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ORIGINAL PAPER

Marker-assisted characterization of durum wheat Langdon–Golden Ball disomic substitution lines

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Abstract The durum wheat cultivar 'Golden Ball' (GB) is a source of resistance to wheat sawfly due to its superior solid stem. In the late 1980s, Dr. Leonard Joppa developed a complete set of 14 'Langdon' (LDN)–GB disomic substitution (DS) lines by using GB as the chromosome donor and LDN as the recipient. However, these substitution lines have not been previously characterized and reported in the literature. The objectives of this study were to confirm the authenticity of the substituted chromosomes and to analyze the genetic background of the 14 LDN–GB DS lines with the aid of molecular markers, and to further use the substitution lines for chromosomal localization of DNA markers and genes conferring the superior stem solidness in GB. Results from simple sequence repeat marker analysis validated the authenticity of the substituted chromosomes in 14 LDN–GB DS lines. Genome-wide scans using the target region amplification polymorphism (TRAP) marker system produced a total of 359 polymorphic fragments that were used to compare the genetic background of substitution lines with that of LDN. Among the polymorphic TRAP markers, 134 (37.3%) and 185 (51.5%) were present in LDN and GB, respectively, with only 10 (2.8%) derived from Chinese Spring. Therefore, marker analysis demonstrated that each LDN–GB DS line had a pair of chromosomes from GB with a genetic background similar to that of LDN. Of the TRAP markers generated in this study, 200 were successfully assigned to specific chromosomes based on their presence or absence in the corresponding LDN–GB DS lines. Also, evaluation of stem solidness in the substitution lines verified the presence of a major gene for stem solidness in chromosome 3B. Results from this research provides useful information for the utilization of GB and LDN–GB DS lines for genetic and genomic studies in tetraploid wheat and for the improvement of stem solidness in both durum and bread wheat.

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Introduction

Solid-stemmed wheat genotypes can provide effective control of wheat stem sawfly (*Cephus cinctus* Nort.), one of the major insect pests of durum (*Triticum turgidum* L., subsp. *durum*, $2n = 4x = 28$, AABB) and bread wheat (*T. aestivum* L., $2n = 6x = 42$, AABBDD) in the Northern Great Plains of North America (Eckroth and McNeal 1953; Weiss and Morrill 1992; Clarke et al. 2002). Early research indicated that stem-solidness in hexaploid wheat was controlled by genes located on five chromosomes of homoeologous groups 3 and 5 (Larson and MacDonald 1959) with mainly additive (McNeal et al. 1974; Wallace et al. 1973) but also epistatic (Hayat et al. 1995; McKenzie 1965) effects. More recently, Cook et al. (2004) mapped a single QTL for solid stems in chromosome arm 3BL, while Lanning et al. (2006) mapped a second QTL in chromosome arm 3DL. In tetraploid durum wheat, stem-solidness has been reported to be controlled by one recessive gene (Engledow 1923), one gene with partial dominance (Putnam 1942), or one dominant gene (Engledow and Hutchinson 1926; Clarke et al. 2002). Houshmand et al. (2007) reported

a QTL associated with a dominant stem-solidness gene on chromosome arm 3BL in durum wheat.

The first solid-stemmed common wheat cultivar produced in the Northern Great Plains was 'Rescue' (Larson and MacDonald 1962). Early attempts to breed new solid-stemmed cultivars derived from Rescue were hampered by a negative correlation between stem solidness and yield (McNeal et al. 1965). This negative correlation has since been discounted (Lebsock and Koch 1968; McNeal and Berg 1979; Hayat et al. 1995; Cook et al. 2004; Lanning et al. 2006). The source of solid stems in common wheat cultivars, including Rescue, was S-615 (Larson and MacDonald 1962). A second source of the solid stem trait is the durum wheat cultivar 'Golden Ball' (GB) (Kemp 1934) which was introduced to the United States from South Africa in 1918 (Clark et al. 1922). Stem solidness in GB differs from that of the common wheat S-615 by having a superior solid stem, particularly in the upper most internode (McNeal 1961). Numerous unsuccessful attempts to transfer stem solidness from GB to hexaploid wheat were made (McNeal 1961; Larson and MacDonald 1963), and the failure to successfully transfer the trait was attributed to an inhibitor gene on chromosome 3D (Larson and MacDonald 1963). However, wheat germplasm derived from GB and having stems as solid as GB has recently been developed and released (Clarke et al. 2005).

In the late 1980s, Dr. Leonard Joppa developed a set of 14 Langdon (LDN)–GB intervarietal disomic substitution (DS) lines using GB as the chromosome donor and LDN as the recipient. Each of the LDN–GB DS lines carries 13 pairs of chromosomes from LDN and one pair from GB. Because each of the GB chromosomes is present in the same LDN background, the substitution lines are useful tools for evaluating the effect of stem solidness on grain and biomass yield, and for locating both major and minor genes governing stem solidness or other traits. Although a dominant gene for stem-solidness has been mapped to chromosome arm 3BL in a doubled haploid population using molecular markers (Houshmand et al. 2007), the availability of LDN–GB DS lines will allow us to more precisely evaluate the effects of this gene.

