

RESEARCH

Genetic Mapping of Foliar and Tassel Heat Stress Tolerance in Maize

James P. McNellie, Junping Chen,[★] Xianran Li, and Jianming Yu[★]

ABSTRACT

The frequency of heat stress events is expected to increase, further complicating the challenge of feeding a growing population. A better understanding of the genetic and molecular mechanisms of heat stress tolerance in maize (*Zea mays* L.) would facilitate the development of heat-tolerant cultivars. To address this knowledge gap, we evaluated two biparental recombinant inbred line (RIL) populations (B73 × NC350 and B73 × CML103) for leaf and tassel heat tolerance traits. Two foliar traits, leaf firing and leaf blotching, were evaluated at three vegetative growth stages. In B73 × NC350, two tassel traits, tassel blasting and reduction in spikelet size, were scored at flowering. We detected 22 quantitative trait loci (QTL), 15 in B73 × NC350 and seven in B73 × CML103. We previously observed that the development of leaf firing was differentiable between parents, and indeed, the different manifestations of the leaf firing trait were not significantly correlated and QTL did not co-localize. Leaf firing and leaf blotching traits were correlated at some vegetative growth stages, and most QTL did not co-localize. Quantitative trait loci number and position for traits measured at multiple vegetative stages were generally consistent. There was a single QTL for tassel blasting on chromosome 5. Heat-induced plant death segregated in B73 × CML103 and a major QTL was detected on chromosome 3, explaining 26.2% of phenotypic variance. Our study indicates that complex genetic mechanisms underlie the heat stress response in maize.

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Abbreviations: CI, confidence interval; EVS, early vegetative stage; HS, heat stress; LB, leaf blotching; LF, leaf firing; LOD, logarithm of odds; LVS, late vegetative stage; MVS, middle vegetative stage; PD, plant death; PVE, phenotypic variance explained; QTL, quantitative trait locus/loci; RIL, recombinant inbred line; RSS, reduction in spikelet size; SNP, single nucleotide polymorphism; TSBL, tassel blasting.

MAIZE (*Zea mays* L.) is a major source of calories and biofuel, with an estimated 187.9 million ha harvested worldwide in 2016 (FAOSTAT, 2016). Maize can be grown in a diverse range of environments, from the equator to the low 50° latitudes (Meng et al., 2014). Although widely adapted, maize is susceptible to abiotic stresses, such as drought, excess water, and heat. Management practices can mitigate certain abiotic stresses; fertilizers to overcome nutrient deficiencies, irrigation to relieve drought, and drainage tiles to remove excess water. Irrigation can also reduce yield losses attributable to heat stress (HS), but most maize grown for grain is produced without irrigation (Shaw et al., 2014; Carter et al., 2016). For dryland production, planting heat-resistant cultivars offers protection against deleteriously high temperatures.

Heat stress can cause significant yield losses by reducing photosynthetic efficiency, resulting in kernel abortion and reduced starch storage (Schoper et al., 1986; Cantarero et al., 1999; Wilhelm et al., 1999; Edreira and Otegui, 2013). Male flowers (tassels) are especially sensitive to HS (Schoper et al., 1987). The effect of HS on foliar tissue is variable, and depending on the genotype and severity of stress, the consequences can range from a temporary

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reduction in photosynthetic output to extensive tissue and plant death (PD) (Karim et al., 1999; Sinsawat et al., 2004; Chen et al., 2010). High temperatures also exacerbate yield losses caused by drought (Mallya et al., 2013; Lobell et al., 2014). Drought and record heat in 2012 reduced yields in the United States by 27% (from pre-drought estimates) and caused an estimated US\$30 billion in losses (Rippey, 2015). The frequency of extreme heat events is expected to increase because of climate change (Duffy and Tebaldi, 2012), and even optimistic models predict that HS events will become more frequent in the future (Anderson, 2011; Coumou and Robinson, 2013).

The physiological and molecular response of plants to HS has been an ongoing area of research, especially in the model organism *Arabidopsis thaliana* (L.) Heynh. (Mittler et al., 2012), but research in the field setting (in situ) is lacking. The majority of in situ HS studies conducted in maize have focused on evaluating and comparing genotypes, not on quantitative trait locus (QTL) mapping (Jodage et al., 2017; Akula et al., 2018; Lizaso et al., 2018). The lack of mapping studies is understandable, as heat waves are sporadic and of variable severity, making heat a difficult stress to study. Drought stress often accompanies HS, thus irrigation is necessary to reduce the confounding effect of drought. Controlled HS treatments in a field setting can be accomplished using portable plastic shelters placed over plots to create a greenhouse effect (Cantarero et al., 1999; Neiff et al., 2016), but such techniques are not practical for evaluating large mapping populations. Researchers have subjected segregating populations of seedlings to HS in growth chambers, or taken tissue from field-grown populations indoors to apply a heat treatment (Ottaviano et al., 1991; Frova and Sari-Gorla, 1994; Frova, 1996; Frey et al., 2015). The drawback of applying a stress ex situ is that the results have a “reduced reality,” meaning that the results from controlled environments (ex situ) may not be pertinent in the field environment (Roy et al., 2011). That “reduced reality” was observed by Chen et al. (2012) when genotypes exhibiting HS sensitivity in the field were found to be tolerant in a controlled environment and vice versa. Only one study has reported in situ mapped QTL for HS tolerance in maize, detecting 11 QTL in six inter-connected $F_{3:5}$ populations representing European flint and dent germplasm (Frey et al., 2016). To our knowledge, there are no reported genetic mapping studies for heat tolerance in North American maize germplasm under well-watered conditions.

The ultimate objective in studying HS tolerance is to increase grain yield, but even in stress-free environments, elucidating the complex genetic networks that influence grain yield is nontrivial. We chose to focus on foliar and tassel stress traits because they are components of yield reduction after HS. After a HS event, susceptible genotypes can exhibit two foliar stress phenotypes, leaf firing (LF) and

leaf blotching (LB). Leaf firing is tissue death starting from the leaf tip, and developing leaves are more susceptible than mature leaves (Zaidi et al., 2016). Leaf blotching is irregularly shaped lesions between leaf veins that may necrose or recover, depending on the severity of HS (Chen et al., 2017). In sorghum [*Sorghum bicolor* (L.) Moench], association mapping has revealed that LF and LB are controlled by unique genomic regions, as well as by shared regions (Chen et al., 2017). We previously observed that LF developed differently among susceptible genotypes, and those differences appeared to segregate in progeny. We hypothesize that genetic factors are responsible for the observed differences in LF and that further dissection of the LF phenotypes may provide an additional strategy to understand HS. When HS occurs immediately before or during tassel emergence, death and desiccation of tassel tissue is observed, referred to as tassel blasting (TSBL). When HS occurs at V8 to V10 (the early developmental stage of the tassel), the amount of pollen produced is reduced because of smaller tassel size and fewer anthers (Chen et al., 2012). The genetic control and molecular mechanisms of these heat intolerance traits are unknown in maize.

