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Plant Genetic Resources

Identification of chromosome-specific simple sequence repeat markers to characterize the Langdon durum D-genome disomic substitution lines

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Abstract

Genetic studies of durum wheat (*Triticum turgidum* L. ssp. *durum* [Desf.] Husnot.) provide valuable information for crop improvement. In the 1980s, D-genome disomic chromosome substitution (DS) lines were developed, which involved the substitution of a pair of D-genome chromosomes from hexaploid wheat (*T. aestivum* L.) for a pair of homoeologous A- or B-genome chromosomes in the durum variety Langdon (LDN). DS lines were developed for each of the durum chromosomes, and they have been used extensively over the past 35 years for genetic studies. However, true DS lines for five chromosomes were not obtained because the substituted chromosomes do not completely compensate for their homoeologous chromosomes. These non-compensating DS lines are maintained with a monosome or telosome, which segregate in progeny and require cytogenetic analyses to determine the chromosome constitution of individual plants before evaluation. Here, our objective was to identify chromosome-specific simple sequence repeat (SSR) markers that could be used to characterize the chromosome constitution of all 14 Langdon disomic substitution (LDN-DS) lines. Toward this goal, we identified two to five SSRs specific to each chromosome that can be used to determine the chromosome constitution of each of the LDN-DS lines using basic polymerase chain reaction and agarose gel electrophoresis. In the process, we found that the LDN-4D(4A) substitution line has been lost and now exists only as a disomic chromosome 4D addition line. This work provides a simplified means for characterizing the LDN-DS lines suitable for most laboratories, which will allow more researchers to use the LDN-DS stocks for various genetic studies.

Abbreviations: CS, Chinese Spring; DS, disomic substitution; LDN, Langdon; LDN-DS, Langdon disomic substitution; NT, nullisomic-tetrasomic; PCR, polymerase chain reaction; QTL, quantitative trait loci; SSR, simple sequence repeat.

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Plain Language Summary

Durum wheat is used to make macaroni and pasta, making it an important crop. Genetic studies of durum wheat provide valuable information for crop improvement. In the 1980s, specific durum lines developed to carry chromosomes from a common wheat line substituted for chromosomes in a durum variety called Langdon were developed. These "chromosome substitution" lines have been widely used for genetic analysis of various traits in durum wheat. However, the genetic integrity of the lines must be monitored and maintained. Until now, maintenance required the use of methods involving microscopic analysis of chromosomes in dividing cells, which was laborious and somewhat inaccurate. Here, researchers developed methods using DNA markers to determine the chromosome constitution of the Langdon substitution lines. The identified markers are suitable for use in any laboratory equipped to do basic DNA analysis and will allow the lines to be used more broadly for genetic analysis of durum wheat.

1 | INTRODUCTION

Common wheat (*Triticum aestivum* L.), which is used to make bread, cookies, crackers, noodles, and other products, accounts for approximately 95% of the wheat grown worldwide. Durum wheat (*T. turgidum* L.), which is used to make products from coarsely ground semolina flour such as pasta, accounts for about 5% of the wheat produced. Both common wheat and durum wheat are allopolyploids with the former being hexaploid and containing two copies each of the A, B, and D genome chromosomes ($2n = 6x = 42$, AABBDD genomes) and the latter being tetraploid containing only the A and B genome chromosomes ($2n = 4x = 28$, AABB genomes). Because of the *Pairing homoeologous 1* (*Ph1*) gene on chromosome 5B, both common and durum wheat demonstrate disomic inheritance where homoeologous chromosomes are prohibited from pairing and recombining. Therefore, even though they are both polyploid, both species exhibit diploid-like inheritance.

Polyploid wheat is somewhat unique among crop plants in that it can tolerate a high degree of aneuploidy, which is defined as an abnormal chromosome number, compliment, or constitution. Beginning in the 1950s, Dr. Ernest Sears developed many aneuploid stocks in the hexaploid wheat landrace Chinese Spring (CS), which included stocks such as ditelosomic, monotelosomic, nullisomic, trisomic, tetrasomic, nullisomic-tetrasomic (NT), and intervarietal chromosome substitution lines (Sears, 1954, 1966; Sears & Sears, 1978). These stocks were invaluable for assigning genes and markers to chromosomes and chromosome arms along with aiding in chromosome identification, understanding chromosome biology and behavior, and understanding gene and quantitative trait loci (QTL) function in homogeneous backgrounds.

In the 1970s and 1980s, Dr. Leonard Joppa (USDA-Agricultural Research Service, retired) began developing similar cytogenetic stocks for durum wheat using the North Dakota durum variety Langdon (LDN) as the primary genetic background. These included double-ditelosomics, dimonotelosomics, D-genome disomic substitutions (DSs), intercultivar chromosome substitution lines, and homozygous recombinant lines (see Joppa, 1993 for review).

Among the aneuploid stocks developed by Joppa, the D-genome DS lines (Joppa & Williams, 1988) have been the most widely used. Dr. Joppa developed these stocks by crossing CS NT stocks (Sears, 1954, 1966), where the absence of one pair of A- or B-genome chromosomes was compensated for by an extra pair of homoeologous D-genome chromosomes, with the durum variety LDN. For example, to obtain a line where chromosome 1D from CS was substituted for chromosome 1A in the LDN background, Dr. Joppa crossed the CS line nullisomic for chromosome 1A and tetrasomic for chromosome 1D (N1AT1D) with LDN. F_1 plants from this cross had a pair of 1D chromosomes from CS, were monosomic for the other D-genome chromosomes, and they were monosomic for chromosome 1A from LDN. Continued self-fertilization of these plants led to the loss of the 1A monosome and the 2D-7D monosomes, and it resulted in the formation of D-genome DS lines. However, because these lines still retained many CS genes on the A- and B-genome chromosomes, they were backcrossed 12 times to LDN to ultimately obtain LDN D-genome DS (Langdon disomic substitution [LDN-DS]) lines with little to no residual CS chromatin on the A- and B- genome chromosomes. Eventually, Dr. Joppa developed a complete set of LDN-DS lines involving all seven D-genome chromosomes from CS substituted for the homoeologous pairs of A- and B-genome chromosomes of LDN for a total of 14 lines.

Since the 1980s, over 45 research studies have been published that utilized the LDN-DS lines (recently summarized by Feldman and Levy [2023]). This set of lines has been used for the development of the widely used LDN-*T. dicoccoides* disomic chromosome substitution lines (Joppa & Cantrell, 1990), development of hybrids and other wheat genetic stocks (Inagaki et al., 1998; Jauhar & Peterson, 2006; Klindworth & Xu, 2008; Klindworth et al., 2006, 2007; Mukai et al., 2015; Othmeni et al., 2022; de. V. Pienaar & Marais, 1986; Takenaka et al., 2020; S. J. Xu & Joppa, 2008a, 2008b; S. S. Xu et al., 2010; L. Zhang et al., 2008), development of bacterial artificial chromosome libraries (Cenci et al., 2003), hybrid necrosis gene mapping (D. Liu et al., 1998; Tsunewaki, 1992), mapping stress tolerance genes (Dubcovsky et al., 1996; Gorham et al., 1987, 1990; Górny, 1999; Luo & Dvořák, 1996; Sun & Quick, 1991; Watanabe et al., 1993), mapping wheat quality genes (Anderson & Morris, 2001; Anderson et al., 2006; Bietz & Burnouf, 1985; Burnouf & Bietz, 1985; Chen et al., 2011; Jimenez & Dubcovksy, 1999; Joppa et al., 1983; Lin et al., 2010; C. Liu et al., 1994, 1995; Morris et al., 2011; S. S. Xu et al., 2005; C. Zhang et al., 2011), mapping disease resistance genes and QTL (Amri et al., 1990; Bai & Knott, 1992; Hussein et al., 2005; H. Li et al., 2023; Yahiaoui et al., 2004; X. Zhu et al., 2016, 2022), mapping various spike traits (Kosuge et al., 2008; Klindworth et al., 1990; Konzak & Joppa, 1988; Watanabe et al., 1996), and development and anchoring markers to specific chromosomes (Gadaleta et al., 2009; J. Li et al., 2006; Wang et al., 1995). There is renewed interest in these lines to be utilized as a bridge for introgressing traits from the D genome of synthetic wheat into durum wheat, along with introgressing traits from *Aegilops tauschii* Coss. ($2n = 2x = 14$, DD genomes), which is the D-genome donor, into durum wheat (Othmeni et al., 2022).