The LDN–GB DS lines were developed using the same procedure for development of the Langdon-*T. turgidum* L. subsp. *dicoccoides* (LDN-DIC) DS lines as described by Joppa and Cantrell (1990). Meiotic pairing analysis with the use of LDN–Chinese Spring (CS) D-genome DS as recurrent parents was the major approach for development and selection of the LDN–GB DS lines. The designation of the substitution lines was based on the recurrent parents, i.e. LDN D-genome DS lines, but the authenticity of substituted chromosome in LDN–GB DS has not been cytologically (i.e. chromosome banding) verified or molecularly characterized. The LDN–GB DS lines were developed prior to 1991 (Joppa, unpublished), at which time the LDN D-genome DS lines had not been improved by additional backcrosses. Thus, residual genetic material of CS in the LDN D-genome DS lines (Du and Hart 1998) might have been incorporated in the LDN–GB DS lines. In addition, some GB genetic components other than the substituted chromosomes in the substitution lines might not have been eliminated through the limited number of backcrosses. Knowledge of the genetic backgrounds of the substitution lines is necessary for utilization of the substitution lines in genetic and genomic studies.

Simple sequence repeat (SSR) and target region amplification polymorphism (TRAP) markers are excellent tools for characterization of inter-varietal DS lines in wheat. Many SSR markers in wheat have been physically assigned to specific chromosomes and they provide a reliable approach to verify the authenticity of inter-varietal DS lines (Korzun et al. 1997; Pestsova et al. 2000). TRAP markers are a relatively high-throughput and efficient marker system (Hu and Vick 2003) that have been proven to be reliable and reproducible in wheat (Xu et al. 2003; Liu et al. 2005; Li et al. 2006; Chu et al. 2008) and can provide useful information on both substituted chromosomes and the genetic background of the DS lines (Xu et al. 2003; Li et al. 2006). In the current study, we characterized the set of 14 LDN–GB DS lines genome wide using SSR and TRAP markers. In addition, the stem-solidness of each LDN–GB DS line was evaluated to verify the chromosomal location of the major gene controlling solid stem in GB.

Materials and methods

Plant materials

Two durum wheat cultivars, LDN and GB, and the complete set of 14 LDN–GB DS lines were used. The LDN–GB DS lines were developed by crossing GB with the set of LDN–CS D-genome DS (Joppa and Williams 1988) where a pair of A- or B-genome chromosomes was substituted by a pair of corresponding homoeologous D-genome chromosomes of CS. Plants that were double monosomic for an A- or B-genome chromosome and a corresponding homoeologous D-genome chromosome derived from the crosses were selected and backcrossed five times with LDN D-genome DS, and the complete set of 14 LDN–GB DS lines were selected after one generation of self-pollination and tested with LDN double ditelosomics. Other plant materials used as controls in molecular marker analysis included CS, the D-genome chromosome donor for the LDN D-genome DS lines, and the three wild emmer (*T. turgidum* L. subsp. *dicoccoides*) accessions

Israel A (ISA), PI 481521 (521), and PI 478742 (742), which were the chromosome donors in three sets of LDN-DIC DS lines (Xu et al. 2004).

Plant culture and stem solidness analysis in the greenhouse

For each of the 14 LDN–GB DS lines, 4–5 seeds (BC_5F_3) from each of two original BC_5F_2 plants were planted for marker analysis and stem solidness evaluation in the greenhouse. They were grown along with LDN, GB, CS, and the three wild emmer accessions in 6-in. clay plots with one plant per pot. The clay pots were filled with Sunshine SB100 Mix (Sun Gro Horticulture Distribution Inc., Bellevue, WA, USA), and fertilized with Osmocote Plus 15-19-12 (Scotts Sierra Horticultural Product Company, Marysville, OH, USA). The greenhouse was maintained with a temperature at approximately 25°C and a photoperiod of 16:8 (L:D) h.

At maturity, stem solidness of the 14 LDN–GB DS lines, LDN, GB, and CS were evaluated using methods described in Houshmand et al. (2007) with slight modification. Eight plants per line were scored. The primary culm of each plant was scored for stem-solidness by cutting the stems transversely through the center of each stem. Stem-solidness was scored on all available internodes from first (apical) to fifth internodes using a scale of 1–5, where one was completely hollow and five was completely solid. A mean stem solidness score for each internode was calculated for each line. Fisher's protected least significant difference (LSD) ($P < 0.05$) was calculated to compare mean scores of the LDN–GB DS lines as well as those of LDN and GB (SAS Institute 2008).

SSR and TRAP marker analysis

Total genomic DNA was isolated separately from two BC_5F_3 plants for each of the LDN–GB DS lines using the method described by Slotta et al. (2008). The DNA concentration was adjusted to 50–100 ng/μl for SSR and TRAP PCR reactions. Based on previously published genetic maps (Somers et al. 2004) and previous marker screening for polymorphisms of parental lines, a total of 35 SSR primer pairs (2–4 SSR markers per chromosome) were used to verify the authenticity of the LDN–GB DS lines. SSR marker analysis was performed on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using the procedures described in Chao et al. (2007) and Somers et al. (2004).

Target region amplification polymorphism marker analysis was conducted as described by Li et al. (2006). TRAP-PCR involved the use of fixed primers in combination with two random primers 5′-end labeled with different infrared dyes (IR-700 or IR-800). A total of 16 fixed primers were used (Table 1), of which four (W01, W11, W13 and W15) were used by Liu et al. (2005), nine (W09,

