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Physical location of a *HSP70* gene homologue on the centromere of chromosome 1B of wheat (*Triticum aestivum* L.)

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Abstract Cereal centromeres consist of a complex organization of repetitive DNA sequences. Several repetitive DNA sequences are common amongst members of the *Triticeae* family, and others are unique to particular species. The organization of these repetitive elements and the abundance of other types of DNA sequences in cereal centromeres are largely unknown. In this study, we have used wheat-rye translocation lines to physically map 1BL.1RS centromeric breakpoints and molecular probes to obtain further information on the nature of other types of centromeric DNA sequences. Our results, using the rye-specific centromeric sequence, pAWRC.1, indicate that 1BL.1RS contains a small portion of the centromere from 1R of rye. Further studies used molecular markers to identify centromeric segments on wheat group-1 chromosomes. Selected RFLP markers, clustered around the centromere of wheat homoeologous group-1S chromosomes, were chosen as probes during Southern hybridization. One marker, PSR161, identified a small 1BS segment in all 1BL.1RS lines. This segment maps proximal to pAWRC.1 in 1BL.1RS and on the centromere of 1B. Sequence analysis of PSR161 showed high homology to *HSP70* genes and Northern hybridization showed that this gene is constitutively expressed in leaf tissue and induced by heat shock and light stimuli. The significance of this work with respect to centromere organization and the possible significance of this *HSP70* gene homologue are discussed.

Keywords Centromere · 1BL.1RS · Translocation, *HSP70* · Wheat

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Introduction

The cloning of several repetitive DNA sequences from cereal centromeres indicate that the primary constriction consists of a complex array of highly reiterated sequences. The isolation of pSau3A9 (Jiang et al. 1996), CCS1 (Aragón-Alcaide et al. 1996) and *cerebra* (Presting et al. 1998) from sorghum, wheat and barley, respectively, and the abundance of all three sequences within several members of the *Triticeae* indicate that some centromeric repetitive DNA families originated from the common cereal ancestor, prior to speciation. The cloning of highly repetitive sequences provides a basis for analysing the DNA sequence organization and complexity of a cereal centromere.

In a recent study, a repetitive family from cereal rye was cloned (Francki 2001) and shown to be localized only to the centromeres of rye A and B chromosomes and not to wheat, barley or rice (Wilkes et al. 1995; Francki 2001). The rye centromeric repetitive DNA is a diverged retrotransposon-like sequence and represents the *Bilby* family of retrotransposons, a new member of a species-specific repetitive element (Francki 2001). The identification of highly repeated centromeric DNA common amongst members of the *Triticeae* indicates that cereal centromeres consist of a combination of common and unique repetitive DNA sequences that have diverged through evolution. The function of each repetitive element, if any, is largely unknown. The presence of other DNA sequences, including single-copy sequences, has not been located on cereal centromeres.

Physical mapping and DNA organization studies of centromeres have been hampered by the presence of large amounts of repetitive DNA. The analysis of centromeric heterochromatin in *Drosophila melanogaster* has been facilitated by using chromosomes that juxtaposed euchromatin and heterochromatin (Karpen and Spradling 1990; Murphy and Karpen 1995; Sun et al. 1997). This enabled access to centromeric heterochromatin using single-copy sequences within adjacent euchromatin and was shown to be useful in isolating and analysing centromeric DNA. A strategy involving rearranged chromosomes in maize was used to localize a B chromosome sequence. (Alfenito and Birchler 1993). Breakpoints within the B centromere and along the chromosome arm in a series of A-B translocations enabled the physical mapping of a repetitive sequence to the B centromere using Southern hybridization. Alfenito and Birchler (1993) demonstrated the value of using translocated chromosomes to physically map DNA sequences to cereal centromeres.

Molecular genetic maps have identified homoeologous group 1S restriction fragment length polymorphism (RFLP) markers linked to the centromere of chromosome 1AS, 1BS and 1DS of wheat (Gale et al. 1995; Van Deynze et al. 1995). RFLPs are sources of low-copy sequences that may provide information on the nature of genes residing on cereal centromeres. However, there is no correlation between genetic and physical map positions with respect to centromeric RFLP markers, and uncertainty exists as to whether markers are located on the centromere or on the proximal region of the chromosome arm. Physical mapping would, therefore, identify genes on centromeres and provide a basis for further studies in centromeric DNA organization. An approach using fluorescence in situ hybridization (FISH) and RFLP markers as probes has not proven reliable due to the insensitivity of detecting single-copy sequences amongst highly condensed regions of metaphase chromatin (reviewed in Jiang and Gill 1994). Therefore, an alternative physical mapping approach is to use wheat chromosomes that have been rearranged within the centromere and to identify centromeric segments using RFLP markers as probes during Southern hybridization.

