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Genetic analysis of heat shock proteins in maize

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Abstract A genetic analysis of heat shock protein (HSP) synthesis was performed in seedling leaf tissue of two maize inbred lines, their F_1 hybrid and F_2 progeny. Protein synthesis following a high temperature treatment was visualized by [^{35}S]-methionine in vivo labelling and two-dimensional gel electrophoresis. The parental lines' HSP synthesis patterns revealed both qualitative and quantitative polymorphisms implicative of differences in HSP structural genes and regulatory factors. The F_1 hybrid HSP profile indicated that synthesis of all parental HSPs conformed to dominant inheritance patterns, including complete dominance, over-dominance and co-dominance. Alleles for six low-molecular-weight HSPs in F_2 progeny assorted according to typical 3:1 Mendelian ratios for dominant gene expression. There is evidence for unlinked gene loci of four different HSP gene pairs, but data for three other HSP gene pairs were inconclusive, perhaps reflecting linkage for one pair and complex regulatory factor interactions for the other two pairs of genes. These results clearly indicate the existence of genetic variability in HSP synthesis and emphasize the potential of partitioning their roles in thermal tolerance using genetic and molecular analyses.

Key words Mendelian genetics · Heat shock response · Stress proteins · Maize

Introduction

The heat shock (HS) response is the most intensely investigated stress protein system. Its hallmark is the synthesis of heat shock proteins (HSPs), which have been correlated with acquired thermal tolerance in many species including bacteria (Sanchez and Lindquist 1990); mammalian fibroblasts (Riabowol et al. 1988); and higher plants such as soybean (Lin et al. 1984), wheat (Krishnan et al. 1989) and maize (Jorgensen et al. 1992).

Although the biochemical functions of some HSPs (HSPs 70 and 60 families) have been defined in bacteria, yeast and *Drosophila melanogaster* (Welch 1993, for recent review), understanding the molecular and physiological bases of heat tolerance in higher plants has proved difficult since it is a complex phenomenon controlled by many genes whose products affect numerous physiological systems (Blum 1985; Ottaviano and Gorla 1988). Potential experimental approaches to investigate the roles of plant HSP genes in thermal tolerance include exploration of intraspecific variation in HSP synthesis and characterization of HSP inheritance. A model higher plant species could accommodate this strategy because of relative ease in advancing generations through the F_1 hybrid and assorting F_2 progeny. A prominent feature of the plant HS response is the synthesis of low-molecular-weight (LMW) HSPs between 15 and 30 kilodaltons (kDa). There is extensive evidence of both qualitative and quantitative intraspecific genetic variability in LMW HSP synthesis, as reported in sorghum (Ougham and Stoddart 1986; Jorgensen et al. 1993); cotton (Fender and O'Connell 1989); wheat (Krishnan et al. 1989; Vierling and Nguyen 1990) and maize (Frova et al. 1988; Jorgensen et al. 1992).

In our laboratory we are evaluating alternative models of structural and regulatory gene interactions that affect protein synthesis in hybrids and assorting progeny. Hypothetically, an F_1 hybrid's gene products could reflect co-dominance (additive inheritance) of parental gene effects. This hypothesis assumes no parental regulatory suppression of gene expression (Leonardi et al. 1988; Marmiroli et al. 1989); alternatively, if one parental genome's structural or regulatory factors exert dominant control in hybrid phenotype

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determination (nonadditive inheritance), equal expression of parental contributions is not attained (Fender and O'Connell 1990). This is the molecular basis of both dominant and epistatic gene expression. F_2 progeny phenotypes would be expected to exhibit assortment of gene control mechanisms.

In maize hybrids during non-stress conditions, two-dimensional (2-D) gel electrophoretic analysis proved useful for demonstrating that protein inheritance was either additive or nonadditive (de Vienne et al. 1988; Leonardi et al. 1988). The definition of additive inheritance pertained to protein spot intensities within the parental range, and nonadditive inheritance was defined as spot intensities similar to those of one parent or outside the parental intensity ranges. Some protein synthesis patterns suggested equal parental contributions to gene expression, but others demonstrated dominant or epistatic control.

To date there have been few reports of HSP gene transmission in plants; indeed there have been no comprehensive analyses of genotype-specific HSP inheritance from parental lines to their F_1 hybrid and assorting F_2 progeny. Heat shock protein inheritance was studied in barley F_1 hybrids using one-dimensional (1-D) gel electrophoresis, and additive inheritance was confirmed for some HSPs. This work also revealed the expression of hybrid-specific HSPs, indicating activation of genes that were suppressed in one parent (Marmiroli et al. 1989).

Heat shock protein synthesis was evaluated in maize inbreds and their F_1 hybrids using 1-D gel electrophoresis (Frova et al. 1988). Both additive and nonadditive inheritance were demonstrated in the F_1 hybrids. It was observed that substantial qualitative variation in HSP17 synthesis existed among the parental lines and that the inheritance of this HSP family in F_1 progeny was dominant or nonadditive. These researchers further studied F_2 maize progeny to correlate the inheritance of HSP17 synthesis and restriction fragment length polymorphism (RFLP) markers, individually, with cellular membrane stability (CMS) and concluded that intraspecific qualitative polymorphism in LMW HSP synthesis is extremely rare (Ottaviano et al. 1991) and that quantitative variation in HSP synthesis may determine relative thermal tolerance levels (Frova and Gorla 1993).