Herein, we report QTL for foliar and tassel heat tolerance in two biparental recombinant inbred line (RIL) populations (B73 \times NC350 and B73 \times CML103). The objectives of this study are: (i) to identify loci involved in tolerance to HS for foliar and tassel traits, (ii) to determine if the different parental forms of LF are controlled by unique loci, (iii) to compare the genetic control of LF and LB, and (iv) to evaluate the effect of vegetative developmental stage on HS tolerance.

MATERIALS AND METHODS

Mapping Populations

We used two RIL populations, B73 \times NC350 (NAM020) and B73 \times CML103 (NAM002), developed for the maize Nested Association Mapping population (Yu et al., 2008; McMullen et al., 2009). Prior evaluation identified the three parental inbreds as having contrasting HS tolerance (Chen et al., 2012). The common parent, B73, is resistant to TSBL and developing leaves (within the whorl) are sensitive to HS at late vegetative stages. NC350 is moderately susceptible to TSBL; the first and second expanding leaves above the youngest mature leaf show sensitivity to HS. CML103 is susceptible to PD and LF at all vegetative stages. Seed for B73 \times NC350 and B73 \times CML103 RILs and parental lines were obtained from the Maize Genetics Cooperation Stock Center (<http://maizecoop.cropsci.uiuc.edu/nam-rils.php>).

Field Trials

Field evaluations were conducted at the USDA-ARS Cropping Systems Research Laboratory in Lubbock, TX (33°35'31.2'' N, 101°53'49.2'' W), in 2010, 2011, and 2012. Using 36°C as a starting point for HS, in 2010, there were 11 d with a maximum air temperature $>36^{\circ}\text{C}$ in the first 90 d after planting; there

were 46 d in 2011, and 19 d in 2012. The stress phenotypes were not reliably observed in 2010 when there were the fewest days above 36°C; therefore, only the data from 2011 and 2012 were analyzed. On 5 May 2011 and 19 Apr. 2012, seeds were planted in single row plots 3.05 m in length, with 0.91 m between plots, and thinned to a density of 0.15 to 0.20 m per plant prior to six fully emerged leaves. Routine practices for insect control and fertilization were used and irrigation maintained well-watered conditions throughout the season. An automated weather station recorded daily meteorological data (<https://www.csrl.ars.usda.gov/wewc/weather-pswc-data.aspx.html>).

Evaluation of Heat Tolerance

Recombinant inbred lines were scored on a whole-plot basis using a 0-to-5 scale in increments of 1. A score of 0 denotes no plants exhibited HS intolerance, 1 denotes <20% of plants showing heat sensitivity, 3 denotes 40 to 60% of plants showing heat sensitivity, 4 denotes 61 to 80% of plants showing heat sensitivity, and 5 denotes ≥80% of plants showing heat sensitivity (Fig. 1). Two foliar HS traits, LF and LB, were scored. Leaf firing is outright tissue death after a HS event, and LB is the development of blotchy lesions. The parental LF phenotypes were differentiable and segregated among RILs and were denoted as LF_B73, LF_NC350, and LF_CML103. LF_NC350 and LB were scored at three vegetative stages: early (EVS), middle (MVS), and late (LVS). The EVS is prior to 10 emerged leaves (V10), MVS is between V11 and V14, and LVS is at tasseling. For clarity, the abbreviation for LF of the NC350 form, scored in the LVS, is LVS_LF_NC350. LF_B73 and LF_CML103 were measured once in the LVS. CML103

is particularly intolerant to high temperatures, to the point of PD. Only B73 × CML103 RILs exhibited PD, measured once in the LVS. Two tassel traits were scored, which were death and desiccation of tassel tissue (TSBL) and reduction in spikelet size (RSS). The severity of PD in B73 × CML103 prevented accurate scoring of TSBL and RSS.

In B73 × NC350, EVS_LB had two replications scored in 2011 and one in 2012. Measurements for MVS_LB were taken from two replications in 2011, and one measurement for LVS_LB in 2012. All other traits in B73 × NC350 had two replications scored in both 2011 and 2012. In B73 × CML103, scores for LF and PD were recorded from three replications in 2011 and two replications in 2012. One repetition for EVS_LB was measured in 2011 and 2012, three replications for MVS_LB were measured in 2011, and three replications were measured for LVS_LB in 2011 with one replication in 2012.

Least square means were calculated in R, with individual lines (genotypes) treated as fixed effects, and years, replications within years, and genotype × year interaction treated as random effects (Bates et al., 2015; Kuznetsova et al., 2016; Lenth, 2016; R Core Team, 2017). To obtain entry mean-based heritability (h^2) estimates, all effects were treated as random effects. The equation for h^2 is

$$h^2 = \frac{\sigma_G^2}{\sigma_G^2 + (\sigma_{GY}^2/\gamma) + (\sigma_\epsilon^2/r)}$$

where σ_G^2 is the genetic variance, σ_{GY}^2 is the genotype × year interaction variance, σ_ϵ^2 is the residual variance, γ is the harmonic mean of number of years per family, and r is the harmonic mean of number of plots per family replications (Holland et al., 2003).



Fig. 1. Heat-induced damage of foliar tissue and the scale used to score recombinant inbred lines: (A) unstressed phenotype representing a score of 0, (B) leaf firing (LF), (C) leaf blotching (LB), (D) example of the 0-to-5 scale used to score LF starting from 1 (first image from the left) to 5.

The above model was modified as needed for traits without measurements in multiple years and replications.

