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Molecular markers (RFLPs and HSPs) for the genetic dissection of thermotolerance in maize

E. Ottaviano, M. Sari Gorla, E. Pè and C. Frova

Department of Genetics and Microbiology, University of Milan, Via Celoria 26, I-20133 Milan, Italy

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Summary. Cellular membrane stability (CMS) is a physiological index widely used to evaluate thermostability in plants. The genetic basis of the character has been studied following two different approaches: restriction fragment length polymorphism (RFLP) analysis, and the effects of segregating heat shock protein (HSP) loci. RFLP analysis was based on a set of recombinant inbreds derived from the T32 × CM37 F_1 hybrid and characterized for about 200 RFLP loci. Heritability of CMS estimated by standard quantitative analysis was 0.73. Regression analysis of CMS on RFLPs detected a minimum number of six quantitative trait loci (QTL) accounting for 53% of the genetic variability. The analysis of the matrices of correlation between RFLP loci, either within or between chromosomes, indicates that no false assignment was produced by this analysis. The effect of HSPs on the variability of the CMS was tested for a low-molecular-weight peptide (HSP-17) showing presence-absence of segregation in the B73 × Pa33 F_2 population. Although the genetic variability of the character was very high ($h^2 = 0.58$) the effect of HSP-17 was not significant, indicating either that the polypeptide is not involved in the determination of the character or that its effect is not statistically detectable.

Key words: Thermotolerance – Membrane stability – Restriction fragment length polymorphism – Heat shock proteins – Maize

Introduction

High temperature tolerance is one of the main components of yield stability in crop plants (McWilliams 1980). It relates to the ability to protect the plant metabolism and cell structures when they are exposed to high temperatures (Levitt 1980).

The basic mechanisms controlling within- and among-species variability remain essentially unknown. However, the analysis of molecular and physiological responses to high temperature stresses and the study of material adapted to different climatic conditions indicate that the phenomenon can be caused by a large number of molecular and metabolic components (Ottaviano and Sari Gorla 1988; Jones and Qualset 1984) and, consequently, that the variability is the result of a very complex genetic architecture.

When the analysis is based on the overall response of plants grown either in the field or in controlled environments, a precise evaluation of the character is difficult to obtain because of its low heritability and repeatability (Blum 1986). To avoid this difficulty, whether to investigate the biological basis of the trait or for breeding purposes, a number of physiological tests have been proposed (Blum 1985; Ottaviano and Sari Gorla 1988). Cellular membrane stability (CMS) is one of the tests most extensively used (Martineau et al. 1979a; Sullivan 1972). It is evaluated as electrolyte leakage (Sullivan 1972), measured as the increase of electrical conductance produced when the electrolytes are released in distilled water by the injured tissue. Besides permitting a very simple test, suitable for application to very large populations, CMS is considered a major component of temperature and drought stress tolerance (Blum and Abercon 1981; Bewley 1979). Although the molecular basis of the variability of the character is not known, structural analysis of the membranes suggests that differential thermostability can be produced by membrane lipids, membrane protein and protein-lipid ratios, protein-lipid configuration, substances (protein, phenols, growth regulators) possessing natural affinity to membranes, and enzy-

matic fatty acid desaturation (see McDaniel 1982 for a review). The complexity of the character has been confirmed by the results of the analysis of the segregating F_2 population obtained by crossing two divergent lines in soybeans (Martineau et al. 1979 b).

