Chromosome location and characterization of genes for grain protein content in *Triticum dicoccoides*

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ABSTRACT

Three sets of Langdon (LDN) durum-*Triticum dicoccoides* (LDN-DIC) disomic chromosome substitution lines were previously developed by L. R. Joppa using *T. dicoccoides* accessions Israel A (IsA), PI 481521 (521), and PI 478742 (742) as the chromosome donors and LDN durum as the recipient. The set based on Israel A was well characterized previously, resulting in the identification and isolation of *Gpc-B1*, a major gene for high grain protein content (GPC) on chromosome 6B. This study was conducted to identify and characterize genes for high GPC in the two sets based on PI 481521 and PI 478742. The two sets of substitutions lines and controls were grown at Fargo and Prosper, ND, in 2005 and 2006 using a randomized complete block design with four replications. The GPC analysis from the trials showed that the substitution lines LDN(742-6B) and LDN(521-7B) had the highest GPC within their respective sets, suggesting that chromosome 6B of PI 478742 and 7B of PI 481521 carry high GPC genes. In addition, six other substitutions including 1A, 2A, and 5B of PI 481521 and 7A, 5B, and 7B of PI 478742 had significantly higher GPC than LDN. To determine if the GPC gene in PI 478742 is the same as *Gpc-B1*, we screened three *T. dicoccoides* accessions and their 6B substitution lines using an allele specific marker *Xuhw89* for *Gpc-B1*. Marker analysis indicated that chromosome 6B of PI 478742 carried the same *Gpc-B1* allele as Israel A. A comparison of the chromosomal locations of other GPC genes in the three *T. dicoccoides* accessions suggested that chromosomes 2A and 7B of PI 481521 and 7A of PI 478742 are likely candidates as sources of new high GPC genes.

INTRODUCTION

Grain protein content (GPC) of durum wheat (*T. turgidum* L. var. *durum*) is an important factor in pasta product quality. High GPC increases nutritional value and pasta firmness (Samaan et al. 2006) and decreases pasta stickiness (Del Nobile et al. 2005). Thus, high GPC has been one of the major goals in durum wheat breeding. *Triticum dicoccoides*, a wild progenitor of durum wheat, has accessions with high GPC ranging from 150 to 250 g kg^{-1} (Mansur-Vergara et al. 1984). To uncover the genetic controls of high GPC in *T. dicoccoides*, Joppa and Cantrell (1990) substituted chromosomes of *T. dicoccoides* accession Israel A into a 'Langdon' (LDN) background to produce LDN-*T. dicoccoides* (LDN-DIC) substitutions. Joppa and

Cantrell (1990) located GPC genes to chromosomes 6B, 2A, 5B, 3A, and 6A, with chromosome 6B having a particularly strong effect. Joppa et al. (1997) studied recombinant inbred chromosome lines derived from the LDN(IsA-6B) substitution and found that a single locus lying between *Xabg387-6B* and *Xmwg79-6B* accounted for 66% of the variation in GPC. In subsequent studies, this locus was named *Gpc-B1* (Olmos et al. 2003); was linked to a marker (*Xuhw89*) lying only 0.1 cM distal to the gene (Distelfeld et al. 2006); and, using positional cloning, *Gpc-B1* was identified as a nitrogen assimilation control (NAC) gene, with modern cultivars having non-functional copies of the gene due to a 1 bp insertion (Uauy et al. 2006).

In addition to the LDN-DIC (IsA) substitutions, two additional sets of LDN-DIC substitutions based on *T. dicoccoides* accessions PI 481521 and PI 478742, were produced to transfer resistance to Fusarium head blight (FHB) from *T. dicoccoides* to durum wheat. These sets are proving to be useful both as sources of new genetic variability and for genetic studies. Kumar et al. (2007) mapped a QTL (*Qfhs.fcu-7AL*) which conferred moderate FHB resistance in LDN(742-7A) to the proximal region of the 7AL arm. Xu et al. (2003) used the LDN-DIC (521) set to developed chromosome-specific molecular markers. Novel glutenin and gliadin proteins have been located to the Group 1 and Group 6 chromosomes of all three LDN-DIC sets (Xu et al. 2004). Fuerst et al. (2008) used the LDN-DIC (IsA) and LDN-DIC (521) sets to show that polyphenol oxidase activity is controlled by a gene(s) on the Group 2 chromosomes, with chromosome 2A having the greater effect. In this paper, we report the chromosomal location and characterization of genes for GPC using the LDN-DIC (521) and LDN-DIC (742) sets of substitutions.

