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Development of a self-fertile ditelosomic line for the long arm of chromosome 4B and its characterization using SSR markers

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The ditelosomic line for the long arm of chromosome 4B (4BL) of Chinese Spring (CS) wheat is not available because it is completely male sterile. Since all deletions in the 4B short arm (4BS) cause male sterility in the homozygous condition, a male-fertility gene should be located in a distal region of 4BS. Among the selfed progeny of a hybrid between a male-sterile 4BS deletion plant (4BS-8) and a Japanese common wheat cultivar Norin 61 (N61), we obtained self-fertile 4BS-8 homozygous deletion plants. We also found fertile nullisomic-4B plants among the selfed progeny of a hybrid between a monosomic line 4B of N61 and CS. This fact suggested that N61 has a novel male-fertility gene on a chromosome other than 4B. We established a self-fertile ditelosomic 4BL line after backcrossing a fertile 4BS-8 plant to CS monotelodisomic 4BL. By cytological observation and PCR analysis using 16 SSR markers, we verified that this ditelosomic line lacked the entire 4BS arm and found that three of the markers were on 4BS. We conducted deletion mapping using three 4BS homozygous deletion lines and three 4BS-specific markers.

Key words: wheat, ditelosomic, 4BL, SSR

The improvement of wheat started with simple selection and has progressed by molecular breeding or marker-assisted selection. Among various molecular markers that have been developed in wheat, SSR markers have been extensively used in genetic mapping (Röder et al., 1998; Somers et al., 2004; Song et al., 2005; Torada et al., 2006) and deletion mapping (Sourdille et al., 2004) because they are highly polymorphic among wheat cultivars. In wheat the chromosomal confirmation of molecular markers depends on the aneuploid lines of common wheat cultivar Chinese Spring (CS). In general a molecular marker can be mapped to a chromosome and to either of the chromosome's arms by the use of the nullitetrasomic lines (Sears, 1954) and ditelosomic lines (Sears and Sears, 1978), respectively. However, ditelosomic lines for some chromosome arms have not been established because the missing arms of the critical chromosomes are indispensable to the fertility or viability of the plants. Ditelosomic 4BL, which would have only the long arm of chromosome 4B (4BL), is one such unavailable ditelosomic line: The lack of the short arm of chromosome 4B (4BS) causes complete male sterility (Sears and Sears, 1978). The fact that homozygotes for all nine 4BS (4BS-1~9) deletions become male sterile indicates that a gene responsible for male fertility should be located in the 4BS distal region (Endo and Gill, 1996; Endo et al., 1991). Neither ditelosomic 4BL nor any 4BS deletion-homozygous stocks have been available due to their male sterility, and consequently this has hampered the assignment of molecular markers to 4BS so far.

Some years ago, we happened to find self-fertile homozygous deletion plants among the selfed progeny from a cross between a male-sterile homozygous deletion for one of the 4BS deletions (4BS-8) and a Japanese common wheat cultivar Norin 61 (N61). Also we found fertile nullisomic-4B plants among the selfed progeny of a hybrid between N61 monosomic 4B and CS. Therefore, N61 should have a novel male-fertility gene or genes besides the one on chromosome 4B. This fact suggested that we could develop a self-fertile ditelosomic 4BL line by transferring the N61 novel male-fertility gene to CS.

Development of a male-fertile ditelosomic 4BL line We started to introduce the novel male-fertile gene or genes into CS by the breeding scheme shown in Fig. 1. For this cross-breeding we used CS monotelodisomic 4BL (4B'/4BL') and a CS double deletion line (4BS-8"/4BL-11') that is self-fertile thanks to the presence of a 4BL deletion

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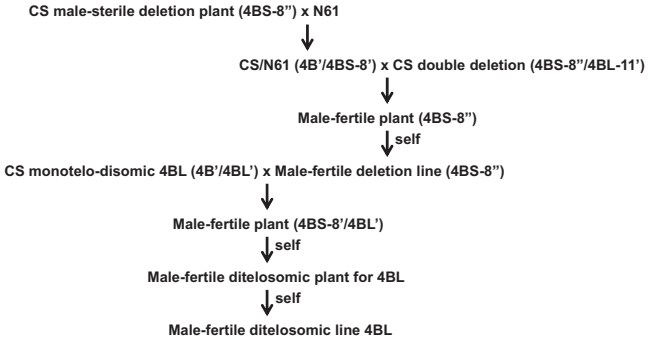

Fig. 1. A breeding scheme to develop a male-fertile ditelosomic line 4BL. In the crosses, the lines on the left were used as the female parent.

