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

Qi LL, Friebe B, Gill BS

Wheat Genetic and Genomic Resources Center, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506-5502, USA

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[3, 7, 8]. Previous studies have identified expressed genes and transcripts in the flanking regions of some centromeres[1] and reported active genes and their normal transcription in a human neocentremere[5]. Discovery of active genes in the sequenced centromere was first reported in rice chromosome 8[3]. 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[8]. 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

Plant materials: The following species were used: *Triticum urartu, T. monococcum subsp. aegilopoides, T. monococcum subsp. monococcum, Aegilops. speltoides, Ae. tauschii, Hordeum vulgare* cv. Betzes, *Secale cereale* cv. Imperal, *Haynaldia villosa, Oryza sativa* sp. *Japonica* cv. Nipponbare, *Oryza sativa* sp. *Indica* cv. IRBB7, *Zea mays* cv. B73, *Glycine max* cv. Jack, *Lycopersicon esculentum* cv. Pto.R, and *Arabidopsis thaliana Columbia*. Genetic stocks used in the study included 21 wheat nullisomic-tetrasomic (NT) lines, six ditelosomic (Dt) lines, and 19 deletion (del) lines of homoeologous group 7 chromosomes.

DNA extraction and PCR: Genomic DNA was extracted from leaves as reported in Qi et al.[4]. Sequences of the primer of 6730t11 were according to Nagaki et al.[3]. PCR reactions were performed in a final volume of 25 µl containing 50 ng DNA, 1,5 mM $MgCl_2$, 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 $(dT)_{20}$ primer with SuperScript™ III 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

chromosome 8 centromere, were assigned to the centromeric regions of homoeologous group-7 chromosomes (our unpublished data). The RT-PCR primers of these clones were used to amplify DNA fragments from genomic DNA of CS wheat. Two pairs of primers, 6729.t09 and 6729.t10, amplified multi-fragments, while primer 6730.t11 amplified a unique DNA fragment. When the PCR products from a set of wheat nulli-tetrasomic lines amplified by RT-PCR primer 6730.t11 were separated on a SSCP gel, three fragments could be distinguished from chromosomes 7A, 7B, and 7D. A 202-bp sequence was found in these chromosomes. The rice genomic DNA recovered by the 6730.t11 RT-PCR primer has a 211-bp sequence (http://www.tigr.org/tdb/e2k1/osa1/pseudomolecules/info.shtml), which shares 100 percent and 87 percent similarity in first 38 and last 72 nucleotides, respectively, with the 202-bp sequence amplified from CS-7A. Amplification from rice cultivar IRBB7 with the 6730.t11 RT-PCR primer gave two sequences, 202-bp and 211-bp. However, only the 211-bp sequence was recovered from rice cultivar Nipponbare, an original source for rice genomic sequencing. Further, PCR amplifications with the 6730.t11 RT-PCR primer were conducted in *T. uratu*, *T. monococcum subsp. aegilopoides* and *monococcum*, *Ae. speltoides*, *Ae. tauschii*, barley, rye, *H. villosa*, maize, soybean, tomato, and *Arabidopsis*.

CS-7A and A-genome species: The CS-7A and two accessions, TA709 and TA829, of *T. urartu* have a 202-bp sequence that is identical with the 202-bp sequence in rice IRBB7. Both *T. monococcum* subspecies also have a 202-bp sequence, that share a common SNP; subspecies aegilopoides has an additional SNP (Fig. 1).

CS-7B and *Ae. speltoides*: The amplified sequences from CS-7B and two accessions of *Ae. speltoides* are heterogeneous. Most clones sequenced have a 204-bp sequence with an insertion of two nucleotides (AT) in position 94 and a SNP in position 38 compared with the rice 202-bp sequence. A 202-bp sequence, identical with that of rice IRBB7, is also present in CS-7B and *Ae. speltoides* accession TA2780 (Fig. 1).

CS-7D and *Ae. tauschii*: The 202-bp sequences among the CS-7D and two accessions, TA1691 and TA10132, of *Ae. tauschii* are identical and share a common SNP in position 157 with *Ae speltoides* accession TA1770, barley, rye, and soybean compared with the 202-bp sequence in rice IRBB7 (Fig. 1).