Although most of the LDN-DS lines can be maintained as true disomic lines, five of the 14 lines could not be maintained in such a way due to the D-genome chromosomes failing to completely compensate for the loss of the homoeologous A- or B-genome chromosomes (Joppa & Williams, 1988). These five lines had to be maintained with an extra monosome or telosome, and cytogenetic techniques such as counting chromosomes at metaphase from root tip preparations was necessary to identify plants lacking the extra monosome or telosome for use in genetic studies. Today, few labs are equipped to conduct cytological analysis, and such techniques can be laborious and time-consuming. As the curators of the LDN-DS genetic stocks, we aimed to make the process of determining the chromosome constitution of the LDN-DS lines less laborious, more efficient, and more suitable to general molecular genetics or plant breeding laboratories that might not have access to expensive high-throughput instrumentation. Therefore, objective of this research was to identify multiple chromosome-specific polymerase chain reaction (PCR)-based simple sequence repeat (SSR) markers that distinguished between CS and LDN chromosomes and could be used to monitor the integrity of the true LDN-DS lines and identify the mono/telosomes in the non-compensating LDN-DS lines.

2 | MATERIALS AND METHODS

2.1 | Plant material

The 14 LDN-DS lines developed by Leonard Joppa (Joppa & Williams, 1988) were the subject of evaluation in this study. Our goal was to identify SSR markers specific to the replaced A- or B-genome chromosomes in LDN as well as the substituted D-genome chromosome from CS so that users could validate the chromosome constitution of all lines. As mentioned above, Dr. Joppa was unable to develop compensating lines for the LDN-DS lines LDN-3D(3B), LDN-4D(4A), LDN-5D(5A), LDN-5D(5B), and LDN-6D(6B). The chromosome constitutions of the five non-compensating lines are described in Table 1, and a copy of the original document that Dr. Joppa wrote and provided with seed shipments regarding his observations of those lines is included as Figure S1. For the nine fully compensating DS lines, we grew at least three plants of each line for marker evaluation. For the five non-compensating lines, we planted at least 10 plants of each. Additionally, LDN, CS, and CS NT lines (Sears, 1954, 1966) were grown to use as controls to determine chromosome specificity of markers. All plants were grown in 15-cm diameter pots in a greenhouse with 16-h photoperiod and a temperature of 21°C. Heads were covered with glassine bags to ensure self-pollination to obtain pure seed of true substitution lines for future use. In some cases, non-compensating lines underwent multiple plantings to evaluate seed of different seed sources until DS lines could be identified through SSR marker analysis.

2.2 | SSR marker analysis

Total genomic DNA was extracted from leaf tissue as described by Faris et al. (2000), and the DNA concentration was adjusted to ~100 ng/μL using distilled water. DNA fragments were amplified using PCR and the markers chosen in Table 2 based on polymorphism between LDN and CS. The polymorphic markers were selected from the following libraries: BARC (Song et al., 2005), CFA (Sourdille et al., 2004), CFD (Sourdille et al., 2004), GDM (Pestsova et al., 2000), WMS (marker designation = "gwm"; Röder et al., 1998), and WMC (Somers et al., 2004). Markers were chosen for the initial survey based on searching the GrainGenes database for chromosome-specific SSR markers (Yao et al., 2022) and then tested for polymorphism between

TABLE 1 Description of the non-compensating Langdon disomic substitution (LDN-DS) lines that cannot be maintained as true disomic substitution lines.

LDN-DS line	Chromosome constitution to maintain line	Notes
LDN-3D(3B)	$13'' + 1''_{3D} + 1'_{3B}$	Chromosome 3B contains a gene that is necessary to prevent asynapsis at MI of meiosis
LDN-4D(4A)[a]	$13'' + 1''_{4D} + 1'_{4A}$	Nullisomic 4A plants do not germinate
LDN-5D(5A)	$13'' + 1''_{5D} + 1'_{5A}$	Nullisomic 5A plants have very low fertility.
LDN-5D(5B)	$13'' + 1''_{5D} + 1'_{5B}$	*Ph1* gene is on chromosome arm 5BL and needed to prevent pairing between nonhomologous chromosomes.
LDN-6D(6B)	$13'' + 1''_{6D} + 1t'_{6BS}$	Nullisomic 6B plants have low fertility/pistilloid heads. Need to maintain with telosomic 6BS chromosome.

[a]According to Joppa and Williams (1988), this line was developed with the noted chromosome constitution. However, we were not able to identify any such plants from multiple seed sources in this research. Instead, only disomic addition lines with $13'' + 1''_{4D} + 1''_{4A}$ were identified.

LDN and CS before testing on the corresponding aneuploid lines.

PCR solutions consisted of 200 ng of template DNA, 1× NH_4 PCR buffer, 2 mM $MgCl_2$, 0.2 mM deoxynucleotide triphosphates, 0.4 μM forward and reverse primers, and 0.5 units of BIOTAQ DNA polymerase (Thomas Scientific), with nuclease-free water added to a final volume of 10 μL. PCR was performed using either a MiniAmp Thermal Cycler (Thermo Fisher Scientific) or a GeneAmp PCR system 9700 machine (Applied Biosystems). The PCR cycle used for all markers was a touchdown method which includes an initial denaturation at 94°C for 5 min, followed by 35 cycles of 30 s of denaturation at 94°C, 30 s of annealing step starting at 65°C and incrementally decreases 0.2°C each cycle, and 90 s of extension at 72°C, and finishing with an incubation for 7 min at 72°C and cooling to 4°C. PCR products were separated on 4% agarose gels with a current of 90 volts for 85 min (unless different time noted in Table 2), stained with ethidium bromide, and scanned using an ENDURO GDS Touch II Gel Documentation System (Labnet International). The fragment lengths for the amplified products (Table 2) were calculated using a 1 Kb Plus DNA ladder (Thermo Fisher Scientific), logarithmic formula, and calibration curve as demonstrated in the video at: https://www.youtube.com/watch?v=uCfWruPMQ0w.

Primer sequences listed in Table 2 were obtained from the GrainGenes database (Yao et al., 2022; https://wheat.pw.usda.gov/GG3/). Sequences of the SSR amplicons were obtained using the GrainGenes database (Yao et al., 2022; https://wheat.pw.usda.gov/GG3/), WheatIS website (Alaux et al., 2018; http://www.wheatis.org/), and the WheatOmics platform (Ma et al., 2021; http://wheatomics.sdau.edu.cn/). The physical position of each fragment in the CS RefSeq v1.0 genome assembly (IWGSC, 2018), CS RefSeq v2.1 genome assembly (T. Zhu et al., 2021), and Svevo Rel. 1.0 pseudomolecules (Maccaferri et al., 2019) was determined using BLASTn searches on the GrainGenes database to verify the chromosome arm assignment for each SSR marker (Table 2).

3 | RESULTS AND DISCUSSION

3.1 | SSR marker criteria and informativeness

SSR markers were considered diagnostic if they met these criteria:

For D-genome diagnostic markers: (1) they did not amplify in LDN or in CS NT lines nullisomic for the specific D-genome chromosome, (2) they were present in CS NT lines nullisomic for A- or B-genome chromosomes but tetrasomic for the specific D-genome chromosome, and (3) they were present in CS and in LDN DS lines with the specific D-genome chromosome substitution.

For A- or B-genome diagnostic markers: (1) they were present in both CS and LDN and showed polymorphism between them, (2) They were absent in LDN DS lines where a D-genome chromosome replaced the specific A- or B-genome chromosome, and (3) They were absent in CS NT lines missing the specific A- or B-genome chromosome.

