAGATTATTGGCAACAAGACAAGT	GCGTAAGAAATATATCACGCTAGTT	Os03g53670
XBF474164	SSCP	5A/5B	AGACTTTCTCGTCCCGATACTT	CAACATATGTCTGGCCTACTACTCT	Os03g53720
XBG262450	SSCP	5A	GATAATTTCAGAACAATGCCATTAC	AAGAGTAGCCAATCTGTAGTTGATG	Os03g51020
XBG274700	SSCP	5A	CAGAAGACAGTGAAGAACCAAAAC	AACTCTCAAGTCACTCATCTCAATC	Os03g55950
XBG313229	CE	5B	CTTGCTGTCCTCGAGAAGTTT	ATAGTATCCCATCAATTGTAAGCTG	Os03g58470
XBG607162	SSCP	5B	ATGCATACAAGGACCGCTAC	AATCACACCCTTGCGAATAAT	Os03g63140
XBF293297	SSCP	5A/5B	TGGCCGCGCCCTTCTTCTCCA	TTGTCCTGCGGCTTCACCATC	Os03g53600
XBF474090	SSCP	5B	GAGGCCATGGACCCCAACTTT	GGACAGGAGAACCTGAAGGAT	Os03g53670
XBF145263	SSCP	5A	ACGTGGACGACTACTTGGAGT	CAGGTCATAAGCTTGGCGTGC	Os03g53700
XUsw15	SSCP	5B	ACCAGCAGGACATTGGGAACA	GAACCTTGGACGATTGCTAAC	Os03g53590
Genes associated with *Vrn-B1*					
Xwg644	SSCP	5A	GACTTGTTCAGTCATCTCATA	GCAGCTTGTGTCTGATGTGAA	Os03g54790
Xwg644	SSCP	5B	GCTCTTAAGCAGGCTTTCTGA	CTGTAAGGCTGTATAAGATGA	Os03g54790
MC	SSCP	5A	AGTCGGTGTTCAAGCAACAGG	GCGATCAATCTTCTAACTACC	Os03g54760
CSFs-1	SSCP	5B	TCGGCACCAATGCCGTGGATT	AGAACTTAATGGATGTGTCCC	Os03g54770
CSFs-1	SSCP	5A	CCAGTAGCTCATCTCTATGAT	ACTCGTAGCTTCTACAGATCC	Os03g54770
PCS2	CE	5B	TCAACTACCAGCAGTTCCGAC	GTAGGCCTGCCAACAAGAGCA	Os03g54750
PHY-C	SSCP	5B	ACTGGAAGCAGGCTATCCTGG	AACATAGTCGCCTTGTATCCG	Os03g54084
MTK4	SSCP	5A	CGTGGTGGAACAGGACGAGGG	CATCATTCCCAGGTAGAACAC	Os03g53880

SSCP single strand conformational polymorphism gel electrophoresis, *CE* capillary electrophoresis

[a] M13 (CACGACGTTGTAAAACGAC) tag attached to 5′ end of forward primer

Tris–HCl, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.1 μM of M13 sequence-modified forward ESM primer (M13 tag, CACGACGTTGTAAAACGAC, attached to 5′ end of forward primer), 0.4 μM of reverse ESM primer, 0.152 μM of Universal dye-labeled M13 primer (Schuelke 2000), 1.75 U of Taq DNA polymerase and 100 ng of genomic DNA. The universal primer was labeled with either HEX, FAM, or NED fluorescent dyes. The total PCR volume was 25 μL. Temperature cycling was 94°C for 5 min followed by 3 cycles of 94°C for 30 s, 56°C for 45 s, 72°C for 45 s, 94°C for 30 s, 54°C for 45 s, 72°C for 45 s, 94°C for 30 s, 52°C for 45 s, 72°C for 45 s, 94°C for 30 s, 50°C for 45 s, 72°C for 45 s, then 32 cycles of 94°C for 30 s, 51°C for 45 s, 72°C for 45 s, then a final extension at 72°C for 10 min before cooling to 10°C. Primers were first assessed for polymorphisms using capillary electrophoresis (CE) (ABI3100xl; Applied Biosystems). For CE, 1 μL of diluted PCR product (diluted 1/20 or 1/10 in deionized water) was combined with 9.0 μL HiDi formamide (ABI, Foster City, CA), and 0.08 μL of 500(-250) ROX size standard. The samples were run on a 36-cm array, processed with Applied Biosystem Data Collection Software version 2.0, and genotyped using GeneMapper version 3.0. Monomorphic ESMs were further analyzed using single strand conformational polymorphism (SSCP). For SSCP analysis, 4 μL of the PCR product were mixed with 20 μL of loading buffer containing 95% formamide, 0.05% bromophenol blue, and 0.05% xylene cyanol. The samples were heated at 94°C for 5 min and then immediately placed on ice to allow single strand folding. The fragments were resolved on a 0.6X MDE gel (Lonza, Rockland, ME, USA) run at room temperature for 17 h (6 W) using 0.6X TBE buffer. The Bio-Rad Sequi-Gen GT System (38 × 50 cm) was used for electrophoresis. Gels were visualized by silver staining as described previously (Bassam and Gresshoff. 2007).

Genetic mapping and QTL analysis

Revised genetic linkage maps of the CS/CS-DIC 5B (Lu et al. 2006) and W9262-260D3/Kofa (Knox et al. 2009) populations were constructed using the Haldane mapping function of JoinMap 4.0 (van Ooijen and Voorrips 2004) at a minimum LOD score of 3.0. Only the W9262-260D3/

Kofa population was used for QTL analysis using grain Cd concentration collected previously (Knox et al. 2009). QTL analysis was performed using a multiple locus model (MLM) in MapQTL Version 5.0 (van Ooijen 2004) and the significance threshold ($P < 0.01$) of the LOD score was determined as described previously (van Ooijen 1999). For QTL analysis, the least square means for each DH line was used and were estimated from data collected from two environments (Knox et al. 2009). The average QTL effects (one half the difference between parental marker class means) were estimated by MapQTL Version 5.0.

Colinearity with the rice and *Brachypodium* genomes

For comparative analysis with the rice and *Brachypodium* genomes, the reported sequences of the ESMs linked to *Cdu1* were subjected to BLASTn searches of the rice and *Brachypodium* (http://www.brachybase.org/blast) genomes and filtered for sequences with e-values $<10^{-7}$ and $\geq$80% nucleotide identity for at least 60 bases. When several significant hits were found, only the best hit (lowest e-value) is reported. Markers were developed for rice genes within the *Cdu1* co-linear region using the same procedures described above. A total of 44 sequence tagged site (STS) primer pairs were designed based on 14 collinear genes. These markers were analyzed as per the ESMs.

