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The influence of the rye genome on the accumulation of HSP18 and HSP70 transcripts in a wheat genetic background

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Abstract The influence of the rye genome on the accumulation of HSP18 and HSP70 transcripts in a wheat genetic background was examined in the wheat/rye hybrid triticale (*Triticum aestivum* cv Chinese Spring × *Secale cereale* cv Imperial). To quantify the amount of transcript accumulation in wheat, rye, triticale, and in the disomic and the ditelosomic rye addition lines to wheat, we used two independant methods, namely (1) Northern dot-blot hybridizations and (2) an exami-nation of the in-vitro translation products. Both the HSP18 and HSP70 transcripts were expressed at similar levels in Chinese Spring wheat, Imperial rye, and triticale. The HSP18 and HSP70 transcript levels of the disomic and the ditelosomic addition lines to wheat were compared to the transcript levels in wheat. With the exception of 5R, increased levels of HSP18 and/or HSP70 transcripts were expressed in all six of the remaining disomic addition lines. A neutral or suppressed level of HSP18 and HSP70 transcripts accumulated in addition lines 5R, 5RL, 5RS and 6RL. Wheat/rye genomic interactions influenced the level of heat-shock gene transcript accumulation in triticale. Rye chromosome 5R, and in particular both arms of rye chromosome 5R (5RL and 5RS), had a strong suppressive influence on the accumulation of wheat HSP18 and HSP70 transcripts. The genes controlling rye HSP expression appeared to be widely distributed throughout the rye genome.

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Introduction

The expression of rye genes in a wheat genetic background is often precluded by genomic interaction. Genomic interaction in triticale may result in a suppression of the gene expression that is common to the wheat and/or rye genomes. For example, expression of the rye nucleolar organizer regions (NORs) is weak in a wheat genetic background (Appels et al. 1986, Gustafson et al. 1988). The presence of any or all of the wheat NORs suppresses the expression of the rye NOR genes (Appels et al. 1986).

The degree of DNA methylation is associated with NOR expression in each genome of triticale. Wheat NORs that are expressed in triticale are under-methylated relative to the unexpressed rye NORs (Thompson and Flavell 1988).

Wheat HSP expression is suppressed in the presence of the rye genome (Somers et al. 1992). In our previous study involving wheat/rye genomic interactions, the suppression of wheat HSP expression was observed with the addition of rye chromosomes 1R, 3R and 5R. The addition of 5R to wheat shows the expression of the fewest number of HSPs; 6 of 14 HSP18 isoelectric variants, which are unique to wheat, are not expressed in triticale (Somers et al. 1992).

The present research was directed at analysing further the influence of wheat/rye genomic interactions on the accumulation of heat-shock transcripts in triticale. The rye (cv Imperial) disomic and ditelosomic addition lines to wheat (cv Chinese Spring) were used to determine which rye chromosomes had the most influence on the expression of wheat heat-shock genes. DNA probes of a corn HSP70 gene and a wheat HSP17 gene were used to quantify the level of transcript accumulation in each of the experimental genetic stocks.

Materials and methods

Plant material

The genetic stocks used in this study included *Secale cereale* L. cv Imperial (IR) (2n = 14), *Triticum aestivum* L. cv Chinese Spring (CS) (2n = 42), Imperial × Chinese Spring (triticale, TR) (2n = 56), the seven Imperial disomic addition lines to Chinese Spring, and 11 Imperial ditelosomic addition lines to Chinese Spring consisting of 1RL, 1RS, 2RL, 3RS, 4RL, 4RS, 5RL, 5RS, 6RL, 7RL and 7RS. The remaining three ditelosomic addition lines were not available. The ditelosomic addition lines were acquired from Dr. J. P. Gustafson and the remainder of the genetic stocks came from the late Dr. E. Sears, both from the Department of Agronomy, University of Missouri, USA.

Heat stress

Seedlings of each genetic stock were soil grown in an environmental chamber for 3 weeks at 20 °C (ambient temperature) with a 16-h day at a light intensity of 850 μMol $m^{-2} s^{-1}$. These seedlings were divided into two groups, one was transferred to a second environmental chamber set at 40 °C (ambient temperature) for 2 h in the light and the other remained at 20 °C. Two samples of leaves from each heat-stressed and unstressed genetic stock were then excised and frozen in liquid nitrogen.