Linkage Map Construction and QTL Mapping

Quantitative trait loci detection was performed using 185 lines of B73 × NC350 and 195 lines of B73 × CML103. All NAM RILs were previously genotyped using 1536 single nucleotide polymorphism (SNP) markers on the Illumina GoldenGate Assay system. We used the publicly available 1144 SNPs that passed quality control to create our linkage maps (McMullen et al., 2009). Single nucleotide polymorphism marker data were downloaded from <https://www.panzea.org/>. The genetic map for B73 × NC350 is composed of 519 markers across 1384.6 cM, and the genetic map for B73 × CML103 contains 493 markers across 1321.0 cM. Both linkage maps have a mean distance of 2.7 cM between markers. Inclusive composite interval mapping detected QTL using a step width of 1 cM in ICIMapping (Meng et al., 2015). A QTL was declared when the logarithm of odds (LOD) score exceeded the significance threshold established via 1000 permutations. Quantitative trait loci detection was performed in individual populations rather than joint population analysis because only four of the measured traits can be compared across populations (LVS_LF_B73 and the three vegetative stages of LB). A consensus linkage map was created and the joint population QTL analysis detected one novel QTL. Synteny between maize and sorghum was analyzed using CoGe (<https://genomevolution.org/coge/>) (Lyons and Freeling, 2008).

RESULTS

Maximum air temperature was $>36^{\circ}\text{C}$ for 46 d in the first 90 d after planting on 5 May 2011, and the average maximum temperature on those days was 38.3°C (Fig. 2). The average maximum air temperature on non-HS days was 32.4°C . The first 90 d after planting on 19 Apr. 2012 saw 19 d with a maximum air temperature $>36^{\circ}\text{C}$, and the average maximum temperature on those days was 37.6°C . The average maximum air temperature on non-HS days in 2012 was 30.7°C . The environmental (i.e., year) and genotype × environment interaction variance were low for a majority of traits (Table 1). Overall heritability estimates were high, ranging from 0.81 for EVS_LB to 0.96 for LVS_LF_NC350 and LVS_LF_B73 in B73 × NC350. In B73 × CML103, heritability estimates ranged from 0.29 (EVS_LB) to 0.96 (LVS_LF_B73).

Two traits were measured in both populations, LVS_LF_B73 and LB. The severity of LVS_LF_B73 and EVS_LB was greater in B73 × NC350 RILs; MVS_LB and LVS_LB was more severe in B73 × CML103 RILs (Table 1). The greater frequency of HS events and higher overall maximum air temperature in 2011 resulted in greater mean HS scores than in 2012 for all traits except those scored in the EVS (Supplemental Table S1). The largest difference in trait means between years was 0.63 for LVS_LB in B73 × CML103. Trait distributions were generally right skewed, and the full range of HS scores (0–5) was observed for all traits (Supplemental Table S1).

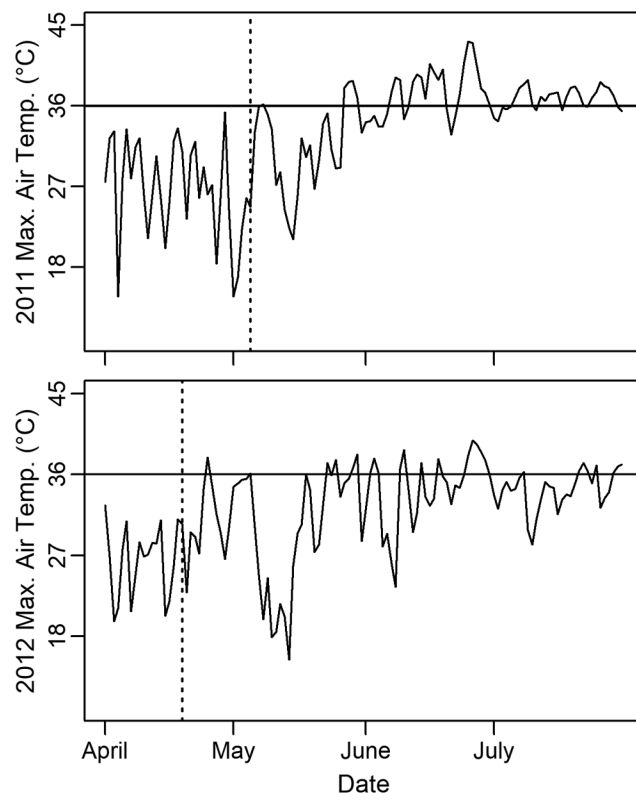


Fig. 2. Maximum air temperature profile and heat stress occurrence in Lubbock, TX. Dates shown are between 1 April and 31 July for 2011 (top) and 2012 (bottom). Vertical dotted lines denote planting date.

QTL in B73 × NC350

Fifteen QTL were detected across eight of the nine traits scored in B73 × NC350 (Fig. 3, Table 2). Quantitative trait loci for LF_NC350 were detected on chromosomes 5, 9, and 10, explaining between 22.8% (two QTL in EVS) and 30.6% of phenotypic variance (three QTL in MVS). The QTL on chromosome 10 had the highest phenotypic variance explained (PVE), and the B73 allele conferred heat tolerance. NC350 donated the tolerance allele for the other two QTL on chromosomes 5 and 9. Quantitative trait loci effects for LF_NC350 were consistent across developmental stages, the biggest change being a decrease of 2.9% from the EVS to LVS for the QTL on chromosome 10 (Table 2). The two LF phenotypes (LF_NC350 and LVS_LF_B73) were not correlated and QTL did not co-localize (Table 3). One QTL for LVS_LF_B73 was detected on chromosome 1, with a PVE value of 8.0%.

For LB, QTL were detected on chromosome 1 in the EVS and MVS, on chromosome 10 in the EVS and LVS, and on chromosome 8 in LVS. The variance explained by the QTL on chromosome 1 decreased from 15.2% in the EVS to 8.7% in the MVS, and finally to 6.3% in the LVS, where the LOD score of 2.8 was no longer above the threshold (Fig. 3E–3G). The heat tolerance allele for LVS_LB on chromosome 8 was donated by NC350; all other LB tolerance alleles came from B73. Leaf blotching

Table 1. Mean, variance components and heritability (h^2). Variance components include genotype (σ^2_G), year (σ^2_Y), genotype \times year interaction (σ^2_{GY}), and residual (σ^2_ϵ) components. For parameters that could not be estimated, a dash is inserted as a placeholder.