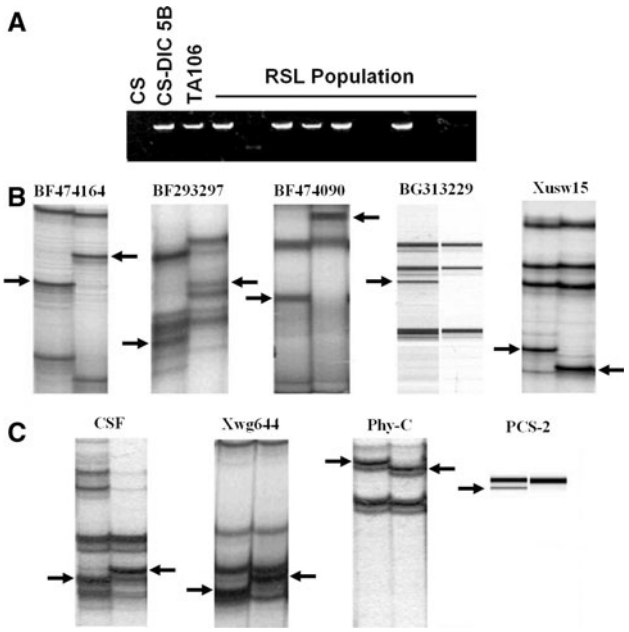

Fig. 1 **a** Polymorphisms detected at *ScOpc20* in Chinese Spring (*CS*) and CS-DIC 5B and a subsample of RILS from the CS/CS-DIC 5B RSL population; **b** polymorphic EST-derived markers (*ESM*) associated with *Cdu1* in the population W9262-260D3/Kofa; **c** polymorphic markers associated with the *Vrn-B1* locus. For gel images in **b** and **c**, Kofa is lane 1 and W9262-260D3 is lane 2. All polymorphisms were detected using SSCP, except for BG313299 and *PCS2* which were detected using capillary electrophoresis. *Arrows* indicate those polymorphic fragments that localized to 5B

Results

We previously localized *Cdu1* (Penner et al. 1995) on 5BL (Knox et al. 2009) approximately 3 cM distal to *ScOpc20* and 12 cM distal to *Xfcp2,* a marker linked to *Tsn1*. A 5BL map derived from the CS/CS-DIC 5B RSL population is well-saturated in the *Tsn1* region (Lu et al. 2006), so we first attempted to map *Cdu1* and associated markers in that population. *ScOpc20* primers amplified the expected 394-bp fragment from CS-DIC 5B, but no fragment was amplified from CS (Fig. 1a). *XBG608197* and *Xrz575* were mapped previously in the CS/CS-DIC 5B population, and *ScOpc20* was found to co-segregate with these markers at a position 4.5 cM proximal to *Xwg644* (Fig. 2a). Despite segregation at *ScOpc20*, grain Cd concentration for CS and CS-DIC 5B was similar (Table 2). The shoot, root, and whole-plant Cd concentrations of seedlings were significantly higher in CS than in CS-DIC 5B (Table 2), However, there was no difference between CS and CS-DIC 5B in the percentage of whole-plant Cd accumulation transported to the shoots, so both CS and CS-DIC 5B would be classified as low Cd accumulators because high Cd accumulating durum genotypes transport 40–50% of whole-plant Cd accumulation to the shoots after 14 days growth (Harris and Taylor 2004; Hart et al. 2006). Even though it was not possible to localize *Cdu1* in this population, we were able to associate the *Cdu1* linked markers *ScOpc20* and *Xwg644* to *XBG608197* and *Xrz575,* which were previously localized to deletion bin 5BL9 0.76–0.79 (Lu et al. 2006; Fig. 2a). Therefore, *Cdu1* is also located within deletion bin 5BL9 0.76–0.79.

Having established the physical location of *Cdu1*, we then designed and evaluated 120 primer pairs from the sequences of 54 wheat ESTs previously localized to bin 5BL9 0.76–0.79. Twenty-five of these primers produced amplicons that were polymorphic between Kofa and W9262-260D3 (Table 1) and 13 were mapped in the DH population. X*BF474090* was polymorphic (Fig. 1b) and co-segregated with *Cdu1* in the DH population (Fig. 2b, c). Primers designed from the sequences of BF293297 and BF474164 each produced two polymorphic fragments (Fig. 1). *XBF293297* co-segregated with *Cdu1* and *XBF474164* mapped 0.2 cM distal (Fig. 2b, c). *XBG313229* mapped 7 cM proximal to *Cdu1*. None of these markers were polymorphic between CS and CS-DIC 5B (Data not shown). Primers for the ESMs X*BE604920*, X*BE426348*, X*BF474090*, *XBF145263,* X*BE494515*, X*BG262450*, and X*BG274700* were all polymorphic among Kofa and W9262-260D3, but all clustered

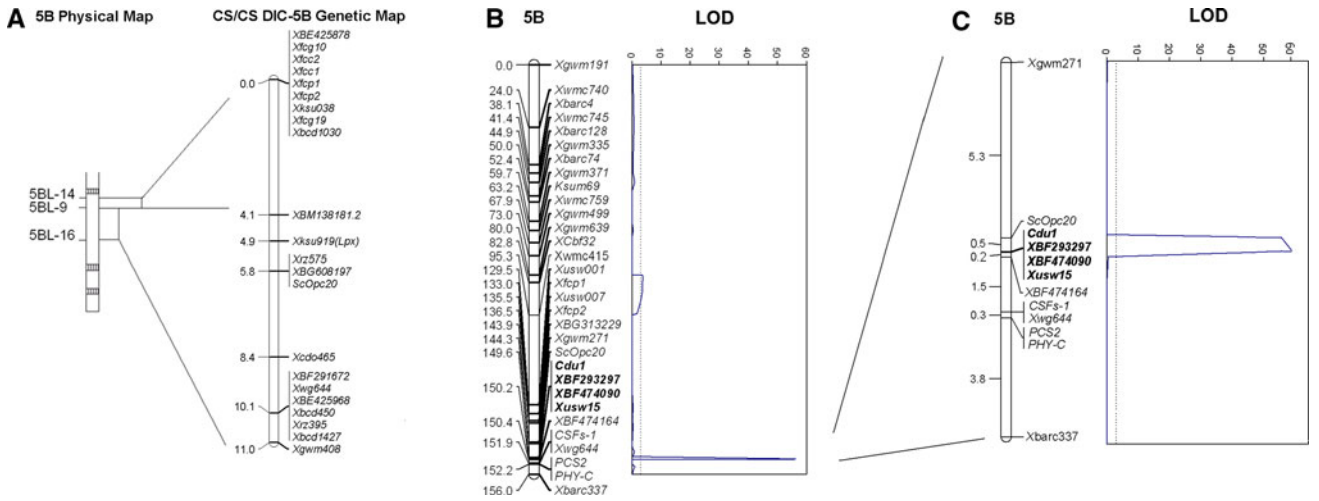

Fig. 2 **a** Physical map of chromosome 5BL and corresponding genetic map of CS/CS-DIC 5B. **b** Genetic map of 5B in W9262-260D3/Kofa population. **c** Genetic map of *Cdu1* region in the W9262-260D3/Kofa population. Distance between markers is in cM