A significant number of wheat varieties harbour a 1RS translocation on either chromosome 1A, 1B or 1D. The most common translocation source, Kavkaz, a hard red winter wheat (HRWW), contains the short arm of chromosome 1R from rye fused to chromosome 1BL of wheat, with the breakpoint located within the centromere. The introgression of 1RS has enabled the transfer of valuable disease resistance genes from rye to wheat (reviewed in Baum and Appels, 1991), and Kavkaz has been used as a parental source for the introgression of disease resistance genes into other wheat classes. The cultivars Grant, Freedom and GR876 of the soft red winter wheat class (SRWW) contain the 1BL.1RS translocation derived from Kavkaz (Lafever and Berzonsky 1993; Ohm et al. 1995; Berzonsky and Francki 1999). In this study, 1BL.1RS translocations were used to physically map an RFLP marker to the centromere constriction of chromosome 1B. The rye centromeric sequence pAWRC.1 (Francki 2001) was used as an important chromosomal landmark to characterize the 1BL.1RS centromeric breakpoint region and align the RFLP marker to the centromere of 1B. The physical location of a single-copy sequence on the centromere of chromosome 1B and its application in studying centromere structure and function is discussed.

Materials and methods

Plant material

Wheat cvs. Kavkaz, Grant, Freedom and GR876, all of which contain 1BL.1RS, have been described previously (Berzonsky et al. 1991; Lafever and Berzonsky 1993; Ohm et al. 1995). Seed was obtained from certified stocks. Cultivar Kavkaz seed was originally obtained from Dr. A. Lukaszewski (University of California-Riverside, USA). Seed of the 1RS ditelosomic and 1R disomic addition lines was originally obtained from Dr. E.R. Sears (University of Missouri, USA). Seed of the nullisomic-tetrasomic lines for wheat group-1 chromosomes and ditelosomic for 1BS and 1BL chromosomes were obtained from the Wheat Genetics Resource Center (Kansas State University, USA).

DNA probes

Various homoeologous group-1 cDNA clones, including PSR161, were obtained from Prof. M. Gale (John Innes Center, UK) and Dr. M. Sorrells (Cornell University, USA).

Fluorescence in situ hybridization

Seeds of various lines were surface-sterilized and placed on moist filter paper at 4°C overnight, followed by 2–3 days at 23°C in the dark. Root tips were incubated in ice-cold water overnight and fixed in ethanol:acetic acid (3:1, v/v). They were subsequently squashed onto a microscope slide in 45% acetic acid. The coverslips were frozen in liquid nitrogen, removed and dried by gentle heating. The probe pAWRC.1 was labelled with biotin-16-dUTP (Boehringer Mannheim, Indianapolis, Ind.) using a nick translation kit (Gibco-BRL, Gaithersburg, Md.). Efficiency of biotin incorporation was determined using a dot-blot detection system (Gibco-BRL).

Hybridization, washing and detection using avidin-FITC and biotinylated anti-avidin have been previously described (Francki and Langridge 1994) for chromosomes from rye and 1R addition lines. An additional amplification step during the detection procedure was required for all 1BL.1RS preparations to obtain a signal. Chromosome preparations were counterstained with propidium iodide and viewed on an Olympus model BHS microscope equipped with a blue excitation prism and filter. Photographs were taken with Kodak Ektachrome 160T film using an attached Olympus PM-C35DX camera.

DNA isolation and Southern hybridization

Plant DNA isolation, Southern transfer, probe labelling and the hybridization and washing of filters were done according to methods previously described (Francki et al. 1997). Filters were exposed to X-ray film for 5 days at –80°C.

DNA sequencing

Restriction maps of PSR161 and BCD1072 were determined. Templates of overlapping subclones were prepared using the

Wizard DNA mini-prep kits (Promega, Madison Wis.). Sequencing reactions were done using the Cy5 Autoread sequencing kit (Pharmacia Biotech, Piscataway, N.J.) and reactions run on an ALF*express* DNA sequencer. Sequencing information was analysed using the *DNAsis* programme (Hitachi Software Engineering, Japan).