RNA isolations

RNA isolations were performed according to Manning (1991). The tissue (1–2 grams fresh weight) was powdered in liquid nitrogen and then thawed in TBE extraction buffer [0.2 M Tris-boric acid (pH 7.6);20mM EDTA] with 0.5% (w/v) SDS and 2% (v/v) 2-mercaptoethanol added fresh. This mixture was warmed to room temperature and extracted with an equal volume of phenol/chloroform prepared by adding 0.2 vol of chloroform to water-saturated phenol and then saturating with TBE. Following centrifugation at 12 000 g for 15 min, the aqueous layer was removed and the remaining homogenate was re-extracted with the organic mixture. The two aqueous layers were combined and then re-extracted with the organic mixture.

The aqueous layer containing nucleic acids was then diluted 2.5 times with water and the Na^+ concentration was raised to 80 mM by adding 1 M sodium-acetate/acetic acid (pH 4.5). Polysaccharides were precipitated and removed by adding 0.4 vol of 2-butoxyethanol (2BE) to the dilute mixture for 30 min at 4 °C followed by centrifugation at 12 000 g for 20 min. Crude nucleic acids were recovered from the supernatant by adding 2BE to 1 vol for 30 min at 4 °C followed by centrifugation at 12 000 g for 30 min. The final pellet was washed sequentially with a mixture of TBE/2BE (1:1), 70% ethanol containing 0.1 M potassium-acetate/acetic acid (pH 6.0), and with absolute ethanol.

The nucleic acid pellet was resuspended in sterile water to a concentration not less than 1 mg/ml. The RNA was selectively precipitated for 1 h at 4 °C in 3 M LiCl, and was collected by centrifugation at 12 000 g. The RNA pellet was washed with 3 M LiCl and then with absolute ethanol.

Northern analysis

Total RNA (20 μg) was separated by electrophoresis in 1% (w/v) agarose gels and was capillary blotted to nylon membranes (Zeta-probe, BioRad, Mississauga, Ontario). The RNA electrophoresis, capillary blotting, Northern hybridization and blot washing protocols were performed according to Fourney et al. (1989). The hybridization was for 16 h at 42 °C in [5 × SSPE (pH 7.2), 5 × Denhardt's 1% (w/v) SDS, 50% (v/v) formamide, and 180μg/ml of salmon sperm DNA]. The blots were washed as follows: 3 × 15 min at 20 °C in 1 × SSC; 0.1% SDS (pH 7.0), then 3 × 15 min at 55 °C in 0.1 × SSC; 0.1% SDS (pH 7.0). The DNA probes consisted of HSP70 from corn [pMON9502, 4.0 kilobase pairs (kbp); Rochester et al. 1986], HSP17 from wheat (C5-8, 742 bp; McElwain and Spiker 1989) and a 26*s* rDNA fragment from rice (RR217.9, 900 bp; Department of Agronomy, University of Missouri). The probes were nick translated with ^{32}P-dCTP to a specific activity > 1.0 × 10^6 cpm/μg of DNA. Autoradiograms were produced by exposing blots to Kodak X-Omat AR film for 1–4 days.

We refer to the transcripts detected by the wheat HSP17 probe as HSP18 transcripts because (1) there is no direct evidence as to the exact M_r of the wheat protein derived from this probe, and (2) several in-vitro translation products centered at 18 kDa were observed in this study.

Dot blot and analysis

Since we encountered difficulties in quantifying the transcript levels from the autoradiograph hybridization signals with spectrophotometry, we quantified the accumulation of the heat-shock transcripts using Northern dot blotting and scintillation counting.

The total RNA was transferred to a nylon membrane with a dot-blot apparatus (Biodot, BioRad, Mississauga, Ontario) according to the manufacturer's recommendations. Dot blots to be hybridized with either heat-shock genes or rDNA contained 9 μg of RNA/dot and 0.1 μg of RNA/dot respectively. Dot blots also contained a range of standard amounts of Chinese Spring heat-shock RNA. The hybridization signals from the sample RNA were compared to the hybridization signals from the RNA standards to guage the relative amounts of detected transcripts. Following hybridization and washing (Fourney et al. 1989), each dot was cut out and the associated radioactivity was quantified using liquid scintillation counting.