Table 1 List of the TRAP fixed primer sequences and source EST accession numbers

Fixed primer[a]	Fixed primer sequence	Wheat EST[b]	
		Accession number	Chromosome location
W01	ATCCATCATCTCCAGAGC	BE637568	1AS, 1BS, 1DS
W03	GGAGATACCTGCCATCAT	BE406450	1AS, 1BS, 1DS
W07	GATGATCGAGGAGAAGGA	BE406918	1AL, 1BL, 1DL
W09	TATCGTCACTTACGCCAG	BE425566	2AL, 2BL, 2DL
W10	CGTTCCTCAAGTGGTACA	BE638034	2AL, 2BL, 2DL
W11	GAAACTTCCAGTTACCCG	BE426431	2AS, 2BS, 2DS
W13	GGTGAAAGAGTTTCCGAC	BE406551	3AL, 3BL, 3DL
W14	CCTCTTGACAAAGGAAGC	BG606778	3AL, 3BL, 3DL
W15	GGAGGATCATGACCAGTT	BE426356	3AS, 3BS, 3DS
W17	AGTACAGCTTCAGCAACG	BE406618	4AS, 4AL, 4BS
W19	TCATGCCCAGTGATACCT	BE490658	3AL, 3BL, 3DL, 4AS, 4AL, 4BL, 4DL
W22	GCTGACCTTCCATTGAGT	BE500894	5AL, 5BL, 5DL
W33	ACTGCTCTAACGGGAAAC	BE405692	1AL, 1BL, 1DL
W43	GGCATTATCCACTGTCCT	BE426317	4AL, 4BL, 4DL
W54	GAATTCAGCTTCACGGAC	BE405227	6AS, 6BS, 6DS
W55	GCTTCCCTACAACAAACC	BE405234	7AS, 7BS, 7DS

[a] W01, W11, W13 and W15 were used by Liu et al. (2005); W09, W10, W14, W17, W19, W22, W33, W54 and W55 were used by Li et al. (2006); and W03, W07 and W43 were used by Chu et al. (2008)

[b] Wheat EST data are from the GrainGenes-SQL (2004)

W10, W14, W17, W19, W22, W33, W54 and W55) were used by Li et al. (2006), and three (W03, W07 and W43) were used by Chu et al. (2008). The two arbitrary primers, T03 (5′-CGTAGCGCGTCAATTATG) and T13 (5′-GCGCGATGATAAATTATC) that were 5′-end labeled with 700-IR and 800-IR, respectively, were described by Xu et al. (2003). TRAP gel images were obtained using a Li-Cor Global DNA Sequencer (Lincoln, NE, USA).

TRAP markers were designated following the format of the prefix-chromosome name-size in base pair, where the prefix of LDN-, GB-, and CS-indicated that the marker was only present in LDN, GB, or CS, respectively. The prefix of LDN–GB-, LDN–CS-, or GB–CS- were correspondingly used for markers that were simultaneously present in any two of them, and the prefix of All- and N- was used for the markers that were present or not present in all three parental lines, respectively. The markers present only in DS lines were classified as "novel" since their derivations were not able to be determined. The markers present only in parental lines were considered as "unassigned". The chromosome name in the marker designation indicated that the marker was assigned on the corresponding chromosome, and a "/" was added between two chromosome names when the marker was simultaneously assigned to two chromosomes, or no chromosome name is indicated when a marker was simultaneously assigned on three or more chromosomes (e.g., GB-1A-413 indicates a 413 bp fragment from GB only present on chromosome 1A, LDN-3A/3B-345 indicates a 345 bp fragment from LDN present on chromosomes 3A and 3B, and N-164 indicates a 164 bp fragment that was not present in LDN, GB, and CS but was present on more than three chromosomes).

Results

Confirmation of donor chromosome authenticity via SSR markers

Among the 35 SSR markers used to test the authenticity of the 14 LDN–GB DS lines, 28 revealed polymorphisms between GB and LDN (Table 2). Because each of the 14 LDN–GB DS lines differed from LDN for one pair of the substituted chromosomes from GB, theoretically, the GB allele of a polymorphic marker locus on a specific chromosome should appear only in the substitution line (targeted DS line in Table 2) for that chromosome and the LDN allele should be present among the remaining 13 LDN–GB DS lines (other DS lines in Table 2). For example, the marker *Xbarc17* on chromosome 1A detected a 301 bp fragment in GB and the line LDN–GB(1A), and a 283 bp fragment in LDN and the remaining 13 LDN–GB DS lines (Table 2). SSR marker analysis showed that 27 GB alleles among 28 polymorphic loci were present only in the targeted LDN–GB DS lines and thus designated for specific chromosomes.

The only SSR marker present on more than one chromosome was *Xgwm427* (231 bp), which appeared not only in LDN–GB(6A) as expected, but also in LDN–GB(6B). However, the GB allele of *Xwmc201* (250 bp) in chromosome arm 6AS appeared only in LDN–GB(6A), and the GB allele of *Xwmc494* (232 bp) in chromosome arm 6BS appeared only in LDN–GB(6B) (Table 2), indicating that the designations of LDN–GB(6A) and LDN–GB(6B) were accurate. Thus, the chromosomal locations of the 27 SSR markers were consistent with the genetic maps reported by Somers et al. (2004) and the SSR marker data agreed very well with the cytogenetic profiles of the LDN–GB DS lines (Table 2).

In addition to the GB and LDN alleles, nine additional alleles were detected in some of the substitution lines (Table 2). Markers *Xgwm312* (252 bp), *Xbarc212* (203 bp), *Xwmc73* (211 bp), and *Xwmc494* (238 bp) were apparently derived from CS but were also present in LDN–GB(5B), LDN–GB(6A) and LDN–GB(7B), respectively (Table 2), indicating that CS chromatin still remained in some of the LDN–GB DS lines. The other five marker alleles, including *Xbarc212* (232 bp), *Xwmc532* (178 bp), *Xwmc327* (208 bp), *Xgwm427* (207 bp) and *Xgwm219* (171 bp), were present only in some of the LDN–GB DS lines but not in LDN, GB and CS. Thus, they represented "novel" markers. For example, the "novel" marker *Xwmc327* (208 bp) was present only in line LDN–GB(4B) and marker *Xwmc532* (178 bp) was present in both LDN–GB(7A) and LDN–GB(6B). However, "novel" markers of *Xgwm427* (207 bp), *Xgwm219* (171 bp), and *Xbarc212* (232 bp) appeared in four (2A, 4A, 5A, and 3B), six (4A, 5A, 6A, 1B, 4B, and 5B), and nine (1A, 3A, 4A, 5A, 1B, 3B, 4B, 5B, and 6B) LDN–GB DS lines, respectively (Table 2).

