Theor Appl Genet (1987) 74:209–213



# Genetic variability for heat shock proteins in common wheat

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Received December 11, 1986; Accepted February 17, 1987
Communicated by H. F. Linskens

**Summary.** The response of the common wheat line 'Chinese Spring' to heat shocks of different time lengths was studied by the two-dimensional (2D) electrophoresis of denatured proteins. After a heat shock of 5 h, 33 heat shock proteins (HSPs) accumulated in an amount sufficient to be revealed by silver stain. Two other wheat lines ('Moisson' and 'Selkirk') were then submitted to a heat shock of 5 h, and the responses of the 3 lines were compared: of a total of 35 HSPs, 13 (37.1%) were quantitatively or qualitatively variable. This variability concerns low-molecular-weight and high-molecular-weight HSPs. The three genotypes showed thermal tolerance but 'Chinese Spring's' response to heat treatments was slightly different from those of the other two lines The possibility of a relationship between HSP patterns and thermal sensitivity is discussed.

**Key words:** Genetic variability – Heat shock proteins – Wheat – Two-dimensional electrophoresis – Thermal tolerance

## Introduction

In organisms as different as bacteria, animals, and higher plants, an abrupt elevation of temperature results in a similar response: synthesis of a specific set of proteins called heat shock proteins, decrease or stop of the synthesis of the other proteins, and development of the ability to withstand higher temperatures that would otherwise be lethal (Kimpel and Key 1985; Schlesinger et al. 1982).

Several works on mutants (Lin et al. 1984; McAlister and Finkelstein 1980; Yamamori and Yura 1982) and on the localization of HSPs in cells (Loomis and Wheeler 1982; Velasquez and DiDomenico 1980) support the idea that these proteins play a part in the development of this thermal tolerance.

Although high homologies have been found between HSPs of species belonging to different kingdoms (Kelley and Schlesinger 1982; Moran et al. 1983), a diversity exists: plants synthesize more low-molecular-weight (LMW) HSPs than other organisms (Key et al. 1985) and, among higher plants, an interspecific diversity has been reported (Key and Nobel 1986; Lopato and Gleba 1985; Vierling et al. 1986).

In this study, the existence of intraspecific diversty for HSPs is tested in common wheat (*Triticum aestivum*): HSPs of 3 unrelated varieties are compared by two-dimensionnal electrophoresis (O'Farrell 1975). Their thermal tolerance is then tested.

## Material and methods

### *Plant material and treatments*

Three common wheat lines were studied: 'Chinese Spring' (CS), 'Moisson' (Ms), and 'Selkirk' (Sk). For protein analysis, seedlings were grown on moist paper in Petri dishes, in the dark at 20 °C for 6 days. For heat shock, the Petri dishes were transferred to a temperature of 41 °C, for 1 h, 2 h, or 5 h. The aerial part of seedlings were cut immediately after heat shock and conserved in liquid nitrogen until extraction. No seedling showed any morphological damage after a 5 h-long exposure to this temperature. Control experiments were done using both seedlings kept at 20 °C, and on 7-day-old seedlings, to ascertain that protein inductions observed in treated plants were not due to stage-specific regulation. For thermal tolerance tests, seeds were arranged in rows on moist paper in large boxes so that numerous seddlings could be measured individually.

### *Protein extraction*

Proteins were extracted and denatured according to Zivy (Damerval et al. 1986) except that they were dissolved with 70 instead of 50 µl of sample solution ("UKS") per mg of dry pellet.

*2D-electrophoresis*

Two types (I and II) of 2D-electrophoresis conditions were used: type I gives good resolution for proteins of medium and high molecular weight, and type II for proteins of low molecular weight.

In type I, the mixture of ampholytes is 80% Pharmalyte pH 5–8, 10% Pharmalyte pH 3–10, 10% Servalyt pH 3–10. The acrylamide concentration in the 2nd dimension gel is 11%.

In type II, the resolution of small proteins is increased by using a 13% concentration of acrylamide in the 2nd dimension. The ampholyte composition was changed to 100% LKB Ampholines pH 5–8, since Pharmalyte interact in the silver staining of low molecular weight proteins (Zivy 1986).

In both types, the diameter and length of the IEF gels were, respectively, 1 mm and 22 cm. After a run of 26,000 Volts × hours, they were placed in equilibration buffer for 15 min and both ends were removed: 2 cm of the acidic end and 4 cm of the basic end for type I; 3 cm of both ends for type II. The SDS electrophoresis (gel size: 16 cm × 16 cm) was run in a Nucleonics Isodalt tank. All electrophoresis solutions not described here are as in Colas des Francs and Thiellement (1985). The gels were silver stained according to the method of Granier and de Vienne (1986), i.e. an adaptation of the Oakley method (1980) for staining batches of 10 gels bound to Gel Bond (FMC, Marine Colloids).

