Development of self-fertile deletion homozygous and ditelosomic lines for the long arm of chromosome 2A in common wheat

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Most deletions for the short arm of chromosome 2A (2AS), and the telocentric chromosome for the long arm of chromosome 2A (2AL), are available only in the heterozygous condition in ‘Chinese Spring’ hexaploid wheat. This is due to the female sterility, and therefore self-sterility, of their homozygotes, caused by the partial or entire loss of the 2AS chromosome arm on which genes for normal synapsis and female fertility are located. On the other hand, a D-genome disomic substitution line 2D(2A) of ‘Langdon’ tetraploid wheat, in which chromosome 2D is disomically substituted for chromosome 2A, is available (i.e., self-fertile) despite chromosome 2A being missing in this line. This fact indicates that another gene for female fertility must be present in Langdon 2D(2A). We attempted to develop self-fertile 2AS homozygous deletion and ditelosomic 2AL lines by transferring this female fertility gene, through a series of crosses and cytological screening, from Langdon 2D(2A) to the two aneuploid lines. We finally obtained self-fertile 2AS homozygous deletion and ditelosomic 2AL lines. These lines displayed normal meiotic chromosome pairing and lacked all 12 of the 2AS markers used for PCR analysis.

Key words: aneuploid, chromosome 2A, common wheat, ditelosomic, self-fertile

A series of aneuploid lines were produced in the common wheat cultivar Chinese Spring (CS) (Triticum aestivum L., 2n = 6x = 42, genome constitution AABBDD) (Sears, 1954, 1966; Sears and Sears, 1978). Ditelesomic lines, in which one of the two arms of each chromosome is disomically missing, have been used to allocate genes and DNA sequences to specific chromosome arms. Common wheat has 21 different chromosomes, which are grouped into three genomes (A, B and D) and seven homoeologous groups (1 to 7). Therefore, 42 ditelosomic lines are possible in common wheat. However, six ditelosomic lines are not available (Devos et al., 1999). Sears (1954) reported that the right (= short) arm of chromosome II (= 2A) (2AS) carries genes for normal synapsis and female fertility, and therefore a ditelosomic line for the long arm of chromosome 2A (2AL) is not available due to female sterility (Sears and Sears, 1978). Endo and Gill (1996) also reported that most deletion homozygotes for 2AS have irregular meiosis and are almost sterile.

A complete set of disomic substitution lines was developed in the tetraploid wheat cultivar Langdon (LDN) (T. turgidum L. var. durum, 2n = 4x = 28, genome constitution AABB). In each of these aneuploid lines, a pair of LDN homologous chromosomes are replaced by a pair of D-genome homoeologous chromosomes that were transferred from CS (Joppa and Williams, 1988). Most of these lines are self-fertile, including LDN 2D(2A) in which LDN chromosome 2A is replaced with CS chromosome 2D. This suggested that LDN 2D(2A) has another gene responsible for female fertility on a chromosome other than chromosome 2A, and led us to the idea of developing a self-fertile line of ditelosomic 2AL in common wheat. Here we report the breeding process of self-fertile 2AS homozygous deletion and ditelosomic 2AL lines by transferring the female fertility gene from LDN 2D(2A) to these CS aneuploids.

Plant materials and the breeding scheme We used euploid LDN, a LDN D-genome disomic substitution line LDN 2D(2A) (13”+1”2D(2A)), and two CS aneuploid lines: a deletion 2AS-2 heterozygous line (20”+1’2A+1’del2AS-2) and ditelosomic 2AS (20”+1’2AS). These lines are available at NBRP (The National BioResource Project)-wheat (https://shigen.nig.ac.jp/wheat/komugi/). We also used
monotelodisomic 2AL (20"+t'2AL+t'2A), which had been maintained by the corresponding author. We consulted the website https://wheat.pw.usda.gov/gpgpages/nomenclature.html for the description of chromosome configurations. Figure 1 depicts the breeding scheme of self-fertile lines of deletion 2AS-2 homozygotes and ditelosomic 2AL. The chromosome constitutions of the wheat lines used and their progeny were checked by C-banding and identified based on the previously published banding karyotypes of common wheat (Endo and Gill, 1984; Gill et al., 1991).

**Chromosome constitution of LDN 2D(2A)** Chromosome 2D is disomically substituted for chromosome 2A in LDN 2D(2A) (Fig. 2). Although Joppa and Williams (1977) reported desynapsis in the progeny of a 2D(2A) disomic substitution line, LDN 2D(2A) used in this study had normal meiosis with 14 bivalents (Supplementary Fig. S1A). The desynapsis in LDN 2D(2A) was presumably corrected because such a problem was not mentioned in their later publications (Joppa and Williams, 1988; Joppa, 1993).