As an example, the SSR markers *barc263*, *barc137*, and *cfd282* were specific for chromosomes 1A, 1B, and 1D, respectively, and can be used to characterize the genetic stocks involving homoeologous group 1 chromosomes (Figure 1). The marker *cfd282* was absent in LDN and CS N1DT1A, and it was present in CS, N1AT1D, N1BT1D, LDN-1D(1A), and LDN-1D(1B), which indicated the marker was specific for chromosome 1D and that both LDN-DS lines LDN-1D(1A) and LDN-1D(1B) contained chromosome 1D. Analysis of these lines with marker *barc263* showed that it was specific to 1A because it was absent in CS N1AT1D and present in CS, CS N1BT1D, and CS N1DT1A as well as LDN. Because *barc263* was also absent in LDN-1D(1A), that indicated this line is missing chromosome 1A, which was replaced by chromosome 1D as shown by the results with marker *cfd282*. Similarly for chromosome 1B, marker *barc137* was absent in CS N1BT1D but present in CS, N1AT1D, and N1DT1A as

TABLE 2 Chromosome-specific simple sequence repeat (SSR) markers identified as polymorphic between Langdon (LDN) and Chinese Spring (CS) that can be used to characterize the chromosome constitution of Langdon D-genome disomic substitution lines.

Marker	Chromosome arm	Primer	Primer sequence 5′–3′	Gel run time (minutes)	Fragment length (bp)		Citation
					LDN	CS[a]	
barc263	1AS	Forward	GGAAGCGCGTCAGCACTAGGCAAC	85	207	234	Song et al. (2005)
		Reverse	GGCTTCTAGGTGCTGCGGCTTTTGTC				
wmc24	1AS	Forward	GTGAGCAATTTTGATTATACTG	85	89	113	Somers et al. (2004)
		Reverse	TACCCTGATGCTGTAATATGTG				
wmc312	1AL	Forward	TGTGCCCGCTGGTGCGAAG	85	264	234	Somers et al. (2004)
		Reverse	CCGACGCAGGTGAGCGAAG				
wmc716	1AL	Forward	CATTTATGTGCACGCCGAAG	85	207	128	Somers et al. (2004)
		Reverse	CCATAAGCATCGTCACCCTG				
barc137	1BL	Forward	GGCCCATTTCCCACTTTCCA	85	234	207	Song et al. (2005)
		Reverse	CCAGCCCCTCTACACATTTT				
barc187	1BL	Forward	GTGGTATTTCAGGTGGAGTTGTTTTA	85	207	264	Song et al. (2005)
		Reverse	CGGAGGAGCAGTAAGGAAGG				
wmc44	1BL	Forward	GGTCTTCTGGGCTTTGATCCTG	85	335	378	Somers et al. (2004)
		Reverse	TGTTGCTAGGGACCCGTAGTGG				
cfd282	1DL	Forward	TCTCATCCCTGTTCCTCTGC	85	NA[b]	183	Sourdille et al. (2004)
		Reverse	GTCGACGTCTGCACATTGTT				
cfd63	1DL	Forward	TCCTGAGGATGTTGAGGACC	85	NA	264	Sourdille et al. (2004)
		Reverse	GAGAGAGGCGAAACATGGAC				
cfd72	1DS	Forward	CTCCTTGGAATCTCACCGAA	85	NA	264	Sourdille et al (2004)
		Reverse	TCCTTGGGAATATGCCTCCT				
gdm111	1DL	Forward	CACTCACCCCAAACCAAAGT	85	NA	234	Pestsova et al. (2000)
		Reverse	GATGCAATCGGGTCGTTAGT				
gdm126	1DL	Forward	TCCATCATATCCGTAGCACA	85	NA	297	Pestsova et al. (2000)
		Reverse	CGTGGTTGATTTCAGGAGGT				
barc220	2AL	Forward	CCTCTGCCATAAACATCACCTCTC	85	144	163	Song et al. (2005)
		Reverse	GGCCTCAACATCATGTGAAAGA				
cfa2263	2AL	Forward	GGCCATGTAATTAAGGCACA	85	163	184	Sourdille et al. (2004)
		Reverse	CTCCCAGGAGTACAGAAGAGGA				
wmc177	2A	Forward	AGGGCTCTCTTTAATTCTTGCT	85	207	234	Somers et al. (2004)
		Reverse	GGTCTATCGTAATCCACCTGTA				
wmc332	2BL	Forward	CATTTACAAAGCGCATGAAGCC	85	163	144	Somers et al. (2004)
		Reverse	GAAAACTTTGGGAACAAGAGCA				
barc1064	2BL	Forward	GCGTATTATTCTCAAGATCAGAGGCACTA	85	144	128	Song et al. (2005)
		Reverse	GCGTTCTGGGGATTTCCTCTACCAATTAT				
barc167	2BL	Forward	AAAGGCCCATCAACATGCAAGTACC	85	128	144	Song et al. (2005)
		Reverse	CGCAGTATTCTTAGTCCCTCAT				
cfd239	2DL	Forward	CTCTCGTTCTCTCCAGGCTC	85	NA	264	Sourdille et al. (2004)
		Reverse	GAGAGGAGAGCTTGCCATTG				
cfd53	2DS	Forward	CCCTATTTCCCCCATGTCTT	85	NA	207	Sourdille et al. (2004)
		Reverse	AAGGAGGGCACATATCGTTG				
gwm349	2DL	Forward	GGCTTCCAGAAAACAACAGG	85	NA	298	Röder et al. (1998)
		Reverse	ATCGGTGCGTACCATCCTAC				
barc57	3AS	Forward	GCGACCACCTCAGCCAACTTATTATGT	85	128	163	Song et al. (2005)
		Reverse	GCGGGGAGGCACATTCATAGGAGT				

(Continues)

TABLE 2 (Continued)

Marker	Chromosome arm	Primer	Primer sequence 5′–3′	Gel run time (minutes)	Fragment length (bp)		Citation
					LDN	CS[a]	
cfa2193	3AL	Forward	ACATGTGATGTGCGGTCATT	85	234	207	Sourdille et al. (2004)
		Reverse	TCCTCAGAACCCCATTCTTG				
wmc664	3AS	Forward	GGGCCAACAAATCCAAT	85	128	163	Somers et al. (2004)
		Reverse	TCTACTTCCTTCATCCACTCC				
wmc532	3AS	Forward	GATACATCAAGATCGTGCCAAA	85	184	163	Somers et al. (2004)
		Reverse	GGGAGAAATCATTAACGAAGGG				
wmc559	3AL	Forward	ACACCACGAATGATGTGCCA	85	264	297	Somers et al. (2004)
		Reverse	ACGACGCCATGTATGCAGAA				
gwm493	3BS	Forward	TTCCCATAACTAAAACCGCG	85	144	207	Röder et al. (1998)
		Reverse	GGAACATCATTTCTGGACTTTG				
wmc78	3BS	Forward	AGTAAATCCTCCCTTCGGCTTC	85	297	330	Somers et al. (2004)
		Reverse	AGCTTCTTTGCTAGTCCGTTGC				
wmc231	3BS	Forward	CATGGCGAGGAGCTCGGTGGTC	85	70	62	Somers et al. (2004)
		Reverse	GTGGAGCACAGGCGGAGCAAGG				
cfd141	3DS	Forward	CGTAAAGATCCGAGAGGGTG	85	NA	128	Sourdille et al. (2004)
		Reverse	TCCGAGGTGCTACCTACCAG				
cfd35	3DS	Forward	GGGATGACACATAACGGACA	85	NA	207	Sourdille et al. (2004)
		Reverse	GCACGAGATACGGACAATCA				
cfd55	3DS	Forward	CCAGTAGCCGGCCCTACTAT	85	NA	336	Sourdille et al. (2004)
		Reverse	GCACGAGATACGGACAATCA				
cfd64	3DS	Forward	ACAGTGTTGTTGCCCCTTTC	85	NA	264	Sourdille et al. (2004)
		Reverse	CCCATGTTACAGCTTTGGGT				
gwm397	4AL	Forward	TGTCATGGATTATTTGGTCGG	85	200	189	Röder et al. (1998)
		Reverse	CTGCACTCTCGGTATACCAGC				
cfa2026	4AS	Forward	CTAATCTAAGCAAGGGCCG	85	200	200	Sourdille et al. (2004)
		Reverse	TTACTGCACTGCGAGTTGCT				
wmc468	4AL	Forward	AGCTGGGTTAATAACAGAGGAT	85	163	195	Somers et al. (2004)
		Reverse	CACATAACTGTCCACTCCTTTC				
wmc262	4AL	Forward	GCTTTAACAAAGATCCAAGTGGCAT	85	234	207	Somers et al. (2004)
		Reverse	GTAAACATCCAAACAAAGTCGAACG				
wmc219	4AL	Forward	TGCTAGTTTGTCATCCGGGCGA	85	200	234	Somers et al. (2004)
		Reverse	CAATCCCGTTCTACAAGTTCCA				
gwm495	4BL	Forward	GAGAGCCTCGCGAAATATAGG	90	100	89	Röder et al. (1998)
		Reverse	TGCTTCTGGTGTTCCTTCG				
gwm113	4BS	Forward	ATTCGAGGTTAGGAGGAAGAGG	90	264	207	Röder et al. (1998)
		Reverse	GAGGGTCGGCCTATAAGACC				
gwm368	4BS	Forward	CCATTTCACCTAATGCCTGC	90	427	335	Röder et al. (1998)
		Reverse	AATAAAACCATGAGCTCACTTGC				
wmc125	4BL	Forward	ATACCACCATGCATGTGGAAGT	90	234	335	Somers et al. (2004)
		Reverse	ACCGCTTGTCATTTCCTTCTGT				
cfd106	4DS	Forward	ACGGGTGGTTTTGCTCAGT	85	NA	234	Sourdille et al. (2004)
		Reverse	ACTCCACCAGCGGAGAAATA				
barc1183	4DL	Forward	TCCCGCCCTCTCTAGACGAAACTG	85	NA	234	Song et al. (2005)
		Reverse	CGGGAAAGGAAAGGAGCTTACGGT				

