Physical Mapping of Puroindoline b-2 Genes in Wheat using ‘Chinese Spring’ Chromosome Group 7 Deletion Lines

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ABSTRACT
The puroindoline genes (Puroindoline a-D1 and Puroindoline b-D1), located very near to the distal end of the short arm of chromosome 5D (distal to fraction arm length of 0.78) have a significant effect on grain hardness. Puroindoline b-2 (Pinb-2) is another puroindoline gene family that exists as a homoeologous series on group 7 chromosomes. However, a more detailed localization (physical mapping) of the Pinb-2 genes has not been conducted. In the present study, 24 group 7 long-arm chromosome deletion stocks of ‘Chinese Spring’ were used to physically map three Pinb-2 variant genes: Pinb-2v1, Pinb-2v2, and Pinb-2v4. All three genes were found to be physically located on the most distal 0.11 to 0.16 fraction arm length of chromosomes 7AL, 7BL, and 7DL in Chinese Spring. These results contribute insight into wheat (Triticum aestivum L.) genome syntenY, structure, and organization and provide a useful metric for germplasm and population relationships. Future studies may further resolve the physical mapping of Pinb-2 genes at the ends of group 7 chromosomes and contribute to a better understanding of the molecular and genetic basis of kernel hardness.

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Abbreviations: CS, Chinese Spring; Del., deletion; FL, fraction arm length; LDN, Langdon; PCR, polymerase chain reaction.

Grain hardness plays an important role in end-use quality and usage and consequently is a major factor affecting trade of hexaploid (common) wheat (Law et al., 1978; Mattern et al., 1973). Wheat is normally classified into soft, mixed, or hard types on the basis of grain hardness (Morris, 2002). Giroux and Morris (1997, 1998) showed that grain hardness is controlled by two tightly linked major genes Puroindoline a (Pina) and Puroindoline b (Pinb) that are located on the short arm of chromosome 5D in common wheat. The deletion stocks of Endo and Gill (1996) in Chinese Spring
(CS) and a BC7-derivative in ‘Alpowa’ (Morris and Beecher 2012) facilitated the placement of *Pina* and *Pinb* in the most distal 0.22 fraction arm length (FL) of 5DS. Minor genes for grain hardness have been identified on chromosomes 1A, 2AL, 2DL, 5BL and 6DS (Law et al., 1978; Mattern et al., 1973; Perretant et al., 2000; Pomeranz and Williams, 1990; Sourdille et al., 1996).

The *Grain Softness Protein-1* (*Gsp-I*) gene is tightly linked to *Pina* and *Pinb* on chromosome 5DS (Chantret et al., 2005) and was the first puroindoline-like gene identified (Blochet et al., 1993; Morris, 2002). In contrast to the *Pin* genes, the *Gsp-I* gene is retained as a homoeologous series with copies on the 5A, 5B, and 5D chromosomes in common wheat (Blochet et al., 1993; Chantret et al., 2005). To date, a correlative relationship between *Gsp-I* genes and grain hardness has not been established (Gollan et al., 2007; Massa et al., 2004; Tranquilli et al., 2002), even though the GSP-1 protein shows approximately 40% identity and 60% similarity to *PINA* and *PINB* proteins, respectively (Chantret et al., 2005; Turner et al., 1999). Although a definitive function of GSP-1 proteins is still unknown, they may possibly play a role in plant defense (Gollan et al., 2007).

Chantret et al. (2005) found evidence for ancient evolutionary duplications of puroindoline-like genes in wheat. Located at the 5DS *Hardness* locus were sequences termed *Pinb-relic* and *PseudoPinb*. No other information on these apparent vestigial genes has been forthcoming.

Puroindoline *b*-*2* genes were discovered by Wilkinson et al. (2008) by “mining” the dbEST (expressed sequence tag database) (http://www.ncbi.nlm.nih.gov/dbEST/) with *Pinb* sequence. Three unique sequences were identified and designated “Pin b variant 1,” “Pin b variant 2,” and “Pin b variant 3” (abbreviated PinBv1, PinBv2, and PinBv3, respectively). They subsequently isolated and sequenced complementary DNA clones that provided corresponding gene sequences. The deduced amino acid sequences of these genes, compared to *Pinb-Dla*, were 57, 57, and 60% identical (PinBv1, PinBv2, and PinBv3, respectively). Genetic mapping studies were interpreted as indicating that PinBv1, PinBv2, and PinBv3 were probably “encoded by the same *Pinb*-locus on 7A, termed *Pinb*-A2” (Wilkinson et al., 2008). In contrast, Chen et al. (2010a) physically mapped PinBv1, PinBv2, and PinBv3 to 7DL, 7BL, and 7B, respectively, using CS ditelosomics, nullisomic-tetrasomics, and disomic substitution lines. In addition, a fourth *Pinb*-2 variant was discovered (PinBv4), which mapped to 7AL. These results supported a homoeologous series model with PinBv2 and PinBv3 possibly allelic; PinBv3 was not present in CS. To simplify the nomenclature and more closely conform to the Catalogue of Gene Symbols for Wheat (McIntosh et al., 2009; Morris and Bhave, 2008), these genes were redesignated *Pinb*-2v1, *Pinb*-2v2, *Pinb*-2v3, and *Pinb*-2v4. Chen et al. (2011a, 2011b) corroborated the previous chromosome assignments and extended them by showing that Langdon durum [*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.] lacked *Pinb*-2v1. This gene, however, was present in Langdon (LDN) disomic substitutions of CS, LDN 7D(7A) and LDN 7D(7B), supporting its 7D location in CS. *Pinb*-2v2 was assigned to 7B in CS but was not found in LDN. *Pinb*-2v3 was again found to be absent in CS but was present in LDN. *Pinb*-2v4 was localized to 7A in CS and also in LDN.