All this information is useful in formulating hypotheses for the genetic-molecular dissection of the character, while the experimental approach for the verification of some of the hypotheses can follow two different strategies. One is based on the molecular analysis of mutants for genes controlling the character or, alternatively, on the analysis of correlation between polymorphism of molecules indicated as causal factors and the variability of the character. The other strategy consists in the use of molecular-genetic markers allowing the chromosomal localization of the major genetic factors (quantitative trait loci: QTL) controlling the character to be detected. With regard to the first approach, mutants for genes controlling CMS are not yet available and are likely to be very difficult to select. On the other hand, for the correlation studies, a number of molecular factors, as reported above, have been indicated as causal components. In particular, a large body of data indicates that heat shock proteins (HSPs) can play an important role in the acquisition of thermotolerance. In plants, this role is confirmed mainly by indirect correlative evidence, such as the correlations between heat hardening and HSP synthesis and between the kinetics of HSP synthesis and those of thermotolerance induction (Mascarenhas 1984; Lin et al. 1984; Sachs and Ho 1986; Lindquist 1986; Krishnan et al. 1989). However, although genetic variability for HSPs has been observed in a few plant species, including maize (Zivy 1987; Frova et al. 1988), a more direct relationship, such as that between HSP polymorphism and thermotolerance variability, has not been investigated.

The efficiency of the second approach is confirmed by a number of experimental results (Tanksley et al. 1982; Kahler and Wehrhahn 1986; Edwards et al. 1987; Weller et al. 1988). For example, isozyme markers used in selection studies have allowed detection of chromosomal segments carrying genes controlling tolerance to cold and salinity in tomato (Zamir et al. 1981; Ottaviano et al. 1990). In the last few years, a much more powerful technology has been developed for this approach. It is based on the restriction fragment length polymorphisms (RFLPs): the entire genome of any species can be densely saturated with markers, allowing precise localization of QTL on fragments of DNA and the first step towards gene isolation (Osborn et al. 1987; Nienhuis et al. 1987; Tanksley and Hewitt 1988; Burr and Burr 1988; Martin et al. 1989; Paterson et al. 1988; Ganal et al. 1989; Helentjaris and Burr 1989).

In this paper, we report the genetic dissection of CMS as response to heat treatments in maize. The variability of the character has been analyzed by standard quantitative genetic procedure. The RFLP approach has been used to detect QTLs controlling the character and the cosegregation between HSPs and CMS to study the effect of segregating HSP loci on CMS. The results are discussed also in relation to the efficiency of the two methodologies.

Materials and methods

RFLP analysis of CMS variability was based on a set of recombinant inbred lines (RI) kindly provided by Dr. B. Burr. The material is derived from the F_1 hybrid obtained by crossing two contrasting inbreds (T32 and CM37) and selfing (Burr et al. 1988). For this work the experiment was carried out on 44 F_6 RIs and the two parental lines grown in the greenhouse in seven complete replications (randomized blocks) sown at different times. Sowing took place at weekly intervals. Measurement of the injury was obtained from a single plant at the two-leaf seedling stage, using two leaf disks of 8 mm diameter excised from the same leaf.

Relationships between HSPs and heat injury was analyzed on F_2 plants obtained by crossing two inbreds, Pa33 and B37, showing different HSP patterns. The experimental design included the two inbred lines, their F_1 and F_2. The parental genotypes, Pa33 (tolerant, HSP-17$^+$) and B37 (sensitive, HSP-17$^-$), were chosen on the basis of their degree of thermotolerance and ability or inability to synthesize HSP-17 under heat shock conditions. 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 of the genotypes considered (Pa33, B37, F_1, F_2), the primary roots of 4-day-old intact seedlings were soaked in wells containing 0.75 ml distilled water and incubated in the dark either at 25 °C (control) or 40 °C for 3 h. Four roots were pooled in each well. One hundred microliters ^{35}S-Methionine (specific activity > 1,000 Ci/mmol) was added after the 1st h of incubation. After labeling, 1 cm long primary root tips were excised, rinsed in water, blotted dry, and homogenized singularly in 200 µl 0.05 *M* TRIS-HCl, pH 7.5, 1. m*M* phenylmethylsulphonylfluoride. 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). Following excision of the primary root tip, the seedlings were transferred to large pots with sand and grown until the second leaf was fully expanded. Two leaf disks, 8 mm diameter, were excised from each leaf.