In addition to the polymorphic markers between LDN and GB, the 35 SSR markers used in this study were also highly polymorphic between LDN and the wild emmer accessions. Totally, there were 32, 28, and 27 polymorphic markers generated between LDN and accessions Israel A, PI 481521, and PI 478742, respectively (Table 2). Thus, these SSR markers are diagnostic for the characterization and maintenance of all the intervarietal DS lines based on LDN.

Genetic background analysis and chromosomal assignment of TRAP markers

The 32 TRAP primer combinations (16 fixed primers and two arbitrary primers) detected a total of 359 polymorphic fragments among LDN, GB, CS and the 14 LDN–GB DS

Table 2 Chromosome-specific SSR markers analyzed in Langdon (LDN), Golden Ball (GB), LDN–GB disomic substitution (DS) lines, Chinese Spring (CS), and the three wild emmer lines Israel A (ISA), PI 478742 (742), and PI 481521 (521)

SSR marker			Fragment size (bp) of PCR products amplified from							
Marker	Chr[a]	Targeted DS line	Targeted DS line	Other DS lines[b]	GB	LDN	CS	ISA	742	521
Xbarc17	1AL	LDN–GB(1A)	301	283	301	283	292	292		
Xcfa2153	1AS	LDN–GB(1A)	225	221	225	221	215	223	225	
Xgwm312	2AL	LDN–GB(2A)	202	240, *252(5B)*	202	240	252	227	225	256
Xbarc212	2AS	LDN–GB(2A)	207	*203 (6A)*, 222 (7A, 2B, 7B), ***232***	207	222	203	222	192	
Xgwm636	2AS	LDN–GB(2A)	124	104	124	104	126	92		106
Xwmc522	2AS	LDN–GB(2A)	209	199	209	199	145	163	205	199
Xwmc532	3AS	LDN–GB(3A)	176	***178 (7A, 6B)***, 205	176	205	195	191	194	182
Xbarc314	3AL	LDN–GB(3A)	296	290	296	290	294	288		295
Xwmc428	3AL	LDN–GB(3A)	282	288	282	288	276	242	286	283
Xwmc219	4AL	LDN–GB(4A)	178	168	178	168		168	168	182
Xwmc420	4AS	LDN–GB(4A)	148	148	148	148	143	134	152	132
Xwmc327	5AL	LDN–GB(5A)	202	***208 (4B)***, 214	202	214	174	171	194	186
Xgwm595	5AL	LDN–GB(5A)	161	167	161	167	203	196	196	
Xwmc201	6AS	LDN–GB(6A)	250	265	250	265	265	240	248	243
Xgwm427	6AL	LDN–GB(6A)	231	205, ***207 (2A, 4A, 5A, 3B[c])***, **231 (6B)**	231	205	229	236	231	208
Xcfa2049	7AS	LDN–GB(7A)	162	162	162	162	153	171	162	
Xgwm332	7AL	LDN–GB(7A)	217	226	217	226	250	279	213	205
Xbarc80	1BL	LDN–GB(1B)	120	120	120	120	126	120	126	115
Xwmc626	1BL	LDN–GB(1B)	190	188	190	188	220	176	183	203
Xbarc181	1BS	LDN–GB(1B)	202	204	202	204	210	212	210	192
Xwmc361	2BL	LDN–GB(2B)	246	235	246	235	235		235	233
Xgwm429	2BS	LDN–GB(2B)	232	236	232	236	244	212	247	225
Xgwm566	3BS	LDN–GB(3B)	144	140	144	140	148	138	146	133
Xgwm247	3BL	LDN–GB(3B)	171	157	171	157	177	182	182	
Xgwm285	3BS	LDN–GB(3B)	247	245	247	245	227	246	241	241
Xbarc163	4BL	LDN–GB(4B)	169	169	169	169	184	171	194	186
Xbarc227	4BL	LDN–GB(4B)	204	239	204	239	207	226	226	207
Xwmc73	5BS	LDN–GB(5B)	207	207, *211 (7B)*	207	207	211	209	205	209
Xgwm499	5BL	LDN–GB(5B)	190	147	190	147	163	142		153
Xbarc74	5BL	LDN–GB(5B)	186	190	186	190	198	153	174	159
Xgwm219	6BL	LDN–GB(6B)	173	***171 (4A, 5A[c], 6A, 1B[c], 4B, 5B)***,173	173	173	198	169	171	173
Xwmc494	6BS	LDN-GB(6B)	232	230, *238(7B)*	232	230	238	242	223	226
Xgwm626	6BL	LDN–GB(6B)	157	157	157	157	125	123	138	154
Xbarc255	7BS	LDN–GB(7B)	276	276	276	276	247	223	256	253
Xwmc517	7BL	LDN–GB(7B)	207	204	207	204	206	199	207	222

[a] Chromosome arm locations of the SSR markers in the wheat consensus map (Somers et al. 2004)

[b] Other DS lines include all the LDN-GB DS lines except for the targeted DS line. Fragment sizes in the DS lines other than the targeted DS line are presented by different fonts: *Regular, bold*, and *italic fonts* refer to a band from LDN, GB, and CS, respectively, and italic bold font refers to a novel band. A band size followed by the donor (GB) chromosomes in parenthesis indicated the LDN-GB DS line(s) having the band, and a band size alone indicated that all the remaining DS lines that were not specified had the band