The relative quantities of heat-shock mRNA and rRNA for each RNA sample were assessed from the RNA standards on each dot blot. The level of the heat-shock gene transcript accumulation was then calculated as an 'expression index': the ratio between the heat-shock mRNA and rRNA quantities × 1 000. Two replicate heat-shock RNA samples from each genetic stock were each quantified twice and the expression index was an average of all four values. These ratio data were normally distributed and differences in heat-shock gene expression between genetic stocks was verified by a nested analysis of variance and a multiple range test. The expression index for each genetic stock was subsequently reported in histograms.

Poly (A^+) RNA purification/in-vitro translation

Poly (A^+) RNA was purified from total RNA using oligo (dT)-cellulose (Pharmacia, Mississauga, Ontario) chromatography. In-vivo translations included 0.5 μg of Poly (A^+) RNA that was added to a 30-μl reaction of a rabbit reticulocyte lysate in-vitro translation system (GIBCO BRL, Burlington, Ontario) and proteins were radiolabelled with L-[^{35}S]-methionine (ICN Radiochemicals, Mississauga, Ontario). The in-vitro translation products were separated by replicate two-dimensional (2-D) IEF/SDS PAGE (Somers et al. 1992) with equal radioactive counts loaded (300 000 cpm). All gels were treated with a fluor (En^3 Hance, NEN, Boston, Massachusetts) before being exposed to Kodak X-Omat AR film for 2–7 days.

Results

The experiments were designed to assess the relative quantity of heat-shock mRNA accumulation (HSP18

and HSP70) in wheat, rye, triticale, and the disomic and ditelosomic rye addition lines to 6X wheat. The treatment of all genetic stocks at 40 °C for 2 h induced the expression and accumulation of transcripts homologous to the corn HSP70 and wheat HSP17 genes (Figs. 1, 2). The HSP70 and HSP17 DNA probes each hybridized to only a single band, corresponding to transcripts of 2.2 and 0.8 kbp respectively, in all the genetic stocks tested. The control RNA lanes (20 °C) showed no hybridization signal for the HSP17 probe, and a marginally-visible hybridization signal with the HSP70 probe, on autoradiograms of moderate exposure (Figs. 1, 2).

The hybridization signal from the 26*s* gene fragment showed the relative amount of RNA loaded in each lane of the gel. These rRNA transcript levels were not appreciably different between (1) the 20 °C and 40 °C temperature treatments and (2) all of the genetic stocks (Figs. 1, 2). This allowed us to use the 26*s* probe hybridization signal as an internal control. Thus, any comparative differences in heat-shock probe hybridization signal intensity between the different genetic stocks were viewed relative to the 26*s* probe hybridization signal (Figs. 1, 2).

There were: (1) no differences in the expression indices between replicate measurements from each genetic stock and (2) a significant difference between the expression indices of the genetic stocks as shown by a nested analysis of variance (Tables 1, 2). The multiple range test which established genetic stocks had similar levels of HSP70 and HSP18 transcript accumulation. These expression index data were plotted as histograms, where similar levels of heat-shock transcripts were denoted by bars of the same style (Fig. 3).

Similar amounts of HSP70 and HSP18 transcripts accumulated in IR, CS and TR (Fig. 3 A–D). Compared to CS, there were increased levels of expression of HSP70

