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Quantitative expression of maize HSPs: genetic dissection and association with thermotolerance

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Summary. In higher plants, within-species qualitative polymorphism for heat shock proteins (HSPs) is extremely rare, even between genotypes showing different heritable levels of thermotolerance. Here we have explored the amount of quantitative variability in HSP synthesis in maize. We have analyzed the quantitative expression of the typical HSPs in a set of recombinant inbreds (RIs) derived from the F_1 hybrid between a thermotolerant (T232)- and a thermosensitive (CM37)-genotype, characterized for about 200 mapped RFLP loci. Significant differences were detected in the level of expression of five HSPs, and their frequency distribution in the RI population is that of a quantitative trait. Subsequent mapping of loci controlling the characters, based on RFLP analysis, confirmed the multigenic control of HSP expression: the regression analysis of the band intensities of each variant HSP on RFLPs revealed, for the different HSPs, a minimum number of three to eight quantitative trait loci (QTLs) accounting for a high proportion (0.35–0.60) of the genetic variability of these bands. An analysis of the correlation between the variability of HSPs and that of cellular membrane stability, a cellular component of thermotolerance, did not reveal any significant association of the two parameters.

Key words: Heat shock proteins – Quantitative expression – Restriction fragment length polymorphism – Thermotolerance – Maize

Introduction

Significant deviations from optimal growth temperature, and in particular high temperature stress, are among the main factors affecting plant development and growth in nature and can be responsible for severe yield losses in cultivated crops (Boyer 1982, Christiansen 1982).

The ability of plants to withstand high temperature is due to a wide range of mechanisms acting at different levels of plant organization (Levitt 1980). The cellular component of thermotolerance is defined as the mechanism(s) providing protection to metabolism and cell structure when plants are exposed to temperature stress. The molecular and physiological bases of the phenomenon are not yet understood, but a large body of evidence indicates that cell thermotolerance is a complex character, the variability of which is controlled by a large number of genes (Ottaviano and Sari Gorla 1988).

Among the molecular factors involved in the development of thermotolerance, heat-shock proteins (HSPs) are generally assumed to play a prominent role. Their involvement in the short-term adaption of plants to high temperature stress is supported by indirect evidence, such as the correlation between heat hardening and HSP synthesis and between the kinetics of HSP synthesis and of thermotolerance induction (Lin et al. 1984; Nagao et al. 1990).

Genetic variability in thermotolerance is often found both within and between species (Blum 1985). HSP genetic variability, on the other hand, has been observed between species (Mansfield and Key 1987; Vierling 1991), but is much less evident within species, even in the case of genotypes differing in their heritable thermotolerance (Fender and O'Connel 1989). However, the data in the literature refer almost exclusively to qualitative variability, i.e., the presence/absence of specific HSPs or a change in their molecular weight, while the quantitative variations existing in genotypes or species have not been precisely explored. There are, however, some reports of quantitative HSP variability. For instance, in sorghum, a

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thermotolerant species well-adapted to tropical areas, overall HSP synthesis appears to be more pronounced than in maize, although qualitatively very similar, (Frova et al. 1991). In corn, a screening of several genotypes has revealed, to visual inspection, the existence of quantitative differences with regard to several HSPs (Frova et al. 1988; Ristic et al. 1991). Moreover, quantitative HSP variability is to be expected for the following reasons:

(1) Several HSPs are also expressed at normal temperatures. Their synthesis is, however, dramatically increased, upon heat-shock, suggesting that the amount, and not the mere presence, of HSPs may be a critical factor in cellular thermoprotection.

(2) Differences in regulatory sequences and/or genes would result mostly in quantitative rather than qualitative HSP variability.

(3) Many HSP bands detected by 1-D electrophoresis are further resolved into several polypeptides when analyzed in 2-D gels. Furthermore, the characterization of HS cDNA and genomic clones has demonstrated, for most HSPs, the existence of families of strictly related genes. Therefore, even if some of the multiple spots detected in 2-D gels could represent post-translational modifications and/or degradation of a single gene product, several are expected to be individual polypeptides coded by related genes. In that case, the intensity of a single HSP band would be determined both by the rate of expression and by the number of the hs genes involved, and so vary accordingly.