[c] Among the two plants analyzed for each of the DS lines, one had the novel band and another had the band from LDN

lines (Table 3). Among these polymorphic fragments, 134 (37.3%), 185 (51.5%), and 10 (2.8%) were derived from LDN, GB, and CS, respectively. Only 12 (3.3%) fragments were simultaneously amplified from both LDN and GB, and four (1.1%) from LDN, GB and CS, but they were absent in one or more LDN–GB DS lines. Fourteen (3.9%) fragments were only present in some of the LDN–GB DS lines but were absent in LDN, GB and CS and were therefore classified as "novel" markers. The small portion of polymorphic markers that were either "novel" or

derived from CS indicated that each of LDN–GB DS lines has a genetic background similar to LDN.

Based on their absence in one of the 14 LDN–GB DS lines, 89 (66.4%) of 134 fragments amplified from LDN were assigned to specific chromosomes of LDN (Fig. 1: LDN-7A-850 and LDN-3A-639). Similarly, 111 (60.0%) of 185 fragments amplified from GB were assigned to specific chromosomes of GB based on their presence in one of the 14 LDN–GB DS lines (Fig. 1: GB-5A-620 and GB-5B-509). Thus, a total of 200 polymorphic fragments amplified from LDN and GB were designated as chromosome-specific TRAP markers (Table 4). These chromosome-specific TRAP markers provide additional molecular identities for the LDN–GB DS lines and they will be useful tools for characterizing LDN–GB DS lines and other LDN-based aneuploids.

Besides the chromosome-specific markers, 48 and 26 polymorphic fragments amplified from GB simultaneously appeared on two chromosomes (Fig. 2: GB-4A/2B-92) and more than two (multiple) chromosomes, respectively. Similarly, 21 fragments amplified from LDN were absent in two substitution lines and another 21 fragments were absent in three or more substitution lines (Fig. 2: LDN-96).

Of the 16 fragments simultaneously amplified from both LDN and GB or from LDN, GB, and CS, four, two, and 10 were present in one, two, and more than two substitution lines, respectively (Fig. 3: LDN–GB3A/3B-345). One of the 14 novel fragments was present only in one of the LDN–GB DS lines, but the other 13 appeared in more than two substitution lines (Fig. 2: N-164). Among the 10 fragments amplified from CS, five, one, and four were present in one, two, and more than two substitution lines, respectively (Fig. 3: CS-279). In addition, three (0.8%) fragments (Fig. 4: LDN-519) derived from LDN could not be located to any of the chromosomes (Table 3).

The preliminary evaluation of stem solidness showed that of the 14 LDN–GB DS lines, only LDN–GB(3B) had high stem-solidness scores, but was significantly less solid than GB from the first to the fourth internodes (data not shown). The remaining 13 LDN–GB DS lines either had the same scores as LDN or had slightly increased solidness in some internodes. This observation confirmed the presence of a major gene for stem solidness on chromosome 3B in GB reported previously (Houshmand et al. 2007). The result also phenotypically verified the authenticity of the LDN–GB(3B) substitution line.

Discussion

Durum wheat inter-varietal DS lines are excellent tools for genetic and genomic studies in tetraploid wheat. They are particularly useful in determining the chromosome locations of genes controlling complex traits and developing mapping populations consisting of recombinants between single chromosome pairs. Because inter-varietal disomic chromosome substitution lines in both durum and bread wheat lack cytogenetic landmarks for chromosome identification, they are usually characterized based on molecular markers (Xu et al. 2003; Korzun et al. 1997; Pestsova et al. 2000). In this study, we used SSRs combined with TRAP markers to characterize a complete set of 14 LDN–GB DS lines that have not been previously reported. The SSR marker analysis in this study showed that the chromosomal locations of the 27 SSR markers were consistent with the genetic maps reported by Somers et al. (2004) and the SSR marker data were consistent with the cytogenetic profiles of the LDN–GB DS lines. Therefore, the authenticity of a complete set of 14 LDN–GB DS lines was verified with at least two chromosome-specific SSR markers per

Table 3 Chromosomal allocation of TRAP markers derived from Langdon (LDN), Golden ball (GB), Chinese Spring (CS), and LDN-GB disomic substitution (DS) lines

Polymorphic fragment derived from	Number (%) of markers assigned on				Total
	One chromosome	Two chromosomes	Three or more chromosomes	Unassigned	
LDN	89 (24.8)	21 (5.8)	21 (5.8)	3 (0.8)	134 (37.3)
GB	111 (30.9)	48 (13.4)	26 (7.2)	0	185 (51.5)
CS	5 (1.4)	1 (0.3)	4 (1.1)	0	10 (2.8)
LDN/GB[a]	4 (1.1)	2 (0.6)	6 (1.7)	0	12 (3.3)
LDN/GB/CS[b]	0	0	4 (1.1)	0	4 (1.1)
DS (novel)[c]	1 (0.3)	0	13 (3.6)	0	14 (3.9)
Total	210 (58.5)	72 (20.1)	74 (20.6)	3 (0.8)	359

[a] LDN/GB indicates a fragment which was simultaneously amplified from both LDN and GB was absent in one or more LDN-GB DS lines

[b] LDN/GB/CS indicates a fragment which was simultaneously amplified from LDN, GB, and CS was absent in one or more LDN-GB DS lines

[c] DS (novel) indicates a fragment which was presented only in some of the LDN–GB DS lines but was absent in LDN, GB and CS