Useful interpretation of the maize HSP inheritance studies (Frova et al. 1988; Ottaviano et al. 1991; Frova and Gorla 1993) may be difficult due to technical limitations. Certainly, the utilization of 1-D gel electrophoresis restricts protein synthesis analysis. This technique is inadequate for the analysis of complex gene families (Vierling 1991), such as the HSP17 and HSP18 families, which have multiple members that could vary in thermal tolerance capabilities. Another possible shortcoming of the 1-D gel technique combined with densitometric scanning was difficulty in distinguishing protein bands that differed only slightly in molecular weight, e.g. the LMW HSP17 and HSP18 families. Further technical difficulties may have occurred with unequal gel exposure times, which could prevent useful interpretation of 1-D gels due to illusory variability in protein synthesis levels. These factors may account for the absence of the HSP17 family in some parental protein synthesis patterns. Indeed, an analysis of work in our laboratory using 2-D gel analysis of maize inbred lines B37, A188, OH43, B73 and Mo17 detected several HSPs in the 17-kDa molecular weight range (unpublished results, and Jorgensen et al. 1992).

In this paper we report intraspecific polymorphisms and inheritance patterns of LMW HSPs in maize using high-resolution 2-D gel electrophoresis. The primary objective was to determine inheritance modes of LMW HSP synthesis in the F_1 hybrid and F_2 progeny of the heat-tolerant Mo17 and heat-susceptible B73 maize inbred lines (Jorgensen et al. 1992). We hypothesized that HSP synthesis in the B73 × Mo17 F_1 hybrid and its F_2 progeny would conform to Mendelian inheritance patterns. We used a powerful analytical approach that combined classical genetic methodology with the sensitive detection methods of [^{35}S]-methionine in vivo labelling of HSP synthesis and 2-D gel electrophoresis for visualization and analysis of protein profiles. This approach has previously been used for several genetic analyses of plant protein synthesis (Zivy et al. 1984; Damerval et al. 1987; de Vienne et al. 1988; Gottlieb and de Vienne 1988; Leonardi et al. 1988; Bahrman and Damerval 1989; de Vienne and Gottlieb 1990; Leonardi et al. 1991) but never to partition determinants of HSP synthesis. We have performed a comprehensive molecular genetic analysis of HSP gene transmission in maize. Further, we describe fundamental biological principles of the higher plant HS response. These results promise to facilitate investigations of HSP roles in the genetic control of heat tolerance.

Materials and methods

Materials

Seeds of an F_1 hybrid from the cross B73 × Mo17 were obtained from Pioneer Hi-Bred International, Inc., and were sown and grown to maturity in Lubbock, Texas. Hand pollinations for production of F_2 seeds were performed using a standard technique (Neuffer 1982). The F_2 seeds were harvested, completely dried and stored at 4 °C.

Individual F_2 seeds were sown in sterile soil mix and grown in a controlled environmental growth chamber (Conviron Model E-15, Asheville, NC) as previously described (Jorgensen et al. 1992) until temperature treatment at 4–5 days after emergence. The uppermost fully-expanded leaf was clipped from each plant for heat treatment and in vivo protein labelling.

Temperature treatments and in vivo labelling of protein synthesis

Seedling leaf tissue was heat-treated for 2 h to induce HSP synthesis. In vivo protein labelling, and protein extraction and solubilization were as described by Vierling and Nguyen (1990), with the following modifications. Analysis of both parents and the F_1 hybrid were repeated at least twice; however the individual nature of the F_2 plant material prevented replication. Leaf tissue was subjected to either 28 °C (control) or 40 °C HS temperature treatments for 1 h. The [^{35}S]-methionine (*Trans* [^{35}S]methionine-cysteine, 3.7 × 10^{13}

Bq/mmol, ICN Radiochemicals, Irvine, CA) label was vacuum-infiltrated, and the tissue samples were then heat-treated for an additional 1 h. Labelling of proteins was terminated by first washing the tissue twice with nonradioactive ("cold") 1 m*M* methionine and then with cold methionine under vacuum to dry the tissue. Labelled leaf tissue was immediately frozen, first in liquid N_2 and then at $-70\,°C$ until protein extraction. TCA-precipitable counts were determined as previously described (Mans and Novelli 1961).

Two-dimensional gel electrophoresis and fluorography

Two-dimensional gel electrophoresis was performed as described previously (Jorgensen et al. 1992). Protein samples were mixed with "UKS" solubilization buffer (Damerval et al. 1986). An equal number of radioactive counts (225,000 cpm) were loaded in first dimension isoelectric focusing (IEF) 1.5-mm tube gels and electrophoresed for a total of 21,000 volt hours (Tube Cell Unit Model 175, Bio-Rad Laboratories, Richmond, CA) using 0.1 *M* NaOH cathodic and 0.09% H_3PO_4 anodic buffers. Second dimension slab (16 × 20 mm) gels were electrophoresed at 10 mA/gel (Protean II, Bio-Rad Laboratories, Richmond, CA) using a continuous running buffer. Gels were enhanced for fluorography (Skinner and Griswold 1983), and X-Ray films (Hyperfilm, Amersham Corp., Arlington Heights, Ill.) were exposed for equal counts (3.0×10^6 cpm).