Table 2 Cadmium accumulation in seedlings and grain of Chinese Spring (CS) and CS-*Triticum dicoccoides* 5B (CS-DIC 5B) grown for 14 days in chelator-buffered nutrient solution (seedlings) or grown to maturity in potted soil (grain)

Variable	CS	CS-DIC 5B
Seedling Cd conc. (μg g^{-1})		
Shoot	0.52 (0.03)*	0.41 (0.03)
Root	4.69 (0.08)***	4.11 (0.09)
Whole plant	2.01 (0.04)***	1.79 (0.04)
% Shoot Cd[a]	16.4 (0.7)	14.2 (0.7)
Grain Cd conc. (ng g^{-1})	34 (2)	29 (2)

Numbers in parenthesis are SEM, $n = 10$ (seedlings) or 8 (grain)

Significant differences between genotypes as determined by *t* test are indicated by * $P \leq 0.05$ and *** $P \leq 0.001$

[a] Cd content of shoots as a percentage of whole-plant Cd content

on the distal region of chromosome 5AL when mapped in the DH population (data not shown).

BLASTx performed using the sequences of ESMs *XBG608197*, *XBF293297*, *XBF474090*, and *XBF474164* were used to identify the *Cdu1* co-linear regions from rice and *Brachypodium* (Fig. 3). The gene products from the co-linear regions from the rice and *Brachypodium* genomes are listed as supplemental information (Table S1). All four ESMs localized to rice chromosome 3 and the *Cdu1* region were collinear with a 286-kbp region on that chromosome. Three of the four ESMs had similarity to sequences on *Brachypodium* chromosome 1 and the co-linear region spanned 282 kbp. BLASTx was performed on all of the genes present in the co-linear region of rice against the *Brachypodium* genome (Fig. 3). Eight *Brachypodium* sequences were not present in the rice co-linear region and 20 rice sequences were absent in *Brachypodium*. Tandem repeats of the rice gene Os03g53590 were identified in the *Brachypodium* genome and the orthologous sequence of BF474090 was identified on chromosome 4. Twenty-nine sequences showed near perfect colinearity between rice chromosome 3 and *Brachypodium* chromosome 1 (Fig. 3). Primers were designed for 14 genes from the *Cdu1* co-linear region in rice, but only primers for Os03g53590 (designated hereafter as *Xusw15*) were polymorphic between Kofa and W9262-260D3 (Fig. 1b). When mapped in the DH population, *Xusw15* co-segregated with *Cdu1* (Fig. 2c).

Xwg644 is tightly linked to the *Vrn-*A^m*1* locus in *T. monococcum* which has been sequenced (Yan et al. 2003). Given the linkage of *ScOpc20* with *Xwg644* in the CS/CS-DIC 5B) population (Fig. 2), genes known to be physically linked to *Xwg644* where mapped (Fig. 2). The PCR products for *PHY-C* (phytochrome-C) and *PCS2* (phytochelatin synthase 2) were polymorphic (Fig. 1c), and mapped 1.8 cM distal to *Cdu1* in the DH mapping population (Fig. 2c). The primers for *Xwg644* and cleavage stimulation factor subunit 1 (*CSFs-1*) produced two polymorphic fragments each (Fig. 1c), and one from each gene mapped 1.5 cM distal to *Cdu1* (Fig. 2c). Primers for *MTK4* (putative protein kinase tousled), *MC* (mitochondrial carrier protein) produced polymorphic amplicons, but these mapped to 5AL.

The revised genetic map of chromosome 5B from W9262-260D3/Kofa was used for QTL analysis of grain Cd concentration data. A major QTL (LOD = 58) for grain Cd concentration centered at *XBF293297, XBF474090* and *Xusw15*, and was flanked by *ScOpc20* and *XBF474164* (Fig. 2b). This QTL, previously designated as *QCdu.spa-B1* (Knox et al. 2009), was reduced to a 0.7 cM interval (Fig. 2c) and explained 82% of the phenotypic variation in grain Cd concentration. W9262-260D3 contributed the

