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Molecular cloning of the wheat *CK2α* gene and detection of its linkage with *Vrn-A1* on chromosome 5A

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Abstract The casein kinase CK2 is one of the major multifunctional protein kinases in cells that is expressed ubiquitously and is essential for survival. The α-subunit of CK2 is thought to be involved in light-regulated gene expression and rhythmic expression of genes by circadian rhythm in plants. The rice chromosome-3 region containing the photoperiod-response *Hd6* gene, an orthologue of the *CK2α* genes of *Arabidopsis* and maize, is in synteny with the wheat chromosome-5A *Vrn-A1* region. This evidence proposes two possibilities, first the wheat *Vrn-A1* is an orthologue of the rice *CK2α*, and second the wheat *CK2α* which has not yet been identified is located independently but tightly linked to *Vrn-A1*. To clarify whether the wheat *CK2α* gene is conserved in the *Vrn-A1* region and to elucidate the above two possibilities, we attempted to isolate this gene from the wheat cDNA library and to map it on the chromosome-5A region that is syntenous to the rice *Hd6* region. The isolated cDNA clone showed an extremely high homology with the *Arabidopsis CK2α* gene. Using this clone as a probe genomic Southern-blot analyses of the aneuploid lines available in Chinese Spring assigned the wheat homologue of *CK2α* to the long arm of chromosome 5A. Furthermore, a linkage analysis using an F_2 population having recombination in the *Vrn-A1* region revealed that the wheat *CK2α*, designated as *tck2a*, is tightly linked to *Vrn-A1* by 1.1 cM

Keywords Casein kinase 2 alpha · Photoperiod-response · Vernalization · Wheat

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Introduction

The casein kinase CK2 (formerly casein kinase II), one of the major multifunctional protein kinases in cells, is a serine/threonine kinase that is expressed ubiquitously and is essential for survival (Padmanabha et al. 1990). It is involved in the control of DNA replication and transcription, RNA processing and translation, cell metabolism, and motility of cells (Litchfield and Luscher 1993). The activity of CK2 is not directly affected by any second messenger, but it can be stimulated or inhibited by extracellular signals (Tuazon and Traugh 1991).

CK2 is composed of two α-subunits (α and α′) and two regulatory β-subunits (Tuazon and Traugh 1991). The CK2α-subunit itself has some catalytic activity, while the β-subunit has been found to stimulate the catalytic activity of the α-subunit, stabilize the α-subunit, and change the substrate specificity of the α-subunit in vitro. Their primary and quaternary structures are highly conserved and are readily evident in the structure of *CK2* genes from a variety of organisms (Wirkner et al. 1992). All CK2s have several biochemical characteristics in common; a high sensitivity to polyanions such as heparin, the ability to use GTP as well as ATP as phosphoryl donors, a preference for acidic substrates such as phosvitin and casein, and being stimulated by polyamines (Pinna 1990).

Light signals that induce changes in growth and development stimulate phosphorylation in plant cells. Since CK2 has an ability to phosphorylate transcription factors that bind to the promoter regions of light-regulated genes in vitro, this enzyme is thought to be involved in light-regulated gene expression in plants (Klimczak et al. 1995). The study using transformed *Arabidopsis* with an antisense construct of the CK2α-subunit gene suggested the possibility that CK2 might serve one of several negative and quantitative effectors in light-regulated gene expression (Lee et al. 1999).

On the other hand, a wide rage of processes in plants including expression of certain genes is regulated by endogenous circadian rhythms. Two Myb-related genes,

circadian clock-associated 1 (*CCA1*) and the late elongated hypocotyl (*LHY*), have been identified as potential clock genes in *Arabidopsis thaliana* (Schaffer et al. 1998; Wang and Tobin 1998).

Overexpression of the CKB3 protein, a regulatory β-subunit of CK2 that can interact with the *CCA1* protein, cause an increased CK2 activity and shorter periods of rhythmic expression of *CCA1* and *LHY*. This overexpression also alters the flowering time of *Arabidopsis* grown under both long-day and short-day photoperiods (Sugano et al. 1999).

