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The influence of the rye genome on expression of heat shock proteins in triticale*

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Summary. The heat shock protein profiles from *Secale cereale* L. cv Imperial, *Triticum aestivum* L. cv Chinese Spring, *S. cereale* × *T. aestivum* amphiploid, and the seven disomic *S. cereale* addition lines to *T. aestivum* were used to compare the wheat, rye, and triticale Heat Shock Protein profiles and to study the influence of the rye genome on heat shock protein expression in triticale. Three-day-old seedlings were heat shocked for 2 h at 40 °C in the presence of ^{35}S-methionine, and polypeptides from root tissues were subjected to one- or two-dimensional gel electrophoresis. The wheat and rye heat shock protein profiles each consisted of >150 heat shock proteins, of which 94 were sufficiently reproducible to construct a standard map. There were 11 unique rye heat shock proteins compared to 22 unique wheat heat shock proteins. The triticale heat shock protein profile resembled the rye parent more than the wheat parent. There were 22 heat shock proteins expressed uniquely by wheat that were not expressed in triticale. Rye chromosomes 1 and 3 exhibited a substantial repressive influence on the expression of 95% of the unique wheat heat shock proteins in triticale, while rye chromosome 4 appeared to have the least repressive influence on expression of the unique wheat heat shock proteins in triticale.

Key words: Heat shock proteins – Two dimensional Electrophoresis – Disomic addition lines – Chinese Spring wheat – Imperial rye

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Introduction

A short-term, rapid rise in temperature will induce a response, ubiquitous to all organisms, termed the heat shock (HS) response. The most notable feature of the HS response is the expression of a set of proteins referred to as heat shock proteins (HSPs) (for review, see Schlesinger et al. 1982; Baszczynski et al. 1985). Cooper and Ho 1983; Heat shock proteins are highly conserved in all organisms (Schlesinger et al. 1982; Craig 1985) and are linked to the acquisition of thermotolerance; a brief, non-lethal HS can accomplish (1) the induction of HSP expression, and (2) the acquisition of tolerance to normally lethal temperatures (Lin et al. 1984; Abernethy et al. 1989).

The expression of HSPs is easily detected by one-dimensional (1-D) and two-dimensional (2-D) gel electrophoresis, although 2-D gel analysis enables greater resolution of genotypic differences in HSP synthesis. The use of 2-D gel electrophoresis has detected genotypic variation in HSP synthesis in varieties of hexaploid wheat (Damerval et al. 1986; Zivy 1987). The variation in low-molecular-weight (LMW) HSP expression between the wheat variety Chinese Spring and the ditelosomic series of Chinese Spring was detected by 2-D gel electrophoresis and used to map the chromosomal location of LMW HSPs (Porter et al. 1989).

Triticales provide a unique opportunity to study intergeneric genomic interaction between wheat and rye at the level of gene expression. The activity of the rye Nucleolus Organizer Region (NOR) locus on chromosome R1 demonstrates transcriptional activity in hexaploid triticales if other NORs are absent. The transcriptional activity of the R1 NOR is greatly reduced, but variable, when wheat NORs from 1B, 6B or 1B and 6B are present (Appels et al. 1986), which suggests that wheat/rye genomic interactions affect this type of gene expression.

The expression of HSPs represents a complex genetic response in plants where HSP expression may be affected by genomic interaction. The availability of Imperial rye disomic additions to Chinese Spring wheat provides a system to resolve the influence that each rye chromosome may have on expression of HSPs in a wheat genetic background, and to determine the genomic distribution of HS genes in rye.

Materials and methods

Plant tissue and heat shock

Seeds of *Secale cereale* L. cv Imperial (IR) (2n = 14), *Triticum aestivum* L. cv Chinese Spring (CS) (2n = 42), Imperial × Chinese Spring (Triticale, TR) (2n = 56), and the seven disomic IR addition lines to CS were kindly provided by Dr. E. Sears, University of Missouri. Seeds were germinated on moist filter paper in darkness at 20 °C for 3 days, after which each intact seedling was placed in a 1.5-ml microtube with the root tissue submerged in 500 µl of distilled water. The microtube remained at 20 °C or was placed in a constant temperature water bath at 40 °C in darkness for 2 h. Following 15 min of incubation, 50 µCi of L-[^{35}S]-methionine (ICN Radiochemicals, St.-Laurent, Quebec) was added to the microtube and the incubation was continued for 1.75 h. The incorporation of radiolabel into proteins is very sensitive to the HS temperature, Somers et al. 1989; Baszczynski et al. 1982; Mason-Apps et al. 1990). A growth temperature of 20 °C and a HS temperature of 40 °C were used in this study to optimize both the high levels of HSP expression and the specific activity of the isolated polypeptides.