(Continues)

TABLE 2 (Continued)

Marker	Chromosome arm	Primer	Primer sequence 5′–3′	Gel run time (minutes)	Fragment length (bp)		Citation
					LDN	CS[a]	
wmc331	4DL	Forward	CCTGTTGCATACTTGACCTTTTT	85	NA	144	Somers et al. (2004)
		Reverse	GGAGTTCAATCTTTCATCACCAT				
wmc705	5AS	Forward	GGTTGGGCTCCTGTCTGTGAA	85	207	234	Somers et al. (2004)
		Reverse	TCTTGCACCTTCCCATGCTCT				
barc186	5AS	Forward	GGAGTGTCGAGATGATGTGGAAAC	85	264	297	Song et al. (2005)
		Reverse	CGCAGACGTCAGCAGCTCGAGAGG				
barc117	5AS	Forward	TCATGCGTGCTAAGTGCTAA	85	336	378	Song et al. (2005)
		Reverse	GAGGGCAGGAAAAAGTGACT				
cfa2155	5AL	Forward	TTTGTTACAACCCAGGGGG	85	264	297	Sourdille et al. (2004)
		Reverse	TTGTGTGGCGAAAGAAACAG				
wmc28	5BL	Forward	ATCACGCATGTCTGCTATGTAT	85	100	128	Somers et al. (2004)
		Reverse	ATTAGACCATGAAGACGTGTAT				
barc1032	5BS	Forward	GCGAAATAACTAACAGAATACAACAACA	85	184	145	Song et al. (2005)
		Reverse	GCGAGGAAAACTTGACAATGGTA				
barc74	5BL	Forward	GCGCTTGCCCCTTCAGGCGAG	85	163	184	Song et al. (2005)
		Reverse	CGCGGGAGAACCACCAGTGACAGAGC				
cfd18	5DS	Forward	CATCCAACAGCACCAAGAGA	85	NA	264	Sourdille et al. (2004)
		Reverse	GCTACTACTATTTCATTGCGACCA				
cfd78	5DS	Forward	ATGAAATCCTTGCCCTCAGA	85	NA	378	Sourdille et al. (2004)
		Reverse	TGAGATCATCGCCAATCAGA				
cfd183	5DL	Forward	ACTTGCACTTGCTATACTTACGAA	85	NA	163	Sourdille et al. (2004)
		Reverse	GTGTGTCGGTGTGTGGAAAG				
gwm427	6AL	Forward	AAACTTAGAACTGTAATTTCAGA	85	184	207	Röder et al. (1998)
		Reverse	AGTGTGTTCATTTGACAGTT				
cfa2114	6A	Forward	ATTGGAAGGCCACGATACAC	85	184	207	Sourdille et al. (2004)
		Reverse	GCGACTAGTACGAACACCACAAAA				
wmc494	6BS	Forward	GGATCGAGTCTCAAGTCTACAA	85	184	207	Somers et al. (2004)
		Reverse	AGAAGGAACAAGCAACATCATA				
wmc487	6BS	Forward	CAAATTTGGCCACCATTTTACA	85	144	128	Somers et al. (2004)
		Reverse	CGGTTCAATCCTTGGATTTACA				
barc202	6DL	Forward	GCGATGTCCAATTTTTCTCCCGTTGC	85	NA	234	Song et al. (2005)
		Reverse	CGATTCGAGTCACGCTGGTA				
cfd287	6DL	Forward	TCAAGAAGATGCGTTCATGC	85	NA	145	Sourdille et al. (2004)
		Reverse	GGGAGCTTTCCCTAGTGCTT				
barc196	6DS	Forward	GGTGGGTTTTATCGAATAGATTTGCT	85	NA	163	Song et al. (2005)
		Reverse	GCGTTTCGTCAAGATTAATGCAGGTTT				
barc108	7AL	Forward	GCGGGTCGTTTCCTGGAAATTCATCTAA	85	144	163	Song et al. (2005)
		Reverse	GCGAAATGATTGGCGTTACACCTGTTG				
wmc596	7AL	Forward	TCAGCAACAAACATGCTCGG	85	184	144	Somers et al. (2004)
		Reverse	CCCGTGTAGGCGGTAGCTCTT				
wmc65	7AL	Forward	TGGATGGGAAGGAGAATAAGTG	85	297	335	Somers et al. (2004)
		Reverse	ATCCAACCGGAACTACCGTCAG				
wmc525	7AL	Forward	GTTTGACGTGTTTGCTGCTTAC	85	207	234	Somers et al. (2004)
		Reverse	CTACGGATAATGATTGCTGGCT				

(Continues)

TABLE 2 (Continued)

Marker	Chromosome arm	Primer	Primer sequence 5′–3′	Gel run time (minutes)	Fragment length (bp)		Citation
					LDN	CS[a]	
barc1073	7BL	Forward	GCGGGCACAATATTCTAATGGACAAAG	85	144	163	Song et al. (2005)
		Reverse	GCGCAGATGCAGAGGCCAGGGGTCA				
barc255	7BS	Forward	GTGGCGGCTTGCGGGTGGCTGAGTA	85	184	163	Song et al. (2005)
		Reverse	GGGTCGGCTAGCCTTCTTTTATGTT				
barc85	7BS	Forward	GCGAACGCTGCCCGGAGGAATCA	85	195	184	Song et al. (2005)
		Reverse	GCGTCGCAGATGAGATGGTGGAGCAAT				
wmc364	7BL	Forward	ATCACAATGCTGGCCCTAAAAC	85	144	163	Somers et al. (2004)
		Reverse	CAGTGCCAAAATGTCGAAAGTC				
barc111	7DL	Forward	GCGGTCACCAGTAGTTCAACA	85	NA	184	Song et al. (2005)
		Reverse	GCGTATCCCATTGCTCTTCTTCACTAAC				
barc53	7DL	Forward	GCGTCGTTCCTTTGCTTGTACCAGTA	85	NA	427	Song et al. (2005)
		Reverse	GCGCGTCCTTCCAATGCAGAGTAGA				

[a]More information on the SSR markers and their expected amplicon sizes in Chinese Spring can be found at https://wheat.pw.usda.gov/GG3/.
[b]NA: no amplification occurred.