Population	Trait†	Developmental stage‡	Mean	σ^2_G	σ^2_Y	σ^2_{GY}	σ^2_ϵ	h^2
B73 \times NC350	LF_NC350	EVS	0.75	1.02	0.06	0.03	0.45	0.89
		MVS	1.20	1.66	0.05	0.05	0.48	0.92
		LVS	2.22	3.28	0.08	0.13	0.34	0.96
	LF_B73	LVS	1.21	2.46	0.03	0.10	0.22	0.96
	LB	EVS	0.73	1.38	0.40	0.35	0.40	0.81
		MVS	0.74	1.47	0.42	–	0.42	0.87
		LVS	1.46	–	–	–	–	–
	TSBL	LVS	1.30	1.89	0.05	0.23	0.37	0.90
	RSS	LVS	0.60	1.23	0.00	0.03	0.21	0.95
B73 \times CML103	LF_CML103	LVS	2.01	2.73	0.10	0.43	0.57	0.89
	LF_B73	LVS	1.08	2.26	0.00	0.03	0.35	0.96
	LB	EVS	0.29	0.15	0.01	–	0.68	0.29
		MVS	1.76	2.52	–	–	0.97	0.89
		LVS	2.75	2.27	0.05	0.99	1.05	0.74
	PD	LVS	1.15	1.33	0.05	0.25	0.75	0.83

† LF, leaf firing; LB, leaf blotching; TSBL, tassel blasting; RSS, reduction in spikelet size; PD, plant death.

‡ EVS, early vegetative stage; MVS, middle vegetative stage; LVS, late vegetative stage.

was significantly correlated with LF_NC350 at all developmental stages ($P < 0.001$), and with LVS_LF_B73 in the EVS ($P < 0.01$) (Table 3). Co-localization of LF_NC350 and LB QTL was observed on chromosome 10. The EVS_LB QTL on chromosome 1 is 14 cM from the QTL for LVS_LF_B73, and the QTL have opposite additive effects.

Of the two tassel traits scored, TSBL and RSS, one QTL was detected for TSBL on chromosome 5, with a PVE value of 8.0%. A significant correlation exists between TSBL and LF_NC350; however, QTL mapped in separate regions (44 cM for MVS_LF_NC350 vs. 114 cM for TSBL). No QTL were detected for RSS.

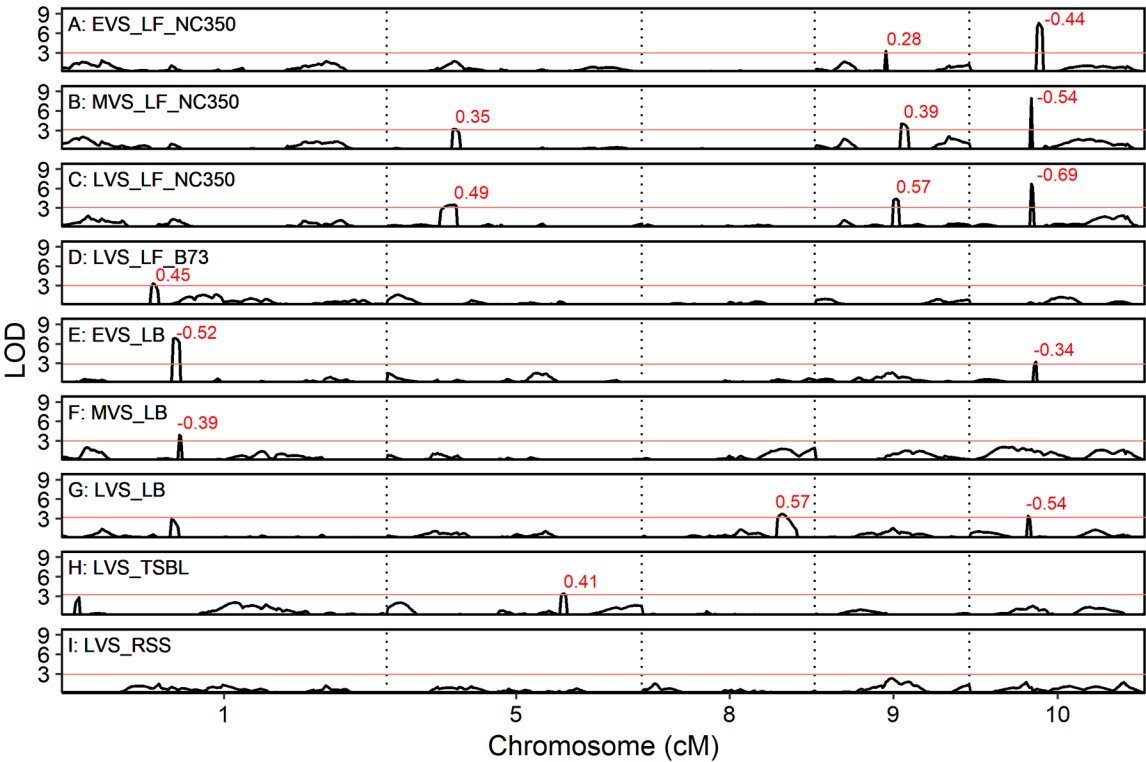


Fig. 3. Genetic mapping results of nine heat stress traits measured in 185 B73 \times NC350 recombinant inbred lines: (A) early vegetative stage (EVS), (B) middle vegetative stage (MVS) and (C) late vegetative stage (LVS) leaf firing NC350 (LF_NC350); (D) LVS_LF_B73; (E–G) EVS to LVS leaf blotching (LB); (H) tassel blasting (TSBL), and (I) reduction in spikelet size (RSS). Logarithm of odds (LOD) is shown for chromosomes containing quantitative trait loci (QTL), and red horizontal lines denote the significance threshold determined by 1000 permutations. Additive QTL effects are reported next to QTL peaks and are negative if B73 donated the heat-tolerant allele and positive if NC350 donated the allele.

QTL in B73 × CML103

Seven QTL were detected in five of the six HS traits scored in B73 × CML103 (Fig. 4). LVS_LF_CML103 mapped QTL to chromosomes 2 and 3, with PVE values of 11.6 and 10.2%, respectively (Table 2). The heat tolerance allele was donated by CML103 for the QTL on chromosome 2, and by B73 for the QTL on chromosome 3. LVS_LF_B73 mapped a single QTL to chromosome 1 at 63 cM, 10 Mb from the QTL for LVS_LF_B73 in B73 × NC350. The allele donated by B73 was for heat intolerance in both populations. LVS_LF_B73 was not significantly correlated with LVS_LF_CML103 and LB (Table 4).