Analysis of gels

Inheritance of individual protein phenotypes in the F_1 hybrid was analyzed by spot intensity comparisons between the two parents and the hybrid according to Leonardi et al. (1988). Two-dimensional gels were visually scored by at least two independent observers using a lightbox and by computer scanning using the Biolmage Visage 2000 (Biolmage Products, Millipore/Biosearch, Ann Arbor, MI). The gels were normalized by comparing replicates of both parents and the F_1 hybrid. The gel scanning data were used to verify visually detectable protein synthesis levels. If quantitative variation in individual protein synthesis levels based on gel scanning was obviously spurious, it was rejected in favor of the null hypothesis. Protein spots were scored for presence or absence (qualitative variation), and for differences in intensity (quantitative variation). Scoring of the F_2 progeny segregation patterns was also based on the above classifications. Biolmage scanning was not applicable since the F_2 progeny were individuals and could not be replicated. No attempt was made to visually quantify continuous variations in protein synthesis levels in the F_2 progeny beyond determination of presence or high intensity, or absence or low intensity, since it was not possible to unambiguously separate intensity levels using visual analysis. Therefore, the discrimination between the homozygotic and heterozygotic classes for protein presence was not attempted.

Chi-square analysis of inheritance

Chi-square (χ^2) analysis was performed to determine goodness of fit of the B73 × Mo17 F_2 progeny HSP inheritance and gene assortment data. Hypotheses for dominant gene assortment and independent gene assortment were tested with critical values corresponding to 95% confidence levels.

Results

Analysis of HSP inheritance in B73 × Mo17 F_1 hybrid

In vivo-labelled HSP synthesis was compared in B73 and Mo17 maize inbred lines and in their F_1 hybrid. This analysis was limited to HSPs in the LMW range (between 15 and 30 kDa) since these proteins are most often correlated with acquired thermal tolerance and variation between these inbreds in mRNA in vitro-translatable LMW HSP synthesis has been demonstrated (Jorgensen et al. 1992). No HSP synthesis was detected in the patterns of control (28 °C) proteins among the two parental lines and the hybrid (data not shown). However, analysis of protein synthesis following a 40 °C HS for 2 h showed that both parents and the hybrid synthesized numerous HSPs, especially in the LMW range corresponding to Classes I and II HSPs between 15 and 18 kDa. Heat shock protein synthesis was compared in inbred lines Mo17 and B73 (Fig. 1a and b). In contrast to previous studies of in vivo-labelled HSP synthesis in maize in which the HSP17 family was conspicuously absent in some parents and hybrids (Frova et al. 1988; Ottaviano et al. 1991), in this study both parents and the F_1 hybrid synthesized several HSPs in the 17-kDa range. Many but not all

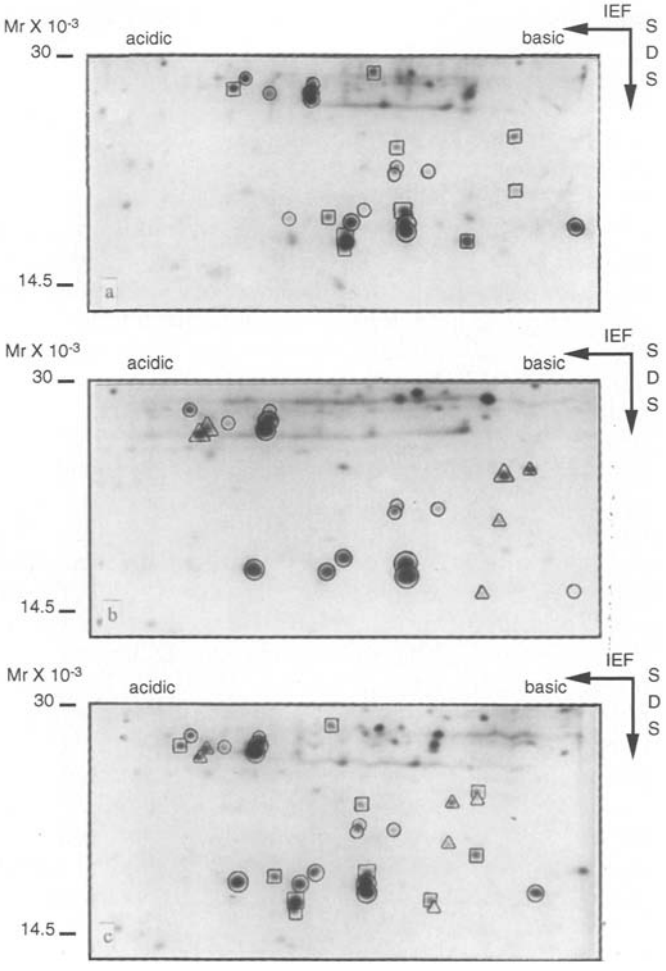

Fig. 1a–c *In vivo*-labelled HSP synthesis at 40°C. *Circles* represent HSPs common to both parental genotypes and the F_1 hybrid, *squares* represent HSPs present in Mo 17 and the F_1 hybrid, and *triangles* represent HSPs present in B73 and the F_1 hybrid. **a** Mo 17, **b** B73 **c** B73 × Mo 17 F_1 hybrid