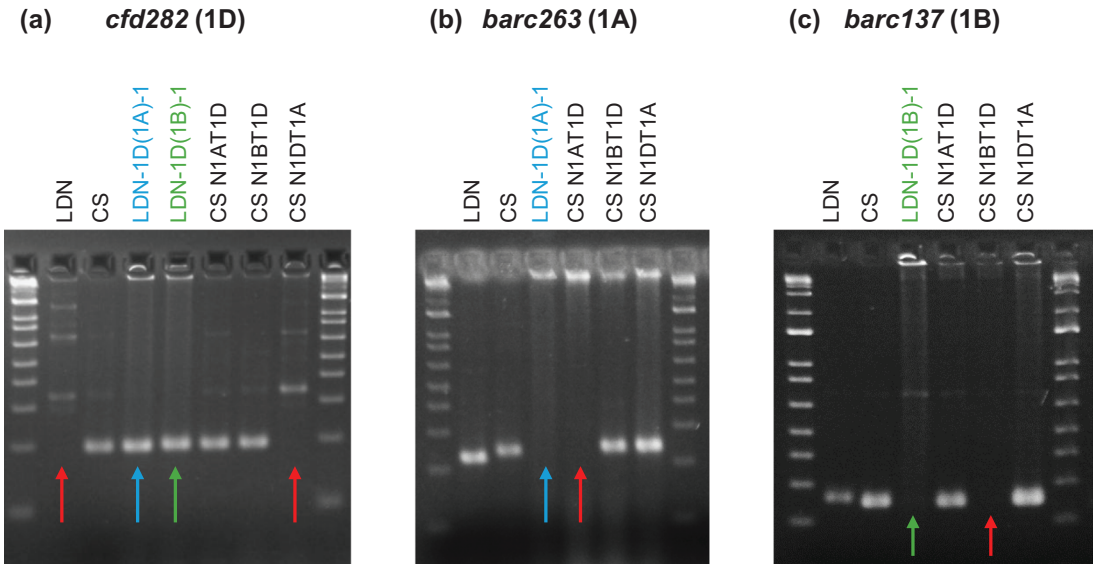


FIGURE 1 Simple sequence repeat (SSR) markers diagnostic for homoeologous group 1 chromosomes involving lines LDN-1D(1A) and LDN-1D(1B), which are both compensating disomic substitution (DS) lines. (a) SSR marker *cfd282* is specific to chromosome 1D based on its absence in the line that is nullisomic for chromosome 1D (CS N1DT1A) and Langdon (LDN) durum (red arrows). Marker *cfd282* was present in both Langdon disomic substitution (LDN-DS) lines LDN-1D(1A)-1 and LDN-1D(1B)-1, indicating that both plants contained chromosome 1D (blue and green arrows). (b) SSR marker *barc263* is specific to chromosome 1A because of its absence in the line nullisomic for 1A (CS N1AT1D) (red arrow). The absence of this marker in LDN-1D(1A)-1 (blue arrow) indicates this plant lacks chromosome 1A and it is a true LDN-1D(1A) disomic chromosome substitution line. (c) SSR marker *barc137* is specific to chromosome 1B based on its absence in the line nullisomic for 1B (CS N1BT1D) (red arrow). The absence of this marker in LDN-1D(1B)-1 (green arrow) indicates this line is a true disomic chromosome substitution line.

well as LDN indicating that it was specific for chromosome 1B. The absence of *barc137* from LDN-1D(1B) showed that chromosome 1B was absent from this line, and it was replaced by chromosome 1D as indicated by the results using marker *cfd282*. We identified three to five diagnostic SSR markers for each chromosome (Table 2) that can be used to assess the chromosome constitution of the LDN-DS lines as described in the example.

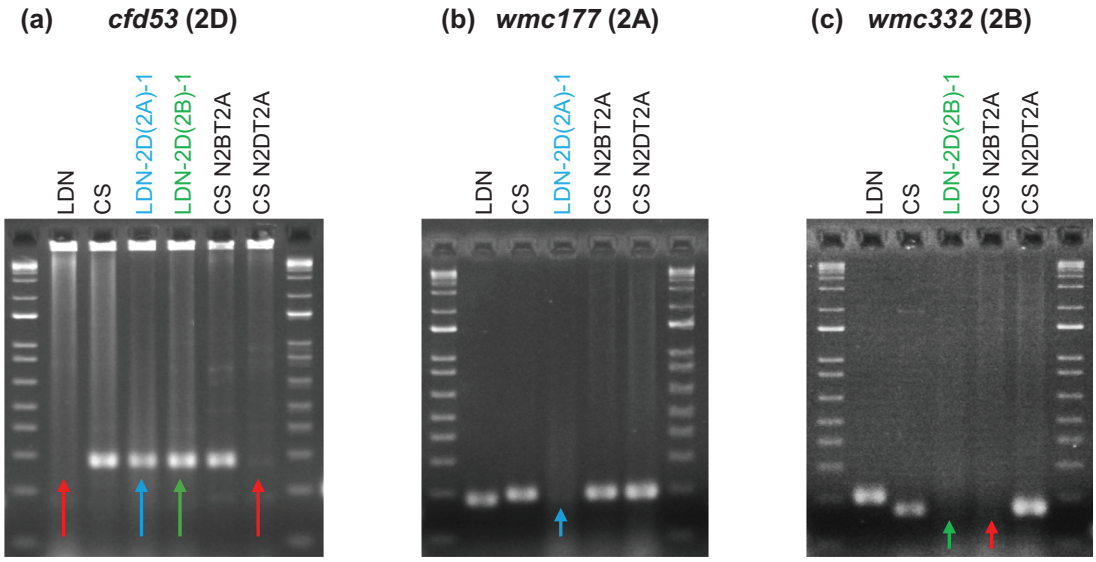


FIGURE 2 Simple sequence repeat (SSR) markers diagnostic for homoeologous group 2 chromosomes involving lines LDN-2D(2A) and LDN-2D(2B), which are both compensating disomic substitution (DS) lines. (a) SSR marker *cfd53* is specific to chromosome 2D based on its absence in the line that is nullisomic for chromosome 2D (CS N2DT2A) and Langdon (LDN) durum (red arrows). Marker *cfd53* was present in both Langdon disomic substitution (LDN-DS) lines LDN-2D(2A)-1 and LDN-2D(2B)-1, indicating that both plants contained chromosome 2D (blue and green arrows). (b) The Chinese Spring (CS) nullisomic-tetrasomic (NT) stock nullisomic for 2A does not exist (Sears, 1954); therefore, it could not be used to verify that SSR marker *wmc177* is specific to chromosome 2A. However, the absence of this marker in LDN-2D(2A)-1 (blue arrow) suggests that it is specific to 2A and that this plant is a true LDN-2D(2A) disomic chromosome substitution line. (c) SSR marker *wmc332* is specific to chromosome 2B based on its absence in the line nullisomic for 2B (CS N2BT2A) (red arrow). The absence of this marker in LDN-2D(2B)-1 (green arrow) indicates this line is a true disomic chromosome substitution line.

3.2 | Identification of markers for characterizing the compensating LDN-DS lines

The nine compensating LDN-DS lines developed by Dr. Joppa included LDN-1D(1A), LDN-1D(1B), LDN-2D(2A), LDN-2D(2B), LDN-3D(3A), LDN-4D(4B), LDN-6D(6A), LDN-7D(7A), and LDN-7D(7B). Good quality diagnostic SSR markers were identified to monitor the critical chromosomes in each of these lines (Table 2), and examples of markers for each critical chromosome for all LDN-DS lines are provided in Figures 1–7. Because these nine lines are fully compensating DS lines, it is not necessary to genotype them on a regular basis, but the markers provided can be used to monitor their integrity should the user have doubts or observe anomalies.