In rice, *Hd6*, one of the quantitative trait loci on chromosome 3 controlling heading date by photosensitivity (Yamamoto et al. 2000), encodes casein kinase II α (Takahashi et al. 2001). Recently, we have demonstrated that the 2.2-cM rice *Hd6* region is in synteny with the 6.7-cM wheat *Vrn-A1* region by the colinearity of four common RFLP markers (Kato et al. 1999a). *Vrn-A1* on chromosome 5A determines the spring/winter growth habit or vernalization requirement, and appears to be the primary determinant in controlling flowering time (Law 1966; Law et al. 1976; Kato et al. 1999b). These lines of evidence suggest two possibilities: first, the wheat *Vrn-A1* is an orthologue of the rice *CK2α*, and second, the wheat *CK2α* is located independently but tightly linked to *Vrn-A1*. To clarify whether the wheat *CK2α* gene which has not yet been identified is conserved in the *Vrn-A1* region, and to elucidate the above two possibilities, we isolated this gene from the wheat cDNA library and mapped it on the choromosome-5A region that is syntenous to the rice *Hd6* region.

Materials and methods

cDNA cloning of the wheat *CK2α* gene

The CK2α-specific probe based on the *A. thaliana* CK2α, accession number D10246 (Mizoguchi et al. 1993), was obtained by PCR from *Arabidopsis* cDNA libraries (ZAPII, mRNA from 7-day old seedlings) using primers Atck2aF (5′ -ATGTCGAAA-GCTCGT-GTGTACACC-3′) and Atck2aR (5′ -GAAGCTTACGC-AGCTCAT GGTCAA-3′). Amplified PCR fragments (499 bp) were labeled by random oligonucleotide priming and used to screen the wheat cDNA library constructed from mRNA from 7-day old seedlings of Chinese Spring. Approximately 5×10^4 recombinants were screened by plaques hybridization and the hybridizing plaque were isolated. After three cycles of plaque purification, in vivo excisions of the pBluescript SK⁻ vector were performed in the *Escherichia coli* K-12 strain XL-1 Blue. The nucleotide sequences of the inserts were determined using the Thermo Sequenase Cycle Sequencing Kit (Amersham Pharmacia Biotech UK) with M13 universal and reverse primers.

Plant materials for mapping of *CK2α*

Triticum aestivum cv Chinese Spring (CS) and its nullisomic-tetrasomic derivatives N5AT5B, N5BT5A and N5DT5B (Sears 1954) were analyzed for assigning *CK2α* to wheat chromosome 5A. In addition, three deletion stocks, q23, q36 and q14, for chromosome 5A of wheat, produced in the background of CS (Ogihara et al. 1994) using the gametocidal genes *Gc1a* or *Gc1b* (Tsujimoto and Noda 1989), were analyzed to assign *CK2α* to the long arm of wheat chromosome 5A.

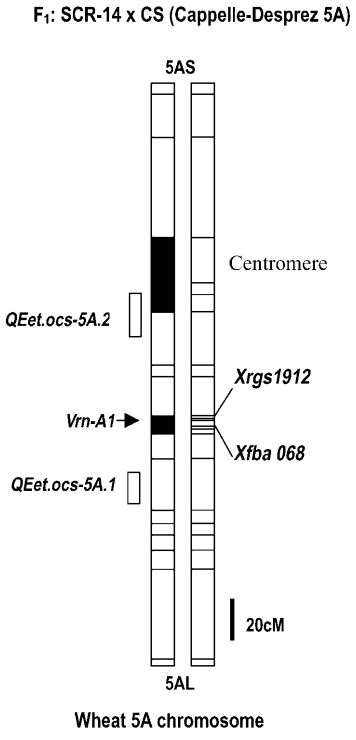

Fig. 1 Graphical genotype of the F_1 plant of the cross between SCR-14 and CS (Cappelle-Desprez 5A) based on the linkage map (Kato et al. 1998, 1999a). *Black and white* regions represent segments of chromosomes derived from *T. spelta* and Cappelle-Desprez, respectively. Three loci controlling ear emergence time, *Vrn-A1*, *QEet.ocs-5A.1* and *QEet.ocs-5A.2*, are represented on *the left side* (Kato et al. 1999b, c)