parental HSPs observed in the synthesis patterns of in vitro-translatable poly $(A)^+$ RNA (Jorgensen et al. 1992) were also synthesized in vivo. As predicted from electrophoretic analysis of HSP in vitro translation products, major qualitative and quantitative polymorphisms were detected between B73 and Mo17 in vivo HSP synthesis patterns (Fig. 1). Analysis indicated 15 qualitative HSP variants between the inbreds, in which a protein spot was detected in one genotype but was apparently absent in another. Several other HSPs varied quantitatively between the two inbreds; it is likely they are encoded by allelic genes. The genetic nature of the qualitative parental variation is ambiguous since assignment as alleles or members of different families is difficult. Structural or regulatory gene variations could result in null alleles, nonfunctional proteins or proteins with reduced activity; allelic variation could be caused by nucleotide changes that alter the protein coding region, resulting in pI or molecular weight polymorphism (Damerval et al. 1987; Leonardi et al. 1988; de Vienne et al. 1988; Marmiroli et al. 1989).

Two-dimensional electrophoretic patterns of HSP synthesis in the F_1 hybrid indicated that all HSPs synthesized in the parents were also synthesized in the hybrid (Fig. 1c), and a composite schematic diagram shows that all LMW HSPs were synthesized in the hybrid (Fig. 2). The F_1 hybrid's HSP synthesis levels relative to both parents were analyzed, and examples of relevant optical densities were reported (Table 1). Several maize hybrids analyzed for organ-specific protein synthesis variability demonstrated the contribution of both parent's genomes to the F_1 hybrid's protein patterns (de Vienne et al. 1988; Leonardi et al. 1988). This is in direct contrast to electrophoretic patterns of protein synthesis in an interspecific tomato hybrid in which it was hypothesized that multiple allelic regulatory factors completely inhibited the expression of certain parental genes (Fender and O'Connell 1990). These examples demonstrated that allelic regulatory factors

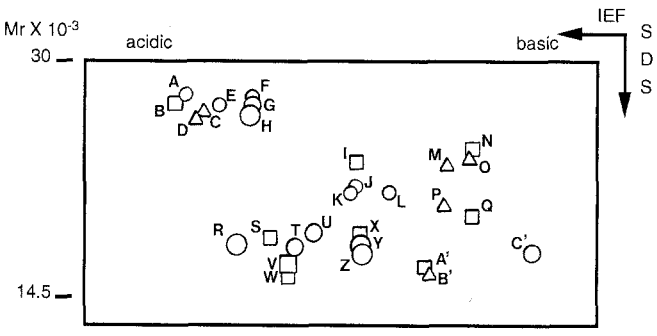

Fig. 2 Schematic of HSP synthesis. HSPs synthesized in Mo17 and B73 parents were the references. *Circles* indicate common HSPs, *squares* indicate HSPs synthesized in Mo17 and the hybrid, and *triangles* indicate HSPs synthesized in B73 and the hybrid

Table 1 Analysis of in vivo-labelled HSP synthesis in maize parents and their F_1 hybrid. Classes of parental variation and F_1 hybrid inheritance were determined.

[a] The symbols "plus" (+) indicated presence or high intensity, and "minus" (−) indicated absence or low intensity of a spot. Within the (+) designation for presence or high intensity of a protein spot, a single "plus" (+) indicated a protein spot similar to one parent, a "plus" with "minus" superscript (+ −) indicated an intermediate protein amount, and a "plus" with a "plus" (+ +) indicated a protein amount greater than either parent

[b] Optical densities for specific HSPs determined by Biolmage scanning, as discussed in the text

[c] When there was no variation between the parents and the F_1 hybrid, the inheritance was not discussed

HSP	Mo 17	B73	F_1	Parental variation	F_1 inheritance
A	+[a]	+	+	None	nd[c]
B	+	−	+	Qualitative	Dominant
C	−	+	+	Qualitative	Dominant
D	−	+	+	Qualitative	Dominant
E	+	+	+	None	nd
F	+	+	+	None	nd
G	+	+	+	None	nd
H	+	+	+	None	nd
I	+	−	+	Qualitative	Dominant
J	+	+	+	None	nd
K	+	+	+	None	nd
L	+	+	+	None	nd
M	−	+(3.932[b])	+ −(1.970)	Qualitative	Co-dominant
N	+	−	+	Qualitative	Dominant
O	−	+(2.512)	+ −(0.951)	Qualitative	Co-dominant
P	−	+	+	Qualitative	Dominant
Q	+(0.615)	−	+ +(2.876)	Qualitative	Over-dominant
R	−	+	+	Quantitative	Dominant
S	+	−	+	Qualitative	Dominant
T	+	+	+	None	nd
U	−(0.177)	+(7.259)	+ −(4.655)	Quantitative	Co-dominant
V	+	−	+	Qualitative	Dominant
W	+	−	+	Qualitative	Dominant
X	+(6.283)	−	+ −(2.730)	Qualitative	Co-dominant
Y	+	+	+	None	nd
Z	+	+	+	None	nd
A′	+(5.230)	−	+ −(1.473)	Qualitative	Co-dominant
B′	−	+	+	Qualitative	Dominant
C′	+(7.788)	−(0.130)	+ −(4.094)	Quantitative	Co-dominant