3.3 | Identification of markers to characterize the non-compensating LDN-DS lines

3.3.1 | LDN-3D(3B)

Sears (1966) reported that wheat chromosome 3B contains a gene necessary to prevent asynapsis during metaphase I of meiosis. In line with this, Joppa and Williams (1988) reported that LDN-3D(3B) plants required the long arm of chromosome 3B to be present making it necessary to maintain LDN-3D(3B) plants with a 3B monosome ($13'' + 1''_{3D} + 1'_{3B}$). We identified four markers (*cfd35*, *cfd55*, *cfd64*, and *cfd141*) specific for chromosome 3D and three markers (*gwm493*, *wmc78*, and *wmc231*) specific for chromosome 3B suitable for characterization of LDN-3D(3B) plants (Table 2). As expected LDN-3D(3B) plants segregated for presence of chromosome 3B. Figure 3a shows that *cfd64* is specific for chromosome 3D and that all three of the LDN-3D(3B) plants shown (LDN-3D(3B)-1, LDN-3D(3B)-2, and LDN-3D(3B)-3) harbor chromosome 3D, and use of the chromosome 3B-specific marker *gwm493* shows that, among the three LDN-3D(3B) plants, LDN-3D(3B)-3 harbors chromosome 3B (most likely monosomic) in addition to chromosome 3D (most likely disomic), whereas plants LDN-3D(3B)-1 and LDN-3D(3B)-2 contain copies of chromosome 3D but not 3B and are likely to be true DSs (Figure 3c). Therefore, one would employ plants LDN-3D(3B)-1 and LDN-3D(3B)-2 in genetic studies and evaluations and use LDN-3D(3B)-3 for further maintaining the line.

3.3.2 | LDN-4D(4A)

Joppa reported that LDN plants nullisomic for chromosome 4A did not germinate, and therefore LDN-4D(4A) plants were maintained with a monosome of chromosome 4A ($13'' + 1''_{4D}$

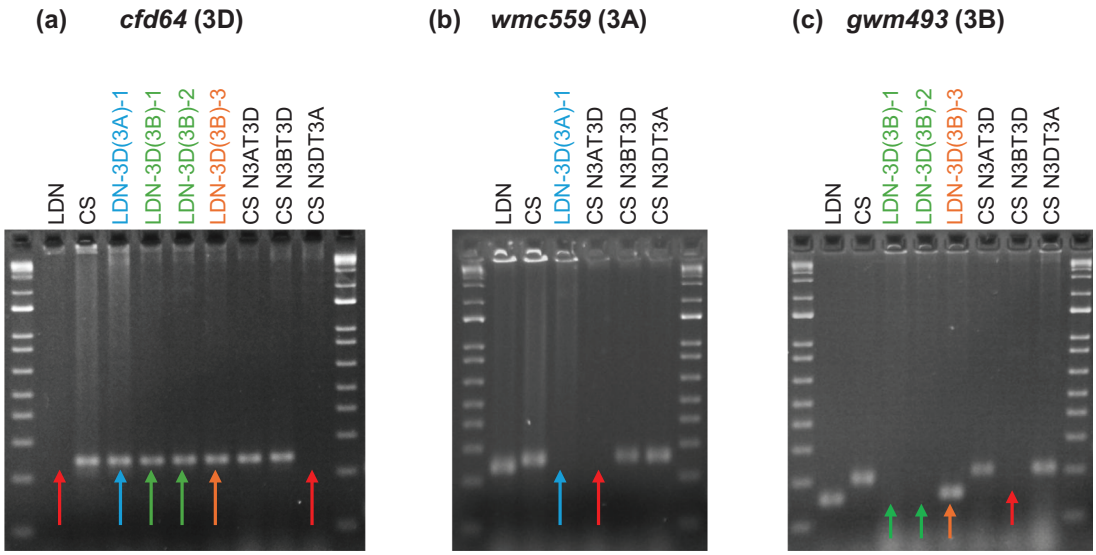


FIGURE 3 Simple sequence repeat (SSR) markers diagnostic for homoeologous group 3 chromosomes involving lines LDN-3D(3A) and LDN-3D(3B), where the former is a true compensating disomic and the latter is not because it segregates for a 3B monosome. (a) SSR marker *cfd64* is specific to chromosome 3D based on its absence in the line that is nullisomic for chromosome 3D (Chinese Spring [CS] N3DT3A) and Langdon (LDN) durum (red arrows). Marker *cfd64* was present in all Langdon disomic substitution (LDN-DS) plants on this gel indicating they all contained chromosome 3D (blue, green, and orange arrows). (b) SSR marker *wmc559* is specific to chromosome 3A because of its absence in the line nullisomic for 3A (CS N3AT3D) (red arrow). The absence of this marker in LDN-3D(3A)-1 (blue arrow) indicates this plant lacks chromosome 3A and it is a true LDN-3D(3A) disomic chromosome substitution line. (c) SSR marker *gwm493* is specific to chromosome 3B based on its absence in the line nullisomic for 3B (CS N3BT3A) (red arrow). The absence of this marker in LDN-3D(3B)-1 and LDN-3D(3B)-2 indicates these plants are nullisomic for 3B (green arrows), whereas the presence of a *gwm493* amplicon in LDN-3D(3B)-3 indicates this plant contains a 3B monosome (orange arrow).

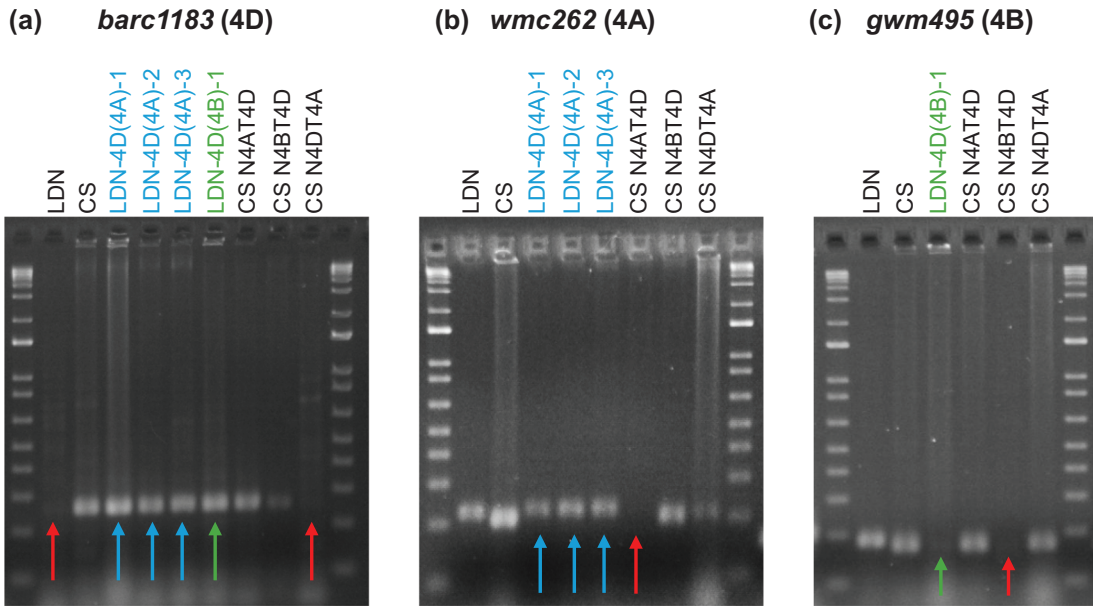


FIGURE 4 Simple sequence repeat (SSR) markers diagnostic for homoeologous group 4 chromosomes involving lines LDN-4D(4A) and LDN-4D(4B). (a) SSR marker *barc1183* is specific to chromosome 4D based on its absence in the line that is nullisomic for chromosome 4D (Chinese Spring [CS] N4DT4A) and Langdon (LDN) durum (red arrows). Marker *barc1183* was present in all Langdon disomic substitution (LDN-DS) plants on this gel indicating they all contained chromosome 4D (blue and green arrows). (b) SSR marker *wmc262* is specific to chromosome 4A because of its absence in the line nullisomic for 4A (CS N4AT4D) (red arrow). This marker was not absent in any of the LDN-4D(4A) plants on the gel (blue arrows) indicating none of the three plants were true disomic substitutions. (c) SSR marker *gwm495* is specific to chromosome 4B based on its absence in the line nullisomic for 4B (CS N4BT4D) (red arrow). The absence of this marker in LDN-4D(4B)-1 indicates this plant is a true disomic substitution line (green arrow).