RNA isolation

Leaf material (approximately 0.5 g) was frozen in liquid nitrogen and ground to a fine powder using a mortar and pestle. The powder was mixed with 10 ml extraction buffer (4 *M* guanidine thiocyanate, 25 m*M* sodium citrate, 0.5% sarcosyl and 0.1 *M* β-mercaptoethanol), and the slurry was centrifuged in a Sorval JA20 rotor for 5 m at 5000 rpm and 4°C. The supernatant (8.5 ml) was transferred to 1.7 g CsCl, and the solution was layered on a 5.7 *M* CsCl cushion. The preparations were spun at 80000 *g* for 16 h at 20°C. The RNA pellet was resuspended in 0.5 ml 0.05% SDS, treated with phenol/chloroform/iso-amyl alcohol (25:24:1) and ethanol-precipitated. After washing with 70% ethanol, the RNA pellet was resuspended in TE buffer.

Northern hybridization

Total RNA (15 μg) was separated on a 1.5% agarose denaturing formamide/formaldehyde gel, transferred to a Hybond N+ membrane (Amersham Life Sciences, Arlington Ill.), and fixed to the membrane by UV light. Prehybridization and hybridization were done at 42°C in a solution containing 5× Denhardts, 0.5% SDS, 5× SSPE and 50% formamide. Inserts from PSR161 and 18S rDNA from wheat were labelled as previously described (Francki et al. 1997). Filters were placed in 2× SSC, 1× SSC and 0.5× SSC at 60°C for 20 m for each wash. Membranes were exposed to X-ray film for 5 days after probing with PSR161 and 1 h after probing with 18S rDNA.

Results

Molecular-cytological analysis of the centromere breakpoint in 1BL.1RS translocation lines

The clone pAWRC.1 has recently been isolated and found to be a member of a rye centromeric repetitive family (Francki 2001). FISH using pAWRC.1 as a probe with high stringency hybridization and washing conditions showed the specificity of this sequence to rye chromosomes. In situ hybridization to chromosomes from a wheat-rye 1R addition line showed the centromeric sequence hybridizing to four rye chromosomes in two metaphase cells, whereas yellow fluorescence was not detected on any wheat chromosomes (Fig. 1a). Consequently, pAWRC.1 is confirmed to be rye-specific using high-stringency FISH conditions.

To investigate the physical position of centromeric breakpoints in 1BL.1RS, four translocation lines were probed with the rye centromeric repetitive sequence. The FISH results in Fig. 1b shows pAWRC.1 hybridizing to each 1BL.1RS translocation, confirming that a rye centromere has been translocated onto wheat chromosome 1B in each line. The intense yellow fluorescence on chromosome 1R and comparatively less intense signal detected on all 1BL.1RS lines indicates that only a portion of the rye centromere has been translocated.