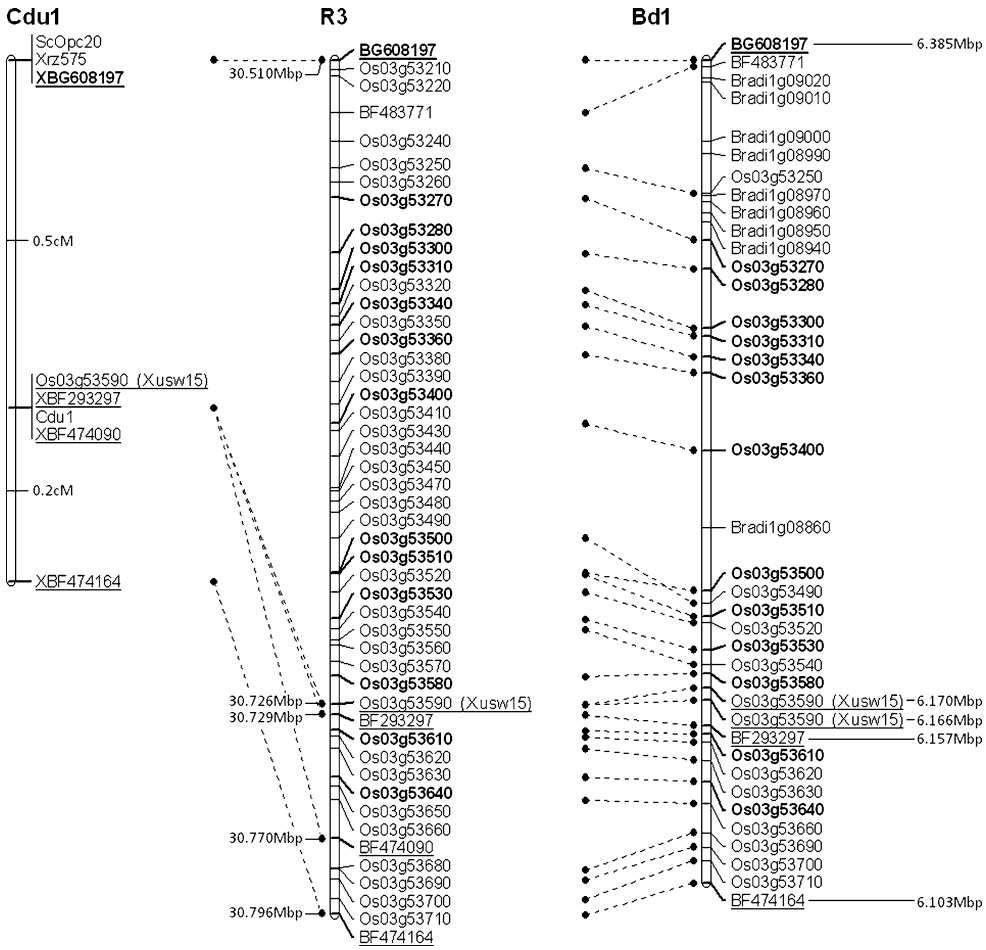

Fig. 3 The genetic map of the *Cdu1* region from W9262-260D3/Kofa and its co-linear region on rice chromosome three (*R3*), and *Brachypodium* chromosome 1 (*Bd1*). The positions of *Xrz575* and *XBG608197* (*bold* and *underlined*) relative to *Cdu1* were inferred from the CS/CS-DIC 5B genetic map (see Fig. 2). *Bold* (*not underlined*) indicates co-linear genes which were evaluated for polymorphisms in Kofa/W9262-260D3

allele for low Cd with an additive effect of 47 ng g^{-1} (Table 3). Using MLM, a second minor QTL (LOD = 4.1) not previously reported by Knox et al. (2009) was also detected on 5BL around *XCbf32* (Fig. 2b) and was designated as *QCdu.usw-B2*. The QTL effect was small relative to *QCdu.spa-B1*, but the low grain Cd parent W9262-260D3 contributed the allele for low grain Cd (Table 3). The interactions between *XCbf32* and the three markers linked to *Cdu1* were not statistically significant, but compared to only *QCdu.spa-B1*, the combined effect of the W9262-260D3 molecular variants at *XCbf32* and *QCdu.spa-B1* reduced grain Cd by 17 ng g^{-1} (Table 3). No other chromosomes were associated with phenotypic variation in grain Cd.

Table 3 Least square means of grain Cd concentrations (ng g^{-1}) from two environments (Knox et al. 2009) for three markers associated with *Cdu1* and *XCbf32*

Molecular variants	*ScOpc20*	*XBF474090*	*XBF474164*	*XCbf32*
Least square means of genotypic groups				
Kofa	160	157	160	121
W9262-260D3	71	67	72	101
Difference[a]	89**	90**	88**	20**
Effect of *XCbf32* in lines homozygous for low Cd uptake at *Cdu1*				
Kofa	75	74	80	
W9262-260D3	63	58	63	
Difference[a]	12*	16**	17**	
Effect of *XCbf32* in lines homozygous for high Cd uptake at *Cdu1*				
Kofa	166	165	165	
W9262-260D3	149	148	149	
Difference[a]	17**	17**	16*	

[a] Differences between genotypic classes were significant at * $P < 0.05$; ** significant at $P < 0.01$

Discussion

Our long-term goal is to identity the genetic factor(s) at *Cdu1* responsible for phenotypic variation in grain Cd accumulation in durum wheat. To achieve this, a dense genetic map of the *Cdu1* region in durum wheat is required. We previously localized *Cdu1* as a Mendelian factor to chromosome 5BL near *ScOpc20* (Knox et al. 2009). Mapping of *ScOpc20* in the CS/CS-DIC 5B population suggested that *Cdu1* localizes to wheat bin 5BL9 0.76–0.79,

so available ESTs previously mapped to this bin were converted to ESMs and mapped relative to *Cdu1*. Two ESMs were identified that co-segregated with *Cdu1*. Colinearity between rice and wheat revealed a third STS marker that also co-segregated with *Cdu1*. QTL analysis confirmed that these three markers were strongly associated with grain cadmium concentration, explaining greater than 80% of the observed phenotypic variation. The additional markers reduced the *Cdu1* interval to 0.7 cM (Fig. 2) and represent a significant step towards positional cloning of *Cdu1*.

ScOpc20 was mapped in the CS/CS-DIC 5B population, but no statistical differences in grain Cd concentration or shoot-to-root partitioning in 3-week-old plants was detected. Both grain cadmium content and shoot-to-root partitioning are associated with the *Cdu1* gene (Hart et al. 2006). The three co-segregating markers were not polymorphic in the CS/CS-DIC 5B population, and both parents showed banding patterns identical to W9262-260D3. Other than the polymorphism for *ScOpc20,* these results suggest that *Cdu1* was not segregating between the parents. Alternatively, the possibility that another gene, perhaps on 5D that, is compensating for the presence of a high Cd allele at *Cdu1* cannot be ruled out. A functional gene present on chromosome 5D could compensate for a loss of a functional gene or high Cd allele on chromosome 5B and therefore a low Cd phenotype.