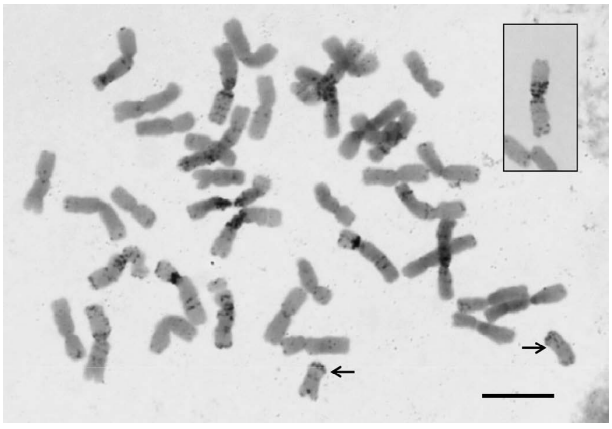

Fig. 2. C-banding image of a mitotic metaphase cell showing a pair of 4BL arms (indicated with arrows). The inset shows a normal 4B chromosome. Bar = 10 μm.

chromosome. Among the selfed progeny of the latter line we can easily obtain male-sterile 4BS-8 homozygotes (e.g. 25 plants out of 50 plants examined). First, we developed a male-fertile homozygous line 4BS-8, and then we crossed it to the CS monotelodisomic 4BL to obtain male-fertile plants with a chromosome constitution of 4BS-8'/4BL'. From the selfed progeny (30 plants) of these plants (4BS-8'/4BL') we obtained two male-fertile ditelosomic 4BL plants (20" + 4BL"); one of which we selected as the parent of a male-fertile ditelosomic 4BL line because its spike morphology was more similar to that of CS; the other plant had awned spikes like N61.

We used C-banding to identify the chromosome constitutions of all derivatives of the cross-breeding and confirmed that the male-fertile ditelosomic 4BL plants (2n = 42) had a pair of 4BL chromosomes (Fig. 2). We confirmed that the male-fertile ditelosomic 4BL line was homozygous for the novel male-fertility gene based on the fact that all selfed progeny examined (10 plants each for two generations) were male fertile. This line had high pollen fertility (82.5%) and seed setting (355 seeds on 20 bagged spikes from eight plants) in a greenhouse. We will release this fertile ditelosomic line 4BL through NBRP-Wheat (http://www.shigen.nig.ac.jp/wheat/komugi/).