Electrolyte leakage was evaluated according to Martineau et al. (1979 a). The two paired leaf disks from the same leaf of each plant were washed and placed in two separate test tubes with 4 ml distilled water. They were incubated in a thermostatically controlled water bath for 2 h at 25 °C (Control = C) and 47 °C (Treatment = T), and then both control and treatment tubes were frozen at −80 °C overnight to allow complete electrolyte release. Conductivity was measured after incubation (C1 and T1) and after freezing (C2 and T2). The effect on CMS was expressed as a percent of injury:

$$I = \{1 - [1 - (T1/T2)]/[1 - (C1/C2)]\} \times 100 .$$

For the RI population, the heritability of the character was estimated as the ratio between the genetic variance due to the between-line variance (σ^2_B) and the phenotypical variance ($\sigma^2_B + \sigma^2_R$), where σ^2_R is the residual component in the standard analysis of variance for randomized block design. For the F_2

Table 1. RFLP loci showing significant effect on membrane injury. b and R^2 value are, respectively, the estimated effects and the proportion of between-lines variability. The denomination of the loci is according to Burr et al. (1988)

Chromosome	Locus	b	R^2
1	7.21	−0.096	0.099
2	5.21B	0.104	0.138
	6.20	0.116	0.131
	5.61B	0.118	0.154
	NPI298	0.126	0.157
4	ZPL2A	−0.087	0.099
8	NPI220	0.136	0.229
9	3.06	−0.124	0.155
	WX1	−0.133	0.170
	5.04	−0.114	0.131
	7.13	−0.109	0.124
10	NPI269B	−0.118	0.142

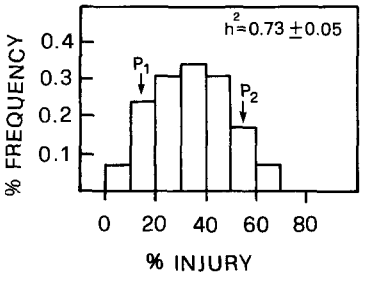

Fig. 1. Recombinant inbred frequency distribution for temperature injury in maize. *Arrows* indicate mean values of the two parental lines: P_1 = Pa33, P_2 = B37

population, the heritability was estimated as the ratio between the genetic variance (σ_G^2) and the phenotypic variance ($\sigma_P^2 = \sigma_G^2 + \sigma_E^2$), where σ_G^2 was the difference between the variance in F_2 and the environmental variance ($\sigma_F^2 - \sigma_E^2$) and σ_E^2 was the mean value of variance of non segregating populations. The procedure to estimate heritability standard error was derived from the general formula for the estimation of the variance of variance components given by Anderson and Bancroft (1952).

QTL detection and mapping were based on the regression of line mean value on the value attributed to the RFLP polymorphic loci in the RI population. The two parental inbreds differed in more then 200 loci, which saturate most of the maize genome, and all RIs used were characterized according to the allele of all segregating loci (Burr et al. 1988). For each locus, the value 1 or 2 was attributed to the T32 or CM37 allele, respectively. For heterozygous combinations, the value was 1.5.

Results

RFLP analysis

Figure 1 reports the distribution frequency of the RI population, the parental mean values, and the heritability of the character. The results obtained indicate that the variability of the character is largely genetically controlled. The pattern of the variability is that of typical quantitative traits, indicating a large number of segregating genetic factors. The difference between the two parental lines is 4.6 standard deviations. The estimate of minimum number of genes, according to Taylor (1976), is 2.