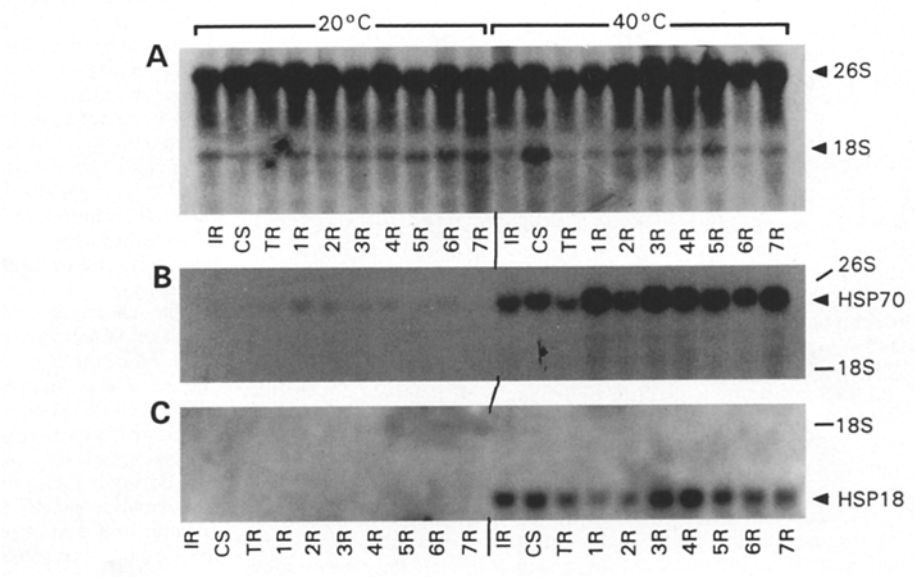


Fig. 1A–C A Northern-blot analysis of leaf tissue total RNA (20 µg) from rye-IR, wheat-CS, triticale-TR and the seven disomic IR additions to CS probed with 26*s* rDNA (**A**), HSP70 (**B**) and HSP17 (**C**). Total RNA was isolated from 3-week-old plant leaves following a 2 h heat-stress (40 °C) or from 3-week-old plant leaves left unstressed (20 °C). Northern analysis was used to detect homologous heat-shock gene and rDNA transcripts. Labels below the lanes indicate the genetic stocks. Labels on the right show the relative position of the detected transcripts

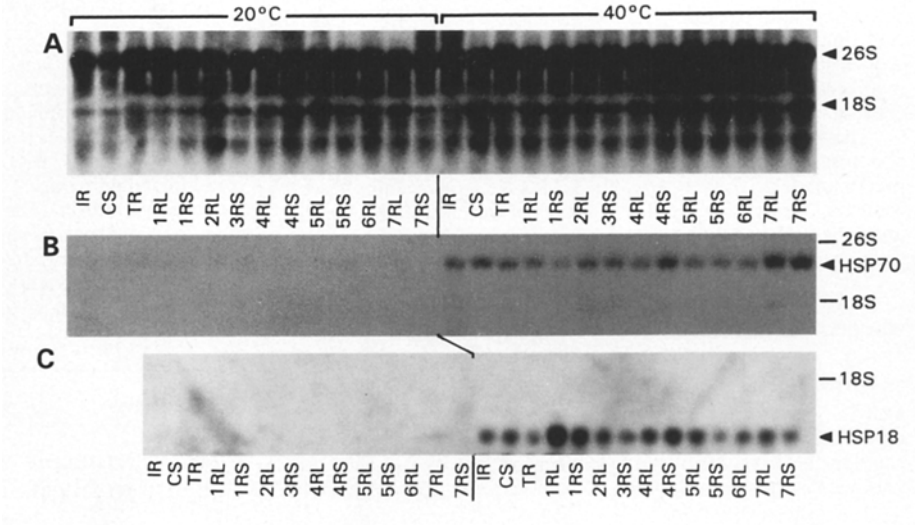


Fig. 2A–C A Northern-blot analysis of leaf tissue total RNA (20 µg) from rye-IR, wheat-CS, triticale-TR and 11 detelosomic IR additions to CS probed with 26*s* rDNA (**A**), HSP70 (**B**) and HSP17 (**C**). Total RNA was isolated from 3-week-old plant leaves following a 2 h heat-stress (40 °C) or from 3-week-old plant leaves left unstressed (20 °C). Northern analysis was used to detect homologous heat-shock gene and rDNA transcripts. Labels below the lanes indicate the genetic stocks. Labels on the right show the relative position of the detected transcripts

Table 1 A summary of the nested analysis of variance showing the source of variation in the HSP18 expression index data of Fig. 3