Previous results (Chen et al., 2010a, 2011a, 2011b) were consistent with *Pinb*-2v2 and *Pinb*-2v3 being allelic at the 7B locus. Variety surveys of Chen et al. (2010b), Geng et al. (2012), and Chen et al. (2012) were consistent with this model. In addition, two sequence polymorphic forms of *Pinb*-2v3 were recently observed in durum and hexaploid wheat varieties (Chen et al., 2012).

The physical position of *Puroindoline b*-2 genes has not been resolved further than the whole chromosome or whole chromosome arm. In the present study, 24 group 7 long-arm chromosome deletion stocks of CS (Endo and Gill, 1996) were used to refine the physical map of the three known *Pinb*-2 variants in CS: *Pinb*-2v1, *Pinb*-2v2, and *Pinb*-2v4.

**MATERIALS AND METHODS**

**Deletion Lines**

Twenty-four group 7 wheat homozygous deletion (Del) lines in CS were used and included 10 Del lines of chromosome 7AL (CS Del7AL), seven Del lines of chromosome 7BL (CS Del7BL), and seven Del lines of chromosome 7DL (CS Del7DL). Seed of these genetic stocks was obtained from Prof. B.S. Gill (Kansas State University); plants were grown in the greenhouse at Washington State University, Pullman, WA. Wheat chromosome bin maps can be accessed at the Group–7 long-arm deletion lines in Chinese Spring wheat (http://www.k-state.edu/wgrc/Germplasm/Deletions/grp7L.html [accessed 12 Apr. 2012]). The chromosome arm cytological FL value in a given Del line identifies the physical breakpoint in the deleted chromosome and the length of the remaining chromosome arm from the centromere relative to the total length of the euploid arm.

**Physical Mapping of the Wheat Puroindoline b-2 Variants**

Genomic DNA was extracted from leaves of individual seedlings for each Del line about 3 wk after emergence, according to the procedures described by Sharp et al. (1989), and was analyzed for *Puroindoline b*-2 variants. To obtain physical mapping of *Puroindoline b*-2 variants in wheat, *Pinb*-2v1, *Pinb*-2v2, and *Pinb*-2v4 were identified using gene specific primers (Table 1). Amplification of *Pinb*-2v1, *Pinb*-2v2, and *Pinb*-2v4 was performed as described by Chen et al. (2010a, 2011a), with minor modifications.

Polymerase chain reaction (PCR) reactions were performed in an MJ Research PTC-200 thermalcycler in a total volume of 25 μL including 250 μM of each deoxyribonucleotide triphosphate, 10 pmol of each primer, 100 ng of genomic DNA, 1x reaction buffer (50 mM KCl, 10 mM Tris-Cl, and 1.5 μM MgCl2, pH 8.4), and 1 U *Taq* DNA polymerase (Promega). Polymerase chain reaction cycling conditions were 94°C for 5 min followed by 45 cycles of 94°C for 50 s, 63 to 68°C (for
primer-specific annealing temperatures, see Table 1) for 50 s, and 72°C for 1 min with a final extension of 72°C for 10 min. The PCR products were separated by electrophoresis on a 1.5% (w/v) agarose gels. The bands were stained with ethidium bromide and visualized using ultraviolet light.

RESULTS

**Physical Mapping of Puroindoline b-2 Variant 1**

*Pinb-2v1* was found to be present in all 17 CS Del lines for 7AL and 7BL but absent in the seven Del lines for the long arm of chromosome 7D (with break points at Del7DL-6, FL = 0.01; Del7DL-1, FL = 0.14; Del7DL-5, FL = 0.30; Del7DL-2, FL = 0.61; Del7DL-4, FL = 0.76; Del7DL-8, FL = 0.77; and Del7DL-7, FL = 0.84) (Table 2). This result indicates that *Pinb-2v1* is located in the distal 0.16 FL portion of 7DL, between breakpoint FL 0.84 (Del7DL-7) and the undeleted (FL 1.00) chromosome of euploid CS (Fig. 1a).

