

## Characterization of maize inbred lines for drought and heat tolerance

J. Chen, W. Xu, J. Velten, Z. Xin, and J. Stout

**Abstract:** Drought and high temperature are two major environmental factors that severely limit plant productivity in the United States and worldwide, often causing extensive economic loss to agriculture. As global climate change progresses, agricultural production worldwide faces serious threats from frequent extreme weather conditions. Integrated approaches that improve the efficiency of agricultural water use and development of plant varieties that can alleviate the negative impacts of environmental stresses to maintain yield stability are essential to sustain and increase agriculture production. Maize (*Zea mays* L.) is a major crop in the United States and worldwide. Its production and yield stability are greatly affected by drought and high temperature stresses. Improving drought and heat tolerance in maize has become one of the top priorities for maize breeding programs in both private and public sectors. Identification of maize germplasm with superior drought and/or heat tolerance is essential and prerequisite for such propose. In this report, we evaluated a selection of maize inbred lines for drought and heat stress tolerance under field conditions in 2009 and 2010 and identified several inbred lines that showed high tolerance to drought. Tolerant inbred lines (Tx205, C2A554-4, and B76) were able to maintain relatively high leaf relative water content when subjected to drought stress, while sensitive lines (B73 and C273A) showed a rapid reduction in leaf relative water content at very early stage of drought. The tolerant lines also showed significantly greater ability to maintain vegetative growth and alleviate damage to reproductive tissues under drought conditions compared to the sensitive lines. Maize inbred lines and hybrids were also evaluated for tolerance to high temperature under well-watered conditions through field observations following the occurrence of major heat events. Maize inbred lines of distinct heat tolerance phenotype were identified. Furthermore, genetic and phenotypic analysis showed that maize hybrids made from inbred lines with superior heat tolerance inherited an enhanced tolerance to elevated temperatures. The tolerant germplasm accessions, like those identified in this study, are essential materials for breeding drought- and/or heat-tolerant maize hybrids. Study for the potential use of such materials to produce maize hybrids that are able to alleviate the negative impacts of drought and heat stress on the growth and development of maize plants is underway.

**Key words:** climate change—crop production—drought—germplasm—high temperature—maize

**Water and temperature are two critical environmental factors that continually influence the growth and development of plants.** Drought and temperature extremes can cause extensive economic loss to agriculture (Boyer 1982; Peng et al. 2004; NCDC 2011), an effect that is likely to increase as global climate change progresses (Stern 2006; Keane et al. 2009). The 2011 drought and heat waves (heat stress) have caused more than US\$5 billion in direct losses to agriculture (NCDC 2011) in the Southern Plains

and Southwest regions of the United States. Drought and heat stresses in 2010 reduced Russian wheat production by more than 30% (USDA 2010). It is anticipated that the negative impacts of drought and heat stress events on agricultural production are likely to be exacerbated in the future as these events become more frequent, intense, and erratic (Giorgi et al. 2001; Cayan et al. 2010; Sanderson et al. 2011). Compounding the problem, the availability of fresh water and land for agricultural use continues to decline

at an unsustainable rate (Hamdy et al. 2003; Perkins 2002; US CCSP 2008). It is predicted that by 2050, cropland could be reduced by 8% to 20% (UNEP GRID-Arendal 2009). Consequently, worldwide agricultural production will face challenges of unfavorable environmental conditions and water limitation (water deficit), underlining the need for comprehensive and fully integrated approaches to sustain and enhance agricultural productivity in the future (Parry and Hawkesford 2010; Tester and Langridge 2010; Delgado et al. 2011). While improvements in soil conservation and water management practices can enhance the efficiency of agricultural water use and the control of soil erosion (Bucks 1990; Hamdy et al. 2003; Delgado et al. 2007), development of stress-tolerant plant varieties will play an important role in alleviating the negative impacts of abiotic stresses on agricultural production (Tester and Langridge 2010). Screening genetic resources to identify plant germplasm with superior drought and/or heat tolerance is one of the foremost steps toward the success of such breeding programs.

Maize (*Zea mays* L.) is a major cereal crop worldwide, serving as a major staple for both human consumption and animal feed. It has also become a key resource for industrial applications and bioenergy production. Maize is highly productive under optimal environmental and crop management conditions. However, maize plants are also very susceptible to drought and heat; each year, an average of 15% to 20% of the potential world maize production is lost due to these stresses (FAO STAT 2006-2008; Lobell et al. 2011). The total yield loss depends on when the stress occurs (plant growth stage), as well as the duration and the severity of the stress. Early season drought reduces plant growth and inhibits plant development (Shaw 1983; Heiniger 2001). Drought that occurs at V8

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to V17 stages has significant impact on plant growth, architecture, ear size and kernel numbers (Westgate and Boyer 1985; Farré and Faci 2006). Drought occurring between two weeks before and two weeks after the silking stage can cause significant reductions in kernel set and kernel weight (Westgate and Bassetti 1990; Schussler and Westgate 1991), resulting in an average of 20% to 50% yield loss (Nielsen 2007; ACIAR n.d.).