At the physiological level, cellular membrane stability (CMS) is considered to be a major component of cell thermotolerance: it is in fact one of the best physiological indicators of heat and drought tolerance, and, as such, is widely used to predict a plants performance under heat stress. CMS variability has been detected in several plant species. In maize, it has been shown (Ottaviano et al. 1991) that the character exhibits the typical features of a quantitative trait and has a high heritability ($h^2 = 0.73$). The genetic dissection of CMS, carried out by RFLP analysis, has indicated that its genetic variability is controlled by at least six quantitative trait loci (QTLs), the chromosomal locations of which have been identified (Ottaviano et al. 1991).

The recently reported chaperon-like function of some HSPs (Pelham 1986, 1990; Vierling 1991), i.e., their role in preventing protein misfolding, denaturation, or aggregation during stress, suggests that this could be a factor involved in maintaining CMS. An initial study in maize (Ottaviano et al. 1991) failed to detect any correlation between qualitative HSP polymorphism and CMS variability. However, the level of thermotolerance, evaluated as CMS, may be largely due to quantitative, rather than qualitative, expression of the genes controlling HSP synthesis. Consequently, this aspect has to be taken into account when considering the possibility of the existence of causal relationships between HSP synthesis and thermotolerance.

In the present paper we have analyzed the quantitative expression of the major HSPs in a set of 44 recombinant inbred lines (RIs) of maize, for which a wide CMS variability has already been demonstrated. Evidence is presented of quantitative polymorphism for five HSPs. Furthermore, by RFLP analysis, several QTLs accounting for a considerable proportion of the genetic variability of these bands were identified and their chromosomal location detected. A correlation analysis of HSP and CMS variability was performed: the results do not point to any major involvement of HSPs in the determination of CMS.

Materials and methods

Experimental design, HSP induction and quantitative evaluation

A set of recombinant inbred lines (RIs) was used in this study. These lines, derived from repeated selfing of the F_1 between two highly diversified lines (T232 and CM37), were kindly provided by Dr. B. Burr, who has characterized each RI for about 200 RFLP loci (see Burr et al. 1988). Quantitative evaluation of HSP synthesis was carried out on 44 F_6 RIs, the two parental lines, and their F_1. The experimental design included all the genotypes in three complete replications. Seeds were surface sterilized in 7% NaOCl, rinsed with distilled water, and germinated in the dark at 25 °C in glass trays on moistened filter paper. For each genotype, the primary root of 4-day-old intact seedlings was soaked in wells containing 0.75 ml distilled water and incubated in the dark at 41 °C for 3 h. Five roots were pooled in each well. Ten microliters of ^{35}S-Methionine (specific activity >1.000 Ci/mmol) was added after the first hour of incubation. A set of parental and F_1 seedlings were labelled in the same way but maintained at 25 °C throughout the treatment and served as a control to identify HSP bands on the gel. After labelling, 1-cm long primary root tips were excised from five seedlings/genotype/treatment, rinsed in water, blotted dry and homogenized in 1 ml 0.05 M Tris-HCl pH 7.5, 1 mM phenylmetylsulphonylfluoride. All extraction steps were carried out at 4 °C. Processing of the homogenate and determination of label incorporation into protein, gel electrophoresis and autoradiography were performed as previously described (Frova et al. 1989), with the following modifications: 100,000 cpm were loaded in each lane, and the dried gels were exposed with an Amersham RPN 7 film for 3–4 days at −80 °C. Exposure time was critical since overexposed films yielded confusing results in reading the bands. In each complete replication, three 20-lane gels were run. Each gel included, besides a marker lane, one control (25 °C) F_1 and three heat-shock (P_1, P_2, F_1) lanes; extracts from the RIs were loaded on one of the three gels in a randomized order. Thus, in the whole experiment, P_1, P_2 and F_1 were represented nine times and the RIs three times each.