Several QTL for Cd concentration and translocation have been identified in other species. In rice, three putative QTL for Cd concentration have been identified on chromosomes 3, 6 and 8 (Ishikawa et al. 2005; 2010) and on chromosome 11 (Ueno et al. 2009). Kashiwagi et al. (2009) identified three QTL for Cd concentration in vegetative tissues of rice; two on chromosome 4 and a third on chromosome 11. In maize (*Zea mays* L.), a QTL for leaf Cd accumulation has recently been identified on chromosome 2 (Soric et al. 2009). The genetic complexity across species suggests that several different physiological mechanisms are responsible for phenotypic variation in Cd accumulation in plants. In durum, the low cadmium phenotype is the result of restricted root-to-shoot Cd translocation (Harris and Taylor 2001, 2004), which limits the size of the shoot Cd pool for remobilization to the grain. Our current hypothesis is that low Cd is the result of a functional transporter or chelator that transports or aids in the transport of Cd to root organelles, thus preventing subsequent translocation to shoots for remobilization to the grain. Sequestration of Cd into chemical complexes or physical compartments, such as the vacuole, could occur in root tissues thereby reducing its availability for loading into xylem and phloem. Recent studies have shown the potential of several ABC transporters to sequester cadmium in plants by transporting cadmium conjugates (glutathione or phytochletain) into the vacuole (Song et al. 2003; Klein et al. 2006; Wojas et al. 2009). Many higher plants synthesize phytochelatins (PCs) in response to Cd and bind Cd to form Cd–PC complexes which then accumulate in the vacuole (Vogeli-Lange and Wagner 1990) and can be transported across the tonoplast (Salt and Rauser 1995). Therefore, it is possible that transport of Cd–PC complexes via one (or more) ABC transporters into the vacuole of root cells might limit Cd translocation to the shoot for subsequent remobilization to the grain (Stolt et al. 2003). The low Cd phenotype is dominant (Clarke et al. 1997), and thus the presence of a functional transporter or chelator that sequesters Cd in roots would result in a low cadmium phenotype. In this study, we mapped *Cdu1* near *PCS2*, a gene coding for phytochelatin synthase and *Xwg644*, which codes for a half-sized ABC transporter (Dubcovsky et al. 2001). Although logical candidates, both genes are ruled out because they mapped distal to the *QCdu.spa-B1* QTL and neither co-segregated with *Cdu1*. This supports the work of Hart et al. (2006) who reported that PC synthesis was not a limiting factor in the differential storage of Cd in roots of high and low Cd accumulating near-isogenic lines. However, the possibility that other ABC-like transporter or metal chelator genes exist in the *Cdu1* region cannot be ruled out. A major QTL associated with grain and shoot Cd concentration on rice chromosome 7 has been identified (Tezuka et al. 2009; Ueno et al. 2009) and much like *Cdu1*, explained a large proportion of the phenotypic variation and low Cd concentration was a dominant trait. In this region of chromosome 7, several putative metal transporter-encoding genes, including OsZIP8, cadmium/zinc transporting ATPase (*OsHMA3*) and *OsNramp1* exist. Some *ZIP* proteins have been implicated in heavy-metal uptake in rice (Ishimaru et al. 2005) and an *Arabidopsis* homologue of OsHMA3 has been shown to transport Cd from the cytosol to vacuoles (Morel et al. 2009). Recently, Takahashi et al. (2009) reported that *OsNramp1* had the capacity to transport both Cd and Fe. BLASTn analysis revealed that wheat ESTs exist for all three genes, but none have been mapped in either hexaploid or durum wheat. Given the similar physiological mechanisms observed, mapping of these genes in durum should be a high priority.

In this study, *Cdu1* was tightly linked to the *Vrn-B1* locus (Fig. 2), which controls vernalization response in wheat (Iwaki et al. 2002). A recent study in hexaploid wheat by Ferenc Bálint et al. (2009) also reported a QTL for copper tolerance that was associated with *vrn-A1* on 5AL. However, the reported map was not well saturated, so it is was difficult to ascertain if *vrn-A1* per se was associated or if linked genes were responsible for the observed variation in copper tolerance. The *Cdu1*/*Vrn-B1* linkage could have implications for breeding low Cd durum varieties, because the presence of vernalization genes in spring

wheat lines can influence flowering time, and thus yield (Iqbal et al. 2007). We did not assay the parents of our mapping population for vernalization requirement, but the markers reported here for *Cdu1* could be used effectively to break any undesirable relationships between the low Cd phenotype and any vernalization response associated with *Vrn-B1*.

To aid in future fine mapping efforts, we established the collinear region in the model plants rice and *Brachypodium* because the available genome sequence data could be used to develop additional DNA markers for further map saturation and to expedite chromosome walking to a gene of interest. The *Cdu1* region was found to be collinear with a 286-kbp region of rice chromosome 3 and a 282-kbp region of *Brachypodium* chromosome 1. We observed a reasonable level of colinearity in the *Cdu1* region of rice and *Brachypodium,* and the genes in this region will serve as an excellent source of additional DNA markers for further fine mapping efforts. In the co-linear regions, no obvious genes coding for known metal transporter or metal chelators could be identified in either rice or *Brachypodium* (Table S1). As well, the QTL for Cd identified on rice chromosome three (Ishikawa et al. 2005) was in a non-collinear region. However, it is not reasonable to expect perfect colinearity between wheat and its model species because multiple breaks in microcolinearity due to inversions, deletions, duplications and other rearrangements have been reported (Bennetzen 2000; Feuillet and Keller 2002; Li and Gill 2002; Sorrells et al. 2003; Francki et al. 2004; Lagudah et al. 2006; Lu and Faris 2006; Valárik et al. 2006; Bossolini et al. 2007; Faris et al. 2008). Indeed the *Xwg644* locus consists of two tandem genes coding for independent half-sized ABC transporters (Ramakrishna et al. 2002), but only a single copy exists in rice and *Brachypodium.* Also, the ESM marker derived from *XBF474090* localized to chromosome 4 in *Brachypodium*.

In a previous study, we only identified a single QTL on 5BL associated with phenotypic variation in grain Cd concentration (Knox et al. 2009). In that study, transgressive segregation for grain Cd concentration was observed, suggesting that additional minor genes influence grain Cd concentration, supporting an earlier hypothesis that other minor genes influence grain cadmium concentration in durum wheat (Clarke et al. 1997). With the improved genetic map of 5BL reported here, we identified a second QTL, designated as *QCdu.usw-B2*, centered at *XCbf32.* Relative to *Cdu1,* the effect of *QCdu.usw-B2* was small, but DH lines carrying the W9262-260D3 molecular variant at *XCbf32* consistently expressed lower Cd content than lines carrying the Kofa molecular variant (Table 3). The primers for *XCbf32* are known to amplify a portion of *CbfIIId-12* (EU194246; Campoli et al. 2009), a gene coding for a C-repeat binding factor (*Cbf*) (Campoli et al. 2009). The *Cbfs* are known transcription factors involved in activation of abiotic stress responsive genes in plants and have been associated with enhanced tolerance to cold (Knox et al. 2008; Campoli et al. 2009) and drought responses (Haake et al. 2002). In rye, we have shown that *Cbf* expression patterns are dependent on the allelic state at *Vrn1* (Campoli et al. 2009) and this has also been shown in wheat (Badawi et al. 2007), and barley (Stockinger et al. 2007). Recently, RT-PCR analysis of several *Cbf* genes revealed transient expression induced by copper stress in hexaploid wheat, suggesting that *Cbfs* may enhance copper tolerance in wheat (Szira et al. 2008). It is possible that the *Cbf* genes have a pleiotropic effect on Cd concentration, possibly by regulating transpiration rates. Higher transpiration rates have been associated with elevated concentrations of several metals and ions in plants, likely the result of increased movement to sink tissue. Indeed, overexpression of an *Arabidopsis Cbf* gene has been shown to improve water use efficiency and reduce transpiration in rice (Karaba et al. 2007). As well, higher expression of *Cbfs* has been associated with reduced transpiration in wheat. Alternatively, we cannot rule out the possibility of linked genes near *XCbf32* that directly influence grain Cd concentrations.