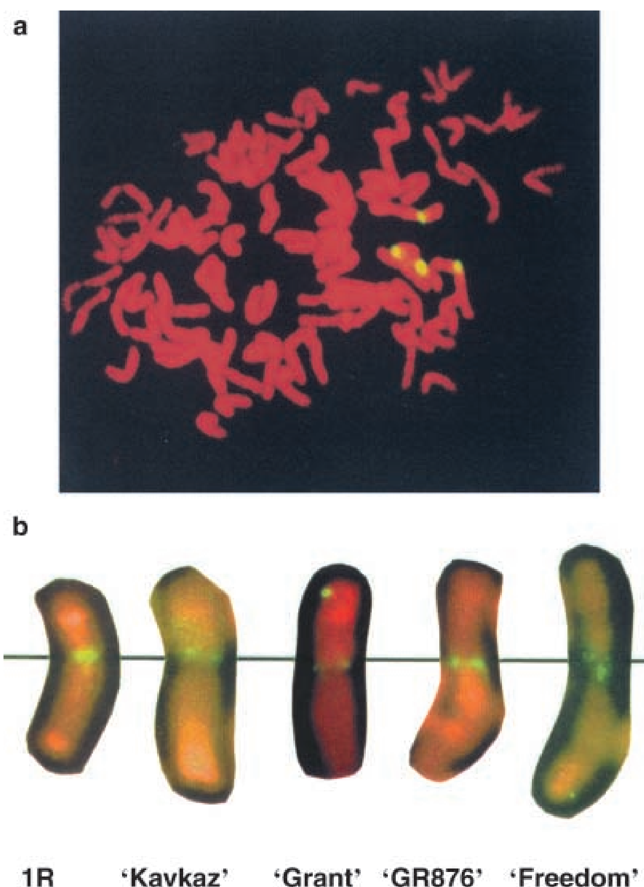

Fig. 1a, b FISH using the rye centromeric repetitive sequence, pAWRC.1, as probe on metaphase chromosomes from a wheat-rye 1R addition line and 1BL.1RS translocation lines. High-stringency hybridization and washing conditions were used as described in the Materials and Methods. **a** The probe is shown hybridizing to the centromeres of two pairs of rye chromosomes in a wheat-rye 1R addition line, and no signal was detected on any of the wheat chromosomes. **b** Hybridization of pAWRC.1 to rye and four 1BL.1RS translocation lines

Localization of an RFLP marker on the centromere of 1B using 1BL.1RS translocation lines

The initial molecular characterization suggested that the 1BL.1RS centromeric region contains a portion of the rye centromere and presumably a portion of the wheat centromere. We have used 1BL.1RS lines to further investigate the molecular organization of the 1B wheat-rye centromeric region. Several RFLP markers were mapped to the centromeres of either chromosome 1AS, 1BS or 1DS of wheat (markers BCD1072, PSR161 and PSR393) and assigned to homoeologous group 1S using ditelosomic aneuploid stocks (Van Deynze et al. 1995; Gale et al. 1995). These markers were selected as probes for Southern hybridization to DNA from 1BL.1RS translocation lines to identify any 1BS wheat segments. Two of the three markers (BCD1072 and PSR393) used in this study showed the absence of the 1BS band and the presence of a 1RS band in each translocation line (data not shown) and confirmed previous cytological observations

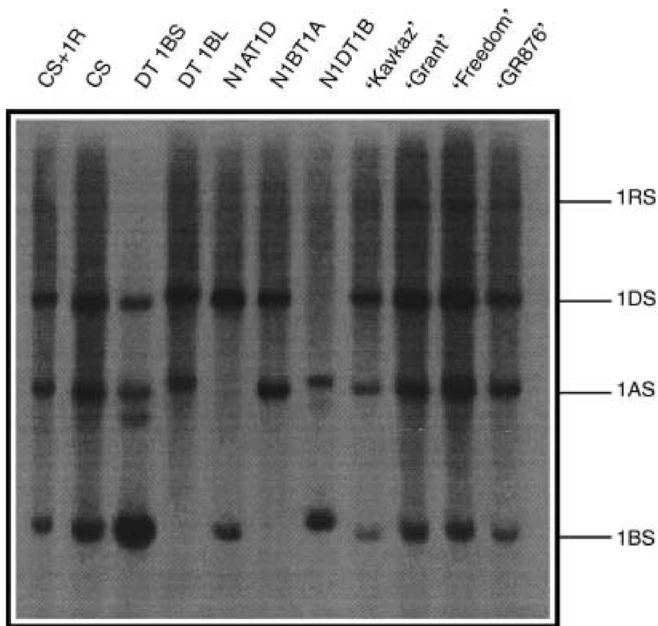

Fig. 2 Southern hybridization using PSR161 as probe on DNA from various aneuploid and translocation lines and identification of a small 1BS segment in 1BL.1RS. Specific bands in wheat (CS) have been assigned to corresponding chromosomes using a 1R wheat-rye addition line (CS+1R) and nullisomic-tetrasomic lines (N1AT1D, N1BT1A, N1DT1B) and are indicated to the *right* of the figure. Lines ditelosomic for 1BS (DT1BS) and 1BL (DT1BL) have been used to assign PSR161 to 1BS. The 1BL.1RS translocations are also included (Kavkaz, Grant, Freedom, GR876). All DNAs were digested with *Hind*III