Protein isolation and electrophoresis

At completion of the 2-h temperature treatment, seedlings were washed in distilled water and the root tissue was excised and homogenized in cold (4 °C) protein extraction buffer [60 m*M* TRIS-HCl (pH 6.8), 2% (w/v) SDS, 10 m*M* dithiothreitol, 1 m*M* phenylmethyl-sulfonyl fluoride, 10% (v/v) glycerol]. Cell debris was removed by centrifugation and the cell extracts were stored at −20 °C.

The radioactive content of isolated polypeptides was determined by spotting a 5-µl aliquot on 3MM chromatography paper and boiling the paper in 10% (w/v) trichloroacetic acid for 10 min. The paper was subsequently washed excessively, first with water, 95% ethanol, then acetone. The precipitated radioisotope was then counted in a LS-150 liquid scintillation counter (Beckman Instruments, Mississauga, Ontario).

For 1-D gels, a 40,000 cpm quantity of each polypeptide sample was loaded in lanes of a 7–17% linear gradient denaturing gel and electrophoresed according to the procedures of Laemmli (1970). For 2-D gels, a 400,000 cpm quantity of each polypeptide sample was loaded on the basic end of tube gels (1.5 mm × 140 mm) containing 3.5% (w/v) acrylamide/piperazine-diacrylamide, 9.5 *M* urea, 2% (w/v) 3-[(cholamidopropyl)dimethylammonio]-1-propanesulfonate, 5% (v/v) LKB ampholines (4/5 pH 5–7, 1/5 pH 3–10), and were focused for 13,000 volt-hours. Tubes were then placed on 7–17% linear gradient denaturing gels for separation of polypeptides in the second dimension according to Laemmli (1970). All of the slab gels were impregnated with a fluor (En^{3}Hance, NEN, Mississauga, Ontario), dried, and exposed to Kodak X-Omat AR film for 20 h (1-D), 2, 3, or 4 days (2-D).

Standard map and data analysis

To study the entire HSP profile in triticale, we chose in vivo labelling with L-[^{35}S]-methionine and 2-D gel electrophoresis of isolated polypeptides. To obtain reproducible 2-D gel results, a minimum quantity of 400,000 cpm was loaded onto each tube, and the gradient slab gels were poured in a multi-gel casting chamber and run simultaneously under similar conditions. Multiple gels of replicate polypeptide samples were run, and variable fluorograph exposures were analyzed to establish a set of reproducible HSPs from all the seed lines, followed by a standard/composite map of the HSP profiles being generated. Each polypeptide was assessed for heat inducibility relative to a control (20 °C) gel, and a reproducible level of expression. The HSPs were then categorized according to their presence of expression in the following combinations of IR, CS, and TR HSP profiles: 'unique to IR', 'unique to CS', 'unique to TR', 'common to IR and CS,' 'common to IR and TR,' 'common to CS and TR,' 'common to IR, CS, and TR', and 'only expressed in addition lines.' The total number of HSPs expressed in each category was recorded, as well as the number of HSPs from each category which were expressed in each of the addition line.

Results

The HS response in wheat and rye at 40 °C consisted of polypeptides ranging from 97 kilodaltons (kDa) to 14 kDa and a pI range of 5.0 to 7.3 (Figs. 1 and 2).

A set of 94 HSPs, which were considered reproducible based on the level of protein expression following an analysis of the ten different seed line HSP profiles (Fig. 2), was included in the standard map (Fig. 3). The HS response was dominated by seven clusters of HSPs