**Production of a self-fertile 2AS-2 homozygous line** Deletion homozygotes for 2AS-2 (1"del2AS-2) were obtained from the self-pollinated progeny of a plant that was disomic for 2AS-2 and monosomic for 2AS (1"del2AS-2+t'2AS) (see Fig. 1). The 2AS-2 homozygotes (1"del2AS-2) were mostly self-sterile (five seeds in 69 self-pollinated florets), while their pollen normally fertilized emasculated florets of euploid CS (perfect seed setting in 23 hand-pollinated florets). This indicated that the self-sterility of the 2AS-2 homozygotes was due to severe female sterility.

We started the production of a self-fertile 2AS-2 homozygous line from a cross between LDN 2D(2A) and (1"del2AS-2+t'2AS) to obtain a 35-chromosome pentaploid hybrid, which lacked t'2AS (see Fig. 1). The 35-chromosome plant was twice backcrossed with (1"del2AS-2+t'2AS), and 42-chromosome 2AS-2 homozygous plants (1"del2AS-2) were selected. One of the 2AS-2 homozygotes was self-fertile and it was self-pollinated three times to obtain a self-fertile 2AS-2 homozygote whose progeny were all self-fertile (15 plants examined), 184 seeds in 230 self-pollinated florets. This plant was probably homozygous for the female fertility gene derived from LDN 2D(2A).

**Production of self-fertile ditelosomic 2AL** We started the production of a self-fertile line of ditelosomic 2AL from a cross between monotelodisomic 2AL (t'2AL+1'2A) and the self-fertile 2AS-2 homozygous line to obtain a double monotelosomic 2A plant (t'2AL+t'2AS) (see Fig. 1). This plant was self-pollinated, and nine out of 27 F1 plants were ditelosomic for 2AL (t"2AL) (Fig. 3). Four of the ditelosomic 2AL plants were self-fertile, while the remaining five were completely self-sterile. The F1 progeny of one of the four self-fertile ditelosomic 2AL plants were all self-fertile (10 plants examined), suggesting that the parental F1 plant was homozygous for the female fertility gene. This F1 progeny was selected to establish a self-fertile line of ditelosomic 2AL. The established self-fertile ditelosomic 2AL line produced a reasonable number of seeds by self-pollination (31 seeds on four spikes).

**Meiotic chromosome configurations of the self-fertile deletion 2AS-2 homozygotes and self-fertile ditelosomic 2AL** Sears (1954) reported that the 2AS chromosome arm carries genes for normal synapsis and for female fertility. Endo and Gill (1996) reported that...
some deletion homozygotes for 2AS had irregular meiosis with many univalents. As expected from these previous studies, the pollen mother cells (PMCs) of the sterile 2AS-2 homozygotes showed irregular meiosis with univalents ranging from 0 to 12 (3.76 on average from 25 PMCs) (Supplementary Fig. S1B). On the other hand, the self-fertile 2AS-2 homozygotes and self-fertile ditelosomic 2AL had normal meiotic pairing with no univalents (Supplementary Fig. S1C and S1D).

**PCR analysis of the self-fertile deletion 2AS-2 homozygotes and self-fertile ditelosomic 2AL**  We selected PCR markers, 12 for 2AS and eight for 2AL, from the microsatellites that had been used to construct a genetic
The result of the PCR analysis with these markers is shown in Table 1 and Supplementary Fig. S2. All the markers were amplified in CS but three of them were not amplified in LDN, suggesting the presence of sequence differences in chromosome 2A between CS and LDN. None of the 2A markers except two of the 2AL markers were amplified in LDN 2D(2A). Two markers (gwm558 and gwm473) could not be located on chromosome 2A because chromosome 2A was missing in LDN 2D(2A). They must have been misassigned to 2A. None of the 2AS markers were amplified in the sterile and self-fertile deletion 2AS-2 homozygous lines, or in self-fertile ditelosomic 2AL, which suggested that all the 2AS markers were located distal to the breakpoint in 2AS-2. Although one of the 2AL markers (gwm372) was missing in the sterile, self-fertile 2AS-2 homozygotes and self-fertile ditelosomic 2AL, it was present in ditelosomic 2AS. This contradiction can be resolved by assuming that gwm372 is on 2AS, somewhere distal to the breakpoint of deletion 2AS-2. Thus, the centromere should be positioned between gwm372 and gwm445.

Chromosomal location of the gene for female fertility The female fertility gene was transferred from LDN 2D(2A) to the self-fertile 2AS-2 homozygous lines and self-fertile ditelosomic 2AL. This gene is most likely located on a chromosome other than chromosome 2A in LDN and lost its function in CS during evolution. Chromosome 2B, a homoeologous group-2 chromosome, is a likely candidate to carry the female-fertility gene in LDN 2D(2A), although there is no direct evidence for this. The loss of multiple genes is likely to happen in hexaploid wheat, as demonstrated for the waxy genes (Yamamori et al., 1994) and for the male-fertility genes (Joshi et al., 2013). Another possibility is that the female fertility gene located on LDN chromosome 2A was transferred onto CS chromosome 2D during the production of LDN 2D(2A). This explanation sounds reasonable because Joppa and Williams (1988) reported