For the mapping of *Vrn-A1* and *CK2α*, 457 $F_{2:3}$ and F_4 progenies from a cross between SCR-14 and CS (Cappelle-Desprez 5A) were used in this study (Fig. 1). SCR-14 was chosen from a homozygous population of 118 single-chromosome recombinant lines (SCRs) for chromosome 5A, which were developed from the cross between single-chromosome substitution lines for 5A: CS (Cappelle-Desprez 5A) and CS (*Triticum spelta* 5A) (Kato et al. 1998). Chromosome 5A of wheat carries at least three loci controlling flowering time (Kato et al. 1999b, c). Among these loci, *Vrn-A1* and *QEet.ocs-5A.1* are the main determinants of genetic variation for ear emergence time under long-day photoperiod conditions without vernalization treatment (Kato et al. 1999c). SCR-14 had been found to carry the Cappelle-Desprez allele at *QEet.ocs-5A.1* and the *Vrn-A1* allele from *T. spelta* (Fig. 1).

RFLP assays

DNA was extracted from young leaves of each line and/or individuals using a modified CTAB method (Murray and Thompson 1980).

To assign the CK2α clone isolated in this experiment to wheat chromosome 5A, RFLP assays were conducted by Southern hybridization with the DNAs of CS, CS N5AT5B, CS N5AT5D, CS q23, q36 and q14 lines digested with *Apa*I, *Bgl*II, *Bam*HI and *Eco*RI, using Gene Images labeling and detection systems (Amersham Pharmacia Biotech, UK).

For the primary screening of the recombinant lines from the F_2 population of 457 lines, two flanking RFLP markers, *Xrgs1912* and *Xfba068*, of *Vrn-A1* (Kato et al. 1999a) were used. Southern hybridization was done with DNA of each $F_{2:3}$ line digested with *Dra*I. Then, for the mapping of the wheat *CK2α* gene, six candidate RFLP markers closely linked to *Vrn-A1* from genetic maps of