High temperature (heat wave) stress at critical developmental stages of maize plants also causes significant yield loss (Shaw 1983; Lobell et al. 2011). Maize plants become susceptible to high temperatures after reaching eight-leaf stage or V8 (Chen et al. 2010). Extremely high temperature causes permanent tissue injury to developing leaves and the injured tissues dry out quickly, a phenomenon called leaf firing. It can also cause desiccation of tassel tissues, a phenomenon called tassel blasting. Plants with severe leaf firing and tassel blasting lose considerable photosynthetic leaf area, produce small ears, and show reduced kernel set and kernel weight. Moderate heat stress occurring at early reproductive stages reduces pollen production, pollination rate, kernel set, and kernel weight, resulting in significant yield loss (Cheikh and Jones 1994; Cantarero et al. 1999; Wilhelm et al. 1999). It has been suggested that each 1°C (1.8°F) increase in temperature above optimum could result in 1% to 2% and up to 3% to 4% of grain yield reduction (Shaw 1983). High temperature coupled with drought during pollination period of maize plants can result in up to 100% yield loss (Heiniger 2001). The dry and hot early summer conditions in much of the United States in 2012 have already caused significant reductions in the prediction of 2012 crop production. According to USDA's July 11 Crop Production report, corn production in the United States has slashed by a staggering 46 million t (1.8 billion bu) due to the dryness and extreme hot weather conditions, from 376 million t (14.79 billion bu) down to 329 million t (12.97 billion bu) (Robinson 2012a and 2012b; USDA 2012). Big losses in US corn production are expected as drought and heat stresses prolong in 2012.

In maize, a large variation for drought and/or heat tolerance exists in germplasm collections (Chen et al. 2010; Lu et al. 2011). Identification and characterization of such variation is a first and essential step in devel-

oping drought- and/or heat-tolerant maize hybrids. In this study, field experiments were conducted to screen and evaluate maize inbred lines for drought and/or heat tolerance. Inbred lines with superior drought and/or heat tolerance traits were identified and their responses to these stresses examined. Heat tolerance of maize inbred lines and their F1 hybrids was evaluated in a well-watered field. The potential use of those identified drought- and/or heat-tolerant lines for the breeding of drought-/heat-tolerant hybrids was discussed.

## Materials and Methods

### *Plant Materials and Experimental*

**Conditions.** Five publically released maize inbred lines (B73, B76, B106, NC350, and Tx205) and six inbred lines developed at Texas A&M (B105C, BR-1, C273A, C2A554-4, C32B, and S1W) were evaluated for drought and/or heat tolerance in 2009 and 2010 at the USDA Agricultural Research Service research field (33°35'62"N, 101°54'19"W, elevation 982 m [3,222 ft]) in Lubbock, Texas, where hot and dry environments during the growing season are ideal for field evaluation of drought and/or heat stress tolerance in maize. In addition, heat tolerance of maize hybrids made from crosses of B76 × B106, B76 × NC350, and NC350 × B106 was evaluated. Due to the sensitivity of B106 and NC350 to high temperature, these two lines were excluded from drought stress evaluation. B73 was included in this study as a control. B73 is the most widely used inbred line in breeding and production since its release (Russell 1972). It has great yield potential despite its susceptibility to insects and moderate sensitivity to abiotic stresses.

Maize seeds were planted on April 15, 2009, and April 28, 2010, by a four-row planter in a split-plot design. Plants were thinned to about 15 cm (5.9 in) apart at three-leaf stage. The distance between rows in all experimental fields was 1.02 m (3.35 ft), and the soil type at experimental site is classified as an Amarillo sandy loam. The meteorological data during these experiments were recorded using an automated weather station (USDA ARS 2012). In 2009, the drought stress experiment consisted of four irrigation levels: well watered (WW, 100% of potential evapotranspiration [PET]), moderately drought stressed (MD, 50% of PET), severely drought stressed (SD, 25% of PET), and permanent stressed (PD, no irriga-

tion during treatment). Field irrigation was provided by a center pivot system, and irrigation levels were program controlled. Maize lines were planted in 5 m (16.4 ft) rows with three replications for inbred lines and four replications for hybrids. Maize plants in all plots were grown under WW conditions prior to stress treatment. Drought treatments started on June 10. The MD and SD treatments were applied by reducing irrigation to 50% and 25% of WW level, respectively, until the end of pollination. For the PD plot, no irrigation was provided after the initiation of drought treatment. The cumulative precipitation received during the first 30 days of drought treatments was 45 mm (1.8 in). The amounts of precipitation of most rainfall events were small and insignificant due to the dry and hot soil surface (figure 1). The total cumulative precipitation during the growing season in 2009 was 140 mm (5.5 in).

In 2010, maize seeds were planted in single 5 m (16.4 ft) row with three replications for inbred lines and double 5 m (16.4 ft) rows with three replications for hybrids (one row for hybrid of normal cross and one row for hybrid made from reciprocal cross). All plots were well watered from planting to the initiation of drought treatment, which started on June 12 and consisted of three irrigation levels: WW, MD, and SD. The cumulative precipitation received at the experimental site for the first 30 days of drought stress treatments was 179 mm (7 in). The multient rainfall with substantial amounts of precipitation prevented imposing the desired drought stress in MD and SD plots. Therefore, the drought stress experiment was terminated two weeks after initiating the stress treatment (June 26). Irrigation for all plots was temporally stopped from July 5 to July 20 due to substantial amount of rainfalls received between July 1 and July 4 (127 mm [5 in], figure 1). Irrigation at 100% PET level was resumed to all plots after July 21.

### *Drought Stress Tolerance Evaluation.*

Drought tolerance of nine maize inbred lines (B73, B76, B105C, BR-1, C273A, C2A554-4, C32B, S1W, and Tx205) was evaluated at set times during the treatment and at the end of growing season. In this experiment, mature leaves were defined as leaves with a visible collar. The leaves were numbered from the top to the bottom of the plant, with the newest leaf with a visible collar assigned as the first mature leaf.