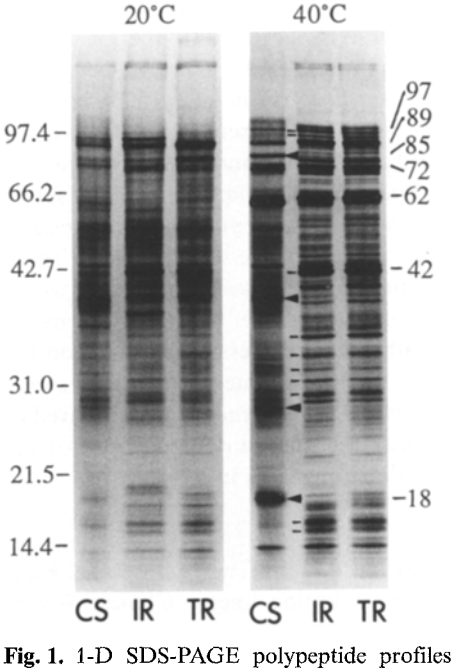


Fig. 1. 1-D SDS-PAGE polypeptide profiles from 3-day-old seedling root tissue, following a 2 h incubation at 20 °C or 40 °C in the presence of L-[^{35}S]-methionine. Equal counts of radioactivity (40,000 cpm) were loaded in each lane. The lanes are designated CS – Chinese Spring, IR – Imperial rye, and TR – triticale. The numbers on the *left* represent M_r standards and the numbers on the *right* represent the M_rs of prominent HSP clusters. *Arrowheads* indicate unique HSPs expressed in CS and the lines indicate unique HSP expression in rye

identified on the gels centered at 97, 89, 85, 72, 62, 42, and 18 kDa (Fig. 2). These major HSP clusters are also indicated on the 1-D gel (Fig. 1).

There were many qualitative similarities between the wheat and rye HSP profiles. The wheat and rye HSP profiles were both composed of the seven major HSP clusters (Fig. 3), within which 48 and 41 HSPs were expressed by wheat and rye, respectively.

The prominent differences between the wheat and rye HS responses were evident in Fig. 1. There were four families of wheat HSPs which showed marginal levels of expression in the rye HSP profile, and ten rye HSP families which were not expressed in the wheat HSP profile (Fig. 1). The triticale HSP profile was most similar to the rye HSP profile at the 1-D level. The differences between the wheat and rye HSP profiles were also observed on 2-D gels. The most evident differences between the wheat and rye HS responses were the expression of HSP62 (lowest M_r in cluster, Figs. 2 and 3) in rye with a corresponding marginal level of expression in wheat, a greater number of HSPs expressed in the 89-kDa HSP cluster in rye as compared to wheat, and the greater number and intensity of the LMW wheat HSPs (<20 kDa) as compared to rye (Figs. 2 and 3). Rye expressed 11 unique HSPs in contrast to the 22 HSPs uniquely expressed in wheat (Fig. 3). Following 2-D gel analysis, the HSP profile from triticale resembled the rye HSP profile.

The set of 94 HSPs was categorized based on their presence of expression in different combinations of IR, CS, and TR, and these data are summarized in Table 1. In the first category of 11 'unique to IR' HSPs, addition lines CS + IR. 1 and CS + IR.3 expressed the least number of unique IR HSPs, one and zero HSPs, respectively. The remaining addition lines expressed from two to seven rye-specific HSPs, with CS + IR.2 and CS + IR.4 expressing the most 'unique to IR' HSPs (Fig. 2, Table 1). The next category, 'unique to CS' HSPs, demonstrated a similar pattern of HSP synthesis within the addition lines. Addition lines CS + IR.1 and CS + IR.3 each expressed only one of the 22 unique CS HSPs, while the remaining addition lines expressed from three to 11 unique CS HSPs (Table 1). There was an absence of HSP expression that could be classified as 'unique to TR' and thus this category is not present in Table 1. There were nine HSPs 'common to IR and CS' which were not expressed in TR. Addition lines CS + IR.1 and CS + IR.3 expressed two and zero HSPs, respectively, in contrast to addition line CS + IR.4, which expressed eight HSPs from this category (Table 1). When 'unique to CS' and 'common to IR and CS' HSPs were combined, addition lines CS + IR.1, CS + IR.3 and, to a lesser extent, CS + IR.5 expressed the least number of HSPs found in these two categories (Table 1). There were nine HSPs expressed that were 'common to IR and TR'. The distribution of HSP expression in the addition lines for this group of HSPs was relatively even, with CS + IR.4 and CS + IR.7 expressing the greatest number (Table 1). The next category of HSPs, 'common to CS and TR,' included 11 HSPs. The majority of this group of HSPs was expressed in all the addition lines, with the exception of CS + IR.6 and CS + IR.7 where only four and three HSPs from this group were expressed respectively (Table 1). The remaining two categories included HSPs expressed by all of IR, CS, and TR or HSPs expressed in the addition lines only (Table 1). Since these groups of HSPs do not show different levels of expression in IR, CS, and TR, these HSPs do not contribute significantly to our understanding of wheat/rye genomic interaction and thus were not studied in detail.