MATERIALS AND METHODS

Two *T. dicoccoides* accessions PI 481521 and PI 478742 and their 5A substitutions had winter growth habit and they could not be used in this study. For the PI 478742 set, the substitutions for chromosomes 2A, 3A, and 3B were not produced (Xu et al., 2004). Therefore, there were 13 LDN-DIC (521) and 10 LDN-DIC (742) substitutions used in this study. In addition, the LDN(IsA-1B) and LDN(IsA-6B) substitutions, the recurrent parent LDN, and four currently grown durum cultivars Lebsock, Maier, Pierce, and Ben were included as checks.

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The LDN-DIC lines and checks were grown at Fargo and Prosper, ND in 2005 and 2006 using a randomized complete block design with four replications. Yield was measured on a whole-plot basis. Because there was only sufficient seed produced from a four-replication yield trial to mill a single sample from each environment, each sample was prepared by taking equal portions of grain from each replication within an environment. Protein content was determined by the Dumas method using the Leco FP-528. For each environment, each entry's PROYLD was determined as (grain protein concentration × each entries mean grain yield)/1000.

For molecular marker analysis, total genomic DNA was isolated from young leaf tissue of each of the LDN-DIC lines and their parents according to Faris et al. (2000). Marker *Xuhw89* was used to screen for the presence of the *Gpc-B1* allele that originated from Israel A. PCR reactions were carried out following the conditions reported by Distelfeld et al. (2006) with the forward primer labeled with a fluorescent dye. The fluorescent dye-labeled PCR fragments were separated on the 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Fragment analysis and allele calling were performed using GeneMapper software v3.7 from Applied Biosystems.

Statistical analysis for yield data was conducted using the SAS Proc GLM (SAS Institute 1999) procedure. The main effects, lines and environment, were treated as fixed effects and random effects, respectively. The line main effect was tested using the mean square for the line by environment interaction. Means were separated by least significant difference (LSD). The data for GPC were analyzed using Proc GLM, with environments treated as replications, and entries being a fixed effect, because each environment produced only a single replication for GPC.

RESULTS AND DISCUSSION

The GPC analysis showed that GPC of LDN was 136.3 g kg^{-1} and nine of the 25 LDN-DIC lines had GPC significantly (LSD$_{0.05}$ = 5.5 g kg^{-1}) higher than LDN. The GPC of LDN(IsA-6B) was 20.2 g kg^{-1} higher than that of LDN, which was similar to the 15 g kg^{-1} estimated in other studies (Chee et al. 2001). Among the other lines having significantly higher GPC than LDN, increases in GPC were 13.5 g kg^{-1} in LDN(742-6B), 9.2 g kg^{-1} in LDN(521-7B), 8.7 g kg^{-1} in LDN(521-5B), 7.7 g kg^{-1} in LDN(742-7A), and 6.7 g kg^{-1} in LDN(742-5B), 6.5 g kg^{-1} in LDN(521-2A) and LDN(742-7B), and 6.2 g kg^{-1} in LDN(521-1A).

Yield should be taken into account when evaluating GPC because it can have negative effect on GPC. Mean grain yields for all of the LDN-DIC lines (212.2 – 310.7 g m^{-1}) were less than the four currently grown cultivars (314.6 - 362.4 g m^{-1}), though five of them did not differ significantly (LSD$_{0.05}$ = 27.5 g m^{-1}) from Ben (314.6 g m^{-1}). Three of the LDN-DIC lines, including LDN(521-3B) (310.7 g m^{-1}), LDN(742-1B) (306.9 g m^{-1}), and LDN(521-6B) (303.9 g m^{-1}), had higher grain yield than their recurrent parent, LDN (303.6 g m^{-1}); though none were significantly higher. Nine LDN-DIC lines did not differ significantly from LDN for grain yield.

The three lines LDN(521-3A) (207.4 g m^{-1}), LDN(742-2B) (212.2 g m^{-1}), and LDN(742-7B) (233.7 g m^{-1}) had the lowest yields among the LDN-DIC lines.. The three lines all had unique characteristics contributing to their low grain yield. LDN(521-3A) produced weak plants and were the shortest in plant height and they had the smallest spikes of the LDN-DIC lines. LDN(742-2B) produced weak stands, with plants significantly shorter than LDN, and had the highest severity of foliar diseases such as tan spot and Stagonospora nodorum blotch among the entries at Prosper in 2005. LDN(742-7B) was the tallest and latest heading entry, being 7.0 d later than LDN. Among the three lines, only LDN(742-7B) had significantly higher GPC than LDN, suggesting that high GPC of LDN(742-7B) might be related to its low yield.