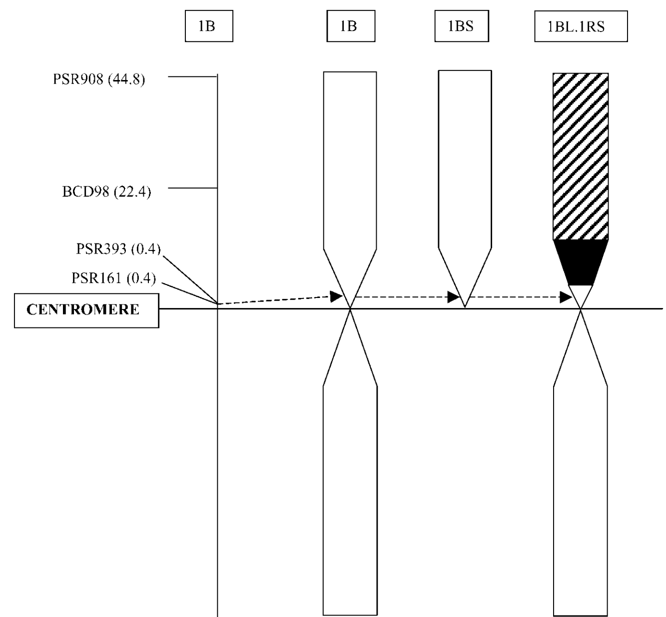

Fig. 3 Comparison of genetic and physical maps for the centromeric region of chromosome 1B from *Triticum aestivum* and 1BL.1RS. The RFLP markers used in this study are shown on a genetic consensus map for chromosome 1B on the left (Gale et al. 1995). Their genetic distances from the centromere are *in parenthesis.* The comparative physical maps for the 1B.1BS and 1BL.1RS translocation lines are shown to the *right* of the genetic map. The *unshaded regions* in the physical maps represent wheat chromatin. The *shaded* and *hatched regions* in 1BL.1RS represent the region containing the rye centromeric repetitive sequence and rye chromatin, respectively. The *arrowed line* shows the physical position of PSR161 on 1BS and 1BL.1RS relative to the rye centromeric repeat

that 1BS has been substituted by 1RS (Berzonsky et al. 1991). However, when probed with PSR161, all wheat bands are present in the 1BL.1RS translocation lines when DNAs are digested with *Hind*III (Fig. 2). PSR161 is present as a single-copy sequence on chromosome 1A, 1B and 1D as determined by nullisomic-tetrasomic analysis (Fig. 2, lanes CS, N1A, N1B and N1D, respectively). The presence of a band in DNA from the 1BS ditelosomic line and its absence in DNA from the 1BL ditelosomic line confirmed that PSR161 is located on the short arm of chromosome 1B (Fig. 2, lanes DT1BS and DT1BL, respectively). The 1BS-specific band is present in DNA from all 1BL.1RS translocation lines, indicating that these lines have a small segment of the short arm of chromosome 1B.

Additional markers mapped to the long arm of wheat group 1 chromosomes were also used to analyze the centromeric breakpoint in 1BL.1RS translocations. Three of the markers chosen mapped within 1.5 cM to the centromere (Van Deynze et al. 1995). BCD207, CDO98 and PSR158 showed the presence of a 1BL-specific band in each of the 1BL.1RS translocations and in the 1BL ditelosomic lines (data not shown), indicating that they are likely markers that map to the proximal or centromeric regions of 1BL.

The identification of a 1BS segment in 1BL.1RS translocation lines using molecular markers allows for fine physical mapping of the centromere of chromosome 1B. The rye centromeric repetitive sequence is an important chromosomal landmark with which to orient the position of the 1BS segment. We have established that each translocation line has a centromeric breakpoint containing a portion of the rye centromere. Therefore, any 1BS band in these lines detected during Southern analysis must map proximally to the rye centromeric repetitive sequence and on the centromere of wheat chromosome 1B. A schematic diagram summarizing the RFLP markers used in this study and the genetic and physical position of the 1BS segment, relative to the rye centromeric sequence in 1BL.1RS, is illustrated in Fig. 3.

Sequence of PSR161 and database homology search

The physical mapping of PSR161 has localized a cDNA marker on the centromere constriction of chromosome 1B. Sequencing data show that PSR161 is 1569 nucleotides long (Genbank accession no. AF074969) and contains a large open reading frame coding for a 376 amino acid polypeptide (Fig. 4). Extensive database searches using the BLASTX, BLASTN and BLASTP programmes identified significant homology of the predicted polypeptide with *HSP70* genes from various organ-