The QTL for MVS_LB (chromosome 2) had a PVE value of 7.1% and the two QTL for LVS_LB (chromosomes 2 and 3) had a combined 16.8% PVE. The allele on chromosome 3 inherited from B73 conferred heat tolerance and the allele on chromosome 2 from B73 conferred intolerance (Table 2). Quantitative trait loci for LB had consistent effects across developmental stages. There was a significant correlation between LB and LVS_LF_CML103 ($P < 0.001$), and QTL for both traits mapped to the same region of chromosome 2. The QTL are 10 cM apart and the one-LOD drop confidence intervals (CIs) did not overlap, suggesting

Table 2. Quantitative trait loci identified for heat tolerance in B73 × NC350 and B73 × CML103. Position and one-logarithm of odds (LOD) drop confidence interval (CI) are shown. Negative additive effect values denote B73 as the source of the heat-tolerant allele, and if positive values denote that the tolerance allele is from the other parent.

Population	Trait†	Developmental stage‡	Chromosome	Position	CI	LOD	PVE§	Additive effect
					cM		%	
B73 × NC350	LF_NC350	EVS	9	46	45.5–46.5	3.21	6.64	0.28
			10	45	43.5–47.5	7.55	16.15	–0.44
	LF_NC350	MVS	5	44	42.5–47.5	3.25	6.60	0.35
			9	57	55.5–60.5	4.01	8.21	0.39
			10	40	39.5–40.5	7.94	15.76	–0.54
	LF_NC350	LVS	5	43	42.5–45.5	3.50	6.87	0.49
			9	52	50.5–54.5	4.39	9.20	0.57
			10	40	39.5–41.5	6.73	13.26	–0.69
	LF_B73	LVS	1	60	58.5–62.5	3.32	7.96	0.45
	LB	EVS	1	74	72.5–77.5	6.85	15.24	–0.52
			10	43	41.5–43.5	3.20	6.54	–0.34
		MVS	1	77	76.5–78.5	3.91	8.73	–0.39
		LVS	8	91	87.5–95.5	3.72	8.73	0.57
			10	38	37.5–39.5	3.41	7.63	–0.54
	TSBL	LVS	5	114	112.5–116.5	3.37	7.96	0.41
B73 × CML103	LF_CML103	LVS	2	113	108.5–115.5	5.55	11.61	0.59
			3	107	104.5–109.5	5.20	10.25	–0.56
	LF_B73	LVS	1	63	60.5–67.5	7.77	15.54	0.61
	LB	MVS	2	123	120.5–124.5	3.27	7.10	0.45
		LVS	2	123	120.5–124.5	3.66	8.39	0.49
			3	134	133.5–138.5	3.84	8.45	–0.49
	PD	LVS	3	106	104.5–107.5	13.03	26.21	–0.65

† LF, leaf firing; LB, leaf blotching; TSBL, tassel blasting; RSS, reduction in spikelet size; PD, plant death.

‡ EVS, early vegetative stage; MVS, middle vegetative stage; LVS, late vegetative stage.

§ PVE, phenotypic variance explained.

Table 3. Pearson correlation coefficient for heat tolerance traits measured in B73 × NC350.

Trait†	MVS_LF_NC350	LVS_LF_NC350	TSBL	RSS	LVS_LF_B73	EVS_LB	MVS_LB	LVS_LB
EVS_LF_NC350	0.93***	0.73***	0.12	0.44***	–0.12	0.56***	0.36***	0.44***
MVS_LF_NC350		0.86***	0.18**	0.44***	–0.12	0.53***	0.41***	0.48***
LVS_LF_NC350			0.18**	0.42***	–0.12	0.51***	0.49***	0.52***
TSBL				–0.16	0.37***	0.04	–0.01	0.22**
RSS					–0.10	0.36***	0.36***	0.26**
LVS_LF_B73						–0.20**	–0.09	0.08
EVS_LB							0.69***	0.63***
MVS_LB								0.54***

***, ** Significant at the 0.01 and 0.001 probability levels, respectively.

† EVS, early vegetative stage; LF, leaf firing; MVS, middle vegetative stage; LVS, late vegetative stage; TSBL, tassel blasting; RSS, reduced tassel size; LB, leaf blotching.