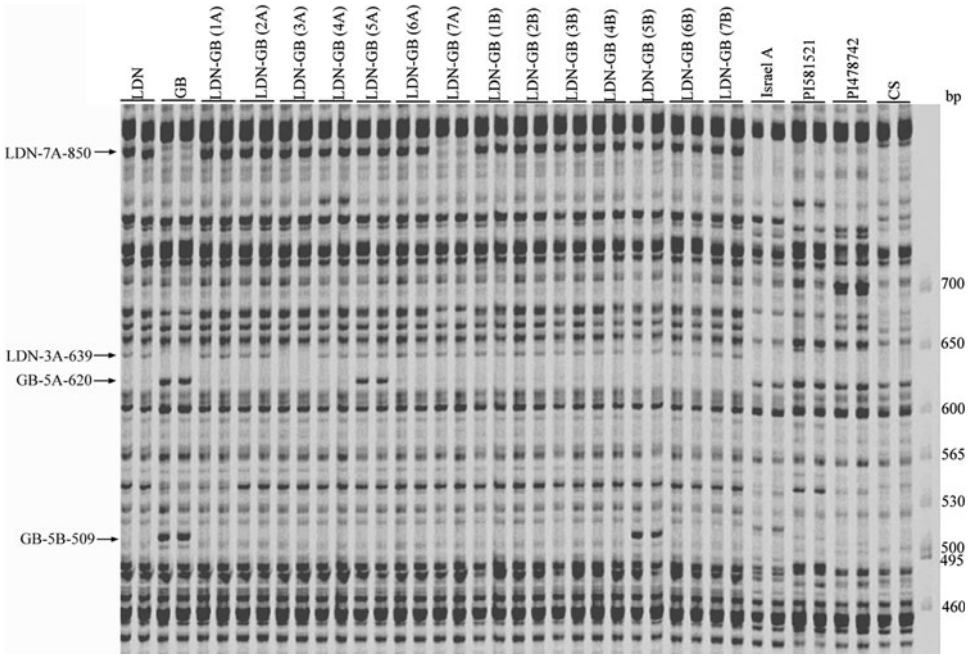

Fig. 1 A portion of a TRAP gel image from primer pair W09T13, showing TRAP markers that were assigned to a single chromosome in Langdon–Golden Ball disomic substitution (LDN–GB DS) lines. On the *top side* of the image, LDN, GB, CS, and LDN–GB(1A) to LDB–GB(7B) are Langdon, Golden Ball, Chinese Spring, and LDN–GB DS lines for chromosome 1A through 7B, respectively. The TRAP markers (*left side* of gel image) are designated on the basis of their origin of derivation, chromosomal location and base-pair size

chromosome except that the substitution lines for 7A, 4B, 6B and 7B were confirmed with one marker.

Amplicons from the SSR marker system are mainly derived from a specific chromosome region, while TRAP marker results in amplicons from multiple genomic regions (Li et al. 2006) and is a high throughput system. Therefore, the TRAP technique is particularly useful for assessing the genetic background as well as providing information on donor chromosomes in inter-varietal DS lines. Among 359 TRAP markers identified in this study, 134 (37.3%) and 185 (51.5%) were derived from LDN and GB, respectively, with only a small portion of polymorphic markers that were either "novel" or derived from CS, indicating that each of the LDN–GB DS lines has a genetic background similar to that of LDN. Therefore, the LDN–GB DS lines are useful tools for assignment of molecular markers and genes of interest to specific chromosomes.

Of 319 TRAP markers derived from LDN and GB, about 63% were present in only one of the 14 LDN–GB DS lines and were designated as chromosome-specific markers. The chromosome-specific markers provided additional molecular identities for each of the LDN–GB DS lines and will be useful tools for characterizing LDN–GB DS lines and/or other LDN-based aneuploids. In addition to the chromosome-specific TRAP markers, 40% of the markers amplified from GB were present, whereas 31% from LDN were absent, in two or more of the substitution lines. Some of these GB markers might be derived from repetitive DNA sequences present in different chromosomes of GB, while the others may result from the genetic residues of GB that were not eliminated through backcrossing to the substitution lines. The duplicate and multiple-chromosome markers derived from repetitive DNA sequences in different chromosomes of LDN could not be detected because absence of one fragment in a substitution line will be masked by another fragment amplified from a different chromosome of LDN (Li et al. 2006). Therefore, a fragment amplified from LDN that was absent in two or more of the substitution lines should indicate that some LDN genetic background was not restored in one or more of these substitution lines. Because 31% of the fragments from LDN were absent in two or more of the LDN–GB DS lines, the genetic background of some LDN–GB DS may not be highly consistent with LDN.

The genetic residues in the LDN–GB DS lines were also revealed by presence of CS-derived markers and the "novel" SSR and TRAP markers. Four of the SSR markers and ten of the TRAP markers were CS-derived, indicating that a small portion of CS background residue was retained in these LDN–GB DS lines. The presence of 19 "novel" markers (5 SSRs and 14 TRAPs), indicated that the sequence of those corresponding genomic regions might be modified slightly during the development of the DS lines. The slight modifications in those regions were most probably due to unequal recombination (Harding et al. 1992) and/or mutation. Unequal recombination has been found to play a major role in creating new alleles at SSR loci in wheat (Huang et al. 2002). Thus, comparative sequence analysis of the "novel" markers would highlight the causes of the modifications that occurred in those regions.