Marker-assisted selection is preferred for selecting breeding lines expressing low Cd because measuring grain Cd is laborious and expensive relative to PCR-based screening (Penner et al. 1995). We have used *ScOpc20* effectively to develop low Cd durum wheat varieties (Pozniak et al. 2009), but it is a dominant marker that is linked in repulsion to the low Cd phenotype, and thus has limited application in backcross breeding (Knox et al. 2009). The three markers reported here that co-segregate with *Cdu1* are co-dominant and could be used as a selection tool in durum wheat breeding programs targeting reduced grain Cd levels. In addition, *XCbf32* has potential for use as a selection tool in further reducing grain Cd levels in durum wheat when combined with *Cdu1* (Table 3). However, these markers should still be validated in a larger germplasm pool to determine their effectiveness for selection of the low grain Cd phenotype in diverse genetic backgrounds. As well, the polymorphisms for these markers were detected using SSCP gels, and conversion of these markers to breeder-friendly, high-throughput markers is a priority.

In conclusion, the three markers identified here that co-segregate with *Cdu1* will serve as the starting point for map-based cloning of *Cdu1*. The physical size of the wheat genome is large, with the largest chromosome (3B) being over twice the size of the entire 370-Mbp rice genome (Itoh et al. 2007). Furthermore, physical mapping of wheat chromosomes has revealed small chromosome segments of high gene density (Faris et al. 2000) and recombination

frequencies are not consistent along chromosomes, with most cross-overs occurring in sub-telomeric regions of wheat chromosomes (Saintenac et al. 2009; Erayman et al. 2004). Together, these two factors can make map-based cloning of genes in wheat a daunting task, and indeed, less than 12 genes have been identified using map-based cloning in wheat (Paux et al. 2008). However, comparison with other high-density maps of 5B suggests that *Cdu1* resides in a gene-rich, recombination hot spot. Saturation mapping of *Tsn1*, which maps proximal to *Cdu1* (see Fig. 2) was estimated to be 400 kb/cM, an 11-fold increase in recombination compared to the genomic average (Faris et al. 2000). The low physical to genetic distance and the observed colinearity between the wheat, rice and *Brachypodium* genomes suggests positional cloning could be used to isolate the *Cdu1* gene from durum wheat. However, we cannot rule out the possibility that the gene responsible for the low Cd phenotype is absent in high accumulators of durum wheat. If this is true, it would have implications for cloning *Cdu1,* because the most utilized BAC library for map-based cloning in durum wheat is derived from Langdon (Cenci et al. 2003), a high-Cd accumulator (C. Pozniak, unpublished results). However, as shown in this study, CS is a low Cd accumulator and showed similar banding patterns to W9262-260D3 at all three *Cdu1* co-segregating markers. Thus, BAC isolation from 5B of CS may be a better alternative for map-based cloning of *Cdu1*.

Acknowledgments We gratefully acknowledge the technical assistance of Ryan Babonich, Russell Lawrie, Charlene Tang, Jay Ross, and Marlin Olfert, and funding of this work by the National Science and Engineering Research Council (NSERC), Western Grains Research Foundation (WGRF), and the Agriculture and Agri-Food Canada Matching Investment Initiative.

References

Alloway BJ, Steinnes E (1999) Anthropogenic additions of cadmium to soils. In: McLaughlin MJ, Singh BR (eds) Cadmium in soils and plants. Kluwer Academic, Dordrecht, pp 97–123

Badawi M, Danyluk J, Boucho B, Houde M, Sarhan F (2007) The CBF gene family in hexaploid wheat and its relationship to the phylogenetic complex of cereal CBFs. Mol Genet Genomics 277:533–554

Bassam BJ, Gresshoff PM (2007) Silver staining DNA in polyacrylamide gels. Nat Protoc 2:2649–2654

Bennetzen JL (2000) Comparative sequence analysis of plant nuclear genomes: microcolinearity and its many exceptions. Plant Cell 12:1021–1029

Bossolini E, Wicker T, Knobel PA, Keller B (2007) Comparison of orthologous loci from small grass genomes *Brachypodium* and rice: implications for wheat genomics and grass genome annotation. Plant J 49:704–717

Campoli C, Matus-Cádiz MA, Pozniak C, Cattivelli L, Fowler DB (2009) Comparative expression of Cbf genes in the *Triticeae* under different acclimation induction temperatures. Mol Genet Genomics 282:141–152

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

Clarke JM, Leisle D, Kopytko GL (1997) Inheritance of cadmium concentration in five durum wheat crosses. Crop Sci 37:1722–1725

CODEX STAN 193-1995 (2009) Codex general standard for contaminants and toxins in foods and feed. Revision 5, 2009. Available at http://www.codexalimentarius.net/download/standards/17/CXS_193e.pdf

Dubcovsky J, Ramakrishna W, SanMiguel PJ, Busso CS, Yan L, Shiloff BA, Bennetzen JL (2001) Comparative sequence analysis of collinear barley and rice bacterial artificial chromosomes. Plant Physiol 125:1342–1353

Dutilleul C, Jourdain A, Bourguignon J, Hugouvieux V (2008) The Arabidopsis putative selenium-binding protein family: expression study and characterization of SBP1 as a potential new player in cadmium detoxification processes. Plant Physiol 147:239–251

Erayman M, Sandhu D, Sidhu D, Dilbirligi M, Baenziger PS, Gill KS (2004) Demarcating the gene-rich regions of the wheat genome. Nucleic Acids Res 32:3546–3565

Faris JD, Haen KM, Gill BS (2000) Saturation mapping of a gene-rich recombination hot spot region in wheat. Genetics 154:823–835

Faris JD, Zhang Z, Fellers JP, Gill BS (2008) Micro-colinearity between rice, *Brachypodium*, and *Triticum monococcum* at the wheat domestication locus Q. Funct Integr Genomics 8:149–164

Ferenc Bálint A, Szira F, Röder MS, Galiba G, Börner A (2009) Mapping of loci affecting copper tolerance in wheat—the possible impact of the vernalization gene Vrn-A1. Environ Exp Bot 65:369–375

Feuillet C, Keller B (2002) Comparative genomics in the grass family: molecular characterization of grass genome structure and evolution. Ann Bot 89:3–10

