Note

Development of Chromosome Segment Substitution Lines Derived from Backcross between *indica* Donor Rice Cultivar ‘Nona Bokra’ and *japonica* Recipient Cultivar ‘Koshihikari’

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Chromosome segment substitution lines (CSSLs), which carry a particular chromosome segment from a donor line in the genetic background of another line, are useful plant materials for genetic studies, such as detection and fine mapping of quantitative trait loci (QTLs) for target traits (Yano 2001, Kubo *et al.* 2002, Ebitani *et al.* 2005). In particular, CSSLs can be used for the detection of QTLs with small additive effects that are masked by QTLs with larger effects in primary populations such as F_2 populations and recombinant inbred lines (Ebitani *et al.* 2005, Keurentjes *et al.* 2007). Several rice CSSLs have been developed and used in QTL mapping (Kubo *et al.* 2002, Ebitani *et al.* 2005, Mei *et al.* 2006). So far, a number of QTLs for traits with biological and economic interest have been detected through the use of these CSSLs, and some have been well characterized (Kubo *et al.* 2002, Ando *et al.* 2004, Ishimaru *et al.* 2005, Ebitani *et al.* 2005, Mei *et al.* 2006). However, a limited number of cross combinations does not cover a wide range of naturally occurring variation in rice. To enhance the detection of QTLs by genetic studies, novel CSSLs derived from various germplasm accessions should be developed.

To achieve this objective, we are currently developing a series of CSSLs by using several *indica* and *japonica* cultivars as donor parents in the genetic background of an elite *japonica* cultivar, ‘Koshihikari’, as a recurrent parent. Here we reported a newly developed CSSL population derived from a cross between Koshihikari and an *indica* cultivar,

‘Nona Bokra’. Nona Bokra is well known as a salt-tolerant donor in conventional breeding (Akbar *et al.* 1986, Ren *et al.* 2005) and shows extremely late heading under long-day conditions (Uga *et al.* 2007). In the present paper, we described the genotype constitution of the CSSLs. In addition, to validate the potential power of QTL detection in the CSSLs, we determined the heading date and identified several chromosomal regions affecting it. By comparison of the QTLs for heading date with previous results obtained in the same cross, we revealed the robust power of QTL detection by the CSSLs.

Development of the CSSLs

The procedure for the development of the CSSLs is summarized in Figure 1. We performed foreground selection (to select target chromosomes entirely heterozygous) until the BC_2F_1 generation, but not background selection (to minimize the occurrence of heterozygous and Nona Bokra homozygous regions in non-target chromosomal regions). In the BC_3F_1 and BC_3F_2 generations, foreground and background selection was combined. Finally, background selection was conducted in the BC_3F_3 and BC_3F_4 generations to select CSSLs.

The F_1 plants derived from a cross between Nona Bokra and Koshihikari were backcrossed to Koshihikari to produce 34 BC_1F_1 plants. These BC_1F_1 plants were again backcrossed to Koshihikari to produce BC_2F_1 seeds. Then, based on the genotype of 98 restriction fragment length polymorphism (RFLP) markers distributed over the whole genome, we selected 17 BC_1F_1 plants that were heterozygous for the entire region of a target chromosome. Subsequently, 317 BC_2F_1 individuals derived from the 17 BC_1F_1 plants were

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Fig. 1. Breeding scheme for the development of chromosome segment substitution lines (CSSLs) carrying Nona Bokra chromosome segments in the Koshihikari genetic background. The numerator and denominator in parentheses stand for the number of plants selected and the number investigated by marker-assisted selection (MAS), respectively.

raised, and 30 BC₂F₁ plants with a heterozygous target chromosome were selected by marker-assisted selection (MAS) for the subsequent backcross to Koshihikari to produce BC₃F₁ plants. PCR-based markers (simple sequence repeat, SSR; and expressed sequence tag, EST) were used in this and later generations to screen plants by MAS. The SSR markers, developed by McCouch *et al.* (2002), are denoted by “RM” followed by a four-digit-number. The EST markers were developed within the framework of the Rice Genome Research Program (2007). In the same way, 88 out of 564 BC₃F₁ plants derived from the 30 BC₂F₁ plants were selected by MAS in the target region. A subsequent whole-genome survey of the 88 BC₃F₁ plants identified 13 plants in which the majority of the genomic regions was homozygous for the Koshihikari alleles, except for the target region. To obtain candidate plants as CSSLs homozygous for the chromosome segments of Nona Bokra in each target region, the 13 BC₃F₁ plants were self-pollinated, and the resultant 1721 BC₃F₂ plants were tested by MAS to select 47 BC₃F₂ plants. Since there were still heterozygous segments in the non-target regions of these plants, additional self-pollination and MAS

were required to minimize their proportion in non-target regions. Finally, 44 plants were selected as CSSLs (Fig. 2). The genotypes throughout the whole genome of the CSSLs were again examined by using 140 SSR and 5 EST markers. Information about the markers used in this study is available at the Web site of the Rice Genome Resource Center (2007). Each chromosome was covered by three or four lines carrying overlapping segments, except for a small region at the distal end of the short arm on chromosome 1 (indicated by the SSR marker RM6887 located on 0.2 Mb from the distal end of the short arm), which was not covered. A small segment at RM1075 (around 3.8 Mb from the distal end of the short arm) on chromosome 2 in lines 504 and 505 remained heterozygous, because plants homozygous for Nona Bokra at RM1075 in the Koshihikari genetic background could not survive (Matsubara *et al.* 2007). The percentage of substituted segment(s) ($100 \times$ total length of substituted donor homozygous segments/total chromosome length) in each CSSL ranged from 2.1% to 11.7%, based on the physical map distance.