Barley: Sequence data from the barley cultivar Betzes are more complicated. Four sequence types were present in barley. Among eight clones sequenced, four have a 198-bp sequence that shows two deletions in positions 124 and 137 and eight independent SNPs compared with the 202-bp sequence in rice IRBB7. Four clones have the 202-bp sequence. Of these, two are identical with the 202-bp sequence of rice IRBB7, and another two share two conserved SNPs in positions 157 and 165 with several other species (Fig. 1).

Rye: Only the 202-bp sequence was amplified from rye cultivar Imperal, but they are heterogeneous. Of six 202-bp sequences, three are identical with the rice 202-bp sequence, one has a SNP in position 157, and two have a SNP in position 165 (Fig. 1).

H. villosa: *H. villosa* is a distant relative of wheat and a cross-pollinating species. A 204-bp sequence found in *H. villosa* is identical with that in CS-7B. Of four 202-bp sequences, two are identical in sequence to rice IRBB7, and the other two have a SNP at position 165 (Fig. 1).

Maize: The sequences amplified from maize cultivar B-73 are divergent. Five of nine clones sequenced have a 184-bp sequence. Three maize clones have a 202-bp sequence identical to the rice 202-bp sequence and one clone has a 211-bp sequence identical with the rice 211-bp sequence.

Soybean: Three soybean clones have a 202-bp sequence identical with the rice 202-bp sequence. The other two 202-bp sequences in soybean have SNPs in positions 82 and 165 (Fig. 1).

Tomato and *Arabidopsis*: The amplified sequence from tomato cultivar Pto.R is unique and has 100 percent similarity to the rice 202-bp sequence. Three of four sequenced clones from *A. thaliana* accession Columbia (Col) have the rice 202-bp sequence. A 204-bp sequence found in Col is the same as the 204-bp sequence of CS-7B except for two additional SNPs in positions 97 and 159.

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 *Eco*RI.