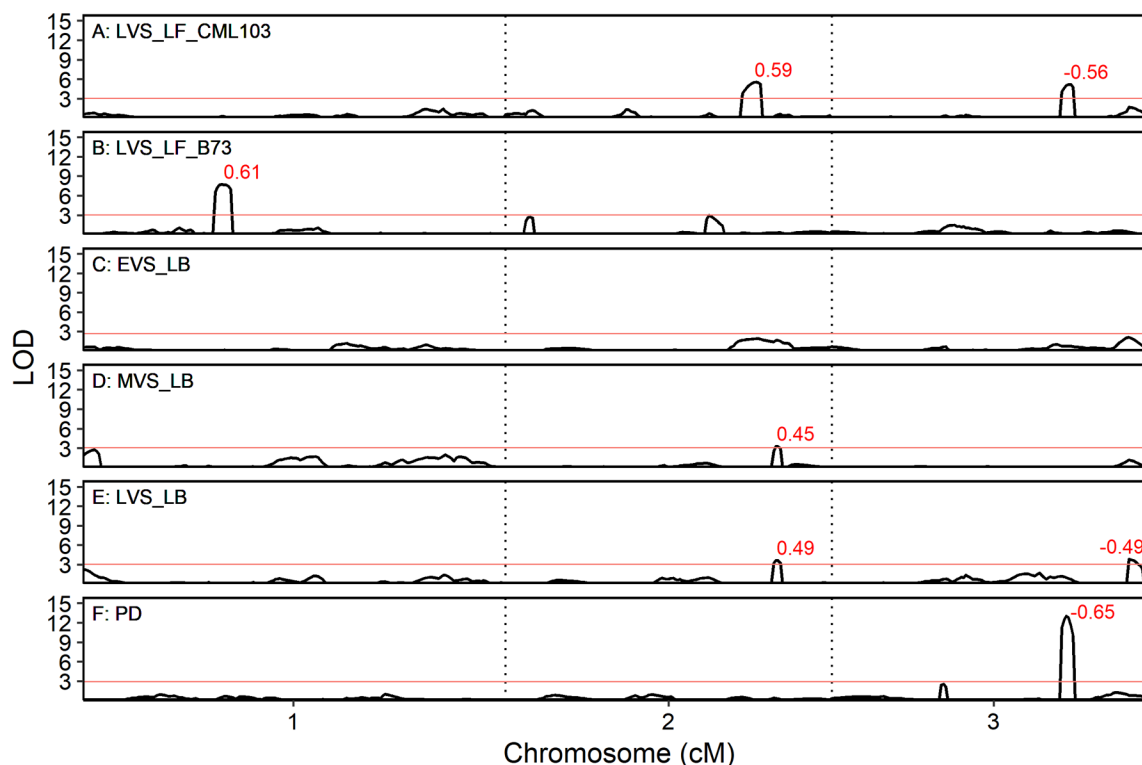


Fig. 4. Genetic mapping results of six heat stress traits measured in 195 B73 × CML103 recombinant inbred lines: (A) late vegetative stage (LVS) leaf firing CML103 (LVS_LF_CML103); (B) LVS_LF_B73; (C) early vegetative stage (EVS), (D) middle vegetative stage (MVS), and (E) LVS leaf blotching (LB); and (F) plant death (PD). Logarithm of odds (LOD) values are shown for chromosomes containing quantitative trait loci (QTL), and red horizontal lines denote the threshold determined by 1000 permutations. Additive QTL effects are reported next to QTL peaks and are negative if B73 donated the heat-tolerant allele and positive if CML103 donated the allele.

that the QTL detected different genetic elements. No QTL were detected for EVS_LB.

The single QTL for PD on chromosome 3 had the largest PVE (26.2%) of any QTL in either population. The QTL for PD and LVS_LF_CML103 appeared to capture the same loci as they were 1 cM apart, and the two traits were significantly correlated ($P < 0.001$), with the B73 allele conferring heat tolerance. This implies that the QTL on chromosome 3 is involved in HS tolerance in both the tassel and leaf.

DISCUSSION

The results of this study represent a starting point to further elucidate the genetic control of HS in North American maize germplasm. The only prior genetic mapping study of HS tolerance in maize under well-watered field conditions used European flint and dent germplasm (Frey et al.,

2016). One foliar HS response was reported in Frey et al. (2016), leaf scorching, and a QTL declared on chromosome 9. The physical positions of the CIs overlap for the leaf scorching QTL and the QTL for LF on chromosome 9 in B73 × NC350. The detection of loci influencing foliar heat tolerance in the distal region of chromosome 9 in different genetic material, evaluated in different continents, makes that genomic region well suited for future research.

Observed differences in the development of LF between inbred parents lead us to ask if the forms of LF are under different genetic control. Quantitative trait loci for the B73 form of LF (LVS_LF_B73) were detected on chromosome 1 in both populations, with the B73 allele conferring heat intolerance, explaining 8.0% of phenotypic variance in B73 × NC350 and 15.5% in B73 × CML103. There was no significant phenotypic

Table 4. Pearson correlation coefficient for heat tolerance traits measured in B73 × CML103.

Trait†	MVS_LB	LVS_LB	LVS_LF_CML103	LVS_LF_B73	PD
EVS_LB	0.51***	0.42***	0.45***	0.05	0.04
MVS_LB		0.71***	0.66***	0.02	0.06
LVS_LB			0.75***	−0.03	0.23***
LVS_LF_CML103				−0.06	0.34***
LVS_LF_B73					−0.03

*** Significant at the 0.001 probability level

† EVS, early vegetative stage; LB, leaf blotching; MVS, middle vegetative stage; LVS, late vegetative stage; LF, leaf firing; PD, plant death.

correlation between LVS_LF_B73 and the other parental forms of LF (LF_NC350 and LF_CML103), and QTL did not co-localize. This gives credence to our hypothesis that multiple, phenotypically differentiable pathways are involved in the LF trait. Future studies measuring LF should look for differences in the development of LF, in addition to measuring the magnitude of heat-induced tissue death.

Having observed that the leaves of susceptible genotypes displayed irregular discolored lesions (LB) that can lead to necrosis, in addition to outright tissue death (LF), we asked if LB is a less severe form of LF (i.e., controlled by the same pathway) or if LF and LB are distinct traits (i.e., controlled by different HS response pathways). Co-localization of LF and LB QTL occurred only on chromosome 10 in B73 \times NC350, and all LVS_LF_CML103 and LB QTL in B73 \times CML103 mapped to the same regions, although the QTL CIs for the latter population were 5 and 24 cM apart (Table 2). We observed equal instances of QTL dissimilarities (non-co-localization) and similarities (co-localization) in the detectable genetic control of LF and LB in the two populations we examined. The dissimilarities suggest that LF and LB are distinct traits under control of different pathways; therefore, cultivar improvement should proceed by pyramiding tolerance alleles for both traits. The observed co-localization suggests that the traits may share regulatory elements and cultivar improvement should focus on identifying and selecting alleles that influence both traits. A sorghum association panel was scored for LF and LB, and similar to our results, there were instances of QTL mapping to unique regions and co-localization (Chen et al., 2017). An objective of our ongoing research is to further clarify the unique and shared genetic components of the LF and LB pathways to facilitate the development of cultivars with improved heat tolerance.

We also sought to determine if the genetic control of HS tolerance changes across vegetative developmental stages. We measured LF_NC350 and LB at three vegetative stages and found few differences in the number of QTL across time. For LB in B73 \times NC350, the QTL on chromosome 1 was detected in the EVS and MVS, the QTL on chromosome 10 was detected in EVS and LVS, and the QTL on chromosome 8 was detected only in the LVS. No QTL were detected for EVS_LB in B73 \times CML103, the QTL on chromosome 2 was significant in the MVS and LVS, and the QTL on chromosome 3 was detected only in the LVS. Phenotypic variance explained by QTL across vegetative developmental stages was also stable, the largest change in PVE being a decrease of 6.5% from EVS to MVS for the LB QTL on chromosome 1 in B73 \times NC350. The aforementioned sorghum association study by Chen et al. (2017) also measured LF and LB at three vegetative stages and, compared with our results, observed more variability in QTL number and position across time. If HS has

consistent genetic control across developmental stages, then the timing of HS during vegetative development is less important than the severity of the stress.