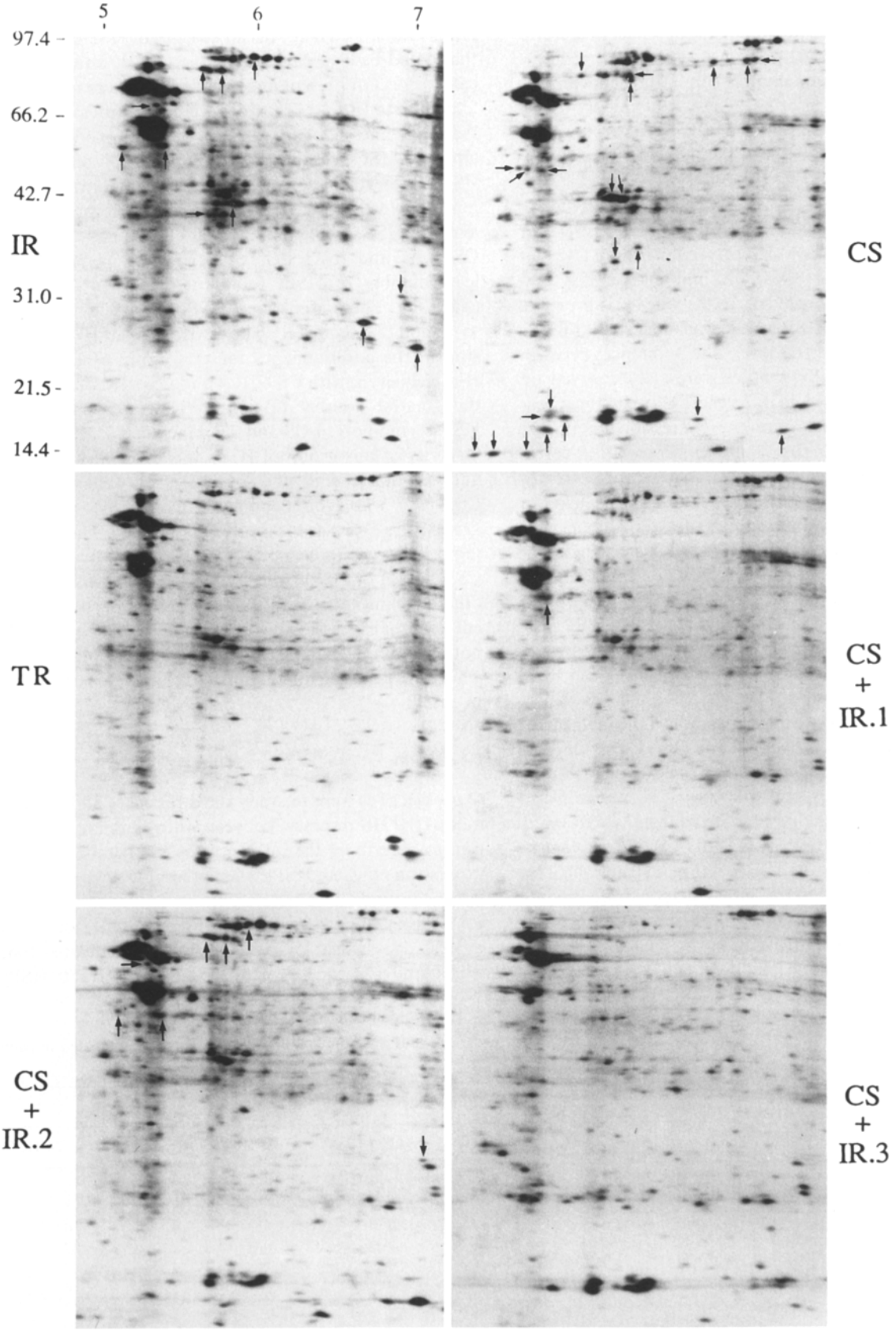
Discussion

Our objectives were to study the differences and similarities of the HS response between wheat and rye, in addition to describing the influence the rye genome has on expression of wheat HSPs when present in a wheat genetic background.