PROYLD is a useful index when evaluating lines initially selected for either GPC or grain yield. PROYLD of the LDN-DIC lines ranged from 29.2 g m^{-2} in LDN(521-3A) to 43.9 g m^{-2} in LDN(IsA-6B). Only LDN(IsA-6B) had a higher PROYLD than LDN, though it was not significantly different from LDN. In a prior study, Cantrell and Joppa (1991) found that LDN(IsA-6B) had a significantly higher PROYLD than LDN. The differing result of this study versus Cantrell and Joppa (1991) may be attributed to the difference between LDN and LDN(IsA-6B) in grain yield; with LDN out-yielding LDN(IsA-6B) in the present study, but the reverse being true in the study by Cantrell and Joppa (1991). The PROYLD rankings of the LDN-DIC lines that had significantly higher GPC than LDN were: LDN(IsA-6B) (43.9 g m^{-2}), LDN(742-6B) (40.0 g m^{-2}), LDN(521-2A) (39.3 g m^{-2}), LDN(742-5B) (37.4 g m^{-2}), LDN(521-1A) (37.3 g m^{-2}), LDN(521-5B) (37.1 g m^{-2}), LDN(742-7A) (37.1 g m^{-2}), LDN(521-7B) (35.2 g m^{-2}), and LDN(742-7B) (33.3 g m^{-2}). Interestingly, LDN(521-6B) (41.9 g m^{-2}), which did not have a significantly higher GPC than LDN, had a higher PROYLD than any other LDN-DIC line except LDN(IsA-6B). The relative PROYLD performance of the LDN-DIC lines indicates that with the exception of LDN(742-6B), none of the other lines combines good grain yield with high GPC.

Because *Xuhw89* is an allele specific marker for the *Gpc-B1* gene, it can be used to test whether any of the LDN-DIC lines in the study carry the same allele on chromosome 6B as Israel A. Lines that carry the *Gpc-B1* allele will produce a 122 bp PCR product, while lines that carry the wild type allele will produce a 126 bp product (Distelfeld et al. 2006). We screened eight of the LDN-DIC lines, including LDN(IsA-6B), LDN(521-6B), LDN(742-6B), LDN(742-5B), LDN(521-1A), LDN(521-5B), LDN(742-7A), and LDN(521-7B), the three parental *T. dicoccoides* accessions, and LDN. Results indicated that PI 478742 and LDN(742-6B) carried the same *Gpc-B1* allele as Israel A and LDN(IsA-6B); however, PI 481521, LDN(521-6B), and the five other LDN-DIC lines had the wild type allele found in LDN. Therefore, the high GPC of LDN(742-

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5B), LDN(521-1A), LDN(521-5B), LDN(742-7A), and LDN(521-7B) was not due to carrying the *Gpc-B1* allele that is present in Israel A.

The fact the LDN(742-6B) was shown to carry the same *Gpc-B1* allele as is present in Israel A suggests that this allele may be common in *T. dicoccoides* collections. If so, this suggests a promising method for selection of new accessions with high GPC genes. *T. dicoccoides* accessions could be screened for the presence of the *Gpc-B1* allele. Accessions that do not possess the allele, but have high GPC would be potential sources of new high GPC genes other than *Gpc-B1*.

Except for the *Gpc-B1* on chromosome 6B, GPC genes from Israel A were also located to chromosomes 2A, 5B, 3A, and 6A (Joppa and Cantrell 1990). Therefore, the genes for high GPC other than the *Gpc-B1* might be present commonly on chromosomes 2A of Israel A and PI 481521 and 5B of all three *T. dicoccoides* accessions. The high GPC in the LDN(IsA-5B) substitution has been shown to be conditioned by three QTLs on chromosome 5B, with the most important one producing an increased GPC of 5 g kg^{-1} (Gonzalez-Hernandez et al. 2004). Because no genes for high GPC were ever located on group 7 chromosomes of Israel A, 7B of PI 481521 and PI 478742 and 7A of PI 478742 are likely candidates as sources of new high GPC genes. Steiger et al. (1996) transferred the high GPC genes from the LDN-DIC (IsA) substitutions to an improved background, and found that chromosomes 6B and 5B had the strongest effect on GPC; that 1A and 6A had a significant, though small effect; and reported no effects of chromosomes 2A and 3A on GPC. Joppa and Cantrell (1990) indicated that high GPC in LDN(IsA-2A) might have been related to its low yield because it had lowest yield among the LDN-DIC (IsA) substitution lines. However, except for the lines with the *Gpc-B1*, LDN(521-2A) had higher PROYLD rankings than the other six LDN-DIC lines with high GPC, indicating that chromosome 2A from PI 481521 might carry gene(s) having strong effects on GPC.

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