### Table 1. PCR analysis of the 2AS-2 deletion homozygous lines and the ditelosomic 2AL line

| Marker | cM | CS  | LDN 2D(2A) | LDN 2AS-2 | Self-fertile 2AS-2 | Self-sterile 2AS-2 | Self-fertile 2AS-2 | Ditelosomic 2AS |
|--------|----|-----|------------|-----------|-------------------|-------------------|-------------------|-----------------|
| cfd036 | 0  | O   | N          | X         | X                 | X                 | X                 | O               |
| gwm512 | 6  | O   | N          | X         | X                 | X                 | X                 | O               |
| gwm636 | 11 | O   | O          | X         | X                 | X                 | X                 | O               |
| wmc407 | 15 | O   | O          | X         | X                 | X                 | X                 | O               |
| wmc177 | 28 | O   | O          | X         | X                 | X                 | X                 | O               |
| wmc522 | 45 | O   | O          | X         | X                 | X                 | X                 | O               |
| gwm339 | 51 | O   | O          | X         | X                 | X                 | X                 | O               |
| gwm122 | 51 | O   | O          | X         | X                 | X                 | X                 | O               |
| gwm275 | 52 | O   | O          | X         | X                 | X                 | X                 | O               |
| gwm425 | 52 | O   | O          | X         | X                 | X                 | X                 | O               |
| gwm249 | 53 | O   | O          | X         | X                 | X                 | X                 | O               |
| gwm095 | 53 | O   | O          | X         | X                 | X                 | X                 | O               |
| centromere | 53–54 | &nbsp; | &nbsp; | &nbsp; | &nbsp; | &nbsp; | &nbsp; | &nbsp; |
| gwm558 | 54 | O   | O          | O         | O                 | O                 | O                 | O               |
| gwm473 | 57 | O   | O          | O         | O                 | O                 | O                 | O               |
| gwm372 | 60 | O   | O          | X         | X                 | X                 | X                 | O               |
| gwm445 | 68 | O   | O          | X         | O                 | O                 | X                 | O               |
| gwm294 | 76 | O   | O          | X         | O                 | O                 | X                 | O               |
| wmc181 | 103| O   | O          | X         | O                 | O                 | X                 | O               |
| gwm356 | 126| O   | N          | X         | O                 | O                 | O                 | O               |
| wmc658 | 140| O   | O          | X         | O                 | O                 | O                 | X               |

Note) ‘O’ and ‘X’ denote the presence and absence of markers, respectively, and ‘N’ denotes no PCR amplification in LDN vs. CS. DNA was extracted from young leaves using a DNeasy Plant Mini Kit (Qiagen). The PCR mixture (15 μl) contained 30 ng of genomic DNA, 1 × GflexDNA Polymerase (TaKaRa), PCR Buffer (TaKaRa), 0.5 mM primers and 0.375 U of Tks Gflex. The PCR conditions were as follows: 94 °C for 1 min followed by 30 cycles of 98 °C for 10 s, 55 °C or 60 °C for 15 s, and 68 °C for 30 s. PCR products were separated on 3% agarose (w/v) gels in TAE buffer.
that pairing between chromosomes 2A and 2D occurred frequently when both chromosomes were monosomic and resulted in plants carrying translocations, and that considerable effort was required to produce LDN 2D(2A) free of translocations. However, the 2D chromosome in self-fertile ditelosomic 2AL did not appear to have any such translocations, i.e., no change in the C-banding pattern of chromosome 2D (Fig. 2B), and also the PCR analysis did not show any evidence of a structural change in chromosome 2D.

Use of the self-fertile ditelosomic 2AL line It is unique to common wheat that ditelosomics are available for most of the chromosome arms. Ditelosomics are useful in localizing genes and DNA markers cytologically to specific chromosome arms. Cytological mapping has often corrected the orders of markers in genetic maps. For example, Joshi et al. (2013) developed a self-fertile ditelosomic 4BL line and, using that line, corrected the marker order on chromosome 4B in the previous genetic maps. So far, cytological mapping has been impossible for the 2AS arm because only ditelosomic 2AS is available and because no nullisomic 2A-tetrasomic 2B or 2D line exists. The self-fertile ditelosomic 2AL line developed in this study enables us to allocate DNA markers to the 2AS arm unambiguously. Indeed, PCR analysis in this study indicated that one of the 2AL markers (gwm372) was located on the 2AS arm (Table 1). Also, deletion mapping for the 2AS arm has been hampered by the unavailability of homozygous deletion lines for most of the 2AS deletions. Nevertheless, we can conduct deletion mapping with 2AS hemizygous deletion plants that can be obtained by crossing the self-fertile ditelosomic 2AL line with 2AS heterozygous deletion lines.

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Supplementary Fig. S1. Phase-contrast photomicrographs of meiotic chromosome pairing. (A) LDN 2D(2A), (B) the sterile 2AS-2 homozygous line, (C) the self-fertile 2AS-2 homozygous line, and (D) the self-fertile ditelosomic 2AL line.
Supplementary Fig. S2. PCR analysis of the 2AS-2 deletion homozygous lines and the ditelosomic 2AL line. Critical bands are indicated with arrowheads to the right of each image.