A quantitative estimate of HSP expression was made using a CAMAG densitometric scanner. Each lane on the autoradiographs was scanned by the instrument with a laser beam capable of distinguishing bands differing by 2 kDa (for instance 84 and 82 kDa), and the intensity of each protein band was expressed as a percentage of the total protein intensity on the lane. HSP peaks were then identified and their relative value used as the measure of quantitative band expression. At the beginning of

the analysis, two non heat-shock proteins present in every lane (control and heat-shocked) were measured and considered as internal controls. The value of each HSP in the lane was then standardized with these two reference bands. However, since the value of the two reference bands did not vary significantly between blocks, gels, or genotypes, this procedure was abandoned and the absolute values (% total proteins) of each band was used for the final analysis.

Statistical and RFLP analysis

Since the band intensities were measured as proportions of the total protein on the lane, the data were submitted to angular transformation.

For the analysis of band intensity variability, an analysis of variance was performed, where block and gel effects were taken into account. The same procedure was used for the comparison of band intensities between the two parental lines and between parentals and F_1s.

The detection of the chromosomal regions where loci controlling the HSPs are located was based on the association between a single band intensity and the allelic composition at each restriction locus. The evaluation was made by regression analysis of the mean value of each band intensity on the allelic composition of each locus. The regressors assumed the numerical value of 1, 2 or 1.5, according to the presence of the two alleles of the first parental line (T232), the two of the second (CM37), or of one of each (heterozygous locus), respectively.

In order to avoid false assignments, the correlation matrices between all the loci that were significant, both within and between chromosomes, were computed. For each band, only the significant loci that proved to be non-correlated were included in a multiple regression analysis, on the basis of which the cumulative contribution of these loci to the trait expression was evaluated.

All the statistical analysis was performed according to SAS GLM procedure, adopting the most suitable model (ANOVA or regression analysis).

Results

Figure 1 shows an example of the pattern of newly synthesized proteins at 41 °C and the relative densitometric profile. The densitometric data were computed by an Hitachi cromointegrator connected with the scanner, and expressed as a percentage of the total protein area. These figures were then used for the statistical analysis.

Of the nine typical maize HSPs (102, 84, 82, 72, 70, 27, 24, 22, 18 kDa), two, 72 and 70 kDa, were not clearly distinguished by the scanner. This is probably due to the fact that the 70 kDa HSP is very dense and tends to overlap with the 72 kDa band. They were considered therefore as a single band termed HSP 70. The mean intensity of each band in the two parental lines and in the F_1 is illustrated in Fig. 2. The analysis of variance, not reported here for brevity, indicated significant differences between genotypes in the intensity of the 84, 27 and 22 kDa HSPs. The difference was significant also when considering only the two parental lines. The F_1 does not show a consistent pattern. In the case of HSP 84 the F_1 value is slightly higher than that of either parent, while for the low molecular weight 27 and 22 HSPs its value is very similar to that of the weaker parent. For these two last HSPs the highest band intensity was found in the inbred T232. It is notable that, of the two parental lines, T232 is characterized by the higher CMS (Ottaviano et al. 1991).

HSP analysis of the 44 RIs revealed variability of the trait (band intensity) for five bands: 84, 82, 27, 22, and 18 kDa, two more than those showing significant differ-