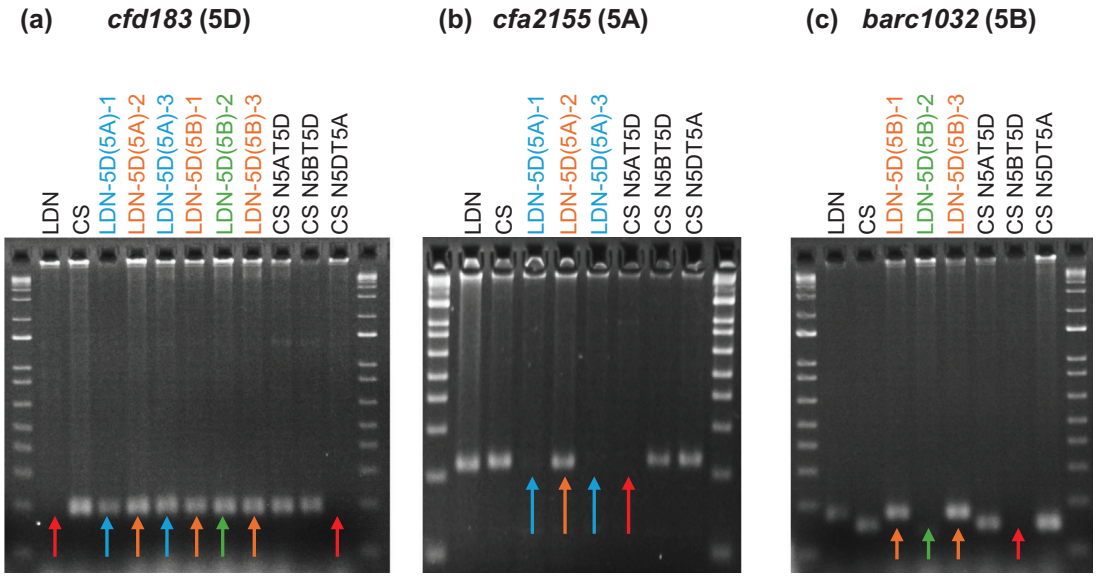


FIGURE 5 Simple sequence repeat (SSR) markers diagnostic for homoeologous group 5 chromosomes involving lines LDN-5D(5A) and LDN-5D(5B). (a) SSR marker *cfd183* is specific to chromosome 5D based on its absence in the line that is nullisomic for chromosome 5D (Chinese Spring [CS] N5DT5A) and Langdon (LDN) durum (red arrows). Marker *cfd183* was present in all Langdon disomic substitution (LDN-DS) plants on this gel indicating they all contained chromosome 5D (blue and green arrows). (b) SSR marker *cfa2155* is specific to chromosome 5A because of its absence in the line nullisomic for 5A (CS N5AT5D) (red arrow). The absence of this marker in LDN-5D(5A)-1 and LDN-5D(5A)-3 indicates these plants are nullisomic for 5A (blue arrows), whereas the presence of a *cfa2155* amplicon in LDN-5D(5A)-2 indicates this plant contains a 5A monosome (orange arrow). (c) SSR marker *barc1032* is specific to chromosome 5B because of its absence in the line nullisomic for 5B (CS N5BT5D) (red arrow). The absence of this marker in LDN-5D(5B)-2 indicates this plant is nullisomic for 5A (blue arrow), whereas the presence of a *cfa2155* amplicon in LDN-5D(5B)-1 and LDN-5D(5B)-3 indicates these plants contain a 5A monosome (orange arrows).

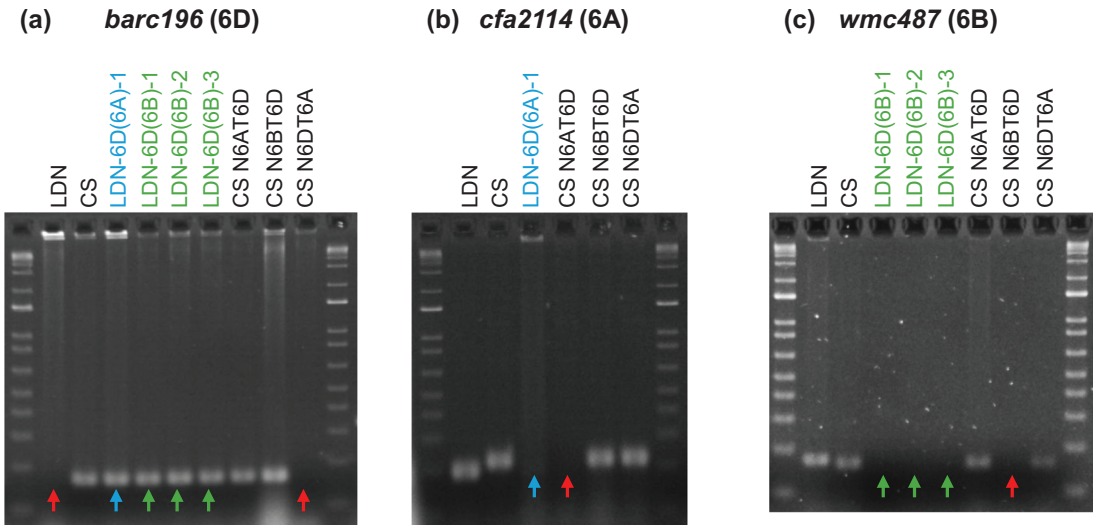


FIGURE 6 Simple sequence repeat (SSR) markers diagnostic for homoeologous group 6 chromosomes involving lines LDN-6D(6A) and LDN-6D(6B). (a) SSR marker *barc196* is specific to chromosome 6D based on its absence in the line that is nullisomic for chromosome 6D (Chinese Spring [CS] N6DT6A) and Langdon (LDN) durum (red arrows). Marker *barc196* was present in all Langdon disomic substitution (LDN-DS) plants on this gel indicating they all contained chromosome 6D (blue and green arrows). (b) SSR marker *cfa2114* is specific to chromosome 6A because of its absence in the line nullisomic for 6A (CS N6AT6D) (red arrow). This marker was absent in line LDN-6D(6A) (blue arrow) indicating plant LDN-6D(6A)-1 was a true disomic substitution. (c) SSR marker *wmc487* is specific to chromosome 6B based on its absence in the line nullisomic for 6B (CS N6BT6D) (red arrow). The absence of this marker in the three LDN-6D(6B) plants indicates they are all true disomic substitution lines (green arrows).