On the day of initiating drought treatment (0 days), plant height was measured ( $H_0$ , height from soil surface to the base of first mature leaf), leaf samples were collected, and relative water content (RWC) of leaf tissue was determined (Barrs and Weatherley 1962). The changes in leaf RWC of maize plants in response to different levels of drought stress were examined at 4 days, 7 days, and 10 days of drought treatment. To measure leaf RWC, a  $2 \times 4$  cm ( $0.8 \times 1.6$  in) leaf segment was collected from the middle section of the second mature leaf of a randomly selected plant and placed into a preweighed (W1) and prelabeled 50 ml ( $3.1$  in<sup>3</sup>) tube. The tube was capped immediately and then placed in a closed cooler ( $\sim 8^\circ\text{C}$  to  $12^\circ\text{C}$  [ $46^\circ\text{F}$  to  $54^\circ\text{F}$ ]). A total of six leaf samples per replicate were collected from six different plants. After returning to the lab, the tubes were weighed immediately (tube and tissue weight, W2), followed by the addition of 40 ml ( $2.4$  in<sup>3</sup>) of distilled water to each tube. The fresh weight (FW) of leaf tissue was calculated as

$$W2 - W1 = FW. \quad (1)$$

The turgid weight (TW) of the fully rehydrated leaf tissue was measured after a 24-hour rehydration period at  $4^\circ\text{C}$  ( $39^\circ\text{F}$ ). The dry weight (DW) of leaf tissue was obtained after drying the samples in a  $65^\circ\text{C}$  ( $149^\circ\text{F}$ ) oven for 3 days. The RWC of leaf tissue was calculated as

$$\left[ \frac{FW - DW}{TW - DW} \right] \times 100 = \text{RWC}. \quad (2)$$

In addition, drought stress phenotypes of maize inbred lines were visually evaluated as the stress treatment progressed.

Plant height was measured again at the end of the growing season ( $H_e$ ), and plant growth ( $H_i$ , posttreatment plant height grown) was calculated as

$$H_e - H_0 = H_i. \quad (3)$$

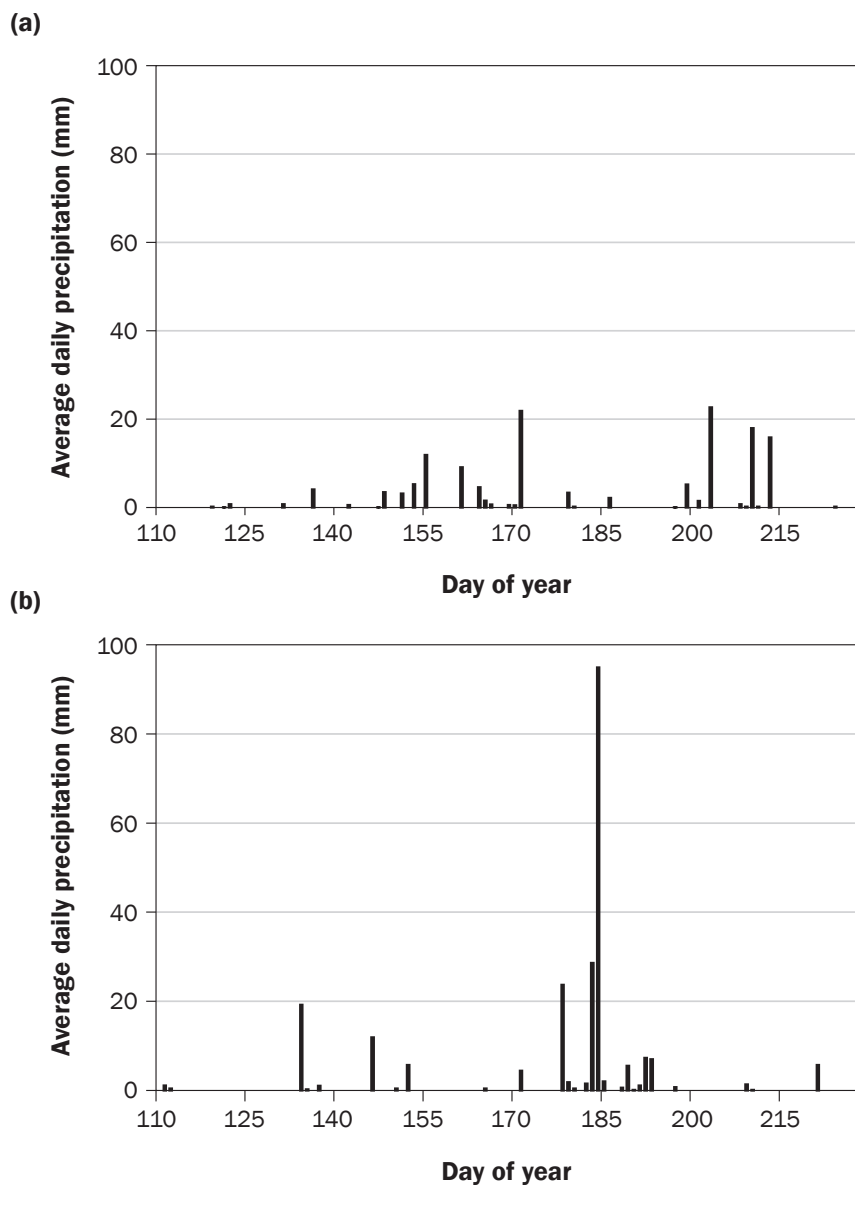
The effect of drought on plant growth was expressed as the percentage height increase compared to that measured in WW plots:

$$\left[ \frac{H_{ed} - H_{od}}{H_{ew} - H_{ow}} \right] \times 100, \quad (4)$$

where  $w$  represents plant height measurements in WW plots and  $d$  represents plant

**Figure 1**

Daily precipitation from planting to the end of the growing season in (a) 2009 and (b) 2010 for maize inbred lines grown at Lubbock, Texas.