The results (R^2 values) of regression analysis of each restriction locus and the degree of injury (cellular membrane stability, CMS) are reported in Fig. 2, where the chromosomal localization of each RFLP locus is graphi-

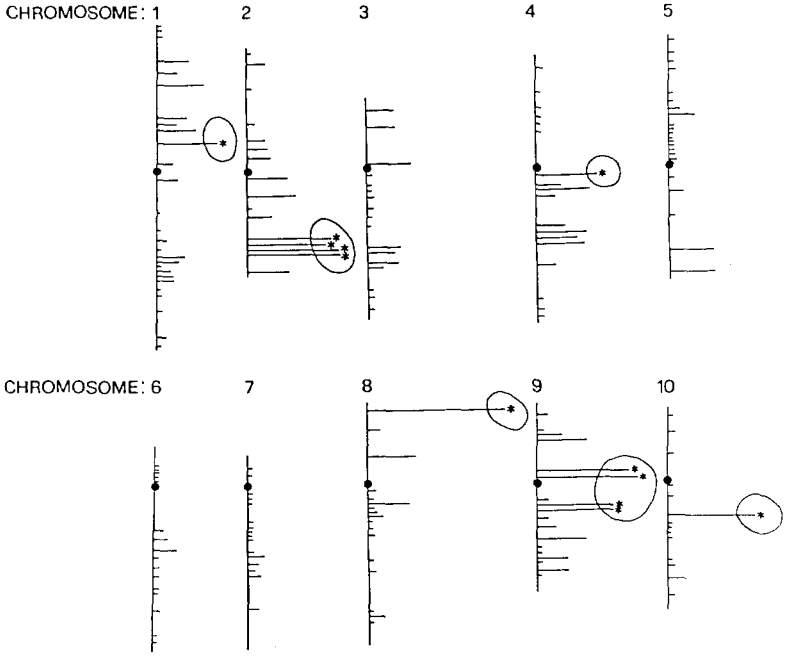

Fig. 2. RFLP analysis for temperature injury in recombinant inbreds from T32 × CM37 F_1 hybrid. *Horizontal bars* indicate degree (R^2) of correlation between RFLP loci and CMS. *: significant values ($p < 0.05$). A cluster, indicated as a circle, of significant values stands for a single QTL

cally indicated. Significant regressions are also listed in Table 1. R^2 values estimate the amount of variation between inbred means explained by the variation of that RFLP specific locus, while the sign of the coefficient of regression indicates the distribution of the QTL alleles in the two parental lines; + or − indicates that the allele which increases the character is in the CM37 or in the T322 parent, respectively.

Significant ($P<0.05$) values cluster in six different chromosomes. However, this information does not allow a straightforward, unambiguous estimation of the number of QTL or a precise mapping: a cluster of significant correlations in one region of the chromosome may be either the result of linkage of one QTL or of a cluster of more then one QTL in the same region. Moreover, when the RI population is not large, random drift can produce correlations among RFLP loci of different segments, either within or between chromosomes, which can result in apparent localization of the same QTLS on two or more unlinked sites.

More information in this regard can be obtained from the study of the correlations between RFLP loci, either within or between chromosomes. For random distribution of parental chromosomes between inbred lines, correlations between loci mapping on different chromosomes have an expected value equal to zero. Large deviations from this expectation indicate that sampling variations during inbreeding have produced apparent linkage between RLFP loci and the consequent possibility of attribution of the same QTL to more than one chromosome. However, the analysis of the matrix of correlation can detect these false assignments. Correlations within chromosomes, on the other hand, measure the degree of recombination between chromosome segments produced in the RI set. For example, in the case of chromosome 9, the inspection of the correlations (not reported here for the sake of brevity) between RFLP loci indicates that the segment including the significant values is transmitted to the RI set as a block with only very rare recombinants. Therefore, on the base of these results, no more then one QTL can be assigned to this cluster.

Complementary information for the estimation of the minimum number of factors within a chromosomal segment is provided by the sign of the coefficient of regression (Table 1). Significant values for a cluster of RFLP loci linked to the same QTL are expected to have all positive or negative signs. Therefore, the analysis of these values can indicate associations between increasing and decreasing alleles in the parents: different signs indicate *trans* associations whereas, when all loci in the cluster have the same sign, one or more QTLs in *cis* cannot be discriminated. The results obtained by the adoption of these criteria of analysis are indicated in Fig. 2: the variability of CMS is attributable to a minimum QTL number not less than 6, unambiguously distinguished. The inspection of the matrices of correlation between chromosomes indicates that in this specific study no false assignments of QTL have been made.

