A rice centromeric sequence is conserved between wheat and rice, as well as between monocots and dicots

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ABSTRACT

The rice centromeric clone 6730.t11, located in the kinetochore region of the rice chromosome 8 centromere, was mapped to the centromeric regions of wheat group-7 chromosomes by Southern hybridization. PCR amplification with an RT-PCR (Reverse Transcription-PCR) primer of 6730.t11 was conducted on genomic DNA isolated from Triticeae species, including T. urartu, T. monococcum subsp. monococcum, T. monococcum subsp. aegilopoides, Ae. speltoides, Ae. tauschii, barley, rye, and H. villosa, the rice cultivars (O. sativa subsp. Japonica) ‘Nipponbare’ and (O. sativa subsp. Indica) ‘IRBB7’, maize, soybean, tomato, and Arabidopsis. A 211-bp sequence was amplified from Nipponbare, which showed 100% identity to the sequenced rice genomic DNA covered by the 6730.t11 RT-PCR primer. Of eight plasmid clones of PCR products sequenced from IRBB7, six had the same 211-bp sequence as found in Nipponbare. Two clones had a 202-bp sequence that shared 100 percent and 87 percent similarity in the first 38 and the last 72 nucleotides, respectively, with the 211-bp sequence amplified from Nipponbare. Surprisingly, the 202-bp sequence amplified from IRBB7 was found in all monocot and dicot species used in this study except Nipponbare. Sequence similarity ranged from 99% to 100% when compared to the 202-bp sequence in IRBB7. A PCR-amplified fragment from genomic DNA of Chinese Spring (CS) wheat using an RT-PCR primer of the clone 6730.t11 was mapped to the same region as clone 6730.t11 in wheat. The RT-PCR results from CS cDNA with primers of 6730.t11 indicated that the rice centromeric gene was expressed in wheat leaf tissue. Our data demonstrate strong selection pressure for the conservation of this DNA element in the kinetochore region of plants, although its functional role remains to be established.

INTRODUCTION

Most eukaryotic centromeres are generally at the megabase level and known to be devoid of genes. Sequencing and assembling such highly repetitive centromeres are a big challenge for genome sequencing projects. Although the genomes of several eukaryotes, including Drosophila melanogaster, human, mouse, Arabidopsis thaliana, and rice, have been sequenced, only three centromeres of rice chromosomes 3, 4, and 8 were sequenced and assembled. Previous studies have identified expressed genes and transcripts in the flanking regions of some centromeres and reported active genes and their normal transcription in a human neocentromere. Discovery of active genes in the sequenced centromere was first reported in rice chromosome 8. Recent research indicated that there are at least 16 active genes within a ~750 kb core domain associated with CENH3 of the centromere of chromosome 8. The ~1,881 kb CENH3 domain of the centromere of rice chromosome 3 also contains 19 transcribed genes. The active genes found in the rice centromere provide a good opportunity to study syntenic relationships between the centromeres of wheat and rice.

MATERIALS AND METHODS


DNA extraction and PCR: Genomic DNA was extracted from leaves as reported in Qi et al. Sequences of the primer of 6730.t11 were according to Nagaki et al. PCR reactions were performed in a final volume of 25 µl containing 50 ng DNA, 1.5 mM MgCl2, 0.3 mM dNTPs, 1X reaction buffer, 12.5 pmol forward and revised primers, and 1 unit Taq polymerase (BIOLINE, Boston).

RNA extraction and RT-PCR: Total RNA was extracted from the CS leaf tissue using TRIzol reagent (Invitrogen Corp., Carlsbad, MA). The first strand cDNA was synthesized using oligo (dT)6 primer with SuperScriptIII Reverse Transcriptase (Invitrogen, Corp., Carlsbad, MA). RT-PCR was conducted using the first strand cDNA as template with gene-specific primers.

RESULTS

A conserved, possible centromeric sequence in monocots and dicots

Three rice centromeric clones, 6729.t09, 6729.t10, and 6730.t11, located in the kinetochore region of the rice
conducted in the wheat cultivar CS. The primer of 6730.t11 amplified a RT-PCR ~95-bp fragment from CS cDNA isolated from leaf tissue with a size similar to that in rice, indicating that the rice gene is expressed in wheat.

**Mapping the 6730t11-CS fragment to the centromere regions of group-7 chromosomes**

The DNA fragment amplified from CS gDNA, named 6730.t11-CS, was mapped to the centromeric regions of the group-7 chromosomes using a set of wheat NT and Dt lines and group-7 chromosome deletion lines by Southern hybridization. The location of this clone in wheat chromosomes is the same as that of rice clone 6730t11, which mapped to the long arm of chromosomes 7A and 7B but to the short arm of chromosome 7D. 6730t11-CS appears to be a single copy clone with three hybridization fragments after CS gDNA was digested with EcoRI.

**Rice centromeric gene expression in wheat**

Rice centromeric clone 6730t11 is an expressed gene, coding a putative cystathionine-β-synthase (CBS) domain containing protein. RT-PCR analysis was conducted in the wheat cultivar CS. The primer of 6730.t11 amplified a RT-PCR ~95-bp fragment from CS cDNA isolated from leaf tissue with a size similar to that in rice, indicating that the rice gene is expressed in wheat.

**DISCUSSION**

Centromeric DNA organization varies widely among species, especially centromeric repeat sequences. Species-specific centromeric DNA was found not only in human but also in mouse, rice, maize, and budding wheat. This suggests that the centromeric DNA is an important target for the evolution of centromeres.
and fission yeasts. Even in Oryza species, centromere-specific satellite repeats are divergent. In spite of the extensive studies of centromeric heterochromatin, knowledge of the conservation of the genes in the centromeric region is still limited because only a few genes are found in the centromere. In this study, a conserved sequence of 202-bp was amplified from monocot and dicot species using the RT-PCR primer 6730.t11, an expressed centromere gene of Cen8. This 202-bp sequence is present in the indica subspecies and absent in japonica subspecies, which has a 211-bp sequence divergent from the 202-bp sequence. The data support the hypothesis of independent domestications of indica and japonica from pre-differentiated pools of a wild ancestor. The 202-bp sequence present in monocots and dicots is an ancestral sequence. Two conserved SNPs were found in position 157 and 165 (Fig. 1). The SNP in position 157 may have occurred before monocot/dicot divergence, because both monocot and dicot species share this SNP. However, the SNP in position 165 is only present in the Triticeae species. Our data demonstrate strong selection pressure for the conservation of this DNA element in the kinetochore region, although its functional role remains to be established.

REFERENCES


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Fig. 1. Multiple alignments of the 202-bp sequences from different species. SNPs were marked with bold and red color. IRBB7, rice; TA709 and TA829, T. urartu; TA141, T. monococcum; TA183, T. monococcum aegilopoides; TA1770 and TA2780, Ae. sativus; TA1691 and TA10132, Ae. tauschii; H.v, H. villosa; B73, maize; Jack, soybean; Pto R, tomato; Col, Arabidopsis.