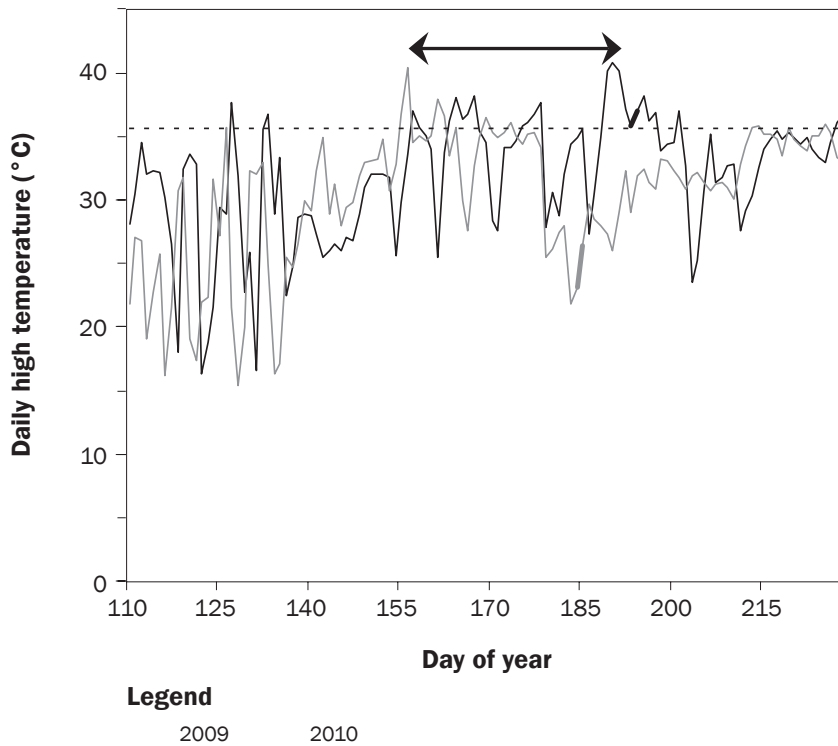
height measurements in drought-stressed plots. Means separation was performed on the lines using the general linear model procedure in SAS (version 9.1.3, SAS Institute, Cary, North Carolina) for all variables. The data was analyzed as a split block design with irrigation treatment as main effect. The Least Squares Means option was used to calculate Tukey's honestly significant difference groupings of treatments and lines.

**Heat Stress Tolerance Evaluation.** Heat tolerance of maize seedlings of all 11 inbred lines was evaluated visually in WW fields

after each natural major heat event (defined as a heat wave lasting for 3 or more days with the air maximum high temperature exceeding  $36^\circ\text{C}$  [ $97^\circ\text{F}$ ] for each given day). Phenotypes, such as leaf firing, leaf blotching, and tassel blasting, were visually inspected 7 to 10 days after the onset of each heat event, and the number of plants showing leaf firing or tassel blasting phenotype was recorded. The effect of heat stress on pollen production was also evaluated in 2009. Under the circumstance where an additional heat wave occurred within the 7 to 10 day time frame

**Figure 2**

Daily air maximum temperature from planting to the end of the growing season in 2009 and 2010 for maize inbred lines grown at Lubbock, Texas. The arrow bracket indicates the time of drought stress treatment in the 2009 season, and the dotted line indicates the threshold for heat wave events, 36°C.



of phenotype evaluation for a previous heat wave, the evaluation was performed 5 to 7 days after the last heat wave. Due to variations in the timing of heat events (figure 2), the developmental stages at which heat tolerance was evaluated in 2009 and 2010 varied.

**Greenhouse High Temperature Treatments and Tassel Phenotype Evaluation.** In nature, heat waves occur sporadically during the growing season, making it difficult to evaluate the impacts of heat stress on tassel development at various tassel developmental stages. For this reason, we evaluated the impact of high temperature on tassel development under controlled environments in a greenhouse. One inbred (B76) representing heat-tolerant lines, two heat-sensitive inbred (B106 and NC350) and one inbred with moderate sensitivity to high temperature (B73) were selected for this experiment. Maize kernels were planted in plastic pots with a diameter of 30 cm (11.8 in) and height of 26 cm (10.2 in), containing Sunshine #1 growth mix soil (Sun Gro Horticulture, Bellevue, Washington), one plant per pot. All maize plants were maintained under WW conditions from planting to the end of

their life cycle. Plants were grown in temperature controlled greenhouses under 14 hour/10 hour photoperiod at 25°C/21°C (77°F/70°F), day/night temperatures to set developmental stages. The light intensity was 400 to 550  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$  during the day, and the pots were rotated twice a week. Maize plants were grown under normal conditions prior to high temperature treatment. At set developmental stages (V8, V9, V10, V11, V12, V13, and V14), six randomly selected plants from each inbred line were transferred to a temperature-controlled, walk-in growth chamber set at 38°C/30°C (100°F/86°F) with a 14 hour/10 hour cycle at a light intensity similar to that in the greenhouse and 50% relative humidity and were subjected to a 3 day high temperature treatment. These plants were also kept under WW conditions by watering the pots twice a day during treatment. After 3 days of treatment, the high temperature treated plants were then transferred back to the greenhouse and continued to grow under normal and WW conditions. Tassel phenotype of maize plant was evaluated at the end of silking stage.