It was difficult to define a finite number of HSPs expressed by wheat or rye, but the data indicated that wheat and rye each expressed in excess of 150 HSPs

Table 1. The number of the HSPs expressed in each of the seven categories and the number of categorized HSPs expressed in each addition line

Category	No. of HSPs in category	No. of categorized HSPs in addition lines						
		CS + IR.1	CS + IR.2	CS + IR.3	CS + IR.4	CS + IR.5	CS + IR.6	CS + IR.7
IR	11	1	7	0	7	2	2	5
CS	22	1	5	1	11	3	9	5
IR, CS	9	2	6	0	8	3	4	4
IR, TR	9	5	4	2	7	3	2	7
CS, TR	11	8	9	7	9	9	4	3
IR, CS, TR	25	21	21	17	24	20	15	14
Addition lines only	6	9	2	0	5	0	2	2


5
6
7
97.4
66.2
42.7
IR
31.0
21.5
14.4
CS
TR
CS + IR.1
CS + IR.2
CS + IR.3

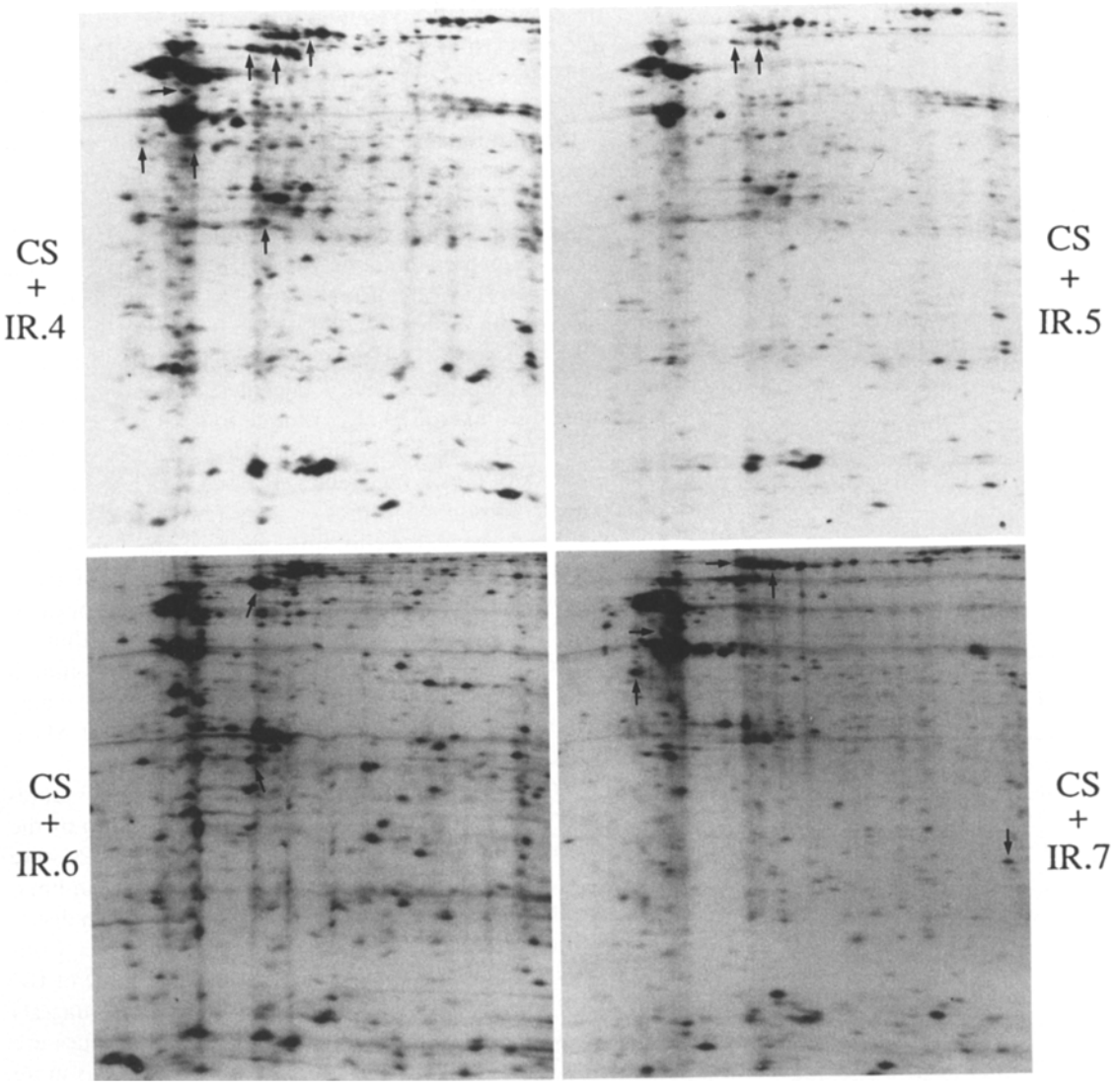


Fig. 2. 2-D SDS-PAGE polypeptide profiles from 3-day-old seedling root tissue, following a 2 h incubation at 40 °C in the presence of L-[^{35}S]-methionine. Equal counts of radioactivity (400,000 cpm) were loaded in each gel. The gels are designated IR – Imperial rye, CS – Chinese Spring, TR – triticale, CS + IR.1 – addition line 1, CS + IR.2 – addition line 2, CS + IR.3 – addition line 3, CS + IR.4 – addition line 4, CS + IR.5 – addition line 5, CS + IR.6 – addition line 6, and CS + IR.7 – addition line 7. The numbers on the *left* of IR represent M_r standards and the numbers *above* IR represent pI standards. *Arrow* in gels IR and the seven addition lines indicate 'unique to IR' HSPs. *Arrows* in CS indicate 'unique to CS' HSPs