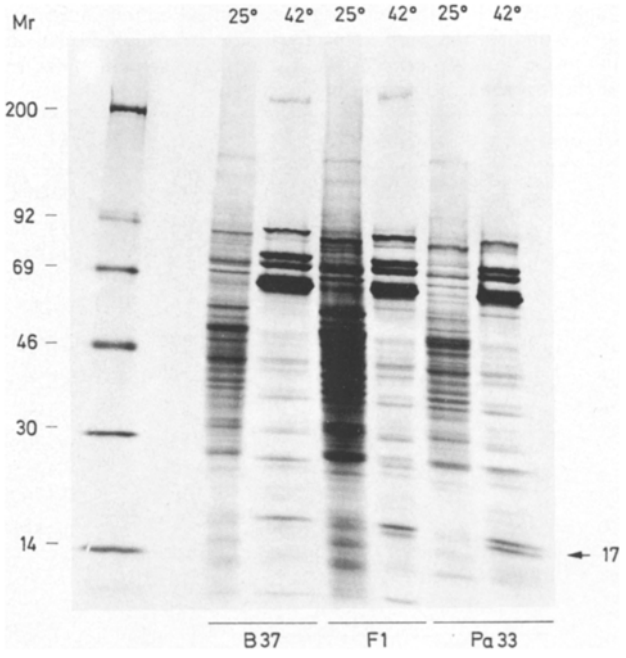

Fig. 3. Pattern of protein synthesis at 25 °C and 42 °C in root tips of two inbreds and their F_1 hybrid

Multiple regression analysis measures the amount of variability monitored by a selected set of RFLP loci. The model, including six selected loci (one for each QTL identified), accounts for 39% of the phenotypic variability between RI and CMS mean values.

Cellular membrane thermostability and HSPs

Figure 3 shows the HSP pattern of inbreds Pa33, B37, and their F_1. It can be seen that the 17-kDa polypeptide, induced in Pa33 but not in B37, represents a major qualitative difference between the HSPs synthesized by the two parental genotypes. The pattern of the F_1 reveals dominance of the 17-kDa band. When the F_2 generation is analyzed (Fig. 4), the 17-kDa polypeptide shows segregation between the progeny seedlings. Each lane shows the HSP pattern of one F_2 seedling, and the whole progeny is easily classified for this character. The results of the analysis are presented in Table 2. Deviation from the expectation 3:1 is significant. Quantitative differences in HSP synthesis that may occur among the F_2 progeny are not revealed by this kind of analysis.

In regard to heat shock injury, the two parental lines reveal a significant difference: the mean values of Pa33 and B37 were 0.21 ± 0.04 and 0.36 ± 0.08, respectively. Also, in this population, the genetic component of the variability of the character is large ($h^2 = 0.58 \pm 0.10$) and the pattern of the frequency distribution is typical of

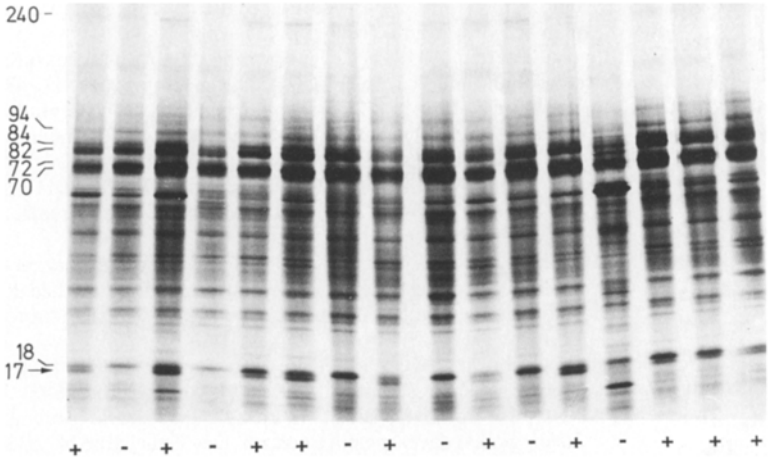

Fig. 4. Pattern of protein synthesis at 42 °C in root tips of the F_2 progeny obtained by selfing the hybrid B37 × Pa33. + and − indicate presence or absence of HSP-17