Source of variation	Disomic additions				Ditelosomic additions			
	df	MS	F	*P*	*df*	MS	F	*P*
Total	39				47			
Among experiments	19				23			
Among stocks	9	93.9	19.0	>0.5	11	50.6	11.3	>0.5
Among replicates	10	4.9	0.4	<0.0005	12	4.5	1.3	<0.0005
Within replicate	20	14.1			24	3.4		

Table 2 A summary of the nested analysis of variance showing the source of variation in the HSP70 expression index data of Fig. 3

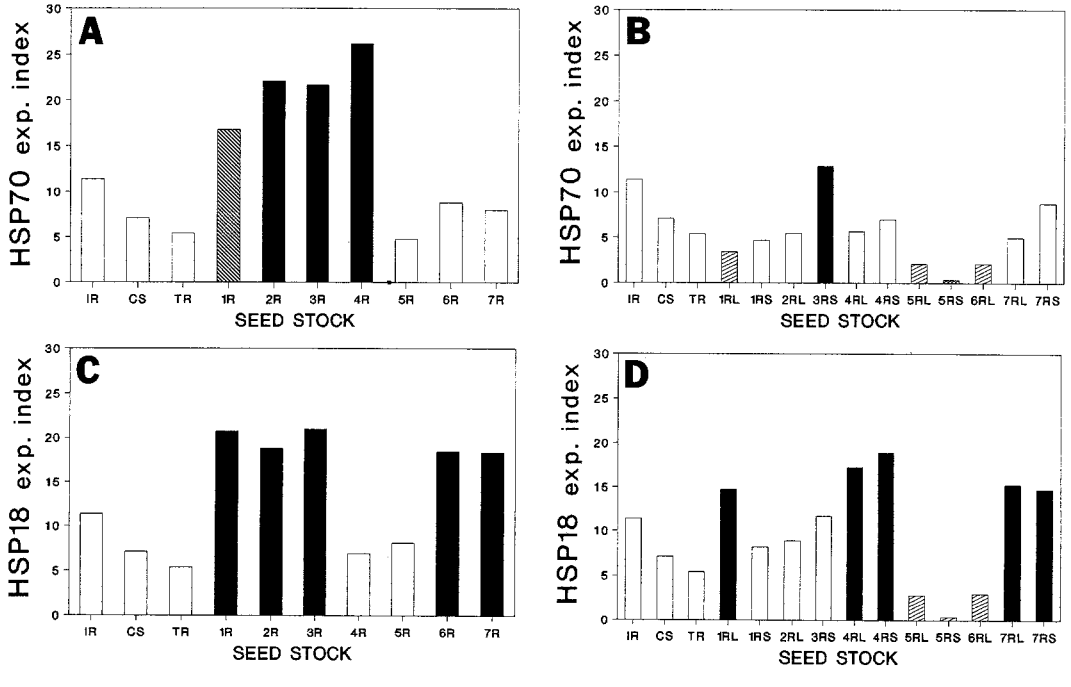
Source of variation	Disomic additions				Ditelosomic additions			
	df	MS	F	*P*	*df*	MS	F	*P*
Total	39				47			
Among experiments	19				23			
Among stocks	9	209.6	13.7	>0.05	11	98.9	34.5	>0.5
Among replicates	10	15.3	2.3	<0.0005	12	2.9	0.9	<0.0005
Within replicate	20	6.6			24	3.2		

transcripts in addition lines 1R, 2R, 3R, 3RS and 4R and increased levels of HSP18 transcripts in addition lines 1R, 1RL, 2R, 3R, 4RL, 4RS, 6R, 7R, 7RL and 7RS (Fig. 3).

The increases in HSP70 and HSP18 transcript accumulation in the disomic addition lines were two-fold greater than in CS (Fig. 3A, C). The degree of HSP70 and HSP18 transcript accumulation remained unchanged, relative to CS, with the addition of 5R (Fig. 3A, C). There were suppressed levels of HSP18 transcripts with addition lines 5RL, 5RS, 6RL and suppressed levels of HSP70 transcripts with addition lines 1RL, 5RL, 5RS and 6RL, relative to CS (Fig. 3B, D).

