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브라키포디움의 핵형분석 및 센트로메릭 시퀀스 분석

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FISH-Karyotyping and Centromeric Sequences from *Brachypodium distachyon*

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Objectives

Brachypodium distachyon, which is a grass species of the tribe Brachypodiaeae in the family Poaceae, is a new model plant for monocot functional genomics. Due to its specific distribution and organization in the genome as well as divergence during speciation, the repeated sequences have been studied as an useful source for the analyses of genetic and evolutionary relationships among cereal crops. Using centromere-specific sequence primers derived from *B. sylvaticum* and Leymus racemosus, we cloned and characterized two centromeric repetitive sequences of *B. distachyon*: BDCS1 (244bp) and BDCS2 (549bp). Data analysis be revealed that sequence homology of the BDCS1 to CCS1 (*B. sylvaticum*) was 71%, followed by RCS1 of rice centromeric sequence 52%. To use BDCS1 and BDCS2 as cytogenetic markers, chromosomal mapping of these sequences is under study.

Materials and Methods

○ Plant Materials

B. distachyon (L.) Beauv (2n=2x=10, Bd21 and Bd21-3) was provided by USDA-ARSC WRRC (Albany, California, USA).

○ Methods

Chromosome preparation

FISH slide was prepared according to a previous method. The fixed root tips were washed thoroughly in distilled water prior to the enzymatic treatment. The meristems of the root tips were macerated with an enzyme mixture (5% cellulase,

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1% pectolyase, 1 mM EDTA, pH 4.5) at 37° C for 40 min. After rinsing, the root tips were spread using a fine forcep on a glass slide with a few drops of fixative, and then air-dried.

Cloning and sequencing of repeated sequences

Genomic DNA was extracted from the young leaves using DNeasy Plant Mini Kit (Qiagen). The repeated sequences were PCR-amplified using the primers (5'-TGCATCTATATTCTTGCTTGTT-3', 5'-CGTCGCTCTAAAATGTACAGA-3' and 5'-GGTGCCCGATCTTTCGATGAGA-3') (Aragón-Alcaide et al., 1996). Amplified rDNA products were isolated from an agarose gel using a gel extraction Kit (LaboPass, COSMO), ligated into the pGEM-T-Easy vector (Promega) and transformed into E. coli DH5α cell. The nucleotide sequences were determined using an automatic DNA sequencer, ABI3730XL, (SolGent, Korea). The DNA sequence data were analyzed using the BLAST network service at the National Center for Biotechnology Information (NCBI).

Results

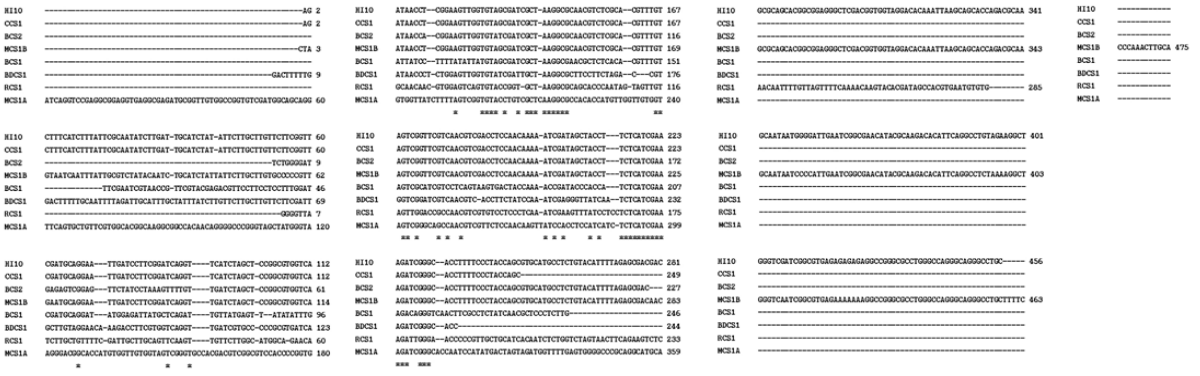

Figure 2. A 244 bp-BDCS1 sequence of B. distachyon and multiple sequence alignment of repetitive cereal centromeric sequences. BDCS1 (Brachypodium distachyon Centromeric Sequence 1) was cloned from B. distachyon using the primer 5'-GGTGCCCGATCTTTCGATGAGA-3' of a clone CCS1 (a part of the clone Hi-10 derived from B. sylvaticum). The clones for multiple sequence alignment are as follows: Hi-10 (B. sylvaticum), CCS1 (a part of the clone Hi-10), BCS1/2 (barley), RCS1 (rice) and MCS1A/B (maize). Sequence data revealed that a 249 bp-CCS1 (B. sylvaticum) showed the highest homology (71%) to the BDCS1, followed by MCS1B (63%) and RCS1 (52%).