Table 2. HSP frequency and cellular membrane stability values (CMS) in F_2 population from Pa33 × B37 cross

	n Plants	Proportion	CMS
HSP-17$^+$	90	0.67	0.303
HSP-17$^-$	45	0.33	0.316

$\chi^2(3:1) = 5.00$ ($P < 0.05$)
$d = 0.013$ ($P > 0.05$)

quantitative traits. The effect of the segregating gene codifying for the HSP-17 is tested by comparing the mean value of HSP-17$^+$ plants with that of HSP-17$^-$ plants (Table 2). The results obtained do not show significant effects of HSP-17 on the variability of the heat shock injury of the membrane.

Discussion

RFLP analysis of thermotolerance in maize measured as CMS indicates that at least six major genetic factors (QTLs) account for a significant portion of the phenotypical variability of the character. For predictive purposes, in selection work, a model including six RFLP loci highly associated with the character would account for 39% of the variability between mean values of RI. As RFLP variability is not affected by environmental factors, and assuming that these factors act at random on the quantitative traits (the experimental design reinforces this assumption), the R^2 value obtained by fitting this model is an estimate of the proportion of the genetic variance between RI means explained by the variability of RFLP loci included in the model. Taking into account the fact that the proportion of genetic variance between RI family means was $h_B^2 = 0.73$, a selection based on the six RFLP markers included in the model will monitor $0.39/0.73 = 0.53$ of the genetic variability of the character. As expected on the basis of the degree of correlation between RFLP loci assigned to the same clusters, this proportion does not increase significantly when all significant loci are included in the model: the proportion of the variability explained by fitting the model is 42% ($R^2 = 0.42$), accounting for 56% ($R^2/h^2 = 0.56$) of the heritability. Therefore, the remaining portion is likely to be due to QTLs of minor effect whose contribution is not statistically detected. Indications for these factors are provided by clusters of effects (see, e.g., that on chromosome 4) having all positive or negative values but falling short of significance.

RFLP analysis based on RI has several advantages when compared with other experimental designs such as those based on F_2 or backcross progeny (Burr et al. 1988). On the other hand, difficulties in the interpretation of the results can be produced by random drift and unconscious selection, especially when the number of random inbreds is not very large. However, the analysis reported in this study shows that a careful inspection of the correlation matrices between RFLP loci can remove most of the indeterminacies in the estimation of the minimum number of QTLs and the detection of false assignments. Another aspect to consider concerns the effect of inbreeding, especially in the case of a cross-breeding population and characters showing heterosis. In fact, differences between RI account only for a portion of the genetic variance (additive variance component). On the other hand, in breeding programs, selection is based on segregating material and the final goal is the production of highly heterozygous cultivars. Although this aspect has not been investigated, it is likely that, by using the information from RI analysis, important genetic components are not monitored. For these reasons, and taking into account the number of generations required to produce RI, selection programs need to be founded on different crossing designs, such as those based on F_2 or F_3 families.

The genetic localization of QTLs controlling the variability of the character can represent the first step for the molecular characterization of the genes underlying the variability (Ganal et al. 1989). In fact, the technology based on chromosome walking requires a genetic marker of the map which is closely linked to the gene to be characterized. In maize, this approach has great limitations due to the large genome size, the large amount of repetitive DNA, and the difficulty of having an efficient transformation system to test whether the RFLP fragment contains the gene. However, the rapid development of molecular technology should soon overcome these obstacles.