Francki M, Carter M, Ryan K, Hunter A, Bellgard M, Appels R (2004) Comparative organization of wheat homoeologous group 3S and 7L using wheat–rice synteny and identification of potential markers for genes controlling xanthophyll content in wheat. Funct Integr Genomics 4:118–130

Gill KS, Gill BS, Endo TR, Boiko EV (1996) Identification and high-density mapping of gene-rich regions in chromosome group 5 of wheat. Genetics 143:1001–1012

Grant CA, Buckley WT, Bailey LD, Selles F (1998) Cadmium accumulation in crops. Can J Plant Sci 78:1–17

Grant CA, Clarke JM, Duguid S, Chaney RL (2008) Selection and breeding of plant cultivars to minimize cadmium accumulation. Sci Total Environ 390:301–310

Haake V, Cook D, Riechmann JL, Pineda O, Thomashow MF, Zhang JZ (2002) Transcription factor CBF4 is a regulator of drought adaptation in Arabidopsis. Plant Physiol 130:639–648

Harris NS, Taylor GS (2001) Remobilization of cadmium in maturing shoots of near-isogenic lines of durum wheat that differ in grain cadmium accumulation. J Exp Bot 52:1473–1481

Harris NS, Taylor GJ (2004) Cadmium uptake and translocation in seedlings of near isogenic lines of durum wheat that differ in grain cadmium accumulation. BMC Plant Biol 4:4

Hart JJ, Welch RM, Norvell WA, Kochian LV (2006) Characterization of cadmium uptake, translocation and storage in near-isogenic lines of durum wheat that differ in grain cadmium concentration. New Phytol 172:261–271

Huo N, Lazo GR, Vogel JP, You FM, Ma Y, Hayden DM, Coleman-Derr D, Hill TA, Dvorak J, Anderson OD, Luo M-C, Gu YQ

(2008) The nuclear genome of *Brachypodium distachyon*: analysis of BAC end sequences. Funct Integr Genomics 8:135–147

Iqbal M, Navabi A, Yang R-C, Salmon DF, Spaner D (2007) Molecular characterization of vernalization response genes in Canadian spring wheat. Genome 50:511–516

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

Ishikawa S, Abe T, Kuramata M, Yamaguchi M, Ando T, Yamamoto T, Yano M (2010) A major quantitative trait locus for increasing cadmium-specific concentration in rice grain is located on the short arm of chromosome 7. J Exp Bot 61:923–934

Ishimaru Y, Suzuki M, Kobayashi T, Takahashi M, Nakanishi H, Mori S, Nishizawa N (2005) OsZIP4, a novel zinc-regulated zinc transporter in rice. J Exp Bot 56:3207–3214

Itoh T, Tanaka T, Barrero RA, Yamasaki C, Fujii Y, Hilton PB, Antonio BA, Aono H, Apweiler R, Bruskiewich R et al (2007) Curated genome annotation of *Oryza sativa* ssp *japonica* and comparative genome analysis with *Arabidopsis thaliana*. Genome Res 17:175–183

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

Karaba A, Dixit S, Greco R, Aharoni A, Trijatmiko KR, Marsch-Martinez N, Krishnan A, Nataraja KN, Udayakumar M, Pereira A (2007) Improvement of water use efficiency in rice by expression of HARDY, an Arabidopsis drought and salt tolerance gene. Proc Natl Acad Sci USA 104:15270–15275

Kashiwagi T, Shindoh K, Hirotsu N, Ishimaru K (2009) Evidence for separate translocation pathways in determining cadmium accumulation in grain and aerial plant parts in rice. BMC Plant Biol 9:8

Kim DY, Bovet L, Maeshima M, Martinoia E, Lee Y (2007) The ABC transporter *AtPDR8* is a cadmium extrusion pump conferring heavy metal resistance. Plant J 50:207–218

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

Knox AK, Li C, Vágújfalvi A, Galiba G, Stockinger E, Dubcovsky J (2008) Identification of candidate CBF genes for the frost tolerance locus *Fr-Am2* in *Triticum monococcum*. Plant Mol Biol 67:257–270

Knox RE, Pozniak CJ, Clarke FR, Clarke JM, Houshmand S, Singh AK (2009) Chromosomal location of the cadmium uptake gene (Cdu1) in durum wheat. Genome 52:741–747

Kumar S, Mohan A, Balyan HS, Gupta PK (2009) Orthology between genomes of *Brachypodium*, wheat and rice. BMC Res Notes 2:93

Lagudah ES, McFadden H, Singh RP, Huerta-Espino J, Bariana HS, Spielmeyer W (2006) Molecular genetic characterization of the *Lr34/Yr18* slow rusting resistance gene region in wheat. Theor Appl Genetics 114:21–30

Li W, Gill BS (2002) The colinearity of the *Sh2/A1* orthologous region in rice sorghum and maize is interrupted and accompanied by genome expansion in the *Triticeae*. Genetics 160:1153–1162

Lindsay WL, Norvell WA (1978) Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Sci Soc Am J 42:421–428

Lu H, Faris JD (2006) Macro- and micro-colinearity between the genomic region of wheat chromosome 5B containing the *Tsn1* gene and the rice genome. Funct Integr Genomics 6:90–105

Lu H-J, Fellers JP, Friesen TL, Meinhardt SW, Faris JD (2006) Genomic analysis and marker development for the *Tsn1* locus in wheat using bin-mapped ESTs and flanking BAC contigs. Theor Appl Genet 112:1132–1142

McLaughlin MJ, Parker DR, Clarke JM (1999) Metals and micronutrients—food safety issues. Field Crops Res 60:143–163

Morel M, Crouzet J, Gravot A, Auroy P, Leonhardt N, Vavasseur A, Richaud P (2009) *AtHMA3*, a P_{1B}-*ATPase* Allowing Cd/Zn/Co/Pb vacuolar storage in Arabidopsis. Plant Physiol 149:894–904

Ouyang S, Zhu W, Hamilton J, Lin H, Campbell M, Childs K, Thibaud-Nissen F, Malek RL, Lee Y, Zheng L, Orvis J, Haas B, Wortman J, Buell CR (2007) The TIGR Rice Genome Annotation Resource: improvements and new features. Nucleic Acids Res 35:D883–D887

Parker DR, Norvell WA (1999) Advances in solution culture methods for plant mineral nutrition research. Adv Agron 65:151–213

Parker DR, Norvell WA, Chaney RL (1995) GEOCHEM-PC: a chemical speciation program for IBM and compatible personal computers. In: Loeppert RH, Schwab AP, Goldberg S (eds) Chemical equilibrium and reaction models. SSSA Spec, Publ. 42, Soil Science Society of America, Madison, WI, pp 253–269