Li et al. (2006) observed the presence of "novel" bands in the LDN–CS D-genome DS lines. Therefore, some "novel" markers in LDN–GB DS lines were attributed to the LDN–CS D-genome DS lines. However, only three

Table 4 Chromosome-specific TRAP markers identified among Langdon–Golden Ball disomic substitution (LDN–GB DS) lines

Primer pair	Chromosome assignments of markers[a]	
	A genome	B genome
W01T03	GB-1A-413, GB-3A-257, LDN-2A-221, LDN-7A-336	GB-1B-255, GB-4B-167, GB-7B-119, GB-7B-152, LDN-1B-338
W01T13	LDN-3A-243, ***LDN-GB-7A-187***, LDN-7A-338	***N-6B-152***, LDN-2B-273, LDN-4B-326, LDN-7B-137
W03T03	GB-5A-241	GB-1B-75, GB-4B-166, GB-6B-174, GB-7B-341
W03T13	***LDN-GB-1A-415***, GB-5A-339	GB-7B-744, LDN-4B-357
W07T03	LDN-7A-750	LDN-2B-685, GB-5B-430, LDN-7B-140
W07T13	GB-6A-338, GB-7A-181, LDN-6A-339	LDN-4B-137
W09T03	GB-1A-411, GB-1A-550	GB-4B-171
W09T13	GB-5A-620, LDN-3A-639, LND-7A-850	GB-5B-509, GB-7B-236, GB-7B-312
W10T03	LDN-2A-563, LDN-7A-335	GB-2B-191, GB-2B-470, GB-4B-395, LDN-1B-598, GB-7B-613, LDN-2B-537
W10T13	GB-1A-50, GB-5A-126, LDN-2A-269, LDN-4A-328, LDN-5A-127, LDN-5A-452, LDN-7A-124	GB-5B-95, GB-7B-559, LDN-7B-135
W11T03	GB-1A-411, LDN-3A-233, LDN-7A-335	GB-1B-368, GB-5B-223, GB-6B-389, GB-7B-249, LDN-2B-297, LDN-4B-271
W11T13	GB-1A-100, GB-1A-416, GB-1A-558, GB-2A-654, GB-4A-357, LDN-3A-236	GB-4B-168
W13T03	GB-1A-627, GB-2A-405, LDN-1A-140, LDN-1A-781, LDN-2A-411	GB-4B-167
W13T13	GB-7A-258, LDN-2A-816, LDN-7A-336	GB-1B-312, GB-1B-329, GB-3B-347, GB-7B-341, LDN-1B-255, LDN-1B-326
W14T03	GB-5A-467, LDN-2A-300, LDN-5A-398, LDN-6A-213	GB-4B-454
W14T13	–	GB-3B-269
W15T03	GB-7A-359, GB-7A-789	GB-1B-519, GB-7B-740, LDN-2B-165 LDN-2B-170, LDN-5B-195, LDN-5B-210
W15T13	GB-3A-352, GB-4A-82, GB-5A-472	CS-7B-84, GB-3B-664
W17T03	CS-2A-436, ***LDN-GB-4A-621***, LDN-2A-523, LDN-7A-151, LDN-7A-336, GB-4A-356	LDN-2B-324, LDN-2B-559, LDN-3B-549, LDN-7B-244
W17T13	GB-7A-228	GB-6B-258, GB-7B-276, LDN-4B-636, LND-1B-238, GB-4B-162
W19T03	GB-4A-358, LDN-2A-295, LDN-7A-338	GB-3B-138, GB-3B-550, LDN-2B-68
W19T13	GB-3A-387, GB-5A-341, LDN-2A-778	GB-1B-197, GB-2B-361, LDN-3B-445, LDN-5B-539
W22T03	GB-1A-555, GB-1A-680, GB-4A-325, GB-7A-693, LDN-7A-336	GB-1B-465, GB-3B-116, GB-3B-131, GB-4B-167, GB-4B-488, LDN-1B-110, LDN-2B-258, LDN-7B-408
W22T13	GB-1A-415, GB-2A-350, GB-2A-412, LDN-3A-234, LDN-5A-161	GB-1B-738, GB-6B-309, LDN-2B-284, LDN-4B-114, LDN-7B-654
W33T03	GB-7A-58	GB-2B-206, LDN-4B-107, LDN-5B-520
W33T13	GB-2A-105, GB-3A-374, LDN-7A-417	LDN-2B-408, LDN-7B-183
W43T03	GB-1A-399, GB-4A-347, LDN-7A-325	CS-4B-350, GB-3B-265, GB-7B-314
W43T13	CS-2A-155, GB-1A-410, LDN-1A-68, LDN-7A-226, LDN-7A-583	GB-5B-50, GB-7B-683, LDN-2B-285
W54T03	GB-4A-278	GB-1B-457, GB-3B-662, GB-5B-213, GB-5B-317, GB-6B-474, GB-7B-667
W54T13	–	GB-2B-286, GB-4B-683, GB-6B-186, LDN-5B-332, LDN-5B-520, LDN-7B-231
W55T03	GB-6A-77, LDN-7A-427	GB-7B-614, LDN-1B-476, LDN-2B-294, LDN-5B-683
W55T13	CS-1A-135, ***LDN-GB-1A-142***, LDN-1A-191	GB-7B-262, GB-7B-353, LDN-7B-534

[a] TRAP markers were designated with the prefix of GB-, LDN- and CS- to indicate they were derived from Golden Ball (GB), Langdon (LDN) and Chinese Spring (CS), respectively, and then followed by the corresponding chromosome name and the fragment size in base pairs. The DS-derived (novel) and LDN/GB-derived markers were shown in bold–italic font style