In addition to foliar response to HS, we scored two tassel traits in B73 \times NC350 (TSBL and RSS) and PD in B73 \times CML103. Tassel blasting is a recognized symptom of heat and drought stress, but studies involving the genetic control of TSBL are few. The estimated heritability for TSBL was 0.90, similar to the estimate of 0.99 in a set of diverse tropical maize inbred lines (Alam et al., 2017). One QTL for TSBL was detected on chromosome 5; no QTL were detected for RSS. The QTL for PD had a PVE of 26.2%, the highest of any trait in either population. A significant phenotypic correlation was observed among PD, LVS_LF_CML103, and LVS_LB, and all three traits had QTL in the same region of chromosome 3. It is unclear if PD is a result of extreme susceptibility to LB and LVS_LF_CML103.

We compared the position of maize genes syntenic to the 15 sorghum genes listed in Table 3 of Chen et al. (2017). Two syntenic maize genes are within, or close to, the physical position of QTL CIs identified in this study (Supplemental Table S2). The sorghum gene *Sb09g026470* is syntenic to *Zm00001d043634* and is a brassinosteroid leucine-rich repeat receptor kinase (*BR11*) (Tůmová et al., 2018). The physical position of *Zm00001d043634* is within the QTL CIs for PD and LVS_LF_CML103 on chromosome 3 in B73 \times CML103. The second gene, *Zm00001d025343*, is a purple acid phosphatase and syntenic to *Sb06g015470* in sorghum. Purple acid phosphatases are a large family of proteins, and in pearl millet [*Pennisetum glaucum* (L.) R. Br.], they have a known role in HS response (Reddy et al., 2017). The two syntenic genes in maize and sorghum that influence foliar response to HS are potential targets for future research.

With HS events predicted to increase in the future, it is critical that maize germplasm with enhanced tolerance be developed (Anderson, 2011; Coumou and Robinson, 2013). Our research used existing mapping populations to show that the parental forms of LF are under different genetic control and that foliar damage in response to HS can manifest itself as LB in addition to LF. Further efforts are needed to resolve the mechanisms of HS in temperate germplasm on both an inheritance and physiological basis.

Conflict of Interest

The authors declare that there is no conflict of interest.

Supplemental Material Available

Supplemental material for this article is available online.

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References

- Akula, D., A.P. Patil, P.H. Zaidi, P. Kuchanur, M. Vinayan, and K. Seetharam. 2018. Line \times testers analysis of tropical maize inbred lines under heat stress for grain yield and secondary traits. *Maydica* 61:4.
- Alam, M.A., K. Seetharam, P.H. Zaidi, A. Dinesh, M.T. Vinayan, and U.K. Nath. 2017. Dissecting heat stress tolerance in tropical maize (*Zea mays* L.). *Field Crops Res.* 204:110–119. doi:10.1016/j.fcr.2017.01.006
- Anderson, B.T. 2011. Near-term increase in frequency of seasonal temperature extremes prior to the 2 C global warming target. *Clim. Change* 108:581. doi:10.1007/s10584-011-0196-4
- Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67(1). doi:10.18637/jss.v067.i01
- Cantarero, M., A. Cirilo, and F. Andrade. 1999. Night temperature at silking affects set in maize. *Crop Sci.* 39:703–710. doi:10.2135/cropsci1999.0011183X003900020017x
- Carter, E.K., J. Melkonian, S.J. Riha, and S.B. Shaw. 2016. Separating heat stress from moisture stress: Analyzing yield response to high temperature in irrigated maize. *Environ. Res. Lett.* 11. doi:10.1088/1748-9326/11/9/094012
- Chen, J., R. Chopra, C. Hayes, G. Morris, S. Marla, J. Burke, et al. 2017. Genome-wide association study of developing leaves' heat tolerance during vegetative growth stages in a sorghum association panel. *Plant Genome* 10(2). doi:10.3835/plantgenome2016.09.0091
- Chen, J., W. Xu, J.J. Burke, and Z. Xin. 2010. Role of phosphatidic acid in high temperature tolerance in maize. *Crop Sci.* 50:2506–2515. doi:10.2135/cropsci2009.12.0716
- Chen, J., W. Xu, J. Velten, Z. Xin, and J. Stout. 2012. Characterization of maize inbred lines for drought and heat tolerance. *J. Soil Water Conserv.* 67:354–364. doi:10.2489/jswc.67.5.354
- Coumou, D., and A. Robinson. 2013. Historic and future increase in the global land area affected by monthly heat extremes. *Environ. Res. Lett.* 8(3). doi:10.1088/1748-9326/8/3/034018
- Duffy, P., and C. Tebaldi. 2012. Increasing prevalence of extreme summer temperatures in the U.S. *Clim. Change* 111:487–495. doi:10.1007/s10584-012-0396-6
- Edreira, J.R., and M.E. Otegui. 2013. Heat stress in temperate and tropical maize hybrids: A novel approach for assessing sources of kernel loss in field conditions. *Field Crops Res.* 142:58–67. doi:10.1016/j.fcr.2012.11.009
- FAOSTAT. 2016. FAOSTAT database. FAO, Rome. <http://www.fao.org/faostat/en/#home> (accessed 24 Apr. 2018).
- Frey, F.P., T. Presterl, P. Lecoq, A. Orlik, and B. Stich. 2016. First steps to understand heat tolerance of temperate maize at adult stage: Identification of QTL across multiple environments with connected segregating populations. *Theor. Appl. Genet.* 129:945–961. doi:10.1007/s00122-016-2674-6
- Frey, F.P., C. Urbany, B. Hüttel, R. Reinhardt, and B. Stich. 2015. Genome-wide expression profiling and phenotypic evaluation of European maize inbreds at seedling stage in response to heat stress. *BMC Genomics* 16:123. doi:10.1186/s12864-015-1282-1
- Frova, C. 1996. Genetic dissection of thermotolerance in maize In: *Physical stresses in plants: Genes and their products for tolerance*. Springer, Berlin, Heidelberg. p. 31–38. doi:10.1007/978-3-642-61175-9_3
- Frova, C., and M. Sari-Gorla. 1994. Quantitative trait loci (QTLs) for pollen thermotolerance detected in maize. *Mol. Genet. Genomics* 245:424–430. doi:10.1007/BF00302254
- Holland, J.B., W.E. Nyquist, and C.T. Cervantes-Martinez. 2003. Estimating and interpreting heritability for plant breeding: An update. *Plant Breed. Rev.* 22:9–112.
- Jodage, K., P. Kuchanur, P. Zaidi, A. Patil, K. Seetharam, M. Vinayan, and B. Arunkumar. 2017. Genetic analysis of heat stress tolerance and association of traits in tropical maize (*Zea mays* L.). *Environ. Ecol.* 35:2354–2360.
- Karim, M., Y. Fracheboud, and P. Stamp. 1999. Photosynthetic activity of developing leaves of *Zea mays* is less affected by heat stress than that of developed leaves. *Physiol. Plant.* 105:685–693. doi:10.1034/j.1399-3054.1999.105413.x
- Kuznetsova, A., P.B. Brockhoff, and R.H.B. Christensen. 2016. lmerTest: Tests in linear mixed effects models. *J. Stat. Softw.* 82(13). doi:10.18637/jss.v082.i13
- Lenth, R.V. 2016. Least-squares means: The R package lsmeans. *J. Stat. Softw.* 69(1). doi:10.18637/jss.v069.i01
- Lizaso, J., M. Ruiz-Ramos, L. Rodriguez, C. Gabaldon-Leal, J. Oliveira, I. Lorite, et al. 2018. Impact of high temperatures in maize: Phenology and yield components. *Field Crops Res.* 216:129–140. doi:10.1016/j.fcr.2017.11.013
- Lobell, D.B., M.J. Roberts, W. Schlenker, N. Braun, B.B. Little, R.M. Rejesus, and G.L. Hammer. 2014. Greater sensitivity to drought accompanies maize yield increase in the US Midwest. *Science* 344:516–519. doi:10.1126/science.1251423
- Lyons, E., and M. Freeling. 2008. How to usefully compare homologous plant genes and chromosomes as DNA sequences. *Plant J.* 53:661–673. doi:10.1111/j.1365-313X.2007.03326.x
- Mallya, G., L. Zhao, X. Song, D. Niyogi, and R. Govindaraju. 2013. 2012 Midwest drought in the United States. *J. Hydrol. Eng.* 18:737–745. doi:10.1061/(ASCE)HE.1943-5584.0000786
- McMullen, M.D., S. Kresovich, H.S. Villeda, P. Bradbury, H. Li, Q. Sun, et al. 2009. Genetic properties of the maize nested association mapping population. *Science* 325:737–740. doi:10.1126/science.1174320
- Meng, L., H. Li, L. Zhang, and J. Wang. 2015. QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. *The Crop J.* 3:269–283. doi:10.1016/j.cj.2015.01.001
- Meng, Q., P. Hou, D.B. Lobell, H. Wang, Z. Cui, F. Zhang, and X. Chen. 2014. The benefits of recent warming for maize production in high latitude China. *Clim. Change* 122:341–349. doi:10.1007/s10584-013-1009-8
- Mittler, R., A. Finka, and P. Goloubinoff. 2012. How do plants feel the heat? *Trends Biochem. Sci.* 37:118–125. doi:10.1016/j.tibs.2011.11.007
- Neiff, N., S. Trachsel, O.R. Valentinuz, C.N. Balbi, and F.H. Andrade. 2016. High temperatures around flowering in maize: Effects on photosynthesis and grain yield in three genotypes. *Crop Sci.* 56:2702–2712. doi:10.2135/cropsci2015.12.0755
- Ottaviano, E., M. Sari Gorla, E. Pè, and C. Frova. 1991. Molecular markers (RFLPs and HSPs) for the genetic dissection of thermotolerance in maize. *Theor. Appl. Genet.* 81:713–719. doi:10.1007/BF00224979
- R Core Team. 2017. R: A language and environment for statistical computing. R Found. Stat. Comput., Vienna.