(Fig. 2). In contrast, Zivy (1987) reported the accumulation of only 33 wheat root HSPs within 2 h at 41 °C; however, the use of silver staining is a less sensitive means of detecting HSP expression than fluorography of radioactive polypeptides.

Many HSPs were expressed in wheat and rye, although some HSPs were not included in the standard map due to inconsistent levels of expression between the ten seed lines. The primary concern in generating the standard map (Fig. 3) was to include HSPs which had consistently identifiable levels of expression. Since the HSP97 and HSP72 clusters were very reproducible and showed little variability in levels of expression between seed lines (Fig. 2), they were used as internal markers to gauge the level of expression of other HSPs.

A comparison of the HSP profiles from 1-D gels indicated that triticale more closely resembled the rye parent than the wheat parent (Fig. 1). The differences between the wheat and rye HS responses were dominated by the unique expression of four HSP families in wheat and ten HSP families in rye. Barley, wheat, rye, and triticale all have similar HSP profiles based on 1-D gels (Necchi et al. 1987), in contrast to different wheat, rye, and triticale HSP profiles reported here. This discrepancy

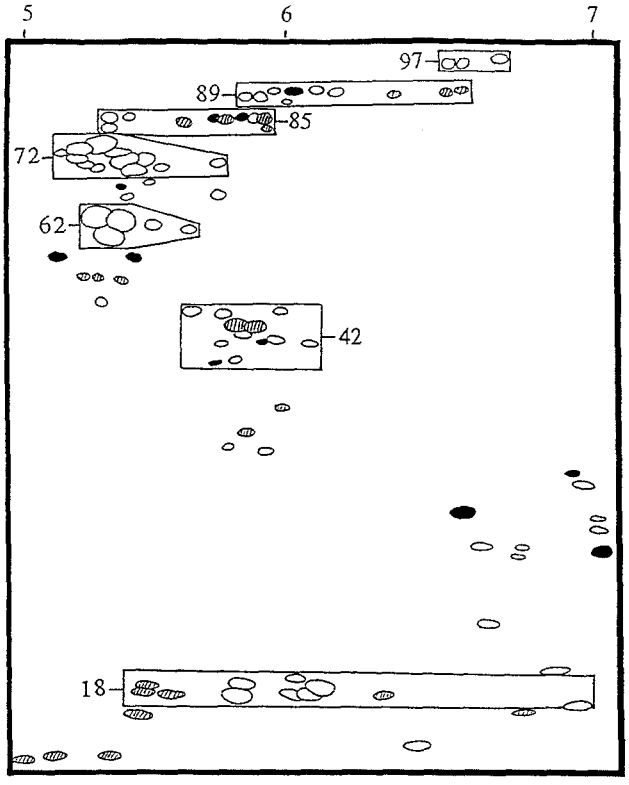


Fig. 3. A standard/composite map of the HSP profiles from all ten seed lines depicted in Fig. 3. The *boxed* HSPs represent HSP clusters and the numbers indicate the approximate M_r of each HSP cluster. The *open spots* represent HSPs expressed by at least one of the ten seed lines, *"hashed"* spots represent unique wheat HSPs, and solid spots represent unique rye HSPs. The pI standards are indicated above the figure