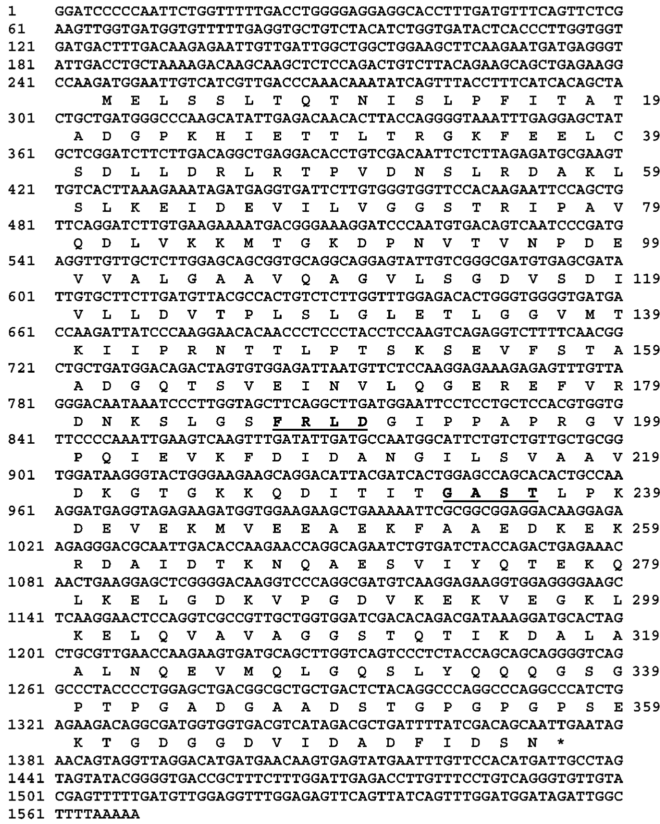

Fig. 4 DNA sequence analysis of PSR161. The DNA sequence is shown in the *upper level* and the predicted amino acid is shown on the *lower level* as a standard one-letter code. Conserved sequence motifs diagnostic for heat shock proteins of chloroplasts and cyanobacteria (Drzymalla et al. 1996) are shown in the predicted polypeptide sequence (*bold* and *underlined*). *Numbers* to the *left* and *right* are shown for DNA and protein sequences, respectively. The *asterisk* denotes the translation stop codon

isms. These include chloroplast localized *HSP70* genes from *Pisum sativum*, *Spinacia oleracea* and *Cucumis sativus*, the *HSP70* gene from *Chlamydomonas reinhardtii* and the DnaK protein from *Synechococcus* species. No significant homology with sequences from monocotyledons was detected in the searches. The results of the protein database search and the levels of homology to PSR161 are summarized in Table 1. Two sequence motifs were identified in the predicted polypeptide sequence considered to be diagnostic for *HSP70* proteins of chloroplasts and cyanobacteria (Fig. 4) and a distinctive difference from mitochondria and eubacteria (Drzymalla et al. 1996). However, at least seven other conserved sequence motifs common for chloroplasts and cyanobacteria (Drzymalla et al. 1996) were not identified in the predicted protein sequence.

Northern analysis

Sequencing data and subsequent database homology search suggested that PSR161 encodes a polypeptide that is a member of the HSP70 family. Further analysis of PSR161 was done to confirm and further characterize the cDNA. Northern blot hybridization experiments were carried out on leaf material obtained from wheat containing a normal chromosome complement under different environmental conditions. In the first experiment, plants were grown in the dark for 4–6 weeks at 18°C and subjected to either light exposure or heat shock, or a combination of both treatments. Etiolated seedlings were subjected to: (1) dark treatment only, (2) continuous light treatment (150 µE m^{-2} s^{-1}) for 6 hours; (3) incubation at 42°C for 3 h in the dark and (4) continuous light treatment (150 µE m^{-2} s^{-1}) for 6 hours followed by incubation at 42°C for 3 h. RNA was extracted after each treatment, separated on a denaturing agarose gel and probed with PSR161. A major transcript (approximately 2600 bp) was detected in RNAs from all treatments, including dark-grown seedlings, and indicates constitutive expression of this gene in leaf tissue (Fig. 5a). A two-to