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

```
IRBB7-2     GTGCGTGAAACTACTAACCTTGAAGATGCTGCAAGGTACGTGTACATCACTGATTGGTTT 60
CS_7A-2     GTGCGTGAAACTACTAACCTTGAAGATGCTGCAAGGTACGTGTACATCACTGATTGGTTT 60
TA709-1     GTGCGTGAAACTACTAACCTTGAAGATGCTGCAAGGTACGTGTACATCACTGATTGGTTT 60
TA829-1     GTGCGTGAAACTACTAACCTTGAAGATGCTGCAAGGTACGTGTACATCACTGATTGGTTT 60
CS_7B-1     GTGCGTGAAACTACTAACCTTGAAGATGCTGCAAGGTACGTGTACATCACTGATTGGTTT 60
TA2780-1    GTGCGTGAAACTACTAACCTTGAAGATGCTGCAAGGTACGTGTACATCACTGATTGGTTT 60
Barley-7    GTGCGTGAAACTACTAACCTTGAAGATGCTGCAAGGTACGTGTACATCACTGATTGGTTT 60
Rye-5       GTGCGTGAAACTACTAACCTTGAAGATGCTGCAAGGTACGTGTACATCACTGATTGGTTT 60
H.v-3       GTGCGTGAAACTACTAACCTTGAAGATGCTGCAAGGTACGTGTACATCACTGATTGGTTT 60
B73-5       GTGCGTGAAACTACTAACCTTGAAGATGCTGCAAGGTACGTGTACATCACTGATTGGTTT 60
C01-1       GTGCGTGAAACTACTAACCTTGAAGATGCTGCAAGGTACGTGTACATCACTGATTGGTTT 60
Jack-5      GTGCGTGAAACTACTAACCTTGAAGATGCTGCAAGGTACGTGTACATCACTGATTGGTTT 60
pto-1       GTGCGTGAAACTACTAACCTTGAAGATGCTGCAAGGTACGTGTACATCACTGATTGGTTT 60
Jack-3      GTGCGTGAAACTACTAACCTTGAAGATGCTGCAAGGTACGTGTACATCACTGATTGGTTT 60
TA183-1     GTGCGTGAAACTACTAACCTTGAAGATGCTGCAAGGTACGTGTACATCACTGATTGGTTT 60
TA141-1     GTGCGTGAAACTACTAACCTTGAAGATGCTGCAAGGTACGTGTACATCACTGATTGGTTT 60
Barley-6    GTGCGTGAAACTACTAACCTTGAAGATGCTGCAAGGTACGTGTACATCACTGATTGGTTT 60
Rye-6       GTGCGTGAAACTACTAACCTTGAAGATGCTGCNAGGTACGTGTACATCACTGATTGGTTT 60
H.v.-8      GTGCGTGAAACTACTAACCTTGAAGATGCTGCAAGGTACGGTACATCACTGATTGGTTT 60
TA1770-4    GTGCGTGAAACTACTAACCTTGAAGATGCTGCAAGGTACGTGTACATCACTGATTGGTTT 60
CS_7D-2     GTGCGTGAAACTACTAACCTTGAAGATGCTGCAAGGTACGTGTACATCACTGATTGGTTT 60
TA1691-2    GTGCGTGAAACTACTAACCTTGAAGATGCTGCAAGGTACGTGTACATCACTGATTGGTTT 60
TA10132-2   GTGCGTGAAACTACTAACCTTGAAGATGCTGCAAGGTACGTGTACATCACTGATTGGTTT 60
Barley-9    GTGCGTGAAACTACTAACCTTGAAGATGCTGCAAGGTACGTGTACATCACTGATTGGTTT 60
Rye-2       GTGCGTGAAACTACTAACCTTGAAGATGCTGCAAGGTACGTGTACATCACTGATTGGTTT 60
Jack-1      GTGCGTGAAACTACTAACCTTGAAGATGCTGCAAGGTACGTGTACATCACTGATTGGTTT 60
            ***************************** ******* ***************
IRBB7-2     CCAGAGATTATATGGACCAACTTGTATGTTAATGTTTCTTAAAGATAACTTAATTAGATG 120
CS_7A-2     CCAGAGATTATATGGACCAACTTGTATGTTAATGTTTCTTAAAGATAACTTAATTAGATG 120
TA709-1     CCAGAGATTATATGGACCAACTTGTATGTTAATGTTTCTTAAAGATAACTTAATTAGATG 120
TA829-1     CCAGAGATTATATGGACCAACTTGTATGTTAATGTTTCTTAAAGATAACTTAATTAGATG 120
CS_7B-1     CCAGAGATTATATGGACCAACTTGTATGTTAATGTTTCTTAAAGATAACTTAATTAGATG 120
TA2780-1    CCAGAGATTATATGGACCAACTTGTATGTTAATGTTTCTTAAAGATAACTTAATTAGATG 120
Barley-7    CCAGAGATTATATGGACCAACTTGTATGTTAATGTTTCTTAAAGATAACTTAATTAGATG 120
Rye-5       CCAGAGATTATATGGACCAACTTGTATGTTAATGTTTCTTAAAGATAACTTAATTAGATG 120
H.v-3       CCAGAGATTATATGGACCAACTTGTATGTTAATGTTTCTTAAAGATAACTTAATTAGATG 120
B73-5       CCAGAGATTATATGGACCAACTTGTATGTTAATGTTTCTTAAAGATAACTTAATTAGATG 120
C01-1       CCAGAGATTATATGGACCAACTTGTATGTTAATGTTTCTTAAAGATAACTTAATTAGATG 120
Jack-5      CCAGAGATTATATGGACCAACTTGTATGTTAATGTTTCTTAAAGATAACTTAATTAGATG 120
pto-1       CCAGAGATTATATGGACCAACTTGTATGTTAATGTTTCTTAAAGATAACTTAATTAGATG 120
Jack-3      CCAGAGATTATATGGACCAACCTGTATGTTAATGTTTCTTAAAGATAACTTAATTAGATG 120
TA183-1     CCAGAGATTATATGGACCAACCTGTATGTTAATGTTTCTTAAAGATAACTTAATTAGATG 120
TA141-1     CCAGAGATTATATGGACCAACTTGTATGTTAATGTTTCTTAAAGATAACTTAATTAGATG 120
Barley-6    CCAGAGATTATATGGACCAACTTGTATGTTAATGTTTCTTAAAGATAACTTAATTAGATG 120
Rye-6       CCAGAGATTATATGGACCAACTTGTATGTTAATGTTTCTTAAAGATAACTTAATTAGATG 120
H.v-8       CCAGAGATTATATGGACCAACTTGTATGTTAATGTTTCTTAAAGATAACTTAATTAGATG 120
TA1770-4    CCAGAGATTATATGGACCAACTTGTACGTTAATGTTTCTTAAAGATAACTTAATTAGATG 120