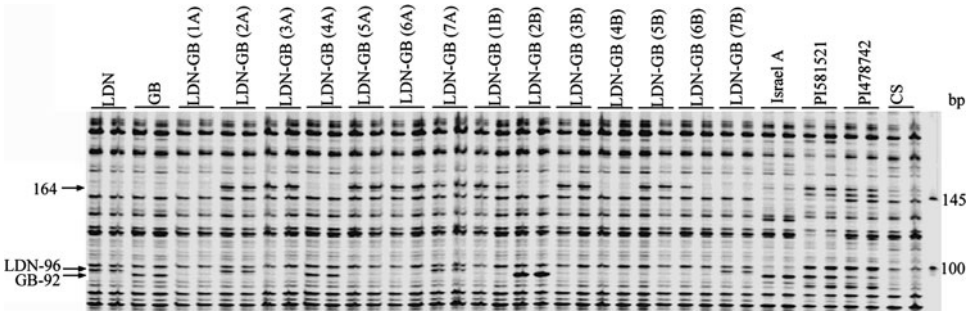

Fig. 2 A portion of a TRAP gel image from primer pair W14T13, showing the bands that were present or absent in more than two of the Langdon–Golden Ball disomic substitution (LDN–GB DS) lines. Genotype notation and TRAP marker designation are the same as in Fig. 1. A DS-derived ("novel") marker (N-164) was present in substitutions for chromosomes 2A, 3A, 5A, 6A, 7A, 1B, 3B, 5B, and 6B; a LDN-derived marker (LDN-96) was present in substitutions for chromosomes 2A, 7A and 7B; and a GB-derived marker (GB-4A/2B-92) was present in substitutions for chromosomes 4A and 2B

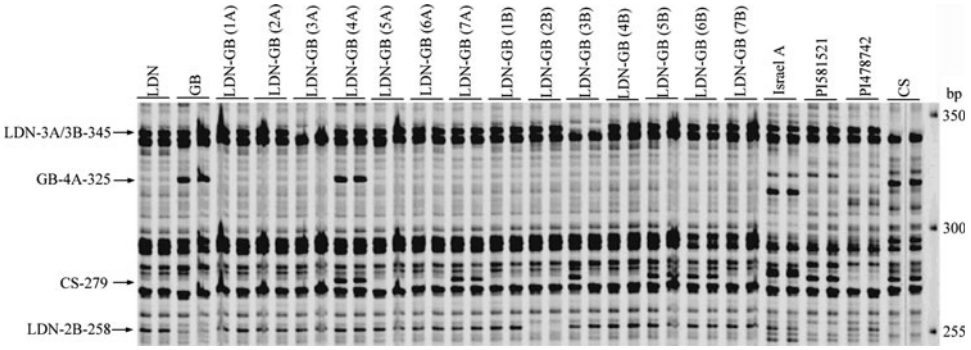

Fig. 3 A portion of a TRAP gel image from primer pair W22T03, showing a marker (CS-279) derived from Chinese Spring (*CS*), which was present on multiple chromosomes, and a marker (LDN-GB-3A/3B-345) present in both Langdon (*LDN*) and Golden Ball (*GB*) that was also present in two of the substitutions. Genotype notation is the same as in Fig. 1

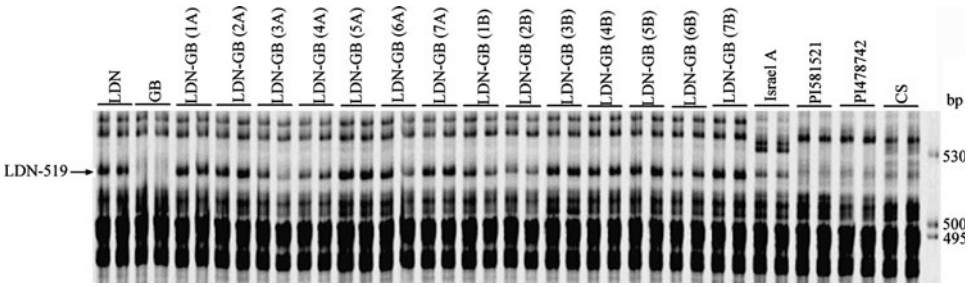

Fig. 4 A portion of a TRAP gel image from primer pair W13T03, showing an unassigned TRAP marker (LDN-519) derived from Langdon (*LDN*). Genotype notation and TRAP marker designation are the same as in Fig. 1

(1.0%) of 307 TRAP markers were considered "novel" markers in the LDN–CS D-genome DS lines, which were recently improved with five additional backcrosses by Dr. Leonard Joppa (Li et al. 2006). Thus, the genetic background of the LDN–GB DS lines could be further improved by additional backcrosses using the newly improved LDN–CS D-genome DS lines as recurrent parents.

Even though a small portion of background residue was detected, marker analysis in our study proved that each LDN–GB DS line has a pair of chromosomes from GB with a genetic background similar to that of LDN, and thus is a useful tool for chromosomal localization of genes governing important traits and molecular markers. Evaluation of stem solidness in the current research demonstrated that the substitution line LDN–GB(3B) had stem-solidness scores significantly higher than that of LDN and the other DS lines. This result is consistent with the report that stem-solidness in GB was controlled by a dominant gene located on chromosome arm 3BL (Houshmand et al. 2007). However, we observed that LDN–GB(3B) had stem-solidness scores significantly less than that of GB, suggesting that some minor genes on other chromosomes might contribute to the superior solid stem in GB. A more comprehensive trial for evaluating the LDN–GB DS lines in multiple environments is needed to verify or even detect these minor genes. Findings in this research would facilitate the utilization of

GB and LDN–GB DS lines for genetic and genomic studies in tetraploid wheat and in improvement of stem solidness in both durum and bread wheat.

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