## Results and Discussion

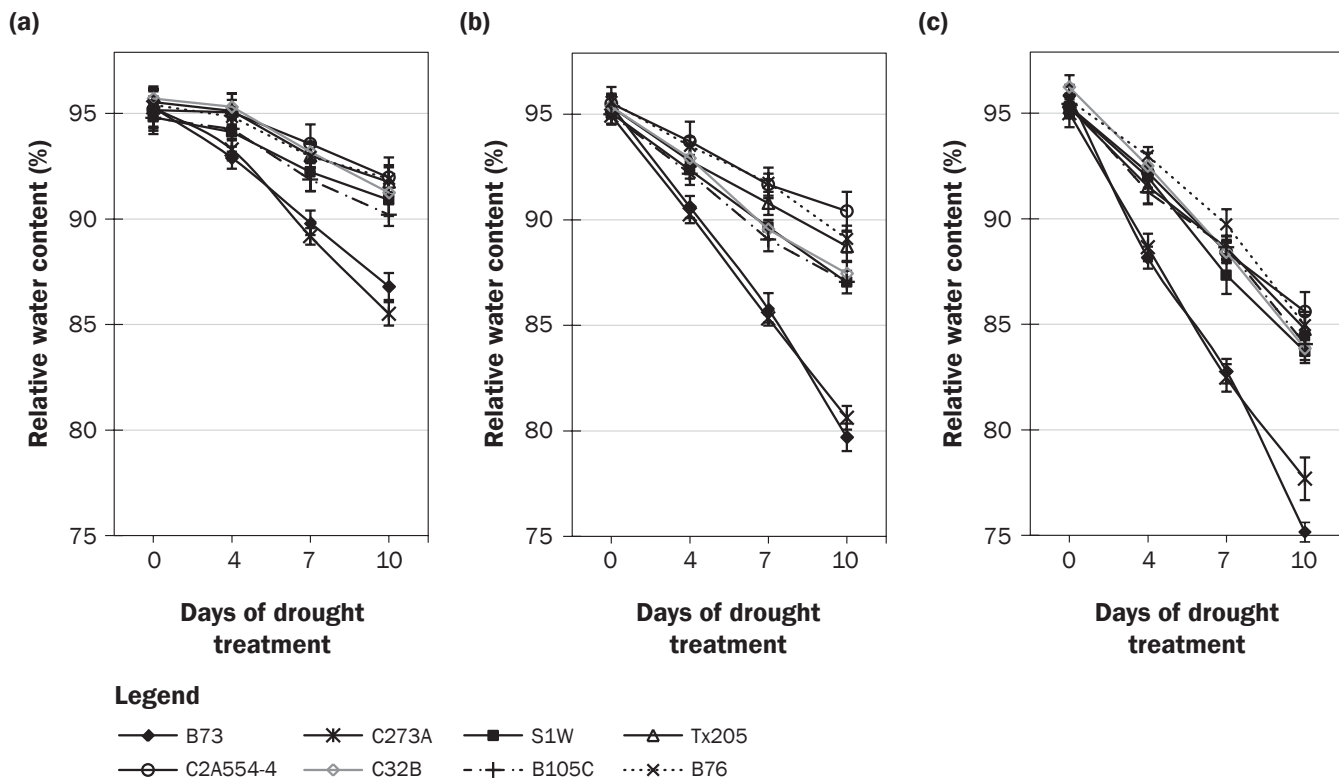
Breeding for drought- and heat-tolerant cultivars/hybrids is an essential step towards sustaining and increasing crop productivity, especially as the negative impacts of climate change on agricultural production escalate. Screening for, and characterization of, plant germplasm with better stress tolerance trait(s) are prerequisites for the success of such breeding programs. This study evaluated a set of maize inbred lines for drought and heat tolerance under field conditions and identified inbred lines with superior drought and heat tolerance traits relative to B73, an inbred line commonly used in breeding programs. The results suggest great genetic variation among maize germplasm for drought and/or heat tolerance. Inbred lines identified for their superior drought and/or heat tolerance will serve as essential genetic material for breeding drought- and heat-tolerant hybrids.

**Response of Maize Inbred Lines to Drought Treatments.** One of the mechanisms associated with drought tolerance is the ability of a plant to retain cellular water under water deficit stress (Kumar et al. 2004; Erice et al. 2010). RWC is a physiological measurement of plant cellular water status influenced by both leaf water potential and osmotic adjustment (Barrs and Weatherley 1962). Leaf RWC is a reliable indicator of leaf water deficit status at the time of sampling. It is often used to examine the response of a plant to the progress of drought stress (Lafitte and Courtois 2002; Erice et al. 2010). To determine the water status changes within plants after initiating drought stress, we measured leaf RWC of maize inbred lines at 4 days, 7 days, and 10 days of drought treatment in the MD, SD, and PD plots and compared these values with RWC of leaf tissue measured prior to treatment (0 days). Phenotypic changes of maize plant induced by drought stress, such as leaf rolling, leaf color change (bleaching), and leaf wilting, were visually evaluated at the time of collecting leaf samples.

In response to drought treatment, leaf RWC in maize plants of nine tested inbred lines decreased over time in all three drought treatment plots. In general, significant reductions in leaf RWC were detected earliest in plants subject to the PD treatment, followed by those grown under SD plots, and then those in MD plots (figure 3; The RWC data for BR-1 was incomplete due to loss of some samples collected and, therefore, was excluded from figure 3). With no additional

**Figure 3**

Changes in leaf relative water content of maize inbred lines in response to (a) moderate drought, (b) severe drought, and (c) permanent drought treatments. The data were collected on the day prior to drought treatment (0 days) and at 4, 7, and 10 days after the initiation of the treatments. The error bars represent standard deviations of measurements on a given sampling day. The result of BR-1 was excluded from this figure due to incomplete data collection.



irrigation provided, plant leaf RWC of all inbred lines examined in PD plots decreased significantly within a few days of the onset of drought stress (figure 3). At a set sampling time point, the degrees of RWC reduction were the largest in plants grown in the PD plots, followed by those in the SD plots, and then those of the MD plots. Phenotype observations in the treatment plots were consistent with RWC measurement. For each tested inbred line, the drought stress phenotype, as indicated by responses such as leaf rolling and leaf wilting, appeared first in plants grown under PD condition.