All results described hereafter (spot presence, absence, or quantitative change) have been visually observed on at least 3 gels (one seedling per gel) of each genotype/treatment combination. Only clearcut and reproducible quantitative differences have been taken into account.

## Results

'Chinese Spring' seedlings were submitted to heat shocks of different time lengths: 1 h, 2 h, and 5 h. The 2D patterns obtained from a control and a seedling submitted to a HS of 5 hours are shown in Fig. 1. After 1 h of HS, the heaviest HSPs appeared as very faint spots (squares 1 and 2 of Fig. 1 C). After 2 h, most of the HSPs shown in Fig. 1 C, D had been detected. Their intensity increased between 2 and 5 h, at which time 33 HS spots could be reproducibly observed. Two of them always appeared as streaks (square 2): these HSPs were never well focused and their positions in the first dimension could not be accurately determined. The molecular weights of the detected HSPs are in the following ranges: 4 of them between 94 and 110 kd (square 1), 3 between 75 and 80 kd (square 2), and 26 between 15 and 30 kd (Fig. 1 D). None of the HS spots were present in the controls, except one (the most acidic in square 2). For this spot, HS resulted in an intensity increase.

The responses of the 3 wheat lines (CS, Sk, and Ms) to 5 h of HS were compared. Qualitative (presence/absence of a spot) and quantitative (intensity changes) variations were found (Fig. 2). Among a total of 35 HS spots detected in the 3 lines, 13 (37,1%) showed between line variability (Fig. 3). Nine of them discriminate Ms from CS and Sk: they are either absent (spots 15, 16, 17, 28, 30, 35) or less intense (spots 22, 29, 34) in Ms. The other 4 variable spots discriminate genotypes in different ways: spots 2 and 14 are specific to CS and Sk respectively; spot 4 is present only in Sk and Ms; spot 13 is present only in CS and Ms.

For testing thermal tolerance, seedlings of the 3 genotypes were submitted to the following 4 treatments: kept at 20 °C (control: number 1), 41 °C for 2 h (number 2), 41 °C for 2 h then 48 °C for 3 h (number

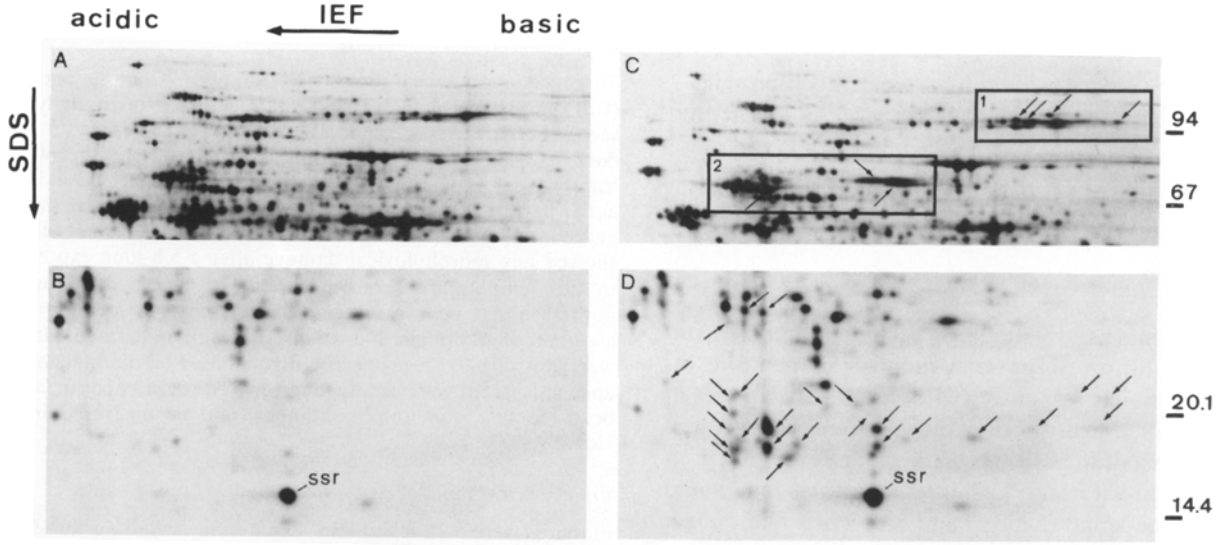


**Fig. 1 A–D.** HSPs in 'Chinese Spring'. **A** and **B** control; **C** and **D** heat shocked seedling (5 h). **A** and **C** are tops of type I gels; **B** and **D** are bottoms of type II gels. *Arrows* show HSPs. Molecular weights in kilodaltons are indicated on the right. ssr: small subunit of ribulose bisphosphate carboxylase/oxygenase