Although data obtained in many plant species suggest that HSPs of minor molecular weight play an important role in thermotolerance (Mansfield and Key 1987), the analysis of the segregation of the gene controlling HSP-17 synthesis did not reveal any effect of this band on CMS. To explain the failure to detect association between the molecular and the physiological variability, it is possible to consider two different hypotheses. First, HSP-17 is not a causal component of CMS, although its implication in the control of other physiological components of thermotolerance cannot be ruled out. Alternatively, the association cannot be detected because of the complexity of the character: the effect of this HSP would represent only a small part of the variability of CMS and would therefore be very difficult to detect by means of the experimental approach adopted in this study. Moreover, in this study, the analysis only considers the qualitative variability in HSP pattern, while the level of thermotolerance may be largely due to quantitative expression of several genes involved in the synthesis of HSPs. The distorted segregation observed for the gene coding for HSP-17 is a phenomenon frequently found in maize (Ottaviano and Mulcahy 1989). In fact, differential transmission of the two alleles may be related to genes controlling male gametophytic competitive ability. Alternatively, it could be the result of interaction between two genes, i.e., structural and regulatory, controlling the synthesis of the polypeptide.

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References

Anderson RL, Bancroft TA (1952) Statistical theory in research. McGraw-Hill, New York

Bewley JD (1979) Physiological aspects of desiccation tolerance. Annu Rev Plant Physiol 30:195–238

Blum A (1985) Breeding crop varieties for stress environments. CRC Crit Rev Plant Sci 3:199–238

Blum A (1986) Methods of plant breeding for drought resistance. In: Monti L, Porceddu E (eds) Drought resistance in plants. EEC, Amalfi, Italy, pp 235–254

Blum A, Ebercon A (1981) Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Sci 21:43–47

Burr B, Burr F (1988) I polimorfismi dei frammenti generati da enzimi di restrizione e il loro impiego nel miglioramento genetico. Agric Ric 93:61–68

Burr B, Burr FA, Thompson KH, Albertson CM, Stuber CW (1988) Gene mapping with recombinant inbreds in maize. Genetics 118:519–526

Edwards MD, Stuber CW, Wendel JF (1987) Molecular marker-facilitated investigations of quantitative trait loci in maize. I. Numbers, genomic distribution, and types of gene action. Genetics 116:113–125

Frova C, Taramino G, Binelli G, Ottaviano E (1988) Heat-shock protein variability in maize. Maydica 33:65–76

Frova C, Taramino G, Binelli G (1989) Heat shock proteins during pollen development in maize. Dev Genet 10:324–332

Ganal MW, Young ND, Tanksley SD (1989) Pulsed field gel electrophoresis and physical mapping of large DNA fragments in the Tm-2a region of chromosome 9 in tomato. Mol Gen Genet 215:395–400

Helentjaris T, Burr B (1989) Development and application of molecular markers to problems in plant genetics. Current communications in molecular biology. Cold Spring Harbor Laboratory Press, Cold Spring Harbor/NY, pp 160

Jones AR, Qualset CO (1984) Breeding crops for environmental stress tolerance. In: Collins GB, Petolino JG (eds) Applications of genetic engineering to crop improvement. Nijhoff M, Junk W Publ, Dordrecht, pp 305–340

Kahler AL, Wehrhahn CF (1986) Association between quantitative traits and enzyme loci in the F_2 population of a maize hybrid. Theor Appl Genet 72:15–26

Krishnan M, Nguyen HT, Burke JJ (1989) Heat shock protein synthesis and thermal tolerance in wheat. Plant Physiol 90:140–145

Levitt J (1980) Responses of plants to environmental stresses. Chilling, freezing, and high temperature stresses. Academic Press, New York

Lin CY, Roberts JK, Key JL (1984) Acquisition of thermotolerance in soybean seedlings. Synthesis and accumulation of heat shock proteins and their cellular localization. Plant Physiol 74:152–160