Additional observations from the HSP18 and HSP70 expression index data include: (1) disomic addition lines 1R, 2R and 3R all showed enhanced levels of both the HSP18 and HSP70 transcripts, (2) the ditelosomic addition lines 5RL, 5RS and 6RL all showed suppressed levels of both the HSP18 and HSP70 transcripts, and (3)

Fig. 3A–D Histograms showing the relative levels of transcript accumulation (Expression Index) homologous to HSP70 (**A, B**) and HSP18 (**C, D**) in rye-IR, wheat-CS, triticale-TR, the seven disomic IR additions to CS, and 11 ditelosomic IR additions to CS. Total RNA, isolated following a 2-h heat shock at 40 °C from each genetic stock, was assessed for the relative amount of accumulated transcripts homologous to heat-shock gene DNA probes by Northern analysis and scintillation counting (see Materials and methods). *Bars of similar style* represent genetic stocks with similar levels of heat-shock transcripts and are significantly different from *bars of alternate style* at alpha = 0.05



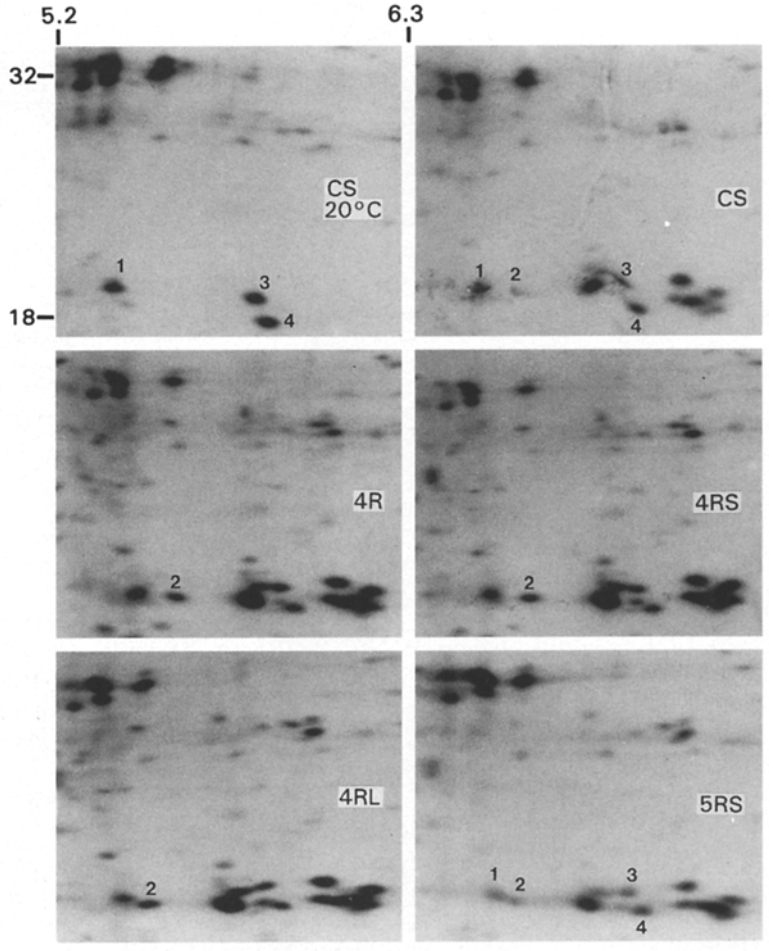


Fig. 4 A 2-D IEF/SDS PAGE separation of in-vitro translation products from wheat control plus heat-shock and various addition line heat-shock leaf tissue poly(A$^+$) RNAs; Poly(A$^+$) RNA was isolated from CS leaf tissue that remained at 20 °C and CS, 4R, 4RL, 4RS and 5RS leaf tissue following a 2 h, 40 °C heat shock and was then translated in vitro. The in-vitro translation products were separated by 2-D IEF/SDS PAGE with equal radioactive counts loaded (300 000 cpm). The HSP18 and 32-kDa polypeptide families are indicated in the top-left panel. The numbers adjacent to particular HSPs are referred to in the text

only rye chromosome-7 addition lines (7R, 7RL, 7RS) showed the ditelosomic and disomic addition lines to have the same level of transcripts for both HSP18 and HSP70 (Fig. 3).