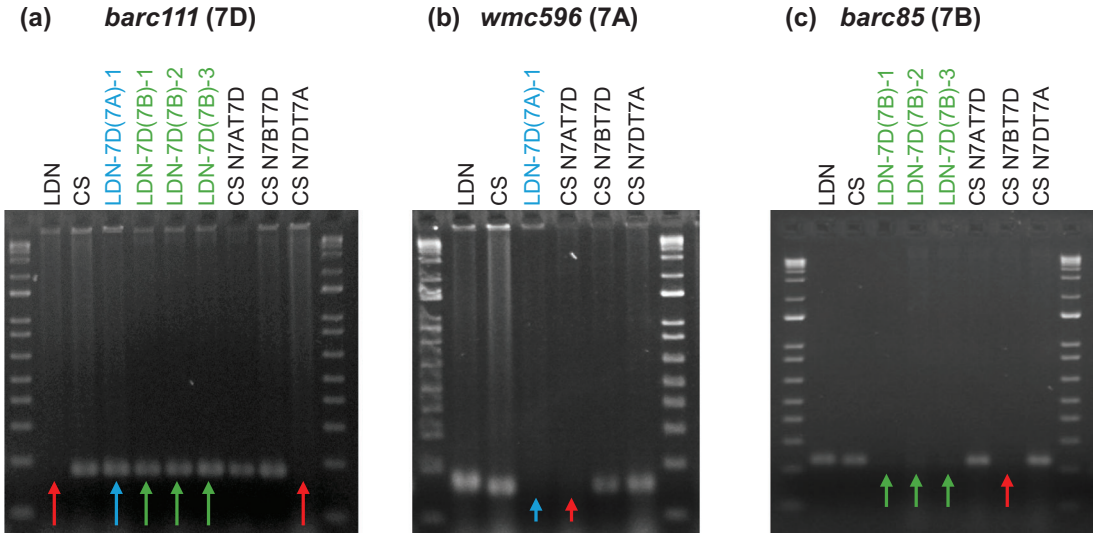


FIGURE 7 Simple sequence repeat (SSR) markers diagnostic for homoeologous group 7 chromosomes involving lines LDN-7D(7A) and LDN-7D(7B), which are both true DS lines. (a) SSR marker *barc111* is specific to chromosome 7D based on its absence in the line that is nullisomic for chromosome 7D (Chinese Spring [CS] N7DT7A) and Langdon (LDN) durum (red arrows). Marker *barc111* was present in all plants of both Langdon disomic substitution (LDN-DS) lines indicating they all contained chromosome 7D (blue and green arrows). (b) SSR marker *wmc596* is specific to chromosome 7A because of its absence in the line nullisomic for 7A (CS N7AT7D) (red arrow). The absence of the marker in LDN-7D(7A)-1 (blue arrow) indicates that this plant is a true LDN-7D(7A) disomic chromosome substitution line. (c) SSR marker *barc85* is specific to chromosome 7B based on its absence in the line nullisomic for 7B (CS N7BT7A) (red arrow). The absence of this marker in the three LDN-7D(7B) plants (green arrows) indicates they are all true disomic chromosome substitution lines.

+ $1'_{4A}$), which would segregate among progeny (Joppa & Williams, 1988). Five markers specific for chromosome 4A (*gwm397*, *cfa2026*, *wmc468*, *wmc262*, and *wmc219*) and three markers specific for chromosome 4D (*cfd106*, *barc1183*, and *wmc331*) were used to evaluate LDN-4D(4A) plants (Table 2). The initial LDN-4D(4A) plants evaluated with these markers all possessed copies of both chromosomes 4A and 4D, and they did not segregate for presence of chromosome 4A as expected (Figure 4). Therefore, we tested more plants grown from 13 different seed sources from as far back as the year 1999, which was the earliest source we could find in Dr. Joppa's inventory. A total of 71 LDN-4D(4A) plants were evaluated, but all had copies of both 4A and 4D chromosomes. Because there was no segregation observed for chromosome 4A among these lines, it appears that plants monosomic for chromosome 4A ($13'' + 1''_{4D} + 1'_{4A}$) no longer exist and that all plants of this line are disomic addition lines for chromosome 4D ($13'' + 1''_{4D} + 1''_{4A}$). Therefore, evaluations of lines where chromosome 4D has been substituted for chromosome 4A in durum are not possible, however one can still evaluate the effects of chromosome 4D when added to the durum genome.

3.3.3 | LDN-5D(5A)

LDN plants nullisomic for chromosome 5A had low fertility making it necessary to maintain LDN-5D(5A) plants with a 5A monosome ($13'' + 1''_{5D} + 1'_{5A}$) and select plants nullisomic for 5A to use for genetic analyses (Joppa & Williams, 1988). We identified three markers specific for chromosome 5D (*cfd18*, *cfd78*, and *cfd183*) and four markers specific for chromosome 5A (*wmc705*, *barc186*, *barc117*, and *cfa2155*) for use in characterizing the chromosome constitution of 5A and 5D in LDN-5D(5A) plants (Table 2). Figure 5a demonstrates the specificity of the chromosome 5D marker *cfd183* and that all three LDN-5D(5A) plants evaluated on the gel harbor chromosome 5D. In Figure 5b, marker *cfa2155* shows there was segregation for the 5A monosome among the three LDN-5D(5A) plants where plant LDN-5D(5A)-2 contains the 5A monosome and can therefore be used to propagate the line, whereas plants LDN-5D(5A)-1 and LDN-5D(5A)-3 are nullisomic for 5A and can be used for genetic analyses.

3.3.4 | LDN-5D(5B)

The *Ph1* gene on the long arm of chromosome 5B prohibits pairing of homoeologous chromosomes in allopolyploid wheat. Therefore, when chromosome 5B is in the nullisomic condition, pairing of homoeologous chromosomes occurs, which leads to chromosomal rearrangements such as translocations, duplications, and deficiencies. This leads to genetic instability, infertility, and other issues after several generations. Therefore, Joppa reported that it was necessary to maintain LDN-5D(5B) with a monosome of 5B ($13'' + 1''_{5D} + 1'_{5B}$) (Joppa & Williams, 1988). As mentioned above for LDN-5D(5A), we identified three chromosome

5D-specific markers to monitor the presence of 5D, and Figure 5a shows *cfd183* as an example to demonstrate several LDN-5D(5B) plants carrying chromosome 5D. For chromosome 5B, we identified three chromosome-specific markers including *wmc28*, *barc1032*, and *barc74* (Table 2). Marker *barc1032* is shown as an example in Figure 5c, demonstrating three LDN-5D(5B) plants that segregate for the presence of the 5B monosome, where plants LDN-5D(5B)-1 and LDN-5D(5B)-3 are monosomic for chromosome 5B whereas plant LDN-5D(5B)-2 is nullisomic for 5B and a true DS that could be used in subsequent genetic analyses. Plants LDN-5D(5B)-1 and LDN-5D(5B)-3 would be used to further propagate the line.

3.3.5 | LDN-6D(6B)

Plants nullisomic for chromosome 6B were found to have very low fertility, and therefore Joppa developed the line to include a telosome of 6BS to maintain it (Joppa & Williams, 1988). Two markers specific to the short arm of chromosome 6B (*wmc494* and *wmc487*) and three markers specific for chromosome 6D (*barc202*, *barc196*, and *cfd287*) were identified (Table 2). As demonstrated in Figure 6a, *barc196* is specific to 6D and shows three LDN-6D(6B) plants that carry chromosome 6D. Further evaluation of these three lines with the chromosome 6B-specific marker *wmc487* indicated that all three LDN-6D(6B) plants lacked the 6BS telosome and could therefore be used for genetic analyses (Figure 6c).

4 | CONCLUSIONS

In this study, our aim was to identify a set of chromosome-specific SSR markers that could be used to quickly and efficiently identify the chromosome constitutions of the LDN-DS lines, especially the non-compensating lines where it is essential to identify plants lacking a monosome or telosome for genetic studies. We were able to successfully identify suitable markers for each chromosome and in the process, found that the LDN-4D(4A) DS line is no longer available, presumably due to the loss of plants monosomic for chromosome 4A, and as such, this line now appears to exist only as a disomic 4D addition line in the LDN background. Nevertheless, the SSR markers identified for the other critical chromosomes in the other LDN-DS lines work effectively. We chose to focus on SSR markers for this work as opposed to another marker platform such as Kompetitive allele-specific PCR (KASP) due to their simplicity of use and low cost. The instrumentation and reagents necessary to run KASP markers is relatively expensive, and evaluating a few plants of each of the non-compensating LDN-DS lines does not require high-throughput capabilities. Most laboratories, even those with modest levels of funding, have access to basic thermal cyclers and agarose gel electrophoresis equipment making SSR markers broadly suitable for almost any lab interested in employing the LDN-DS lines for genetic studies. Thus, the SSR markers identified in this research offer a simpler, faster, and more economical means of characterizing the LDN-DS lines compared to the microscopic-based cytological techniques used in the past.

AUTHOR CONTRIBUTIONS

Heaven S. James: Investigation; methodology; validation; writing—original draft. **Amanda R. Peters Haugrud**: Data curation; formal analysis; writing—review and editing. **Zengcui Zhang**: Investigation; methodology. **Justin D. Faris**: Conceptualization; formal analysis; funding acquisition; project administration; resources; supervision; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data generated in this research are presented in the article. Seed of the LDN-DS lines is available upon request from JDF (justin.faris@usda.gov)

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SUPPORTING INFORMATION

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