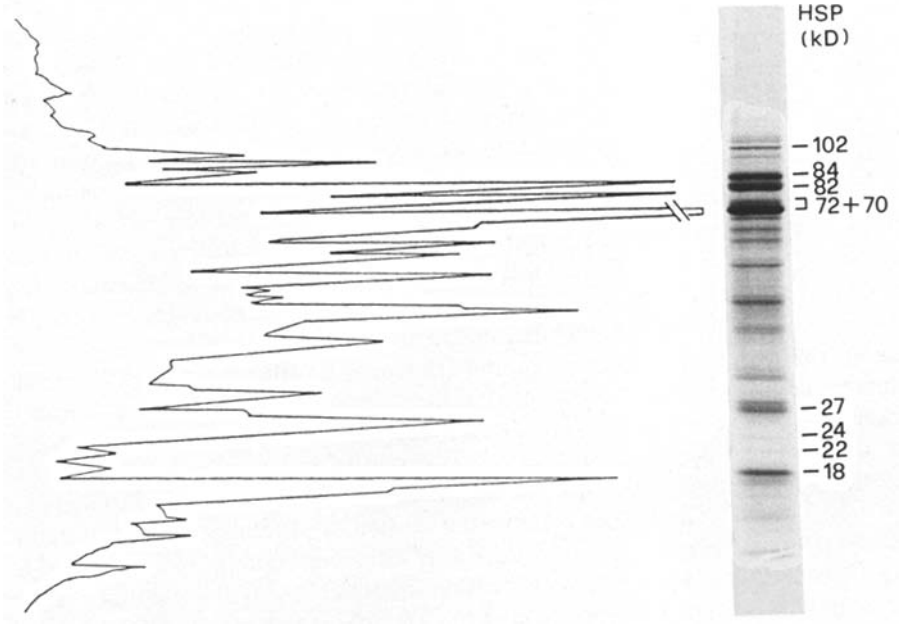

Fig. 1. Densitometric profile of newly synthesized proteins in maize root tips after heat shock (redrawn from the original scanner output). Values of integrated areas not reported

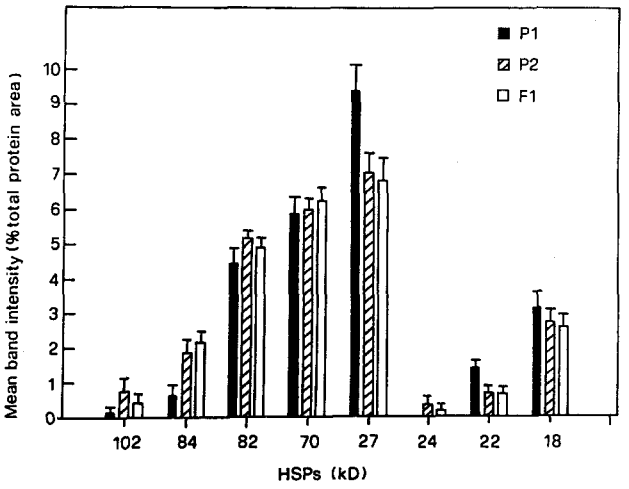

Fig. 2. HSP mean band intensity in P_1 (T232), P_2 (CM37) and F_1. Bars = SE

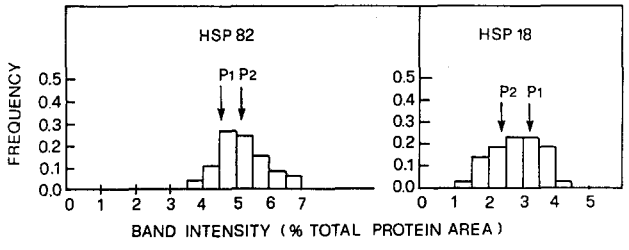

Fig. 3. Recombinant inbred frequency distribution for two HSP band intensities. *Arrows* indicate mean values of the two parental lines: P_1 = T232, P_2 = CM37

Table 1. Mean squares and significance levels from the analysis of variance of the 84, 82, 27, 22 and 18 kDa in HSPs recombinant inbreds

Source	*df*	HSP band (kDa)				
		84	82	27	22	18
Gentoype	43	2.59**	1.26*	3.26*	0.31**	0.92*
Error	78	1.06	0.79	1.89	0.13	0.58

*, $P<0.05$; **, $P<0.01$

ences in the parental genotypes (Table 1). This result can be explained by postulating recombination and segregation of several genes affecting the character during the inbreeding process ($F_1 -> F_6$). In fact, the frequency distribution for these additional bands, shown in Fig. 3, is that of a typical quantitative trait.