CS_7D-2     CCAGAGATTATATGGACCAACTTGTATGTTAATGTTTCTTAAAGATAACTTAATTAGATG 120
TA1691-2    CCAGAGATTATATGGACCAACTTGTATGTTAATGTTTCTTAAAGATAACTTAATTAGATG 120
TA10132-2   CCAGAGATTATATGGACCAACTTGTATGTTAATGTTTCTTAAAGATAACTTAATTAGATG 120
Barley-9    CCAGAGATTATATGGACCAACTTGTATGTTAATGTTTCTTAAAGATAACTTAATTAGATG 120
Rye-2       CCAGAGATTATATGGACCAACTTGTATGTTAATGTTTCTTAAAGATAACTTAATTAGATG 120
Jack-1      CCAGAGATTATATGGACCAACTTGTATGTTAATGTTTCTTAAAGATAACTTAATTAGATG 120
            ********************* ***** ****************************
IRBB7-2     GTGGTGATATTTTGGCATGCAGGTTACTTCTTGTTACTAAATACCGCAGGCTGCCAGTAG 180
CS_7A-2     GTGGTGATATTTTGGCATGCAGGTTACTTCTTGTTACTAAATACCGCAGGCTGCCAGTAG 180
TA709-1     GTGGTGATATTTTGGCATGCAGGTTACTTCTTGTTACTAAATACCGCAGGCTGCCAGTAG 180
TA829-1     GTGGTGATATTTTGGCATGCAGGTTACTTCTTGTTACTAAATACCGCAGGCTGCCAGTAG 180
CS_7B-1     GTGGTGATATTTTGGCATGCAGGTTACTTCTTGTTACTAAATACCGCAGGCTGCCAGTAG 180
TA2780-1    GTGGTGATATTTTGGCATGCAGGTTACTTCTTGTTACTAAATACCGCAGGCTGCCAGTAG 180
Barley-7    GTGGTGATATTTTGGCATGCAGGTTACTTCTTGTTACTAAATACCGCAGGCTGCCAGTAG 180
Rye-5       GTGGTGATATTTTGGCATGCAGGTTACTTCTTGTTACTAAATACCGCAGGCTGCCAGTAG 180
H.v-3       GTGGTGATATTTTGGCATGCAGGTTACTTCTTGTTACTAAATACCGCAGGCTGCCAGTAG 180
B73-5       GTGGTGATATTTTGGCATGCAGGTTACTTCTTGTTACTAAATACCGCAGGCTGCCAGTAG 180
C01-1       GTGGTGATATTTTGGCATGCAGGTTACTTCTTGTTACTAAATACCGCAGGCTGCCAGTAG 180
Jack-5      GTGGTGATATTTTGGCATGCAGGTTACTTCTTGTTACTAAATACCGCAGGCTGCCAGTAG 180
pto-1       GTGGTGATATTTTGGCATGCAGGTTACTTCTTGTTACTAAATACCGCAGGCTGCCAGTAG 180
Jack-3      GTGGTGATATTTTGGCATGCAGGTTACTTCTTGTTACTAAATACCGCAGGCTGCCAGTAG 180
TA183-1     GTGGTGATATTTTGGCATGCAGGTTACTTCTTGTTACTAAATATGCAGGCTGCCAGTAG 180
TA141-1     GTGGTGATATTTTGGCATGCAGGTTACTTCTTGTTACTAAATATGCAGGCTGCCAGTAG 180
Barley-6    GTGGTGATATTTTGGCATGCAGGTTACTTCTTGTTACTAAATATGCAGGCTGCCAGTAG 180
Rye-6       GTGGTGATATTTTGGCATGCAGGTTACTTCTTGTTACTAAATATGCAGGCTGCCAGTAG 180
H.v-8       GTGGTGATATTTTGGCATGCAGGTTACTTCTTGTTACTAAATATGCAGGCTGCCAGTAG 180
TA1770-4    GTGGTGATATTTTGGCATGCAGGTTACTTCTTGTTAGTAAATACCGCAGGCTGCCAGTAG 180
CS_7D-2     GTGGTGATATTTTGGCATGCAGGTTACTTCTTGTTAGTAAATACCGCAGGCTGCCAGTAG 180
TA1691-2    GTGGTGATATTTTGGCATGCAGGTTACTTCTTGTTAGTAAATACCGCAGGCTGCCAGTAG 180
TA10132-2   GTGGTGATATTTTGGCATGCAGGTTACTTCTTGTTAGTAAATACCGCAGGCTGCCAGTAG 180
Barley-9    GTGGTGATATTTTGGCATGCAGGTTACTTCTTGTTAGTAAATACCGCAGGCTGCCAGTAG 180
Rye-2       GTGGTGATATTTTGGCATGCAGGTTACTTCTTGTTAGTAAATACCGCAGGCTGCCAGTAG 180
Jack-1      GTGGTGATATTTTGGCATGCAGGTTACTTCTTGTTAGTAAATACCGCAGGCTGCCAGTAG 180
            ******************************* ******* ****************
IRBB7-2     TCGACAGTTCAGGCAAATTGGT 202
CS_7A-2     TCGACAGTTCAGGCAAATTGGT 202
TA709-1     TCGACAGTTCAGGCAAATTGGT 202
TA829-1     TCGACAGTTCAGGCAAATTGGT 202
CS_7B-1     TCGACAGTTCAGGCAAATTGGT 202
TA2780-1    TCGACAGTTCAGGCAAATTGGT 202
Barley-7    TCGACAGTTCAGGCAAATTGGT 202
Rye-5       TCGACAGTTCAGGCAAATTGGT 202
H.v-3       TCGACAGTTCAGGCAAATTGGT 202
B73-5       TCGACAGTTCAGGCAAATTGGT 202
C01-1       TCGACAGTTCAGGCAAATTGGT 202
Jack-5      TCGACAGTTCAGGCAAATTGGT 202
pto-1       TCGACAGTTCAGGCAAATTGGT 202
Jack-3      TCGACAGTTCAGGCAAATTGGT 202
TA183-1     TCGACAGTTCAGGCAAATTGGT 202
TA141-1     TCGACAGTTCAGGCAAATTGGT 202
Barley-6    TCGACAGTTCAGGCAAATTGGT 202
Rye-6       TCGACAGTTCAGGCAAATTGGT 202
H.v-8       TCGACAGTTCAGGCAAATTGGT 202
TA1770-4    TCGACAGTTCAGGCAAATTGGT 202
CS_7D-2     TCGACAGTTCAGGCAAATTGGT 202
TA1691-2    TCGACAGTTCAGGCAAATTGGT 202
TA10132-2   TCGACAGTTCAGGCAAATTGGT 202
Barley-9    TCGACAGTTCAGGCAAATTGGT 202
Rye-2       TCGACAGTTCAGGCAAATTGGT 202
Jack-1      TCGACAGTTCAGGCAAATTGGT 202
            **********************
```