**Physical Mapping of Puroindoline b-2 Variant 2**

The gene-specific primers for *Pinb-2v2* amplified PCR fragments in all the CS Del lines for chromosomes 7AL and 7DL whereas no PCR fragment was amplified in the seven Del lines for the long arm of chromosome 7B (Del7BL-1, FL = 0.40; Del7BL-9, FL = 0.45; Del7BL-7, FL = 0.63; Del7BL-5, FL = 0.69; Del7BL-10, FL = 0.78; Del7BL-6, FL = 0.84; and Del7BL-3, FL = 0.86) (Table 2). This result indicates that *Pinb-2v2* gene is similarly located in the most distal portion (in this case FL 0.14), distal to the breakpoint in Del7BL-3 (Fig. 1b).

**Physical Mapping of Puroindoline b-2 Variant 4**

The gene-specific primers for *Pinb-2v4* produced a PCR fragment in all of the 14 chromosome 7DL and 7BL Del lines (Table 2). However, no PCR fragment was produced in any of the 10 Del lines for 7AL (Del7AL-4, FL = 0.18; Del7AL-14, FL = 0.31; Del7AL-1, FL = 0.39; Del7AL-11, FL = 0.40; Del7AL-5, FL = 0.63; Del7AL-6, FL = 0.80; Del7AL-8, FL = 0.83; Del7AL-16, FL = 0.86; Del7AL-2, FL = 0.87; and Del7AL-9, FL = 0.89) (Table 2). This result similarly indicates that the *Pinb-2v4* gene is located most distally on 7AL, beyond the FL 0.11 breakpoint in Del7AL-9 (Fig. 1c).

**DISCUSSION**

Previous physical mapping studies using CS ditelosomic, nullisomic-tetrasomic, and CS-Wichita and CS-LDN disomic substitution lines indicated that *Pinb-2v1*, *Pinb-2v2*, *Pinb-2v3*, and *Pinb-2v4* loci were located somewhere on chromosomes 7AL, 7BL, 7B and 7DL, respectively (Chen et al., 2010a, 2011a, 2011b). Using the gametocidal chromosome of jointed goatgrass (*Aegilops cylindrica* Host), Endo and Gill (1996) isolated 436 Del lines in CS. These Del lines are powerful tools for the refined physical mapping of wheat chromosomes and have been extensively used in physical mapping of the wheat genome (Delaney et al., 1995; Qi and Gill, 2001; Weng et al., 2000; Werner et al., 1992a, 1992b). To further localize the *Puroindoline b-2* variants within each of the respective chromosome arms, 24 of these homozygous deletion stocks of CS for group 7 long-arm chromosomes were characterized using gene-specific PCR primers for *Pinb-2v1*, *Pinb-2v2*, and *Pinb-2v4*.

Our study confirms the previous physical mapping data on the existence of *Pinb-2v1*, *Pinb-2v2*, and *Pinb-2v4* loci on the long arms of homoeologous group 7 chromosomes (Chen et al., 2010a, 2011a, 2011b) but also further shows that these *Pinb-2* genes are located on the most distal...
portion of the chromosomes (Fig. 1). The “least” deleted chromosomes included in the study were Del7AL-9, Del7BL-3, and Del7DL-7 with FL ratios of 0.89, 0.86, and 0.84, respectively (Table 2). Within the limits of cytological resolution, it would appear that all homoeologous loci are at similar locations on the chromosome and likely follow a conserved synteny among the A, B, and D genomes.

We may further point out that although the related puroindoline genes, Puroindoline a-D1 and Puroindoline b-D1, are also located very near to the distal end of their chromosome (distal to FL of 0.78), these genes are on a different homoeologous series (group 5) and on the short, as opposed to the long arm.

In this study the location of Pinb-2v3 could not be physically mapped using this set of CS Del lines since Pinb-2v3 is not present in CS (Chen et al., 2010a, 2011a, 2011b). Using disomic substitution lines of Wichita in CS and CS in LDN durum, Pinb-2v3 was localized to 7B and 7BL (Chen et al., 2010a, 2011a, 2011b). In addition, past studies have suggested that Pinb-2v2 and Pinb-2v3 were likely allelic (Chen et al., 2010a, 2010b, 2011a, 2011b, 2012; Geng et al., 2012). Therefore, for now, we assume that both Pinb-2v2 and Pinb-2v3 reside on the distal end of the long arm of chromosome 7B. Future studies will need to more carefully address the allelic status of the two genes.

In genetic mapping, Wilkinson et al. (2008) reported that three Puroindoline b-2 variants (PinBv1, PinBv2, and PinBv3) were probably located on the distal end of 7AL using three doubled haploid populations. In one of these populations derived from ‘Spark’ × ‘Rialto’, PinBv1 was most tightly linked with Xwm116 on the long arm of chromosome 7A. Xwm116 was located at 82 cM and PinBv1 at 87 cM. Based on accumulating data, this result will need to be carefully reexamined.

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References