Lindquist S (1986) The heat shock response. Annu Rev Biochem 55:1151–1191

Mansfield MA, Key JL (1987) Synthesis of the low-molecular-weight heat shock proteins in plants. Plant Physiol 84:1007–1017

Martin B, Nienhuis J, King G, Schaefer A (1989) Restriction fragment length polymorphisms associated with water use efficiency in tomato. Science 243:1725–1728

Martineau JR, Specht JE, Williams JH, Sullivan CY (1979a) Temperature tolerance in soybeans. I. Evaluation of a technique for assessing cellular membrane thermostability. Crop Sci 19:75–78

Martineau JR, Williams JH, Specht JE (1979b) Temperature tolerance in soybeans. II. Evaluation of segregating population for membrane thermostability. Crop Sci 19:79–82

Mascarenhas JP (1984) Molecular mechanisms of heat stress tolerance. In: Collins GB, Petolino JG (eds) Applications of genetic engineering to crop improvement. Nijhoff M, Junk W Publ, Dordrecht, pp 391–425

McDaniel RG (1982) The physiology of temperature effects on plants. In: Christiansen MN, Lewis CF (eds) Breeding plants for less favorable environments. Wiley and Sons, New York, pp 13–45

McWilliams JR (1980) Adaptation to water and high temperature stress: summary and synthesis – adaptation to high temperature stress. In: Turner NC, Kramer PJ (eds) Adaptation of plants to water and high temperature stress. Wiley and Sons, New York, pp 444–447

Nienhuis J, Helentjaris T, Slocum M, Ruggero B, Schaefer A (1987) Restriction fragment length polymorphisms analysis of loci associated with insect resistance in tomato (1987) Crop Sci 27: 797–803

Osborn TC, Alexander DC, Fobes JF (1987) Identification of restriction fragment length polymorphisms linked to genes controlling soluble solids content in tomato fruit. Theor Appl Genet 73: 350–356

Ottaviano E, Mulcahy DL (1989) Genetics of angiosperm pollen. Adv Genet 26: 1–64

Ottaviano E, Sari Gorla M (1988) Selecting cultivars for resistance to high and low temperature. In: Biotechnology in tropical crops improvement. ICRISAT, Hyderabad India, pp 97–105

Ottaviano E, Sari Gorla M, Mulcahy DL (1990) Pollen selection: efficiency and monitoring. In: Markert C, Oghita Z (eds) Isozymes: structure, function, and use in biology and medicine. Wiley-Liss, New York, pp 575–588

Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335: 721–726

Sachs MM, Ho TD (1986) Alteration of gene expression during environmental stress in plants. Annu Rev Plant Physiol 37: 363–376

Sullivan CY (1972) Mechanism of heat and drought resistance in grain sorghum and methods of measurement. In: Rao NGP, House LR (eds) Sorghum in the seventies. Oxford and India Book House, New Delhi, pp 247–264

Tanksley SD, Hewitt J (1988) Use of molecular markers in breeding for soluble solids content in tomato – a reexamination. Theor Appl Genet 75: 811–823

Tanksley SD, Medina-Filho H, Rick CM (1982) Use of naturally occurring enzyme variation to detect and map genes controlling quantitative traits in an interspecific backcross of tomato. Heredity 49: 11–25

Taylor BA (1976) Genetic analysis of susceptibility to isoniazid-induced seizures in mice. Genetics 83: 373–377

Weller JI, Soller M, Brody T (1988) Linkage analysis of quantitative traits in an interspecific cross of tomato (*Lycopersicon esculentum* × *Lycopersicon pimpinellifolium*) by means of genetic markers. Genetics 118: 329–339

Zamir D, Tanksley SD, Jones AJ (1981) Haploid selection for low temperature tolerance of tomato pollen. Genetics 101: 129–137

Zivy M (1987) Genetic variability for heat shock proteins in common wheat. Theor Appl Genet 74: 209–213