Analysis of the association between HSP intensity and each RFLP locus revealed a significant regression for several restriction loci in the case of the five variant bands. The results are listed in Table 2. R^2 values estimate the amount of phenotypic variation between genotypes explained by the allelic substitution of that RFLP-specific locus, while the sign of the coefficient of regression indicates to which of the parental alleles an increase in the character is attributable. Since P_1 and P_2 are T232 and CM37 respectively, a positive sign indicates that the allele increasing the character is that of the CM37 parent, a negative sign that of the T232 parent. R^2 values ranged from 0.09 to 0.30, indicating that a considerable proportion of the variability of band intensity is explained by variation at single RFLP loci. A graphical representation of the data, which also indicates the chromosomal position of the significant RFLP loci for each HSP band, is given in Fig. 4. The genetic map thus obtained shows that, in several cases, significant loci are clustered together.

For a correct interpretation of these results, it must be borne in mind that a cluster of significant correlations does not necessarily mean that several QTLs map in the same chromosomal region. In fact, it could also be the result of physical linkage between adjacent RFLP loci, only one of which associated with a QTL. Moreover, statistical correlation between loci belonging to different chromosomes (in theory expected to equal zero) may also occur, because sampling variation during inbreeding can produce apparent linkage between restriction loci. False assignments can be avoided, however, by the analysis of the correlation matrices including all the significant loci. Following this procedure, loci whose significant association with the quantitative trait could be due to their association with another marker strictly linked to the QTL, were discarded. Additional information for estimating the minimum number of factors within a chromosomal region can be obtained by the sign of the regression coefficient (Table 2). When all the loci in a cluster have the same sign, it is likely that they belong to a linkage block (inherited from the same parent), in which the presence of one or more QTLs cannot be distinguished. On the other hand, a different sign (see, for instance, loci 7.21, 1.556 and YNH20, *Phi* on chromosome 1 for HSP 27), means that the loci increasing the value of the character derive from different parents and should therefore indicate the presence of different QTLs.

According to these criteria, for each character (single band intensity), in each cluster of correlated loci with the same sign, only the locus showing the highest R^2 value was counted in the calculation of the minimum number of QTLs determining each HSP. The same loci were included as regressors in a multiple regression analysis model: the coefficients of determination thus obtained then measure the amount of variability of the trait monitored by the RFLP loci included in the model. The high values of the resulting R^2 (Table 3) indicate that a large proportion of the phenotypic variation in HSP intensity

Table 2. RFLP loci showing a significant effect on HSP band intensity. R^2, proportion of between line variability; b, estimated effect