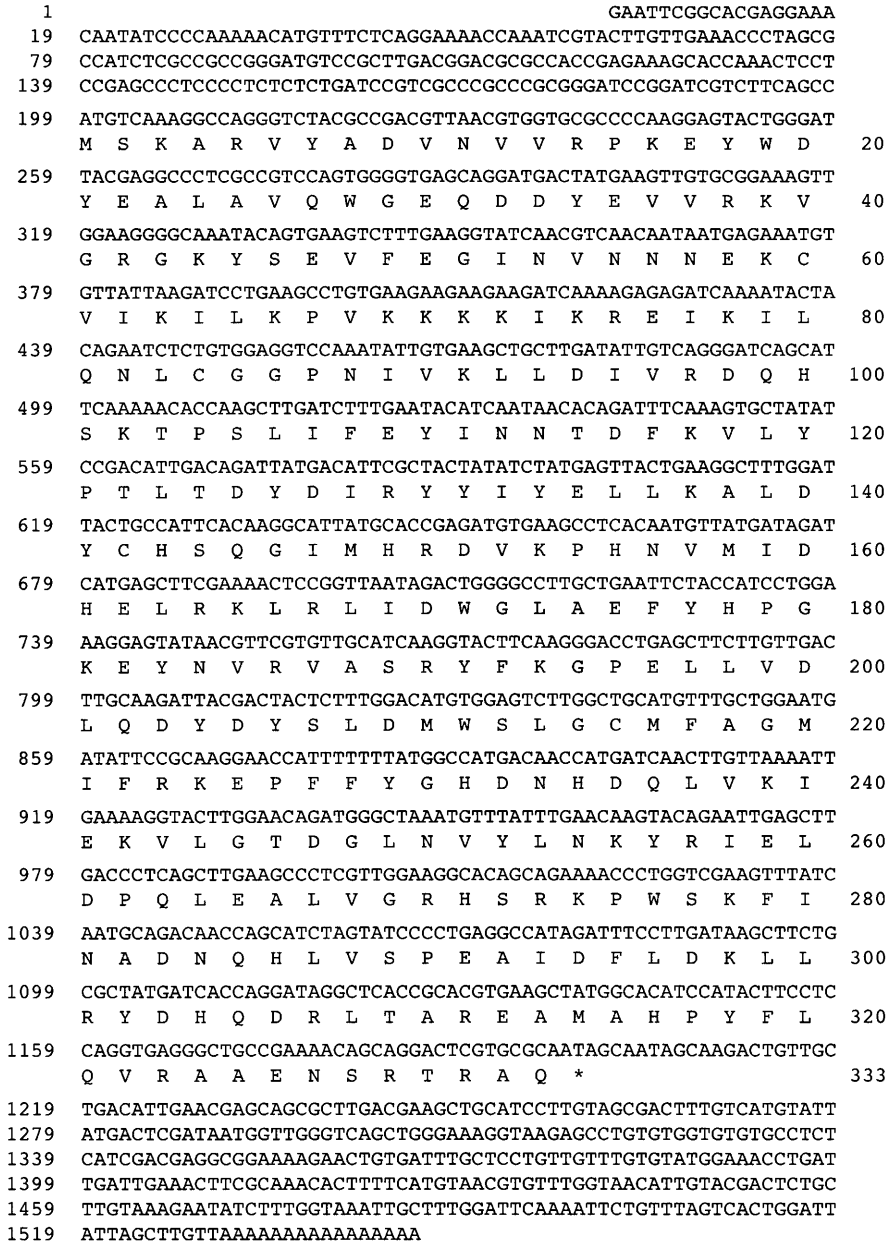

Fig. 2 The nucleotide and deduced amino-acid sequences of *tck2a* encoding the wheat casein kinase 2 α-subunit (CK2α) The position at which the stop codon (TAG) is encountered in the cDNA is indicated by an *asterisk*. The nucleotide sequence data of *tck2a* appear in the DDBJ, EMBL and GenBank database under the accession number AB052133

wheat (Dubcovsky et al. 1998) and barley (Kleinhofs et al. 1993) were employed together with previously mapped markers (Kato et al. 1999a). Polymorphism was identified by southern hybridization with the DNAs of CS (Cappelle-Desprez 5A) and CS (*T. spelta* 5A) digested with 11 restriction enzymes, *Apa*I, *Bam*HI, *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Kpn*I, *Pst*I, *Xba*I and *Xho*I. RFLP markers detected between these two substitution lines were used for genotyping of the screened F_2 lines that have recombination between *Xrgs1912* and *Xfba068*. RFLP assays were conducted using DNA extracted from the young leaves of F_3 and/or F_4 progeny.

Identification of the *Vrn-A1* allele types

To determine allele types at *Vrn-A1*, the heading date of the recombinants identified in Southern-blotting experiments were evaluated and 12–30 F_3 plants derived from each self-pollinated F_2 line were raised in a growth chamber where the temperature was kept at 20 ± 2 °C with a 16-h long-day photoperiod. The evaluation was triplicated. In this experiment, three different phenotypes of heading date were expected with a 1 : 2 : 1 ratio involving homozygous F_2 lines of which all F_3 plants show an earlier heading due to the *Vrn-A1* allele from SCR-14, heterozygous lines that produce a mixture of earlier and later F_3 plants, and homozygous lines with a later heading by the *vrn-A1* allele from CS (Cappelle-Desprez 5A).

Data analysis

The recombination frequencies were converted to centiMorgans (cM) using the Kosambi mapping function (Kosambi 1944).

Results and discussion

cDNA cloning of the wheat *CK2α* gene

Plaque hybridization of a wheat cDNA library identified eight positive cDNA clones. The cDNA inserts were

Fig. 3 Alignment of amino-acid sequences for CK2α from wheat, *A. thaliana* (Mizoguchi et al. 1993), *H. sapiens* (Devilat and Carvallo 1993), *X. laevis* (Jedlicki et al. 1992), *C. elegans* (Hu and Rubin 1990) and *S. cerevisiae* (Chen et al. 1988). Each amino-acid sequence is represented by the standard single-letter code. Gaps (-) are introduced to obtain maximum similarity and the positions at which stop codons are encountered in cDNA are indicated by *asterisks*. The *white box* indicates the putative ATP binding site (amino acid 40–63) and the *shaded box* indicates the putative serine/thereonine Protein Kinase active site (amino acids 147–159)

subcloned into the pBluescript SK⁻ vector by in vivo excisions, and sequenced. The sequence analysis revealed that these cDNA clones encode identical amino-acid sequences. The entire nucleotide and deduced amino-acid sequences of the longest cDNA clone among the eight clones are shown in Fig. 2. This clone consists of 1,545 bases with part of the poly (A) tail (a stretch of 16 adenine residues) and contains an open reading frame (ORF) that starts at base 199 and ends with the TAG stop codon at base 1,198. Thus the ORF of 999-bp was predicted to encode a polypeptide of 333 amino acids.