The accumulation of the HSP18 and HSP70 transcripts was further analysed by in-vitro translation using 2-D IEF/SDS PAGE. In-vitro translation products from the HSP70 mRNA migrated on protein gels to 72 kDa with negligible variation in M_r and a limited pI range of 5.0–5.3 (data not shown). This limited resolution of the HSP70 family precluded a critical assessment of the HSP70 transcript levels by in-vitro translation products.

In contrast, the HSP18 family ranged marginally in M_r and included a pI range of 5.4–6.3 (Fig. 4). The following results are based on visual observations of the 2D gels in Fig. 4. This figure shows the control (20 °C) in-vitro translation products from CS; the remaining five panels are heat-induced in-vitro translation products from the genetic stocks indicated. The 32-kDa polypeptide family is included in each panel because this family showed only small changes in the level of expression for these genetic stocks. The 32-kDa polypeptide family serves as an internal control to guage the relative abundance of HSP18 in-vitro translation products based on visual observations (Fig. 4). Compared to CS, (1) disomic addition line 4R showed a higher level of expression of the HSP18 family and (2) ditelosomic addition lines 4RL and 4RS showed the greatest increase in expression of the HSP18 family (Fig. 4). When compared to the level in CS (Fig. 4) HSP number 2 in addition lines 4R, 4RL and RS appeared to be expressed de novo.

The three polypeptides centered at 18 kDa, numbered 1, 3 and 4 in Fig. 4, were not HSPs since their expression remained unchanged, or was reduced, under heat-shock conditions. The level of expression of all the HSP18 polypeptides in 5RS was similar to the levels in CS while the normally constitutive polypeptide, number 1, was expressed at lower levels in 5RS.

Discussion

Triticale, consisting of Chinese Spring wheat × Imperial rye, was used in this study to examine the influence of the rye genome on the accumulation of HSP18 and HSP70 transcripts in a wheat genetic background.

The strong hybridization signals in the heat-shock lanes of all the genetic stocks indicated that an abundant quantity of heat-shock mRNA had accumulated after a 2 h, 40 °C heat shock (Figs. 1, 2). High levels of heat-shock transcript accumulation in plants, following a heat shock of this nature, is common (Kimpel and Key 1985).

Our attempt at a quantitative, spectrophotometric analysis of the autoradiographs (Figs. 1, 2) was unsuccessful. The problems associated with the spectrophotometric analyses included: (1) the background signal levels in each lane varied, presumably due to the RNA blotting process, (2) the autoradiograph exposures were difficult to standardize from separate hybridizations and (3) the linear range of the signal exposure from the X-ray film was limited.

Quantifying the heat-shock transcript accumulation by Northern dot-blot analyses and scintillation counting provided the following advantages: (a) a complete set of RNA samples for comparison, including replicate measurements, could be assessed from a single blot and hybridization, (2) the RNA transfer (dot blotting) reduced the background signal variation, (3) the hybridization signal range was very broad and near linear, and (4) each blot could be standardized and was made comparable by including a set of standard heat-shock RNA samples.

We used two independant methods to quantify the heat-shock transcripts, namely (1) Northern dot blotting and (2) an in-vitro translation assay. Addition line 4R showed increased expression of HSP18 in-vitro translation products relative to CS (Fig. 4) but showed no change in the level of HSP18 transcripts from the Northern analysis (Fig. 3). This was the only addition line tested that showed some discrepancy in transcript levels between the two assay types.

This discrepancy may be explained by the suggestion that the wheat HSP17 DNA probe may fail to hybridize to some members of the family of gene transcripts due to reduced homology. Further, the in-vitro translation assay is sensitive and detects all of the abundant HSP18 transcripts by translation. Similarly, the in-vitro translation products of corn heat-shock mRNA show that there are six HSP18 isoelectric variants. When mRNAs are hybrid-selected with a corn HSP18 DNA probe, the selected mRNAs translate into only four of the six HSP18 polypeptides (Atkinson et al. 1989; Goping et al. 1991). Therefore, our Northern analysis could underestimate the amount of HSP18 transcripts whereas the in-vitro translation assay would show all of the HSP18 transcripts as translation products.