could be a result of the better resolution by 2-D gel electrophoresis. Differences in HSP expression between closely related genotypes exists, as is demonstrated among different cultivars of maize (Yacoob and Filion 1986).

The differences between the wheat and rye HSP profiles observed on 2-D gels were the presence of a 62-kDa HSP (lowest M_r in cluster) in rye and a greater number of 89-kDa HSPs in rye, which show very reduced levels of expression in wheat (Figs. 2 and 3). In addition, wheat expressed a greater number of LMW HSPs compared to rye (Fig. 2). A similar difference in wheat and rye HSP62 band intensity was observed on 1-D gels by Necchi et al. (1987), but was not completely evident on 1-D gels in this study.

In order to examine the influence of the rye genome on expression of wheat HSPs, a set of 94 HSPs were categorized according to the presence of expression in various combinations of IR, CS, and TR. It was evident that a total of 33 HSPs was expressed uniquely by wheat or rye (Table 1), in addition to nine HSPs 'common to IR and CS'; all of these HSPs were not expressed in triticale. Triticale expressed fewer HSPs than either of the parents; this suggests that genomic interactions between wheat and rye exist at the level of HSP expression. There were 22 HSPs expressed uniquely in CS (Table 1), and expression of these HSPs was greatly reduced in TR and many of the addition lines. These data are a good, and possibly, unique example of the rye genome limiting the expression of wheat genes. The reduction in the number of HSPs expressed in triticale was also observed in CS + IR.3; there was expression of only one HSP by CS + IR.3 in the combined HSP categories 'unique to IR,' 'unique to CS,' and 'common to IR and CS' (Table 1). Similarily, CS + IR.1 and CS + IR.5 expressed just four and eight of the 42 HSPs, respectively, included in combined HSP categories 'unique to IR,' 'unique to CS,' and 'common to IR and CS.' Since disomic addition lines with the presence of rye chromosomes, 1, 3 and, to a lesser extent, rye chromosome 5 expressed very few of the 42 HSPs described above, rye chromosomes 1, 3, and 5 may have a profound influence on reducing the number of HSPs expressed in triticale. In comparison, CS + IR.4 appeared to express the greatest number of HSPs found within the categories 'unique to IR,' 'unique to CS,' and 'common to IR and CS,' and suggests that rye chromosome 4 may have the least infuence on reducing expression of wheat HSPs in triticale.

The HSPs that were 'common to IR and TR' represent rye HSP expression which was not influenced by the presence of the wheat genomes. Since these HSPs were expressed in similar numbers in all of the addition lines, we interpreted this to suggest that there is an even distribution of HS genes throughout the rye genome. Conversely, the more abundant expression of 'unique ot IR' HSPs in CS + IR.2, CS + IR.4, and CS + IR.7 suggests that heat shock structural and/or controlling genes are more abundant on these three rye chromosomes. A more precise gene mapping strategy is required to understand the distribution of heat shock genes in the rye genome.

The analysis of HSP expression categorized as HSPs 'common to CS and TR,' 'common to IR, CS, and TR,' and 'addition lines only' did not further our understanding of wheat/rye genomic interaction or chromosomal location of heat shock genes, due to similar numbers of HSPs being expressed by each addition line or similar expression of HSPs in IR, CS, and TR.

In summary, the present study showed a significant amount of variation in the HSP profiles between wheat and rye. The analysis of 1-D and 2-D gels indicated that the triticale HSP profile was far more similar to the rye HSP profile than it was to wheat. The data strongly suggest that the rye genome, when present in a wheat genetic background, will have a substantial repressive influence on the expression of unique wheat HSPs. The source of this repressive influence appears to be concentrated on rye chromosomes 1 and 3. In contrast, the presence of rye chromosome 4 in triticale appeared to

have the least repressive influence on the expression of unique wheat HSPs.

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