- Reddy, C.S., K.M. Kim, D. James, P. Varakumar, and M.K. Reddy. 2017. PgPAP18, a heat-inducible novel purple acid phosphatase 18-like gene (PgPAP18-like) from *Pennisetum glaucum*, may play a crucial role in environmental stress adaptation. *Acta Physiol. Plant.* 39:54. doi:10.1007/s11738-017-2348-2
- Rippey, B.R. 2015. The U.S. drought of 2012. *Weather Clim. Extremes* 10:57–64. doi:10.1016/j.wace.2015.10.004
- Roy, S.J., E.J. Tucker, and M. Tester. 2011. Genetic analysis of abiotic stress tolerance in crops. *Curr. Opin. Plant Biol.* 14:232–239. doi:10.1016/j.pbi.2011.03.002
- Schooper, J., R. Lambert, and B. Vasilas. 1986. Maize pollen viability and ear receptivity under water and high temperature stress. *Crop Sci.* 26:1029–1033. doi:10.2135/cropsci1986.0011183X002600050038x
- Schooper, J.B., R.J. Lambert, B.L. Vasilas, and M.E. Westgate. 1987. Plant factors controlling seed set in maize the influence of silk, pollen, and ear-leaf water status and tassel heat treatment at pollination. *Plant Physiol.* 83:121–125. doi:10.1104/pp.83.1.121
- Shaw, S.B., D. Mehta, and S.J. Riha. 2014. Using simple data experiments to explore the influence of non-temperature controls on maize yields in the mid-West and Great Plains. *Clim. Change* 122:747–755. doi:10.1007/s10584-014-1062-y
- Sinsawat, V., J. Leipner, P. Stamp, and Y. Fracheboud. 2004. Effect of heat stress on the photosynthetic apparatus in maize (*Zea mays* L.) grown at control or high temperature. *Environ. Exp. Bot.* 52:123–129. doi:10.1016/j.envexpbot.2004.01.010
- Tůmová, L., D. Tarkowská, K. Řehorová, H. Marková, M. Kočová, O. Rothová, et al. 2018. Drought-tolerant and drought-sensitive genotypes of maize (*Zea mays* L.) differ in contents of endogenous brassinosteroids and their drought-induced changes. *PLoS One* 13:e0197870. doi:10.1371/journal.pone.0197870
- Wilhelm, E., R. Mullen, P. Keeling, and G. Singletary. 1999. Heat stress during grain filling in maize: Effects on kernel growth and metabolism. *Crop Sci.* 39:1733–1741. doi:10.2135/cropsci1999.3961733x
- Yu, J., J.B. Holland, M.D. McMullen, and E.S. Buckler. 2008. Genetic design and statistical power of nested association mapping in maize. *Genetics* 178:539–551. doi:10.1534/genetics.107.074245
- Zaidi, P., M. Zaman-Allah, S. Trachsel, K. Seetharam, J. Cairns, and M. Vinayan. 2016. Phenotyping for abiotic stress tolerance in maize: Heat stress. CIMMYT, Hyderabad, India.