Although RWC of leaf tissue decreased over time for all inbred lines subjected to drought treatments, the rates of such decrease differed significantly among the lines. Under all three levels of drought treatments, the decreases in leaf RWC were the slowest for inbred Tx205, C2A554-4, and B76; fastest for B73 and C273A; and slow to moderate for S1W, C32B, and B105C (figure 3). For B73 and C273A, the time at which the decrease in RWC of leaf tissue became significant ( $p \leq$

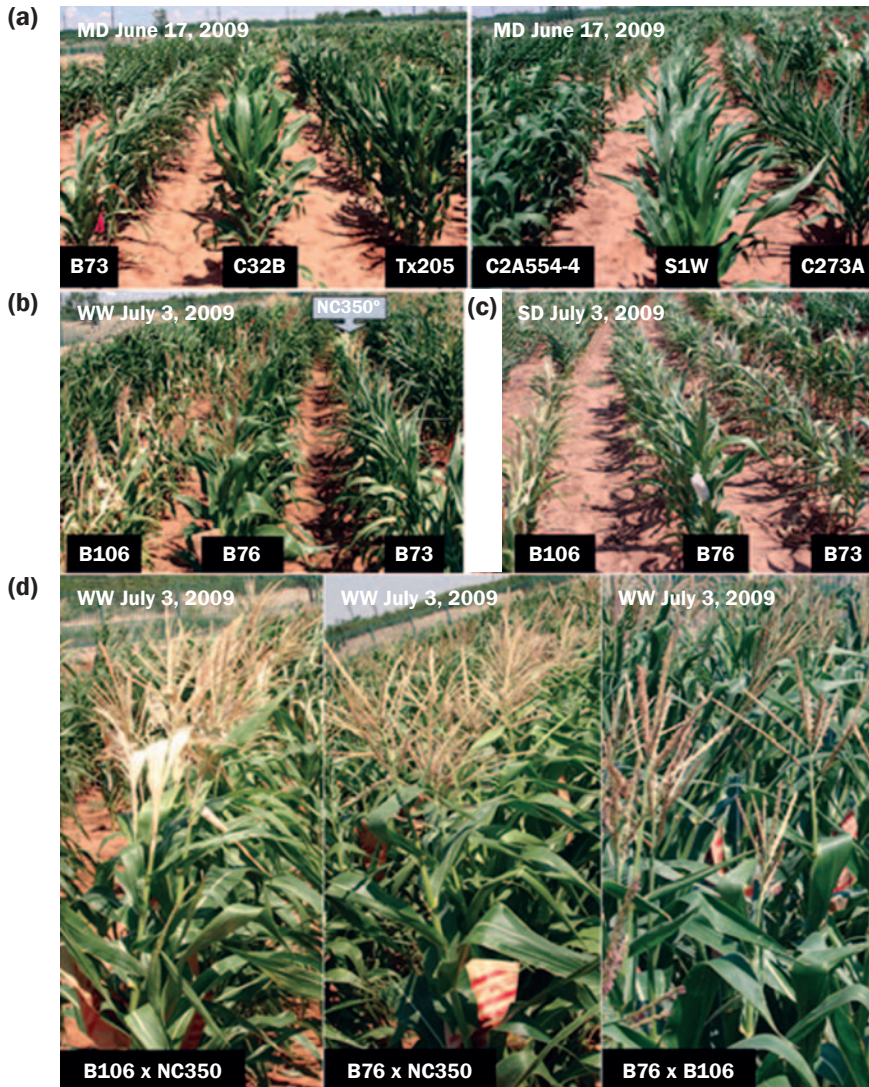
0.05) was 7 days, 4 days, and 4 days after the initiation of drought treatment in MD, SD, and PD plots, respectively; for S1W, C32B, and B105C, it was 10 days, 7 days, and 7 days; and for Tx205, C2A554-4, and B76, the significant RWC changes occurred at > 10 days, 10 days, and 7 days in MD, SD, and PD plots, respectively. No significant decrease in RWC was detected for C2A554-4, Tx205, and B76 plant within the first 10 days of MD treatment. After 10 days of stress treatment, the leaf RWC in C2A554-4 plants were 92.0% in MD plot, 90.4% in SD plot, and 85.6% in PD, comparing to 86.8%, 79.7%, and 75.1% RWC in B73 leaf tissues in corresponding drought treatment plot. Such differences were highly significant ( $p < 0.01$ ), suggesting the capability of C2A554-4 to withstand drought stress and maintain leaf water at a healthier level under water deficit.

Field observations of the appearance and severity of drought stress phenotypes (leaf roll, leaf bleaching, etc.) for inbred lines were consistent with the RWC results. Visual observation also indicated that inbred lines

C2A554-4, Tx205, and B76 were most drought tolerant while B73 and C273A were most drought sensitive. Based on RWC results and phenotype observation, we grouped the maize inbred lines studied into three groups, the drought-tolerant lines (Tx205, C2A554-4, and B76), moderate drought-tolerant lines (S1W, C32B, and B105C), and drought-sensitive lines (B73 and C273A). Figure 4a shows 7 day drought-stressed inbred plants in MD plots. Three lines in each photo represent inbred lines of each drought tolerance group. Under MD treatment, only B73 and C273A plants showed severe leaf rolling (in the afternoon) within the first 7 days of treatment, while the rest of the lines showed no sign of leaf rolling. Under SD treatment, leaf rolling of B73 and C273A was observed around noon on day 4 of the treatment. After 10 days under SD, most of the B73 plants displayed leaf bleaching and wilting. Under PD treatment, severe leaf rolling was observed in 4 day stressed B73 and C273A plants, while no sign of leaf rolling was observed in the rest of the lines. At 7 days, leaves of B73

**Figure 4**

Phenotypic variations of selected maize inbred lines in response to (a) 7-day moderate drought (MD) stress (Phenotypes of B73 and C273A, C32B and S1W, and Tx205 and C2A554-4 represent, respectively, the responses of drought-sensitive, moderate drought-tolerant, and drought-tolerant inbred lines to applied stress), (b) heat stress (well-watered plot [WW]), and (c) combined heat and 3 weeks of severe drought (SD) stresses. (d) Heat-tolerant/sensitive phenotypes of three maize hybrids made with at least one heat-sensitive inbred.



stayed rolled inwards in the morning and were barely able to recover from the rolled state. Leaves of the other 7 day PD-stressed inbred lines also started to show leaf rolling phenotype in the afternoon but were able to recover by the next morning. Eventually, all plants entered a permanent wilted state in SD and PD plots after prolonged treatments.