HSP band (kDa)	Chromosome	Locus	R^2	b
84	1	5.59	0.11	−0.70
		NPI225	0.09	−0.58
		Adh1	0.08	−0.59
	4	ZPLIB	0.17	−0.79
		ZPLID	0.16	−0.78
	5	10.06	0.11	0.72
		7.43	0.10	0.65
	6	*Idh2*	0.12	0.69
		Mdh2	0.10	−0.65
	8	*Mdh1*	0.10	−0.67
		10.39	0.11	−0.69
		9.44L	0.09	−0.61
	9	NPI253	0.16	0.78
		C1	0.11	0.64
		Sh1	0.17	0.82
		Bz1	0.15	0.77
		3.06	0.17	0.84
		Wx1	0.21	0.94
	10	NPI264	0.12	0.73
82	1	YNH20	0.15	0.57
	3	15.20	0.14	0.57
		6.16	0.16	0.67
	4	7.65	0.18	−0.64
		10.05	0.19	−0.64
	7	*O2*	0.09	0.46
		15.21	0.12	0.52
	8	13.05	0.19	0.70
		9.11	0.30	0.83
		Mdh1	0.10	0.51
		7.08	0.13	0.54
		17.01	0.12	0.50
		10.24B	0.15	0.57
		NPI268	0.10	0.48
27	1	7.21	0.16	0.87
		1.556	0.10	0.74
		YNH20	0.12	−0.73
		Phi	0.09	−0.62
	2	NPY239	0.10	0.70
		DIA1	0.12	0.68
	3	5.37	0.10	0.74
	4	NPI451	0.14	0.83
	5	7.56	0.10	−0.66
	7	15.40	0.18	−0.09
		ZP50	0.15	−0.81
		ZPB36	0.12	−0.74
		6.27	0.11	0.73
		13.24	0.11	0.73
		16.06	0.10	0.69
	8	10.24B	0.18	−0.90
		NPI268	0.12	−0.77
22	3	6.06	0.09	0.23
		6.16	0.10	−0.27
		5.33	0.11	−0.24
		1.297	0.09	−0.22
	4	*Adh2*	0.13	0.26
		7.65	0.17	0.31
		10.05	0.20	0.32
		17.05	0.10	0.23
		C2	0.09	0.22
		5.67	0.10	0.22
	7	13.24	0.13	0.27
	8	NPI220	0.10	−0.24
		13.05	0.15	−0.31
		9.11	0.26	−0.38
	9	14.28	0.09	−0.22
		5.09	0.09	−0.21
		7.57	0.10	−0.27
		NPI209A	0.14	−0.27
18	1	*P1*	0.11	0.47
		YNH21	0.14	0.54
	2	NPI271	0.16	0.62
	4	7.65	0.13	0.52
		10.05	0.11	0.48
	7	4.24	0.12	0.51
	8	13.05	0.12	−0.54
		10.24B	0.18	−0.62
		NPI268	0.16	−0.59
		10.12U	0.11	−0.48

is due to association with the QTLs linked to the molecular markers (RFLPs).

The effect of the quantitative expression of heat-shock proteins on cellular membrane stability was investigated by means of an analysis of the correlation between HSP band intensities and the level of membrane injury produced by high temperature in the 44 RIs. This last parameter, measured as ion leakage, had been previously determined (Ottaviano et al. 1991).

No significant correlations were detected for any HSP considered either individually or in two groups according to their high (84 and 82 kDa) or low (27, 22 and 18 kDa) molecular weight. However, in this last group, the r value and sign (−0.116, −0.228, −0.1 for HSP 27, 22 and 18, respectively) indicate the existence of a weak correlation. These data suggest that HSP quantitative expression is not a major factor in determining the variability of heat-shock injury in the membrane, although a minor role of the low molecular weight HSPs can be hypothesized.

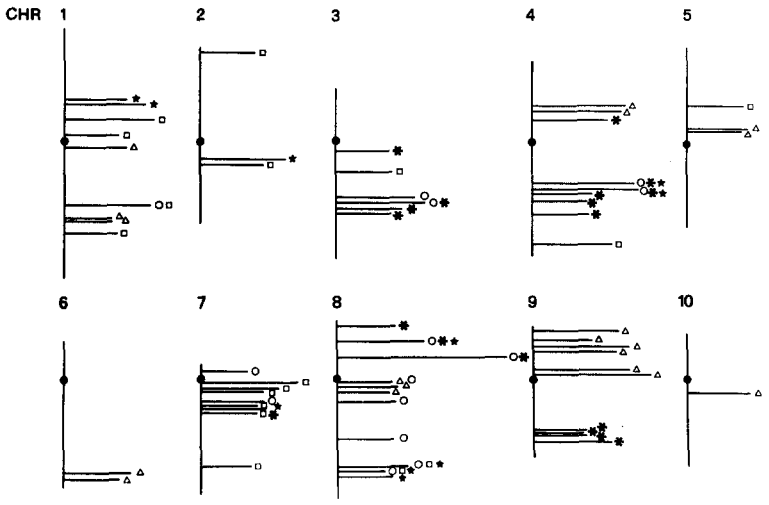

Fig. 4. RFLP analysis of the quantitative expression of five HSPs in recombinant inbreds from T232 × CM37 F_1 hybrid. *Horizontal bars* indicate degree (R^2) of correlation between RFLP loci and HSP band intensity. Only significant loci are reported. Symbols: △ = HSP84 kDa, ○ = HSP82 kDa, □ = HSP27 kDa, * = HSP22 kDa, ✱ = HSP18 kDa