Our previous work demonstrates that the triticale and rye HSP profiles are alike. In addition, the expression of rye HSPs dominates the wheat HSP expression in triticale. The rye genome has a suppressive influence on wheat HSP expression and a partial source of this suppressive influence is from 5R (Somers et al. 1992). In the present experiments, each of the rye disomic additions, when compared to CS, showed an abnormally-enhanced level of HSP18 and/or HSP70 transcript accumulation in the presence of the foreign wheat genomes, with the exception of disomic addition line 5R which remained unchanged (Fig. 3). Since the rye genome is the major source of triticale HSP expression and is capable of suppressing wheat HSPs (Somers et al. 1992), the source of the suppressive influence on HSP18 and HSP70 transcript accumulation here may be located on 5R (Fig. 3).

Studies on triticale NOR activity show that the removal of a wheat NOR enhances the expression of the rye NOR (Gustafson et al. 1988). Also, the addition of the 1U NOR from *Aegilops umbellulatum* into wheat will suppress the expression of the wheat 6B NOR (Flavell 1986). Therefore, these wheat and rye NORs are expressed at abnormal levels when they are in the presence of a foreign NOR or genome.

Similarly, the added heat-shock transcript accumulation in six of the seven disomic addition lines (Fig. 3) could result from rye heat-shock genes carried on the added rye chromosomes. These rye heat-shock genes may be expressed at significantly-enhanced levels due to the lack of proper gene regulation. The up-regulated expression of the rye heat-shock genes is caused by the presence of the foreign wheat genomes and wheat/rye genomic interactions.

An alternative explanation is that the added rye chromosomes may carry genetic elements that enhance the expression of wheat heat-shock genes. With the high degree of conservation among heat-shock genes and heat-shock regulatory mechanisms (Key et al. 1987; Wing et al. 1989; Gurley and Key 1991), it is possible that the rye chromosomes may contribute enhancing elements for wheat heat-shock gene expression.

Since six of the seven rye disomic addition lines showed an enhancement of HSP expression (Fig. 3), the genes controlling expression of rye HSP18 and HSP70 synthesis are most likely distributed throughout these six rye chromosomes. Conversely, the genes that control rye HSP18 and HSP70 expression cannot be located on 5R; no enhanced HSP expression occurred in disomic addition line 5R. Similarly, a study that mapped the genomic location of heat-shock genes in 6X wheat showed a wide dispersal of HSP18 structural and/or regulatory genes throughout the wheat genomes (Porter et al. 1989). In this study, the 2-D gel polypeptide profiles of the HSP18 family from the ditelosomic series of Chinese Spring wheat were examined. When expression of a HSP18 polypeptide was reduced or eliminated from the ditelosomic polypeptide profile, then the missing wheat chromosome arm was considered responsible for expression of that polypeptide. Porter et al. (1989) showed that six different wheat arms in two different homoeologous groups (groups 3 and 4) controlled expression of these six HSP18 polypeptides. In addition, wheat homoeologous group 5 did not encode any HSP18 structural or regulatory genes.

We were able to show that genomic interactions among the wheat and rye genomes influenced the accumulation of HSP18 and HSP70 transcripts. The new information here, coupled with our previous

work, shows that 5R had a suppressive influence on HSP synthesis (Somers et al. 1992). In addition, small segments of the rye genome (chromosomes or chromosome arms) showed different genomic interactions with the wheat genomes in comparison to genomic interactions between the whole rye genome and the wheat genomes. These data strongly suggest that the introgression of smaller segments of the rye genome into a wheat genetic background can dramatically influence the expression of a polygenic character such as HSP synthesis.

It appeared that 5R had a suppressive influence on HSP18 and HSP70 transcript levels; the chromosome arms 5RL and 5RS had the strongest suppressive influence on heat-shock transcript accumulation when either was present as a ditelosomic addition to wheat. In the six disomic addition lines, where the complete 5R chromosome was absent, there were increased levels of HSP18 and/or HSP70 transcripts. The added heat-shock transcript accumulation possibly enhanced wheat HSP expression or additional rye HSP expression.

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