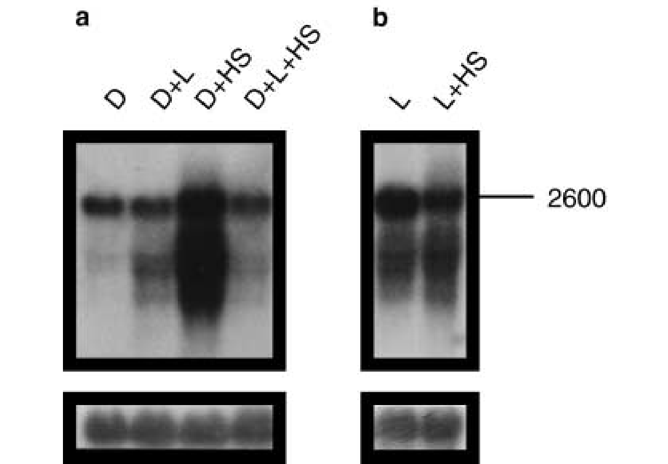

Fig. 5a, b Northern blot hybridization probed with PSR161. **a** Wheat seedlings were grown in the dark and RNA extracted after various treatments: *D* untreated etiolated plants, *D+L* etiolated plants followed by a 6-h light exposure, *D+HS* etiolated plants followed by heat shock for 3 h in the dark, *D+L+HS* etiolated plants followed by six hour light treatment and three hour heat shock. **b** Wheat seedlings were grown during a 16/8-h (*light*/*dark*) photoperiod and RNA extracted after untreated (*L*) and heat shock treatment (*L+HS*). The membranes were probed with 18S rDNA from wheat (*bottom panels*). The size of the major transcript is indicated in nucleotides

Table 1 Summary of the protein homology search for the predicted amino acid sequence from PSR161

Accession number	Sequence	Organism	Percentage protein identity[a]
Q02028	chloroplast HSP70	*Pisum sativum*	76% (289/376)
X73961	Chloroplast HSP70	*Cucumis sativus*	74% (281/376)
Q08080	chloroplast HSP70	*Spinacia oleracea*	73% (277/376)
X96502	HSP70B	*Chlamydomonas reinhardtii*	63% (237/375)
P50021	DanK	*Synechoccus* sp.	57% (215/375)

[a] Fraction in parenthesis represents the number of identical amino acids in the homologous region

threefold accumulation of smaller variant transcripts was observed in etiolated plants after a brief light treatment, and at least a tenfold increase in multiple variant transcripts was detected in etiolated seedlings after treatment at 42°C. Interestingly, the same level of heat shock response was not observed in seedlings subjected to a brief light exposure prior to heat shock treatment (Fig. 5a).

In a second experiment, wheat seedlings were grown at 18°C under a 16/8-h light/dark) photoperiod (light intensity: 150 $\mu Em^{-2} s^{-1}$) and then subjected to heat shock treatment at 42°C. Northern blotting of RNAs extracted from leaf tissue before and after the heat shock treatment showed no significant difference in signal intensity between the two treatments (Fig. 5b). This is a similar result to that found in the first experiment where no heat shock response was detected in etiolated seedlings subjected to a brief light exposure prior to the heat shock treatment (Fig. 5a). Since PSR161 shows very high homology to other *HSP70* genes and a heat shock response is observed in etiolated plants after treatment at 42°C, it can be assumed that PSR161 is likely a member of the *HSP70* family but with different responses to environmental stimuli.

Discussion

A large collection of well-characterized wheat aneuploid lines are available (Sears and Sears 1978; Endo and Gill 1996). Aneuploid lines enable researchers to locate genes to particular chromosome arms and assign molecular markers and repetitive DNA elements to specific chromosome regions. Although extremely useful for these purposes, aneuploid lines are not sufficiently rearranged to enable fine molecular analysis and the manipulation of wheat centromeres. Therefore, we have used 1BL.1RS to physically map a low-copy DNA sequence to the centromere of wheat chromosome 1B. In the investigation reported here, the rye-specific centromeric sequence, pAWRC.1, served as a centromere landmark with which to align and physically map this low-copy DNA sequence.

Alfenito and Birchler (1993) have used A-B translocations to localize a DNA sequence to the centromere of a maize B chromosome. Translocation lines involving maize chromosomes 9 and 10 and B chromosomes were used to localize a B-chromosome-specific sequence. The breakpoints in some translocation lines were located within the centromere or along the arm of the B chromosome (Beckett 1975). Consequently, the probing of B-chromosome-specific bands in lines with or without centromeric breakpoints localized a B-specific repetitive sequence to the centromere (Alfenito and Birchler 1993). In the present study, we used a similar approach to localize an RFLP marker to the centromere of wheat chromosome 1B. In each translocation line, the FISH data using pAWRC.1 as probe showed that the 1BL.1RS centromeric region contains a portion of the centromere of chromosome 1R. The identification of a 1BS-specific band using PSR161 as probe against DNA from 1BL.1RS indicates that a small segment of 1BS was retained during the translocation event. Since 1BS was replaced with a full 1RS arm and fusion was at the centromere, PSR161 must be positioned proximal to the rye centromeric repetitive sequence in 1BL.1RS and on the centromere of chromosome 1B of wheat. The possibility that 1RS had translocated onto another chromosome group, with 1B remaining intact, can be ruled out because C-banding, biochemical markers (Berzonsky et al. 1991) and distal group 1S RFLP markers (data not shown) were clearly able to show that 1BS is replaced with 1RS.