An alignment of the deduced amino-acid sequences of CK2α from wheat *(T. aestivum* in the present study), *A. thaliana* (Mizoguchi et al. 1993), *Homo sapiens* Devilat and Carvallo 1993), *Xenopus laevis* (Jedlicki et al. 1992), *Caenorhabditis elegans* (Hu and Rubin 1990) and *Saccharomyces cerevisiae* (Chen-Wu et al. 1988) is illustrated in Fig. 3. The 333 amino-acids-long of the wheat CK2α-subunit showed a 90.4% amino-acid identity to that of *Arabidopsis* CK2α. On the other hand, the wheat CK2α showed relatively low degrees of similarity to the other reported CK2 α in animals and yeast. It shares a 77.3%, 75.1%, 73.5%, and 64.6% amino-acid identify with that of *H. sapiens*, *X. laevis*, *C. elegans* and *S. cerevisiae*, respectively. However, two regions, one the ATP binding site (residues 40–63 of wheat CK2α), and the other the serine/threonine protein kinase active site (residues 147–159 of wheat CK2α), were highly conserved amongst all the aligned CK2α's including wheat. Hence, we identified this cDNA clone as a wheat *CK2α* gene and designated it as *tck2a* (*Triticum casein kinase 2 alpha*).

Chromosome assignment of *tck2α*

As the rice *Hd6* (*CK2α*) region is in synteny with the wheat *Vrn-A1* region (Kato et al. 1999a), we focused on the group-5 chromosomes to assign the locus of *tck2a*. Genomic Southern-blot analysis was conducted using the *tck2a* clone as a probe and DNAs extracted from CS and nullisomic-tetrasomic lines for group-5 chromosomes. When DNAs were digested with *Bam*HI, *tck2a* hybridized to six fragments in CS indcating that the *CK2α* gene is present as two copies in each of the A, B and D genomes (Fig. 4). One of fragments of approximately 14 kb was absent in the nullisomic line and increased in expression in the tetrasomic line for chromosome 5A. Thus the presence of the *tck2a* orthologue on chromosome 5A was revealed. This homologue, designated as *tck2a*, was further assigned to the long arm in a similar analysis of three deletion lines lacking a part of the long arm. Furthermore, the presence of one *tck2a* homoeolocus on each of the group-5 chromosomes was confirmed by Southern analysis of nullisomic-tetrasomic lines of CS.

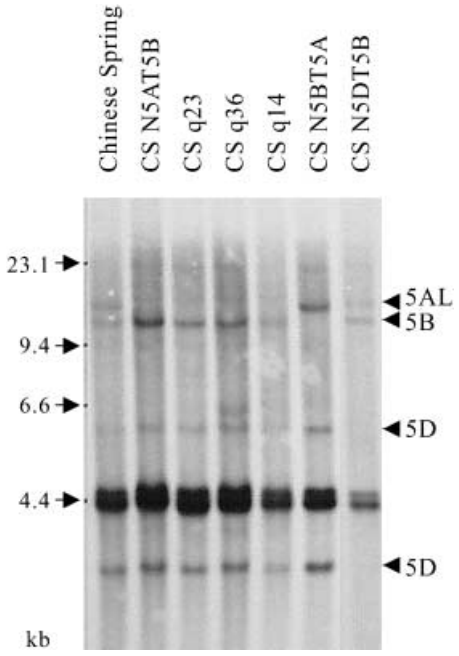

Fig. 4 Southern-hybridization pattern of *Bam*HI-digested Chinese Spring (*CS*), CS nullisomic 5A tetrasomic 5B, CS q23, CS q36, CS q14, CS nullisomic 5B tetrasomic 5A and CS nullisomic 5D tetrasomic 5B probed with wheat casein Kinase 2α (*tck2a*) identified in the present study. *Arrows* on the right hand demonstrate the bands assigned to wheat chromosomes 5AL, and 5B and 5D, respectively