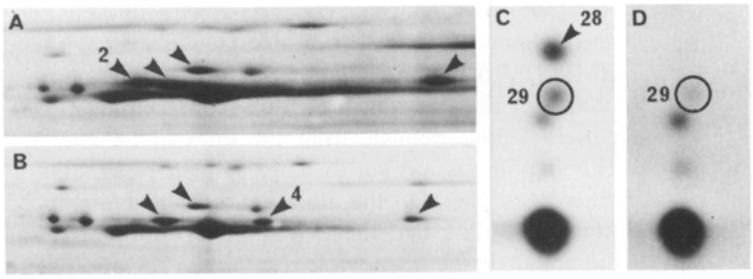


**Fig. 2A–D.** Qualitative and quantitative variations of HSPs. **A** and **B** qualitative variation among HMW HSPs. **A** CS, **B** Sk. **C** and **D** examples of qualitative and quantitative variations among LMW HSPs. **C** CS, **D** Ms. HSPs are labelled with *arrows* and numbers when qualitatively varying, without numbers when not varying, and circled when quantitatively varying

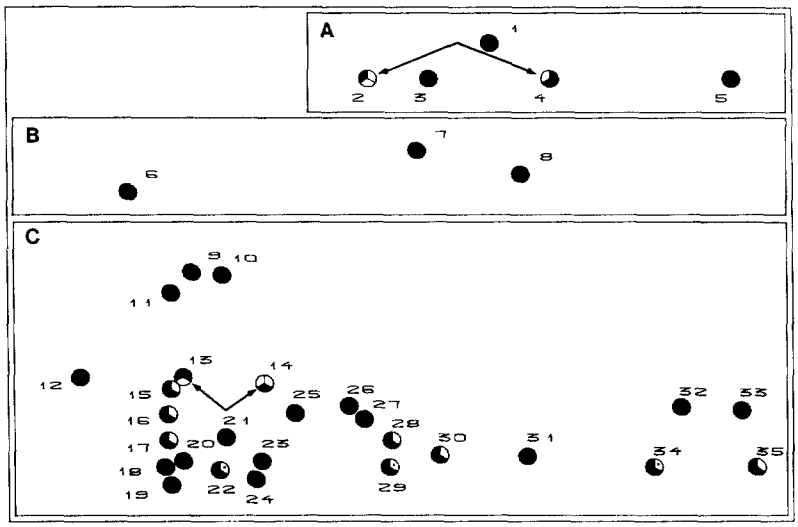


**Fig. 3.** Genetic variation of HSPs. HS spots are symbolized as circles divided into 3 parts corresponding to the 3 genotypes. *Left* CS; *right* Ms; *bottom* Sk. The part is black when the spot is present in the corresponding genotype, white when it is absent, and white with a dot when the spot is less intense than in the other genotypes. Arrows show hypothetical couples of alleles. Squares A and B correspond to squares 1 and 2 of Fig. 1C (HMW HSPs), and square C corresponds to Fig. 1D (LMW HSPs)

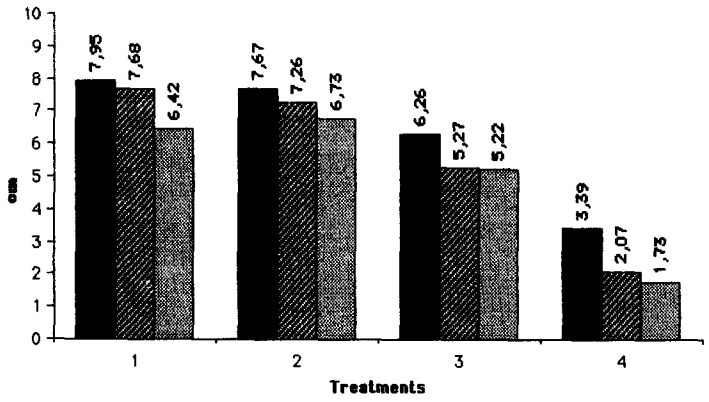


**Fig. 4.** Mean growth of the 3 lines after the 4 treatments. Sk: black; CS: hatched; Ms: gray. For growth and treatment descriptions, see text

3), and directly 48 °C for 3 h (number 4). Their length was measured before treatment and 3 days thereafter, and their growth during these 3 days calculated. The growth means of the 3 genotypes submitted to the 4 treatments are represented in Fig. 4. For each treatment, 25 to 30 seedlings per genotype were measured.