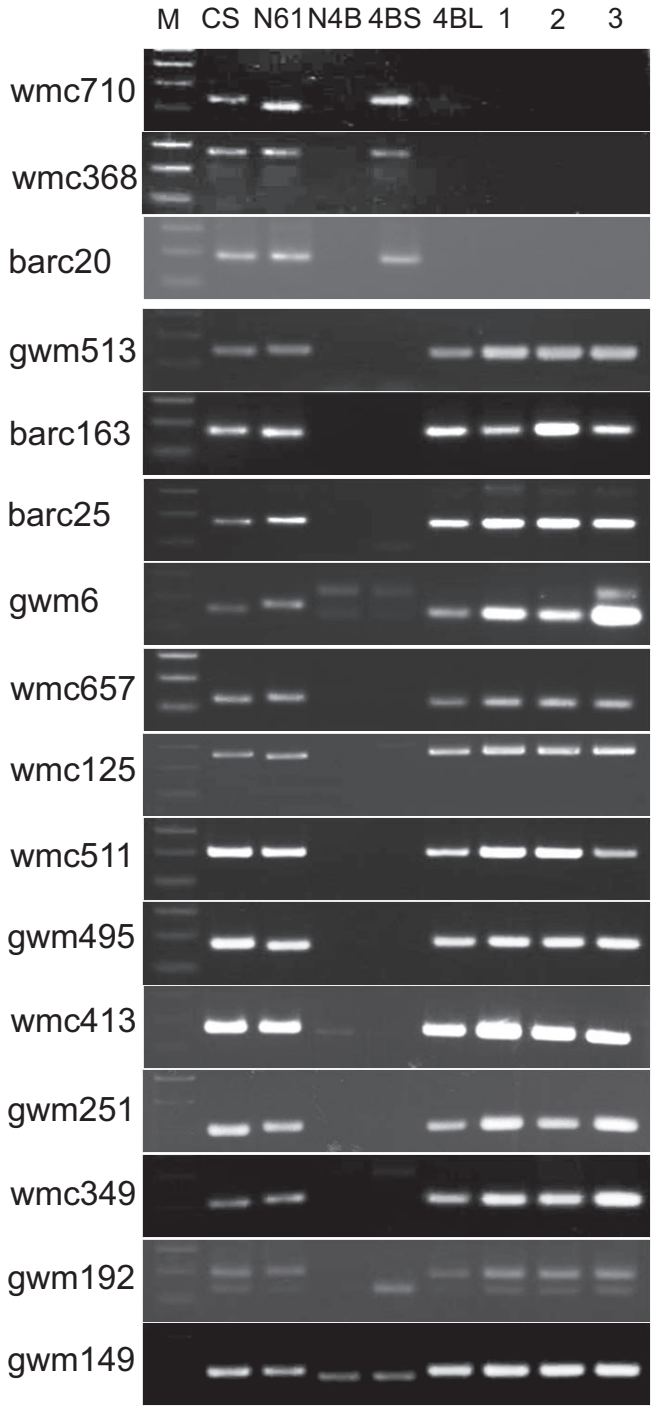

Fig. 3. PCR amplification of the 16 SSR markers. M: Size marker; CS: Chinese Spring; N61: Norin 61; N4B: nullisomic 4B; 4BS: ditelosomic 4BS; 4BL: ditelosomic 4BL; 1–3: three more plants of the ditelosomic 4BL line.

Molecular characterization of the fertile ditelosomic 4BL line In addition to cytology, we verified by PCR that the self-fertile ditelosomic 4BL line lacked the 4BS arm. We selected 19 markers from 24 SSR markers that were reported to be 4B specific (Somers et al., 2004), with excluding markers of the same genetic distances. Using DNA templates of the self-fertile nullisomic 4B, ditelosomic 4BS, self-fertile ditelosomic 4BL, and euploid CS and N61 plants, we conducted PCR analyses with the 19 markers as described below.

We extracted DNA from leaves with the DNeasy Plant Mini Kit (QIAGEN). We added 6 µl of DNA solution (ca. 8 ng/µl) as a template to a PCR mixture consisting of 2.6 µl of 5 x PCR buffer, 0.25 µl of dNTP (10 mM each), 0.75 µl of $MgCl_2$ (25 mM), 0.38 µl of DMSO, 2.50 µl of Betaine (5 M), 0.31 µl of primers (20 pmol/µl), 0.1 µl of KAPA Taq Extra DNA Polymerase (5 U/µl KAPABIOSYSTEMS). The thermal cycling conditions with an iCycler (BioRad) were as follows: 98°C for 5 min, 40 cycles of 98°C for 30 sec, between 50°C and 65°C for 30 sec (depending on the primer combination), and 72°C for 10 min. We checked PCR products by agarose (2.5% Agarose S, Nippon gene) gel electrophoresis (100 V, 25 min).

Among the 19 markers, three markers showed PCR amplification in the nullisomic 4B line as well as in euploid CS and N61. Since this fact suggested that the three markers were not located on chromosome 4B, we did not use them in this study. The remaining 16 markers showed clear PCR amplification in the euploid lines but no PCR amplification in the nullisomic 4B line. Three of the 16 markers amplified only in the ditelosomic 4BS line and 13 only in the ditelosomic 4BL line

Table 1. PCR analysis of the three 4BS-specific SSR makers

SSR markers	Lines							
	Chinese Spring	Norin61	Nullisomic 4B	Ditelosomic 4BL	Ditelosomic 4BS	4BS-1 (FL = 0.84)	4BS-8 (FL = 0.57)	4BS-4 (FL = 0.37)
gwm368	+	+	–	–	+	–	–	–
barc20	+	+	–	–	+	+	–	–
wmc710	+	+	–	–	+	–	–	–

Note) '+' and '–' indicate the positive and negative PCR amplification, respectively. The FL values, calculated as indicated by Endo and Gill (1996), indicate the remaining portions of the deleted 4BS chromosome arm.