**Impacts of Drought Stress on Plant Growth.** Drought stress affects many processes involved in plant growth and development, such as inhibiting plant cell elongation, decreasing photosynthetic rate,

and reducing cell division (Westgate and Boyer 1985; Zinselmeier et al. 1995; Heiniger 2001; Kumar et al. 2004; Nielsen 2007). Maize plants are susceptible to drought stress throughout their life cycle. However, with respect to vegetative growth, maize plants become most sensitive to drought after reaching mid to late vegetative development (V8 to V17), when stem internodes start to elongate rapidly and leaf blades begin rapid expansion (ACIAR n.d). Drought occurring at these stages can significantly reduce leaf size, internode length, overall growth, and

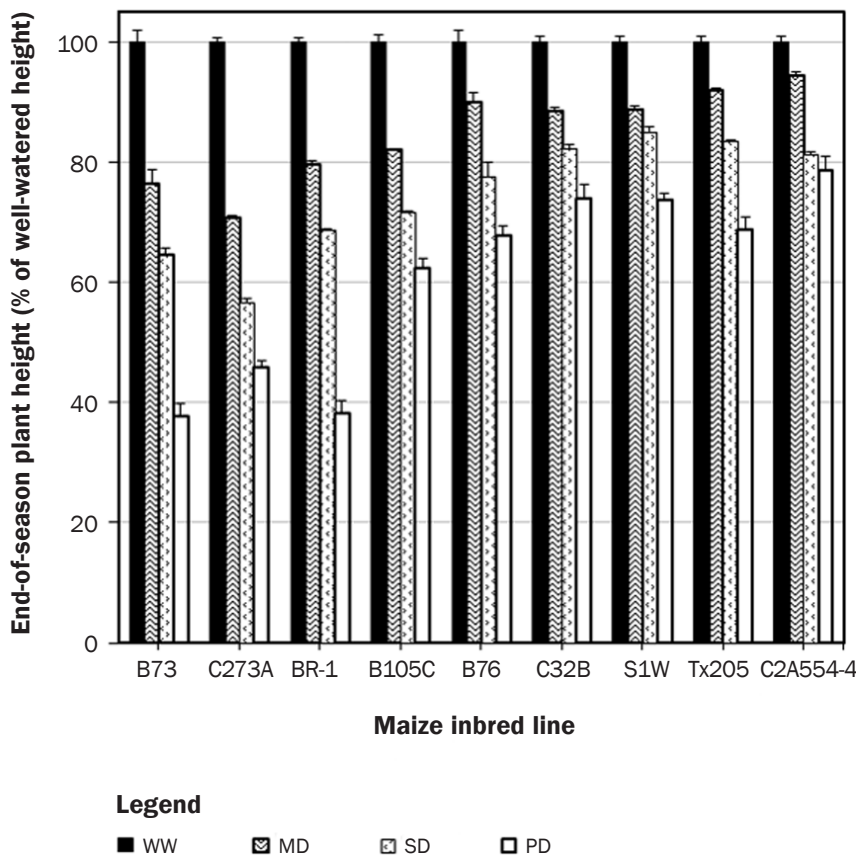
biomass production (Heiniger 2001). It also affects development of reproductive tissues, reduces ear size and kernel numbers, and significantly depresses yield (Herrero and Johnson 1981; Nielsen 2007). Years of breeding experiments at our site also indicate that plant lines that can better stand drought stress during vegetative stress tend to have greater ability to reduce yield loss due to drought stress. Identification and the use of inbred lines with superior drought tolerance traits is the key for the success of breeding drought-tolerant maize hybrids.

In this study, we examined the ability of nine maize inbred lines to withstand such negative impacts of drought stress on its growth and development. The effects of drought stress treatments on plant growth of maize inbred lines was evaluated by comparing plant height measured in stressed plots at the end of the growing season with that in WW plots. Since plant heights varied among inbred lines due to their genetic makeup, the plant height data for each inbred line were expressed as the percentage of stressed plant height relative to the WW plant height of the same line (figure 5). Such data ensures meaningful comparison between different inbred lines. In general, growth of the plants as measured by height was affected by all three drought treatments. The severity of growth reduction caused by drought is positively correlated with the stress level applied. Permanent drought stress significantly ( $p < 0.05$  to  $0.01$ ) reduced plant height in all inbred lines studied (figure 5). The data also showed that, for a given stress level, its impact on plant growth varied greatly among different lines. The effect was most severe on the B73, C273A, and BR-1 lines, with significant reduction in plant height observed at all three treatment levels ( $p < 0.05$ , MD;  $p < 0.01$ , SD and MD). Conversely, C2A554-4 and Tx205 plants showed only slight reductions in plant height under MD stress.