Table 3. Non correlated RFLP loci showing significant effect on HSP band intensity. R^2 = coefficient of determination

HSP band (kDa)	Chromosome	Locus	R^2
84	4	ZPLE	
	6	*Idh2*	
	9	*Wx1*[a]	0.35
82	1	YNH20	
	4	10.05	
	7	15.21	
	8	9.11	0.52
27	1	7.21[a]	
		YNH20	
	2	DIA1	
	3	5.37	
	7	15.40	
		13.24	
		16.06	
	8	10.24B	0.60
22	3	6.06	
		5.33	
	4	*Adh2*	
		10.05	
	7	13.24	
	9	9.11	0.55
18	2	NPI271	
	4	7.65	
	8	10.24B	0.50

[a] Loci associated with QTLs affecting CMS

Discussion

The first point established by this work is the existence of an appreciable amount of within-species quantitative variability in HSP expression. The result is important, given the general lack of qualitative HSP polymorphism in plants, which has so far been a serious obstacle to the discovery of a direct relationship between these proteins and cellular thermotolerance. In fact, the two parental lines here considered, although well differentiated for CMS, a major component of cellular thermotolerance, both synthesize the same typical set of HSPs. However, they reveal significant differences in the intensity of three HSPs, 84, 27 and 22 kDa. As can be seen in Fig. 2, the highest values of band intensity for these HSPs are not clustered all in one parent: CM37 is characterized by a more intense 84 kDa HSP, while T232 shows a higher value for the 27 and 22 kDa HSPs. Since inbred T232 is the more thermotolerant of the two lines, these data support the view that, in plants, low molecular weight HSPs play a particular role in the determination of thermotolerance. Plants differ from most eucaryotes in that they synthesize an unusually large number of low molecular weight HSPs, whose expression is not constitutive but is strongly induced only under high temperature stress (Mansfield and Key 1987; Vierling 1991). A different situation is found in the case of high molecular weight HSPS, in particular the 70 kDa HSP, for which cognate genes actively expressed also at normal growth temperatures have been identified in several plant species (Rochester et al. 1986, Winter et al. 1988; Wu et al. 1988, Chen et al. 1990). This indicates that while high-molecular-weight HSPs may have a more general function in the cell metabolism, the group of low-molecular-weight HSPs may serve more specifically for the protection of cell structure and cell metabolism in stress conditions. From this point of view, it is of interest that HSP 70 does not show significant differences in intensity between the thermotolerant and the thermosensitive parental lines.

The analysis of HSP band intensity in the 44 RIs disclosed a significant difference for two further proteins, the 82 and 18 kDa HSPs. This result, together with the frequency distribution of the band values between lines (Fig. 3), suggests a multigenic control of HSP expression.

Comparable indications come also from the complexity of the HSP electophoretic pattern often detected in 2-D analysis.

The results of the RFLP analysis presented in this paper provide unequivocal proof of the involvement of several genes in determining band intensity variability. For each of the five HSP bands analyzed, three to eight QTLs have been identified. Moreover, the procedure adopted to avoid false assignments (see Materials and methods) gives a very conservative estimate since, in all cases in which the presence of one or more QTLs in the same chromosomal region could not be distinguished, only one was considered. The real number of single QTLs controlling the traits is likely to be larger, as can be seen by careful inspection of the genetic map. For instance, for HSP 22, a cluster of four significant loci all mapping in a small region (approximately 10 cM) on the long arm of chromosome 9, showed a statistical correlation with another cluster on chromosome 4. For this reason they were considered as a single region, but it is reasonable to hypothesize that they represent more than one distinct QTL effect. The same reasoning applies in those cases, such as the RFLP loci on chromosome 8 for HSP 82, 22 and 18, on chromosome 2 for HSP 27, and on chromosome 7 for HSP 82, where the significant molecular markers map to quite different regions of the same chromosome.