QTL mapping for days-to-heading

Forty-four CSSLs, Koshihikari and Nona Bokra were grown in a paddy field located at the National Institute of Agrobiological Sciences in Tsukuba (36°1'N, 140°6'E), Japan, in 2006. Seeds were sown in the seedling nursery on April 17 and 22 plants in each CSSL were transplanted on May 17 with one replication. We determined the number of days from sowing to heading of the first panicle (days-to-heading, DTH) in five plants for each CSSL.

QTLs were identified based on significant differences between Koshihikari and CSSLs, determined by the Dunnett's multiple comparison procedure.

Average value of DTH in Koshihikari was 109.8, whereas in Nona Bokra the value exceeded 200 DTH (Fig. 3) and in CSSLs the value ranged from 107.0 to 138.8 DTH. Significant differences in the DTH values were detected between 18 CSSLs and Koshihikari, and no CSSLs headed significantly earlier than Koshihikari. By using F₂ and advanced backcross populations of crosses between Koshihikari and Nona Bokra, Uga *et al.* (2007) identified 12 QTLs for heading date on chromosomes 3, 4, 6, 7, 8, 11 and 12 (Fig. 4). Nona Bokra alleles at all the QTLs increased the DTH value. The substituted segments of the 18 CSSLs encompassed 10 of these QTLs, except for those on chromosomes 4 and 11. According to the QTL analysis conducted by Uga *et al.* (2007), the additive effects of the Nona Bokra alleles at the QTLs on chromosomes 4 and 11 were only 1.3 and 0.9 days, respectively. These effects might have been too small to be detected by our CSSLs. On the other hand, novel QTLs not detected by Uga *et al.* (2007) were observed on the short arm of chromosome 5 and on the long arm of chromosome 6: lines 515, 516 and 522 headed significantly later than Koshihikari. QTLs for heading date detected in the same regions in different populations (Cai *et al.* 2002, Li *et al.* 2003, Mei *et al.* 2003) may correspond to the novel QTLs

Fig. 2. Graphical genotypes of 44 CSSLs. The black regions denote the homozygous for Nona Bokra alleles; the white regions denote the homozygous for Koshihikari alleles; the gray regions denote heterozygous. The graphical genotypes shown here are based on the physical map distance included in Build 4.0 Pseudomolecules of the Rice Genome (IRGSP 2007). Genotype classes of the 140 SSR and 5 EST markers in the CSSLs can be obtained at the Web site of the Rice Genome Resource Center (2007).

Fig. 3. Days-to-heading of 44 CSSLs and their recurrent parent, Koshihikari, under natural field conditions at Tsukuba, Japan, in 2006. Bars indicate the mean value of days-to-heading with standard deviation. Shaded bars indicate the significant difference between Koshihikari and CSSL at 0.1% level by Dunnett's test.

detected here. Although QTLs with minor effects cannot be detected by the use of CSSLs, these results clearly demonstrated the potential power of QTL detection by the use of the CSSLs.

Implications of the Nona Bokra/Koshihikari CSSLs for rice genetics

Ebitani *et al.* (2005) summarized several advantages of the CSSLs in the genetic analysis of rice. For example, CSSLs can be used as basal materials to develop near-isogenic lines (NILs) for the verification and characterization

Fig. 4. Comparison of QTLs for days-to-heading detected in the CSSLs with those detected in previous studies. Gray bars on the left side of the chromosomes denote the regions most likely to contain putative QTLs detected on the substituted segments in the 18 CSSLs which headed significantly later than Koshihikari under natural field conditions at Tsukuba, Japan, in 2006. Black arrowheads show the QTLs detected using F_2 and advanced backcross populations of crosses between Koshihikari and Nona Bokra (Uga *et al.* 2007). *Hd1* to *Hd14* indicate the QTLs reported from the population of a cross between Nipponbare and Kasalath (Yano *et al.* 2001). The linkage map used here was derived from that constructed by Uga *et al.* (2007).

of QTLs detected. Besides, one additional backcross for CSSLs enables to proceed to fine mapping of putative QTLs. The new CSSLs developed here should display the same advantages. In addition, it is noteworthy that Nona Bokra, with extremely late heading (more than 200 days required to heading under natural field conditions in Japan), was used as the donor cultivar. In general, such late heading makes it difficult to evaluate intrinsic genetic factors underlying several morphological and physiological traits, because late-heading cultivars grow poorly owing to low temperatures at the later developmental stages. Although some CSSLs headed later than Koshihikari, all would have enough time to produce seeds in Japan, except in the far north. Accordingly, the CSSLs developed in the present study may provide an opportunity to explore the potential of Nona Bokra as a gene donor for future rice breeding. Seeds and genotype data of the CSSLs are available at the Rice Genome Resource Center (2007).

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