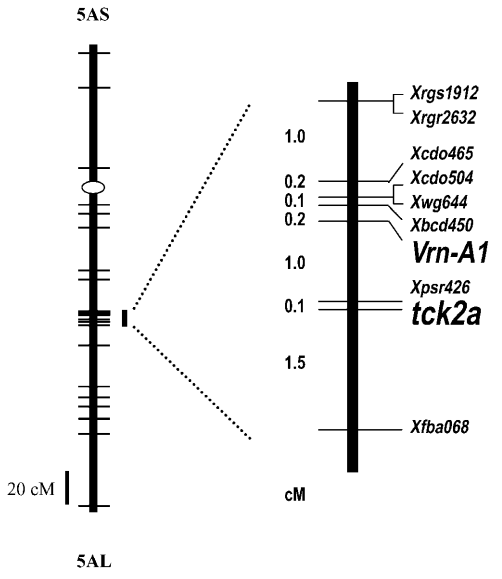

Fig. 5 Linkage maps of the region of wheat chromosome 5A showing the locations of *Vrn-A1* and the wheat casein Kinase 2 α gene (*tck2a*). The *left vertical bar* represents an RFLP linkage map constructed from the 118 single-chromosome recombinant lines developed from the cross between Chinese Spring (CS) (Cappelle-Desprez 5A) and CS (*T. spelta* 5A) (Kato et al., 1998, 1999a). The *right vertical bar* represents the linkage map constructed in the present study. The map distance (cM) were calculated by the Kosambi function (Kosambi 1944) and are shown on the *left side*

The two main fragments with about 4.4 kb were not polymorphic and were not mapped.

Linkage analysis between *Vrn-A1* and *tck2a*

Among 457 $F_{2:3}$ lines screened, 35 were found to be recombinants for the chromosomal region between *Xrgs1912* and *Xfba068*. This also indicated that the map distance between these flanking markers of *Vrn-A1* was 4.2 cM, which is smaller than our previous study using 118 SCRs (Kato et al. 1999a). These 35 recombinant $F_{2:3}$ lines were used for further mapping study of *Vrn-A1* and *tck2a*.

Under the long-day photoperiod condition, there was a 20-day difference for heading time between the early SCR-14 and the late CS (Cappelle-Desprex 5A). In the F_3 progeny test, the expected three phenotypes of heading time were clearly visible: fixed lines of early heading such as SCR-14, segregating lines of both types and fixed lines of late heading such as CS (Cappelle-Desprez 5A). Therefore, it was easy to assess the genotype at *Vrn-A1* of each F_2 plant. The fixed lines of early heading were considered to be homozygous for the *Vrn-A1* allele from SCR-14 or *T. spelta* 5A, the segregating lines were identified as heterozygous *Vrn-A1vrn-A1* and fixed lines of the late heading as homozygous for the *vrn-A1* allele from Cappelle-Desprez 5A. The number of F_2 lines classified into these three groups were 6, 19 and 10, supporting the segregation of a single factor ($\chi^2 = 1.17$, $0.50 < P < 0.75$).

By means of both *Vrn-A1* and RFLP markers, *Vrn-A1* was located between *Xbcd450* and *Xpsr426*. The genetic distances between *Vrn-A1* and *Xbcd450*, and *Xpsr426*, were 0.2 cM and 1.0 cM, respectively (Fig. 5).