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. speltoides*; TA1691 and TA10132, *Ae. tauschii*; H.v, *H. villosa*; B73, maize; Jack, soybean; Pto R, tomato; Col, Arabidopsis.

and fission yeasts. Even in *Oryza* species, centromere-specific satellite repeats aredivergent[2]. 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 centromeric 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[6]. 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

1. Copenhaver, G.P., Nickel, K., Kuromori, T., Benito, M., Kaul, S., et al. 1999 Genetic definition and sequence analysis of Arabidopsis centromeres. Science 286: 2468-2474.
2. Lee, H.R., Zhang, W., Langdon T., Jin, W., Yan, H., et al. 2005 Chromatin immunoprecipitation clone reveals rapid evolutionary patterns of centromeric DNA in Oryza species. Proc Natl Acad Sci 102:11793-11798.
3. Nagaki, K., Cheng, Z., Ouyang, S., Talbert, P.B., Kim M., et al. 2004 Sequencing of a rice centromere uncovers active genes. Nature Genet 36: 138-145.
4. Qi, L.L., Echalier B., Friebe B., Gill B.S. 2003 Molecular characterization of a set of wheat deletion stocks for using in chromosome bin mapping of ESTs. Funct Integr Genomics 3: 39-55
5. Saffery, R., Sumer, H., Hassan, S., Wong, L.H., Craig, J.M., et al. 2003 Transcription within a functional human centromere. Mol Cell 12: 509-516.
6. Sweeney, M., McCouch, S., 2007 The complex history of the domestication of rice. Ann Bot 100: 951-957.
7. Zhang, Y., Huang, Y., Zhang, L., Li, Y., Lu, T., et al. 2004 Structural features of the rice chromosome 4 centromere. Nucleic Acids Res 32: 2023-2030.
8. Yan, H., Jiang, J. 2007 Rice as a model for centromere and heterochromatin research. Chromosome Res 15: 77-84.

ACKNOWLEDGMENTS