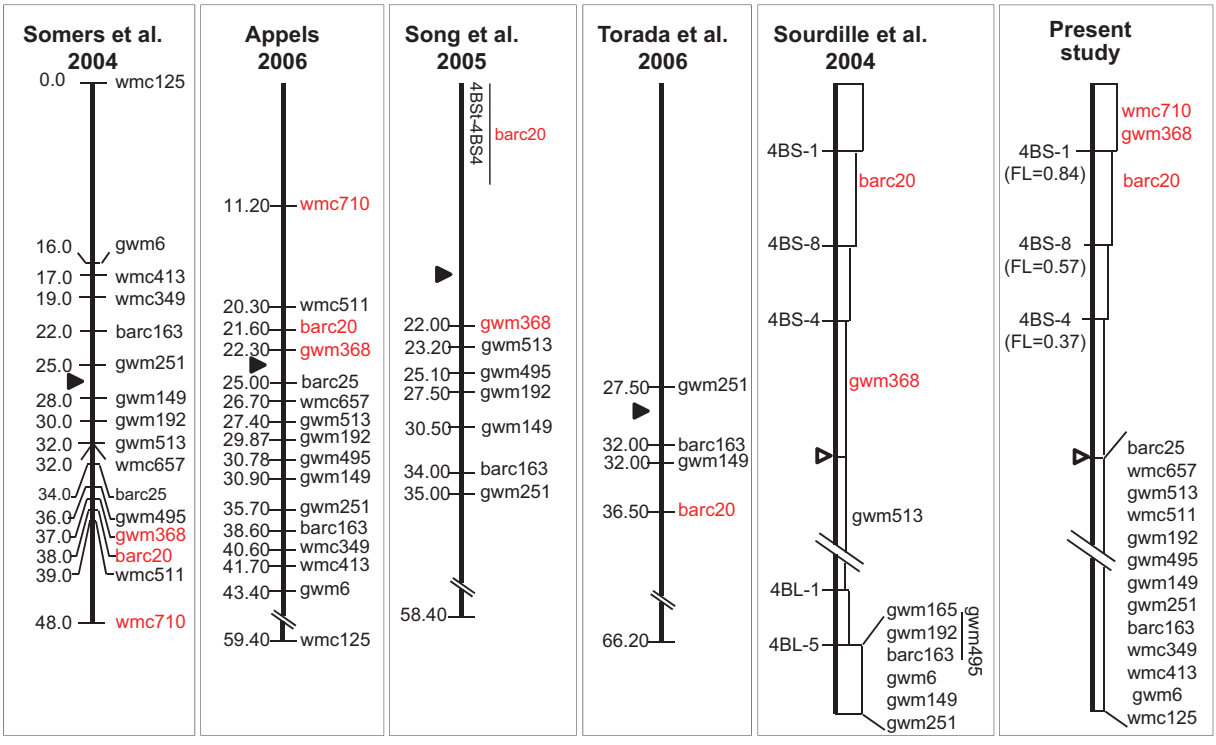

Fig. 4. Comparison of different genetic and deletion maps for chromosome 4B. Only the 16 markers used in the present study are shown in these maps. Markers in red are 4BS-specific ones. These maps other than the one in the present study were drawn based on 'CMap for Wheat' at the website of Wheat Genetic Resources Database KOMUGI (http://map2.lab.nig.ac.jp/cmap/).

(Fig. 3). This clear separation of the markers confirmed the deficiency of the 4BS arm in the ditelosomic 4BL line. With the three 4BS-specific markers we conducted deletion mapping using homozygous deletion plants for 4BS-1, 4BS-4 and 4BS-8 isolated from the selfed progeny of the respective heterozygotes (4B'/4BS-1', 4B'/4BS-4' and 4BS-8"/4BL-11') together with the ditelosomic 4BL line (Table 1, Fig. 3). The PCR analysis showed that one marker (barc20) was in the interstitial region (FL = 0.57 – 0.84) and that the other two markers were in the distal region (> FL = 0.84) (Fig. 4).

We reconfirmed the validity of the fertile ditelosomic 4BL line developed in this study by using the 16 SSR markers that had been assigned to chromosome 4B by Somers et al. (2004). Other investigators used some of these markers for genetic (Song et al., 2005; Torada et al., 2006) and deletion (Sourdille et al., 2004) mapping of chromosome 4B, but the chromosomal locations assigned differed with the study (Fig. 4). For example, although we located a marker gwm368 in the 4BS distal region in this study, Somers et al. (2004) and Song et al. (2005) assigned it to the long arm and Sourdille et al. (2004) mapped it to the 4BS proximal region. Also, Somers et al. (2004) and Torada et al. (2006) assigned barc20 to 4BL and gwm251 to 4BS, but we assigned them to the opposite arms. This discrepancy reflects the poor resolution of genetic maps of wheat chromosomes due to the restricted recombination in the pericentromeric regions, and this is prominently displayed in chromosome 4B whose genetic length is one of the shortest in many mapping studies. Comparing the previous and present studies on the mapping of chromosome 4B, the map of chromosome 4B produced by Somers et al. (2004) should have been inverted. The discrepancy in the map position of the marker gwm368 between the maps of Sourdille et al. (2004) and the present study might be attributed to wrong screening of the three 4BS deletion homozygotes; they are male sterile and need to be selected from the deletion heterozygotes. Prior to the present study, there was no way to allocate DNA markers to 4BS directly by simple aneuploid analysis. We do not know why the above-mentioned contradictions occurred but we believe we correctly determined the chromosomal locations of the 16 SSR markers in this study using the self-fertile ditelosomic 4BL line together with the ditelosomic 4BS line and 4BS homozygous deletions. Also, this ditelosomic 4BL line could be valuable for positional cloning of the novel male-fertility gene because we can create a mapping population from a cross between the self-fertile ditelosomic 4BL line and male-sterile ditelosomic 4BL line.

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