of both Mo17 and B73 determine gene expression in their F_1 hybrid. The results of this study indicated that the inheritance of maize HSPs, using Mo17 and B73 as parents, corresponded to either additive or nonadditive hypotheses of hybrid gene expression. No hybrid-speciic HSPs were detected in the F_1 hybrid, in contrast to an observation in barley (Marmiroli et al. 1989).

Analysis of HSP synthesis indicated that 11 of the hybrid's HSPs were sythesized in both parents and the hybrid at similar levels. It would not be instructive to discuss these HSPs further. Ten of the parental qualitative HSP variants were inherited in a nonadditive or dominant mode, since their observed levels in the F_1 hybrid were similar to those found in one parent (Fig. 3; Table 1). These results suggest that structural genes and genes involved in regulating the expression of these genotype-specific HSPs were inherited in the hybrid and did not interact in an inhibitory manner, since there were no alterations in the apparent HSP synthesis levels or their stability in the hybrid.

Several qualitative HSP variants (M, O, X, and A′) were inherited from the B73 and Mo17 parents in an additive or co-dominant mode, since protein levels observed in the F_1 hybrid were lower than those in the dominant parent (Fig. 4; Table 1). One hypothesis is that no allelic differences existed in structural genes, but the expression levels of this group of HSPs were modified by regulatory factors inherited from the recessive parent. Another cause of the reduced protein level in the hybrid may have been specific *trans*-acting regulatory factors that degraded or reduced the stability of the protein (de Vienne et al. 1988; Marmiroli et al. 1989).

Qualitative variant HSP Q was possibly inherited in an over-dominant mode (Fig. 4). Nonadditive overdominance may reflect interacting complementary regulatory factors inherited from both parents, which results in a higher F_1 hybrid HSP synthesis level or increased stability.

The B73 and Mo17 quantitative HSP variants were synthesized in both parents but at dramatically different levels (Fig. 5). Quantitative variant HSP R was inherited in a dominant mode in the F_1 hybrid. The hybrid's synthesis levels of quantitative HSP variants U and C′ were intermediate to B73 and Mo17 and inherited in a co-dominant or additive pattern (Table 1). Co-domi-

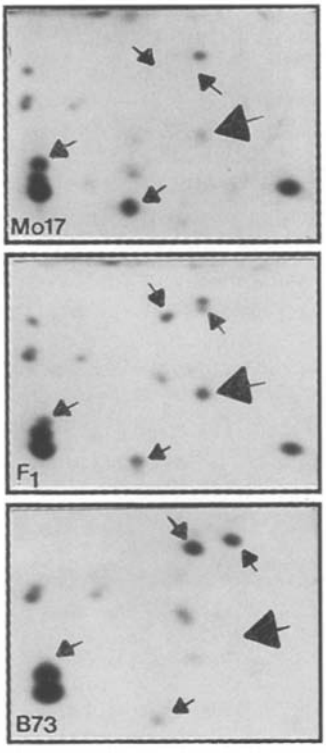

Fig. 4 Qualitative HSP variants inherited in a co-dominant mode in the hybrid. *Small arrows* mark co-dominant qualitative variants and *large arrows* mark the over-dominant qualitative variant