Only one RFLP band of *tck2a-A1* was identified in the *Bam*HI digest of genomic DNAs from CS (Cappelle-Desprez 5A) and CS *(T. spelta* 5A), which was a dominant marker present in Cappelle-Desprez. To identify the genotype at the *tck2a-A1* locus in the 35 recombinant F_2 s we followed two steps. First, in Southern analysis using DNA from bulked leaves of 20 F_3 progenies per each F_2 line and digested with *Bam*HI, we classified 35 F_2 lines into 26 of the Cappelle-Desprez type having the RFLP band of *tck2a-A1*, and nine of the *T. spelta* type lacking the band. The former 26 F_2 lines were expected to include both of the homozygous and heterozygous Cappelle-Desprez types as the RFLP band was a dominant marker. So, in subsequent analysis of Southern hybridization using DNAs from five F_4 individuals per line, the genotypes of the 26 F_2 lines were determined as homozygous for Cappelle-Desprez or heterozygous. As a result, *tck2a* was mapped within the marker interval between *Xpsr426* and *Xfba068*. The genetic distances between *tck2a* and *Xpsr426*, and *Xfba068*, were 0.1 cM and 1.5 cM, respectively. Consequently *tck2a* was found to be linked tightly with *Vrn-A1* that by 1.1 cM (Fig. 5).

Of the six candidate RFLP markers that had been found to link closely to *Vrn-A1* in genetic maps of wheat (Dubcovsky et al. 1998; Kato et al. 1999a) and barley (Kleinhofs et al. 1993), CDO465 (*Xcdo465*), WG644 (*Xwg644*) and CD0504 (*Xcdo504*) were mapped in this genomic region, within the marker interval between (*Xrgs1912*, *Xrgr2632*) and *Xfba068*. Other three candidate markers lacked polymorphism between the parental two lines. We mapped a casein kinase 2a locus in the *Vrn-A1* region that is separate from *Vrn-A1*. Of the other minor three RFLP bands, two were located on 5D and one on 5B, indicating a homoeologous relationship (Fig. 4). For intensively hybridizing bands of about 4.4 kb, however, nullisomic-tetrasomic analysis could not map this second kinase locus on particular group-5 chromosomes when digested with *Bam*HI. Thus, we can not exclude the possibility that a second casein kinase 2a locus is in the same region and could be synonymous with *Vrn-A1*. It needs more mapping study to confirm this possibility.

Adaptability of wheat to diverse environments is mainly due to exploitation of genes controlling heading time. The genetic control of heading time in wheat is extremely complex being determined by the vernalization requirement, the photoperiod response, and earliness *per se* genes. The vernalization genes determine the sensitivity of the plant to cold temperatures and differentiate vernalization-insensitive spring wheats from vernalization-sensitive winter wheats that require an extended period of cool (4–8 °C) temperature before floral primordia are initiated. In hexaploid wheat, *Vrn-A1* on chromosome 5A is the most effective and gives complete insensitivity to vernalization (Law et al. 1976). A series of *Vrn* genes homoeologous to *Vrn-A1* have been mapped on the group-5 chromosomes of the Triticeae; *Vrn-B1* on 5B (McIntosh et al. 1998), *Vrn-D1* on 5D (Law et al. 1976; Nelson et al. 1995), *Vrn-H1* on the barley 5H chromosome (Takahashi and Yasuda 1971; Laurie et al. 1995) and *Vrn-R1* on the rye 5R chromosome (Plaschke et al. 1993). This genomic region is considered as one of the well-conserved regions in the Triticeae. In addition, the colinearity of the wheat chromosome-5A *Vrn-A1* region with the rice chromosome-3 *Hd6* region was proven using common RFLP markers (Kato et al. 1999a). A close linkage of *Vrn-A1* and *Fr1*, a major frost resistance gene, was demonstrated (Galiba et al. 1995). Therefore, the *Vrn* region in the Triticeae and the *Hd6* region in rice are suggested to be of great importance for adaptation and evolution in cereals.

To our knowledge, *tck2a* is the first *CK2α* gene of wheat. The present study demonstrated that *tck2a*, orthologous to the rice heading QTL *Hd6*, is located on the syntenic wheat genomic region and tightly linked to *Vrn-A1*. Further, because of a high homology of the deduced amino-acid sequences between *tck2a* and the *Arabidopsis CK2α* (Fig. 3), it is reasonable to assume that *tck2a* is also photoperiod responsive. However, there is no heading-time QTL(s) or gene(s) linked to *Vrn* so-far reported in the Triticeae. Therefore, the effect of *tck2a* on heading time in Triticeae needs further study.

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