A 2 way-analysis of variance was performed, showing a highly significative effect ($P>0.99$) for treatment and genotype, and a significative effect of the genotype-treatment interaction ($P>0.95$). This interaction is mainly due to CS response: it disappears only when this genotype is removed from the analysis and not when one of the others is removed. When only treatments 3 and 4 are taken in account for the comparison of the 3 genotypes, the interaction is not significant.

A 1 way-analysis of variance was performed, each level of the factor being a genotype-treatment combination. It enabled that the effects of treatments on growth for all genotypes to be ranked as follows: (1 and 2)>3>4 ($P>0.99$). The significant difference between treatments 3 and 4 was what was expected from thermal tolerance. However, the differences be-

tween genotypes were not similar in all treatments: in treatment 1, the comparison of means shows (Sk and CS)>Ms, while in treatments 3 and 4 it shows Sk>(CS and Ms) ($P>0.99$). In treatment 2 Sk>CS>Ms, with $P>0.05$.

## Discussion

Three HS time lengths have been tested on one line to define the optimal conditions for detecting intraspecific HSP differences. All HSPs did not appear simultaneously, and several hours were necessary for most of them to accumulate in a sufficient amount to be reproducibly silver-stained on 2D gels. The HS spot 6 was observed in the control. It could correspond to a HSP normally synthesized at this stage but whose synthesis is enhanced by HS. This kind of regulation has been observed in other species (Cooper et al. 1984; Zimmermann et al. 1983). However, it cannot be ruled out that this observation results from an overlap of a HSP with another protein.

By comparing the HS response of 3 wheat lines, 13 variable HS spots have been found among HMW as well as among LMW HSPs. This allows the differentiation of each genotype from the others on the basis of its HSPs.

Two pairs of spots behave as allelic products, differing by a charge change (see Fig. 2a, b and Fig. 3): CS having a different allele than the other genotypes in one case (couple 2/4 (HMW HSP)), and Sk having the different allele in the second case (13/14 (LMW HSP)).

Variations in the 9 other spots (less intense or absent in Ms) are probably due to variations in factors that control the synthesis of HSPs. It must be pointed out, however, that in this case the number of genetic differences can be smaller than the number of varying spots (Zivy et al. 1984): i) some of them can represent a single protein that is subject to posttranslational modifications (Zannis and Breslow 1981); ii) one mutation in a regulatory gene can result in a quantitative change of several gene products that are under its control.

The test of the hypothesis of allelic variations and the evaluation of the number of variable regulatory genes can be done by looking at F2 segregations and other genotypes.

The 3 genotypes showed a significative thermal tolerance: a pretreatment at 41 °C before treatment at 48 °C allowed them to grow better than no pretreatment. However, CS growth was more slowed down by treatments at 41 °C and at 41 °C followed by 48 °C than growth of the other genotypes. This difference in response is not a difference of thermal tolerance as defined above but it still concerns heat sensitivity, which might be correlated with HSP patterns. Spots 2 and 4 (an assumed pair of allelic HMW HSPs) are possible candidates for this correlation: they are the only spots that separate the CS HSP pattern simultaneously from the ones of Sk and Ms. This hypothesis has yet to be confirmed by looking at other genotypes. On the other hand, it can be deduced from these data that the 11 remaining HSP variations did not affect the phenotypic response to HS.

Nevertheless, a high percentage of variable spots has been found among HSPs (37.1%) compared to the one observed on non-HS spots: only 13% out of 130 reproducible non-HS spots observed in the best resolved region of the gels (LMW proteins on gels of type II) were found variable between the same genotypes (data not shown).

It has been observed in other studies that the patterns of HSPs vary among plant species. The results described here show that diversity for LMW and HMW HSPs also exists within a species. The occurence of genetic variability for the response to heat shock can be helpful in the study and localization of genes encoding HSPs or regulating their synthesis. Moreover, it allows the researcher to look for correlations with phenotypic responses to environmental stresses which might have implications in plant breeding as well as in the study of HSP function.

*Acknowledgements.* I thank J. Blaisonneau, M. Le Guilloux, and M. -P. Digard for their technical assistance. I am grateful to H. Thiellement, D. de Vienne, C. Damerval, and N. Bahrman for comments and suggestions.

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