Paux E, Sourdille P, Salse J, Saintenac C, Choulet F, Leroy P, Korol A, Michalak M, Kianian S, Spielmeyer W, Lagudah E, Somers D, Kilian A, Alaux M, Vautrin S, Bergès H, Eversole K, Appels R, Safar J, Simkova H, Dolezel J, Bernard M, Feuillet C (2008) A physical map of the 1-gigabase bread wheat chromosome 3B. Science 322:101–104

Penner GA, Clarke J, Bezte LJ, Leisle D (1995) Identification of RAPD markers linked to a gene governing cadmium uptake in durum wheat. Genome 38:543–547

Pozniak CJ, Fox SL, Knott DR (2009) CDC Verona durum wheat. Can J Plant Sci 89:321–324

Qi LL, Echalier B, Chao S, Lazo GR, Butler GE, Anderson OD, Akhunov ED, Dvorak J, Linkiewicz AM, Ratnasiri A 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

Ramakrishna W, Dubcovsky J, Park Y-J, Busso C, Emberton J, SanMiguel P, Bennetzen JL (2002) Different types and rates of genome evolution detected by comparative sequence analysis of orthologous segments from four cereal genomes. Genetics 162:1389–1400

Saintenac C, Falque M, Martin OC, Paux E, Feuillet C, Sourdille P (2009) Detailed recombination studies along chromosome 3B provide new insights on crossover distribution in wheat (*Triticum aestivum* L.). Genetics 181:393–403

Salt DE, Rauser WE (1995) MgATP-dependent transport of phytochelatins across the tonoplast of oat roots. Plant Physiol 107:1293–1301

Satarug S, Moore MR (2004) Adverse health effects of chronic exposure to low-level cadmium in foodstuffs and cigarette smoke. Environ Health Perspect 112:1099–1103

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. A poor man's approach to genotyping for research and high-throughput diagnostics. Nat Biotechnol 16:233–235

Song WY, Sohn EJ, Martinoia E, Lee YJ, Yang YY, Jasinski M, Forestier C, Hwang I, Lee Y (2003) Engineering tolerance and accumulation of lead and cadmium in transgenic plants. Nat Biotechnol 21:914–919

Soric R, Loncaric Z, Kovacevic V, Brkic I, Simic D (2009) A major gene for leaf cadmium accumulation in maize (*Zea mays* L.). In: The proceedings of the international plant nutrition colloquim XVI, UC Davis. Available at http://escholarship.org/uc/item/1q48v6cf

Sorrells ME, LaRota M, Bermudez-Kandianis CE, Greene RA, Kantety R, Munkvold JD, Miftahudin, Mahmoud A, Ma X,

Gustafson PJ et al (2003) Comparative DNA sequence analysis of wheat and rice genomes. Genome Res 13:1818–1827

Stockinger EJ, Skinner JS, Gardner KG, Francia E, Pecchioni N (2007) Expression levels of barley Cbf genes at the *Frost resistance-H2* locus are dependent upon alleles at *Fr-H1* and *Fr-H2*. Plant J 51:308–321

Stolt JP, Sneller FEC, Bryngelsson T, Lunborg T, Schat H (2003) Phytochelation and cadmium accumulation in wheat. Environ Exp Bot 49:21–28

Szira F, Ferenc Bálint A, Vágújfalvi A, Galiba G (2008) Are *Cbf* genes involved in copper tolerance? Acta Biol Szeged 52:205–207

Takahashi R, Ishimaru Y, Senoura T, Shimo HM, Nakanishi H, Nishizawa NK (2009) Characterization of OsNramp1, a metal transporter from rice. In: The proceedings of the international plant nutrition colloquium XVI, Department of Plant Sciences, UC Davis. Available at http://escholarship.org/uc/item/2vh9z40

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

Tezuka K, Miyadate H, Katou K, Kodama I, Matsumoto S, Kawamoto T, Masaki S, Satoh H, Yamagucji M, Sakurai K, Takahashi H, Satoh-Nagasawa N, Watanabe A, Fujimura T, Akagi H (2009) A single recessive gene controls cadmium translocation in the cadmium hyperaccumulating rice cultivar Cho-Ko-Kohu. Theor Appl Genet 120:1175–1182

Ueno D, Kono I, Yokosho K, Ando T, Yano M, Ma JF (2009) A major quantitative trait locus controlling cadmium translocation in rice (*Oryza sativa*). New Phytol 182:644–653

Uraguchi S, Mori S, Kuramata M, Kawasaki A, Arao T, Ishikawa S (2009) Root-to-shoot Cd translocation via the xylem is the major process determining shoot and grain cadmium accumulation in rice. J Exp Bot 60:2677–2688

Valárik M, Linkiewicz A, Dubcovsky J (2006) A microcolinearity study at the earliness per se gene Eps-A^m 1 region reveals an ancient duplication that preceded the wheat–rice divergence. Theor Appl Genetics 112:945–957

van Ooijen JW (1999) LOD significance thresholds for QTL analysis in experimental populations of diploid species. Heredity 83:613–624

Van Ooijen JW (2004) MapQTL v5, Software for the mapping of quantitative trait loci in experimental populations. Kyazma BV, Wageningen

van Ooijen JW, Voorrips RE (2004) JoinMap Version 4.0, Software for the calculation of genetic linkage maps. Kyazma BV, Wageningen

Vogeli-Lange R, Wagner GJ (1990) Subcellular localization of cadmium and cadmium-binding peptides in tobacco leaves. Implication of a transport function for cadmium-binding peptides. Plant Physiol 92:1086–1093

Wojas S, Hennig J, Plaza S, Geisler M, Siemianowski O, Sklodowska A, Ruszczynska A, Bulska E, Antosiewicz DM (2009) Ectopic expression of *Arabidopsis* ABC transporter *MRP7* modifies cadmium root-to-shoot transport and accumulation. Environ Pollut 157:2781–2789

Xue D, Chen M, Zhang G (2009) Mapping of QTLs associated with cadmium tolerance and accumulation during seedling stage in rice (Oryza sativa L.). Euphytica 165:587–596

Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J (2003) Positional cloning of wheat vernalization gene *VRN1*. Proc Natl Acad Sci USA 100:6263–6268

Yu GT, Cai X, Harris MO, Gu YQ, Luo MC, Xu SS (2009) Saturation and comparative mapping of the genomic region harboring Hessian Xy resistance gene H26 in wheat. Theor Appl Genet 118:1589–1599

Zook EG, Greene FE, Morris ER (1970) Nutrient composition of selected wheats and 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