The R^2 values obtained taking into account the lowest possible number of QTLs are nonetheless quite high (from 0.35 to 0.60), indicating that a large proportion of the variability of each band intensity is explained by the association with the identified QTLs.

A closer look at the significant RFLP loci reveals that some of them are common to several HSPs (see, for instance, locus 13.05 on chromosome 8), suggesting a possibly regulatory function of the associated QTLs. The fact that no RFLP locus is significant for all HSPs may reflect differences in the mechanisms of regulation. However, it could also be that the regulation of HSP expression is associated with QTLs having a minor effect, whose contribution is not always statistically detectable. This could be the case, for instance, for the QTL located in the region of chromosome 7 identified by a cluster of loci which are significant for HSPs 82, 27, 22 and 18, and fall short of significance for HSP 84 (data not shown).

In plants, the role of HSPs in thermotolerance is still an open question. This is mainly due to the lack of evidence pointing to a contribution of HSPs to the documented heritable differences in resistance to high temperature (see Vierling 1991 for review). An alternative approach to the problem is to determine whether HSPs map in the same position as QTLs for heat tolerance. However, map information about HSPs and these QTLs is, at least in maize, very scant. To our knowledge, only *Hsp1* (coding for HSP 70) has been tentatively located on chromosome 8L (Wright et al. 1987), and the only mapped QTLs controlling heat tolerance components are those affecting CMS variability. A comparison between the chromosomal location of these and that of the QTLs affecting HSP quantitative expression reveals the existence of two common regions on chromosomes 1 and 9 (Table 3), identified by the RFLP loci 7.21 and *Wx1* respectively (for the loci affecting membrane injury see Ottaviano et al. 1991).

The analysis of correlation between membrane injury and HSP variability in the RIs did not reveal any major effect of the qualitative expression of single HSPs on cellular membrane stability. The same result was obtained by Ottaviano et al. (1991) in a study of the effect of quantitative HSP polymorphism (presence/absence of the 17 kDa band in a segregating F_2 population) on CMS. However, the data presented here still support the involvement of low molecular weight HSPs in thermotolerance determination: for HSP 27, 22 and 18 the coefficient of regression, although not significant, had the expected negative value (electrolyte leakage, the parameter adopted as a measure of membrane injury, is inversely related to CMS). Furthermore, as explained above, CMS and a few HSPs could be, at least in part, controlled by the same QTLs. The complex genetical basis of both CMS and HSP quantitative expression could explain the failure to detect a clear association between the variability of the characters: if the variation in one trait (HSP) influences only a component of the second (CMS), then the effect on its overall expression would be too small to be statistically detected.

A final consideration regards the power of RFLP analysis of RIs for the mapping of QTLs. Theoretically, the larger the number of RI families and of RFLP loci, the more accurate the analysis will be. The progeny size (here the number of RIs) required to obtain accurate estimates of the genetic location and phenotypic effects of QTLs depends on a number of parameters, including the difference, measured as standard deviation (SD), between the parental genotypes and the degree of saturation of the RFLP map (Lander and Botstein 1989). In the present study, 180 polymorphic RFLP loci were used, which means, on average, one molecular marker for every 8–10 cM. This can be considered a well-saturated map. With regard to the progeny size, the 44 RIs that were available fall somewhat short of the optimal number. In general, a reduction in the RI families is not expected to greatly impair the accuracy of the information obtained if the parental lines are widely different for the character considered (as was the case for CMS). When, however, the mean values of the parental lines are closer (as in the case of HSPs), then the size of the RI population may be critical, and it is therefore possible that differences between the RIs with regard to some HSPs may have passed undetected.

However, despite this limitation, the quantitative approach, in conjunction with RFLP analysis, to the mapping of HSP genes has proved to be successful. To our knowledge, this is the first study in which QTLs for several HSPs have been identified and localized. Moreover, the data demonstrate very clearly that HSP expression is under the control of several genes, as indicated also by other, more indirect, evidence. Further investigation is needed to establish the precise number and nature (structural or regulatory) of this class of genes.

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