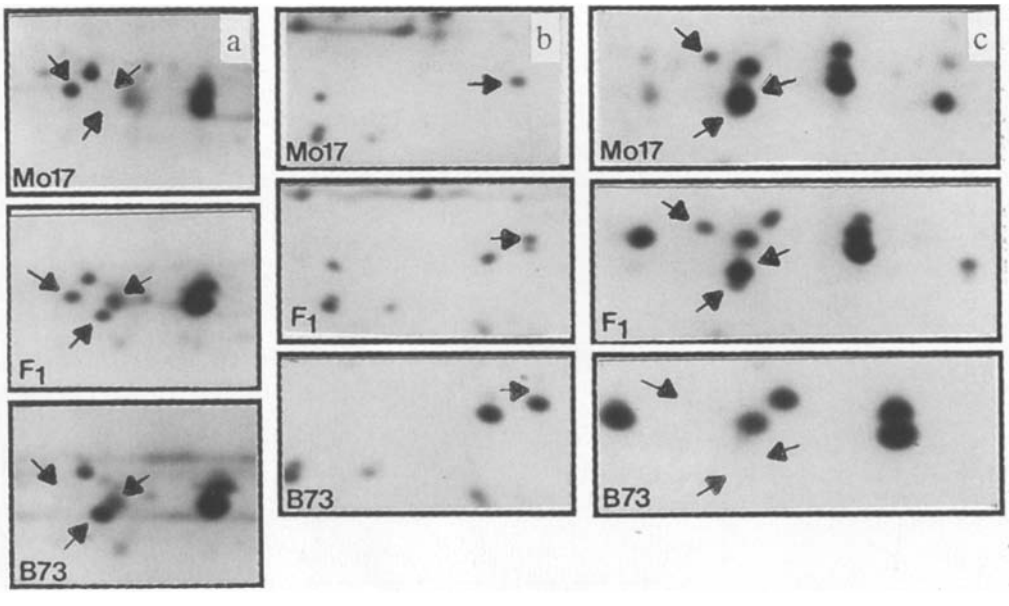

Fig. 3a–c Qualitative HSP variants inherited in a dominant mode. **a** Acidic HSPs of about 30 kDa, **b** basic HSPs of about 22–25 kDa, and **c** Classes I and II HSPs of about 15–18 kDa

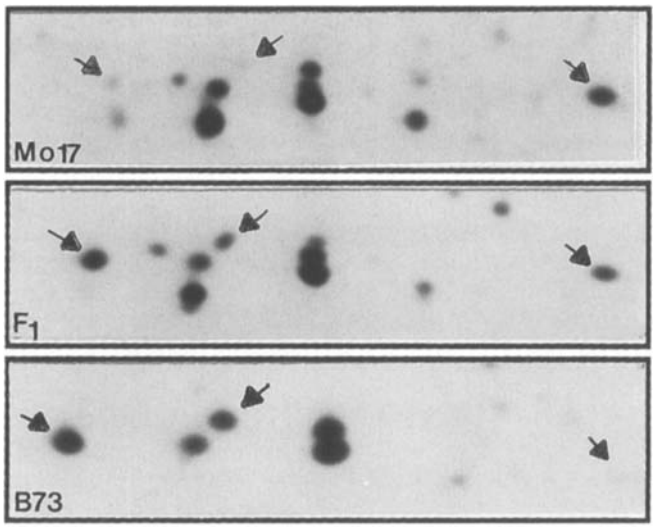

Fig. 5 Quantitative Classes I and II HSP variants, as marked by *arrows*

nant inheritance may be attributed to inherited inhibitory or degrading factors from one parent.

Analysis of HSP inheritance in B73 × Mo17 F_2 progeny

Two-dimensional gel electrophoresis patterns of B73 × Mo17 F_2 progeny were analyzed. Several individuals were utilized for the analysis of [^{35}S]-methionine in vivo-labelled protein synthesis at control (28 °C) temperatures. The results indicated that there were no detectable levels of HSP synthesis in the F_2 progeny during a non-stress temperature treatment (data not shown). Individual F_2 seedlings were heat-treated, and the in vivo-labelled HSP synthesis of 43 seedlings was analyzed. All the F_2 progeny synthesized HSPs, but there were dramatic polymorphisms in their electrophoretic patterns. High levels of HSP gene assortment were observed, and no F_2 individual's pattern of HSP synthesis was the same as another F_2 profile, or either parent's profile. Several examples of protein profiles demonstrate LMW HSP gene assortment in the F_2 progeny (Fig. 6). The qualitative LMW HSP analysis of all F_2 individuals is presented (Table 2).

Chi-square analysis of HSP phenotypic ratios was performed to determine whether HSP assortment patterns in the F_2 progeny follow the rules of simple Medelian genetics for dominant gene expression. Heat shock proteins selected for analysis included four probable Class I and Class II qualitative HSP variants (S, V, W, and X) and two quantitative HSP variants (R and U). All the χ^2 values were below critical values at 95% confidence levels. Therefore, the hypothesis that HSP assortment in B73 × Mo17 F_2 progeny followed a phenotypic 3:1 ratio could not be rejected in any case (data not shown).

A test of whether the same HSP genes assorted independently in the F_2 progeny was evaluated by χ^2