We have used the same physical mapping approach to determine whether PSR161 is positioned on the centromere of 1AS and 1DS. Although 1AL.1RS (var. Amigo) and 1DL.1RS also contain a portion of the centromere from rye chromosome 1, no 1AS or 1DS-specific band was seen when probed with PSR161 during Southern analysis (data not shown). The absence of PSR161 in these lines may be due to two possible reasons. PSR161 may not be in the same physical position as in chromosome 1B but towards the distal end of the centromere or on the short arm. Alternatively, the physical position of PSR161 may be on the constriction of chromosome 1A and 1D, but the wheat-rye breakpoint in both translocation lines may be proximal to this marker. In either case, it appears that the small 1S segment identified by PSR161 in 1BS has been substituted for by 1RS in 1AL.1RS and 1DL.1RS translocation lines. This demonstrates that either the genomic organization of the centromeric regions or the positions of breakpoints are different between translocations involving homoeologous chromosomes. Differences in centromeric breakpoint regions are important factors that must be considered before adopting a similar approach for mapping centromeric genes on other wheat centromeres. Nevertheless, the availability of an extensive range of wheat-rye translocations and the identification of markers clustered around wheat centromeres on genetic maps will likely yield some success in identifying centromeric genes on other chromosome groups.

Sequence analysis and database homology searches showed that PSR161 is likely to represent a gene that codes for a heat shock protein. Interestingly, there was no homology to *HSP70* genes isolated from other monocot species, indicating that PSR161 represents a novel member of the *HSP70* gene family in cereals. HSPs are highly conserved proteins and are thought to function as molecular chaperones with important roles in cell metabolism. These roles include the folding, assembly and moving of proteins and participation in DNA replication (for reviews, see Craig 1989; Craig and Gross 1991; Gething and Sambrook 1992). The significance of a *HSP70* gene located to the centromere of a wheat chromosome is unclear. However, some reports have shown that *HSP70* interacts with components of the mammalian cell cycle (Napolitano et al. 1987; Sánchez et al. 1994) and that the carboxy-terminal region binds to tubulin subunits (Sánchez et al. 1994). Several reports have

shown microtubules of plant cells to be sensitive to light and elevated temperatures (Nick et al. 1990; Smertenko et al. 1997), but no direct evidence supports the interaction of *HSP70* with microtubules or other components important to the cell cycle. It is still unknown whether the *HSP70* gene is specifically expressed from chromosome 1B or derived from 1A and 1D or a combination from all three loci. Additional biochemical and molecular analysis will determine whether the *HSP70* is expressed by the homologue specifically from chromosome 1B or other loci and whether gene expression and the corresponding protein product are involved in chromosome segregation. It is possible that its physical location may not be in any way causally related to its biological role.

C-banding analysis has identified a heterochromatic region within the centromere of chromosome 1B of wheat (Gill et al. 1991). Highly condensed areas of heterochromatin consists of middle to highly repetitive DNA and are generally thought to be inactive. Several repetitive DNA families have been cloned and localized within centromeric heterochromatin of wheat chromosomes, including chromosome 1B (Jiang et al. 1996; Arágon-Alcaide et al. 1996), but the function of these sequences is yet unknown. The localization of a gene on a wheat centromere is analogous to a *light* (*lt*) gene located within centromeric heterochromatin of chromosome 2 of *Drosophila melanogaster* (Devlin et al. 1990a, b; Wakimoto and Hearn 1990). It is thought that repetitive DNA located within and flanking the *lt* gene may have some regulatory role in its expression (Devlin et al. 1990a). The work in *Drosophila* has given some insight into the interaction of repetitive elements with *HSP70* gene expression in wheat. Additional work has commenced to examine the genomic structure, the expression of the centromeric *HSP70* gene and to determine the significance of other sequences within and flanking the gene.

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