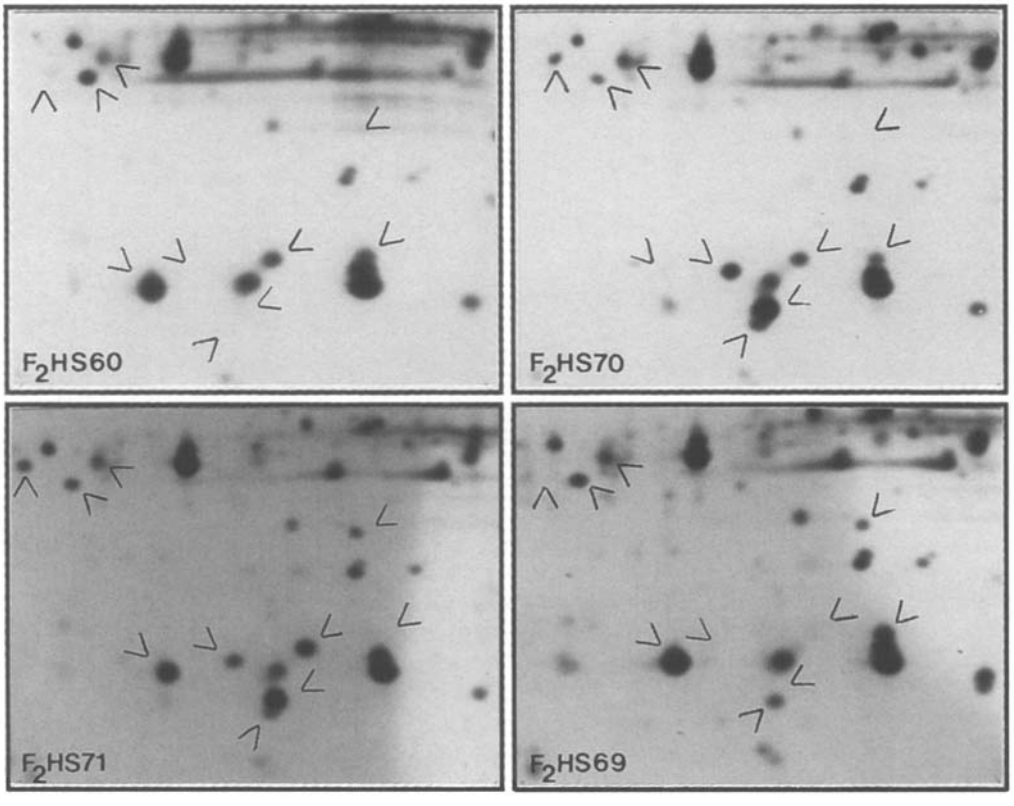

Fig. 6 Examples of HSP gene assortment in F_2 progeny. HSPs are indicated with *arrowheads*

Table 2 Analysis of in vivo-labelled HSP synthesis in B73 × Mo17 F_2 progeny. HSPs R, U, V, W, and X were analyzed for presence or high intensity (+) or absence or low intensity (−)

F_2 progeny[a]	HSPs					
	R	S	U	V	W	X
10	+	+	+	−	+	−
14	+	−	−	−	+	+
16	+	+	+	−	+	+
17	+	−	−	+	+	+
18	−	+	+	+	+	+
19	+	+	+	+	+	−
21	+	+	+	−	+	−
22	+	+	+	−	+	+
23	+	+	−	+	−	+
25	+	+	+	+	−	+
26	+	−	+	−	+	+
27	+	+	+	+	−	+
28	+	+	−	+	−	+
29	+	+	+	+	+	+
30	+	+	+	+	−	+
31	+	−	+	+	+	+
32	-	+	−	+	−	+
33	−	+	+	+	−	+
34	+	−	+	+	+	−
35	+	−	+	−	+	+
36	+	−	−	-	+	+
47	−	+	+	+	−	+
49	−	+	+	+	−	+
51	+	+	+	+	+	na[b]
52	+	−	+	−	+	+
54	+	+	−	+	−	+
55	−	+	+	+	−	−
56	−	+	+	+	−	+
57	−	+	+	+	−	+
58	+	−	+	−	+	−
59	+	+	+	+	+	+
60	+	−	+	−	−	+
61	+	+	+	+	−	−
62	+	+	+	+	+	+
63	−	+	+	+	+	+
64	+	+	−	+	+	+
65	+	+	+	+	+	+
66	+	+	+	+	−	+
67	+	+	+	+	+	+
68	+	−	+	+	+	+
69	+	−	−	−	+	+
70	−	+	+	+	+	+
71	+	+	+	+	+	−

[a] F_2 progeny were named numerically
[b] Data on HSP X were not available (na) in F_2 HS 51

analysis. Every possible pairwise combination of assortment was evaluated if the recessive phenotype indicated by absence or low level of both proteins was observed. In none of the assortment tests for HSP pairs R and U, R and W, S and U, and U and V was the hypothesis of independent assortment rejected. The results suggested that these pairs are products of unlinked gene loci that assort independently.

Chi-square analysis determined that expression of HSPs S and V, V and W, and S and W was inconsistent with a model of independent assortment. In each case the χ^2 value was higher than the critical value, which called for rejection of the independent assortment hypothesis. Alternate hypotheses include joint assortment predicted by two tightly linked gene loci between which meiotic crossover events are minimal, or increased assortment due to trans-acting regulatory inhibition of one gene's synthesis but not another. Linkage results in a higher percentage of parental and a lower percentage of recombinant phenotypes. *Trans*-acing regulatory inhibition of gene assortment at one or more levels of protein synthesis could potentially boost the percentage of recombinants to a higher level.

Heat shock proteins S and V expression in the F_2 progeny was marked by a higher number of parental phenotypes than the independent assortment hypothesis ratios allowed. These results suggested that the structural genes for the HSPs are linked and that there are no regulatory control factors that inhibit their joint expression. Further analysis of the linkage relationship between HSPs S and V genes will require precise physical mapping using RFLP analysis.

The hypothesis of independent assortment of HSP pairs V and W, and S and W was not rejected because of a higher proportion of parental phenotypes, but because of a higher than permitted proportion of recombinants. It was obvious, however, that they did not fit any of the standard ratios for epistatic interactions. HSPs V and W possess approximately the same pI, but a slightly different molecular weight. They are possibly encoded by the same gene, and the molecular weight variation is the result of post-transcriptional or post-translational modifications. Post-translational modification is unlikely, since distinct in vitro-translatable mRNA for both proteins was previously detected in Mo17 (Jorgensen et al. 1992). Heat shock proteins V and W could be products of the same gene, but undergo differentially regulated post-transcriptional processing events. Heat shock protein pair S and W also yielded non-Mendelian independent assortment ratios with χ^2 analysis. An explanation for rejection of both independent assortment and linkage is that regulation of these HSPs is controlled by assorting *trans*-acting factors that interact in the complex expression/repression of protein synthesis. RFLP analysis would be essential for resolving the linkage relationships among these HSP genes.

Discussion

Patterns of HSP electrophoretic profiles in maize inbred lines B73 and Mo17 and their F_1 hybrid and F_2 progeny suggested inheritance modes that conform to simple Mendelien assortment ratios. The results also indicated the complex roles of regulatory factors in HSP gene expression determination, resulting in complex progeny ratios. Interpretation of the genetic meaning of dominant, co-dominant and over-dominant expression of HSP synthesis is limited by our incomplete knowledge

of structural relationships among the HSP genes and the molecular regulation of their transcription and translation to protein (Leonardi et al. 1988; Marmiroli et al. 1989; Fender and O'Connell 1990). Four structural LMW HSP gene families have been identified in plants by nucleotide and amino acid similarities and protein localization criteria (Vierling 1991): one each encoding plastid-localized and endomembrane proteins, and two that encode cytoplasmic proteins (Classes I and II). The HSPs of 17–18 kDa comprise Classes I and II, and in maize several Class II gene sequences (Dietrich et al. 1991; Goping et al. 1991) as well as a Class I cDNA (Jorgensen and Nguyen 1994) have been identified.

Previously reported maize HSP 1-D gel inheritance data yielded oversimplified conclusions concerning intraspecific LMW HSP polymorphism and inheritance as well as the proteins' relationship to thermal tolerance. It was asserted that there is either a single structural gene responsible for HSP 17 synthesis (Frova et al. 1988) or if more than one gene, they are equivalent "strictly related genes" (Frova and Gorla 1993). Little attention was given to the complexity of the LMW HSP families and to the fact that it has not yet been possible to isolate the effects of a single HSP in thermal tolerance. It is certainly premature to functionally equate all members of a specific LMW HSP family. Further, it is unlikely that thermal tolerance can be attributed primarily to increased levels of several constitutively synthesized HSPs. Considerable qualitative intraspecific genetic variability of HSP synthesis has been reported in maize (Jorgensen et al. 1992). This observation should perhaps moderate the concepts that quantitative variation in HSP synthesis is the principle response during heat stress and paramount in thermal tolerance (Frova and Gorla 1993). Since little is known about HSP regulatory factors and their interactions with HSP structural genes, we find it unprofitable to speculate whether variations in parental HSP regulatory control factors would be more likely to affect quantitative variation in HSP synthesis than HSP presence or absence (qualitative variation) (Frova and Gorla 1993).

We were able to draw conclusions from 2-D gels regarding HSP inheritance modes in the F_1 hybrid and gene assortment in the F_2 progeny. This highly sensitive protein analytical approach permitted the detection of numerous HSPs that comprise a phenotype. Synthesis of all parental HSPs in the F_1 hybrid established nonadditive and additive HSP gene expression in a hybrid. The results argue against the hypothesis of regulatory inhibition that suppressed HSP gene expression. The F_1 hybrid synthesized all parental HSPs corresponding to variations of dominant inheritance, including complete dominance, over-dominance and co-dominance. Heat shock protein gene expression in the hybrid may predict a high level of tolerance in agreement with the dominance theory of heterosis. The predicted level of thermal protection could be at least as high as the tolerant genotype Mo17. An investigation of the relationship between HSP synthesis and acquired thermal tolerance in the B73 × Mo17 F_1 hybrid may enhance understanding of the genetic nature of heterosis and the commerical value of this hybrid.

Heat shock protein synthesis patterns in B73 × Mo17 F_2 progeny indicated that HSP alleles assorted according to the 3:1 ratio predicted for dominant gene expression. Detection of patterns corresponding to the predicted ratio is remarkable considering the complex interaction of structural and regulatory genes in protein synthesis. The results indicated unlinked loci for four different pairs of HSP genes. The results of the independent assortment analysis of the other three pairs of HSP genes were inconclusive, perhaps reflecting linkage for one pair and complex regulatory factor interactions for the other two pairs of genes.

Our results demonstrated Mendelian assortment of HSP genes and indicated the potential of partitioning roles of HSPs in thermal tolerance using genetic and molecular analyses. The recombinant inbred lines (RILs) produced from selfing B73 × Mo17 F_2 plants will provide the plant material required for a more extensive analysis of thermal tolerance and HSP synthesis. Perhaps the most effective molecular genetic strategy for investigation of the relationship between HSP synthesis and thermal tolerance in the RILs would be utilization of either gene-specific HSP probes or monospecific HSP antibodies to determine whether a HSP is directly related to thermal tolerance. The RIL material will also be useful for identifying the genetic factors influencing heat tolerance in maize using DNA marker analysis, such as RFLP and random amplified polymorphic DNA.

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