To determine the capacity of maize lines to maintain growth during drought stress, we calculated the posttreatment plant growth in each treatment (height difference between He and the Ho). The posttreatment plant growth in height in the drought stress plots was compared with those in WW plots. The data were expressed as percentages of plant height growth of WW plants (figure 6). As expected, inhibition of plant growth by drought increased as the level of stress intensified. For any of the given inbred lines, plants

**Figure 5**

End of season plant height of maize inbred lines grown in well-watered (WW), moderate drought (MD), severe drought (SD), and permanent drought (PD) treatment plots. The data were expressed as percentage of plant height measured in the WW field. Error bars represent standard deviation of the three replicates. B106 and NC350, two lines that are extremely sensitive to heat stress, were excluded from this figure.



in PD treatment grew the least after initiating drought treatment. It was also evident that the tested maize inbred lines responded differently to the same level of drought treatment (figure 6). Consistent with RWC results, B73 and C273A plants grew less than 20% of their potential height increase after onset of PD stress, while C2A554-4 and B76 reached about 68% of their potential height increase. In SD plot, B73 retained only 49% of its potential growth, and the value was 66% for plants in MD plot. Similar results were observed for C273A in PD and MD treatments. The drought-induced reduction in potential growth of B73 and C273A plants was highly significant ( $p < 0.01$ ) at all three treatment levels. In contrast, C2A554-4, Tx205, B76, and S1W showed extensive tolerance to drought stress. Plants of these inbred lines maintained 60% or more of their growth potential under PD treatment. Surprisingly, C2A554-4 and Tx205

plants sustained about 90% and 75% of their potential height growth under MD and SD treatments, respectively (figure 6).

The data shown in figures 5 and 6 are consistent with leaf RWC measured at an early stage of drought stress (figure 3) and with the field stress phenotypes observed (figure 4a). Compared to B73, a widely used inbred line for breeding, C2A554-4 and Tx205 possess superior drought-tolerant traits that enable the plants to alleviate most of the negative effects of drought stress on plant growth and development.

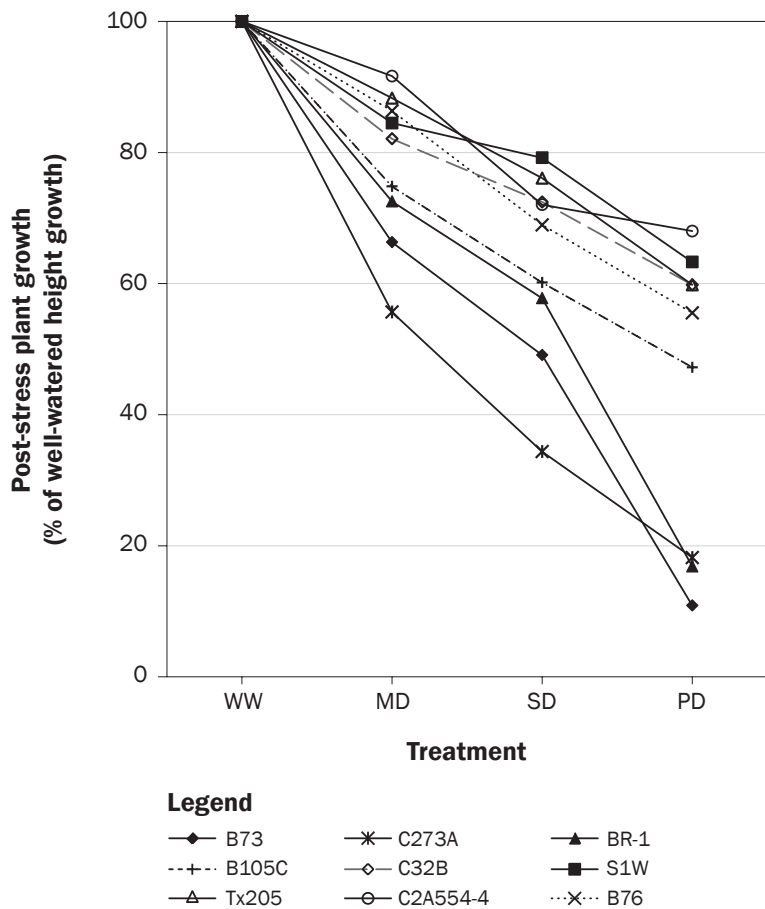
**Response of Maize Inbred Lines to High Temperature.** High temperature affects maize plants at all levels, ranging from molecular and cellular effects to whole plant growth (Huang and Jiang 2001; Burke and Chen 2006; Chen et al. 2010). More importantly, tissue injuries caused by high temperature exposure of crop plants grown under field conditions are mostly irrevers-

ible (Barnabas et al. 2008; Chen et al. 2010). In maize, leaf blotching (chlorosis patches) and leaf firing are two most noticeable heat-sensitive phenotypes of vegetative tissues. Severe heat stress at vegetative developmental stages can cause significant decreases in photosynthetic area and reduction in metabolites production (Wilhelm et al. 1999; Chen et al. 2010). However, in general, maize reproductive tissues are more susceptible to high temperature than vegetative tissues, and tassel blast is the most noticeable field heat-sensitive phenotype of reproductive tissues. Elevated temperature at the reproductive developmental stage reduces pollen shedding, pollen viability, and pollination efficiency, and affects kernel development, resulting in a reduction in seed set, kernel size, and kernel weight (Schooper et al. 1987; Dupuis and Dumas 1990; Cheikh and Jones 1994; Cantareco et al. 1999; Wilhelm et al. 1999; Barnabas et al. 2008). Depending on its timing, duration, and severity, the maize grain yield loss caused by high temperature under drought condition could exceed 40% to 70%, with a possibility of 100% yield loss (Lobell et al. 2011).

In this study, heat tolerance of maize inbred lines was evaluated in WW fields at Lubbock, Texas, the South Plains region of the United States, where sporadic heat waves occur routinely during the growing season (figure 2). Leaf firing and tassel blasting of maize inbred lines were visually evaluated after each of the major heat events in 2009 and 2010. The heat tolerance of maize inbred lines was assessed by the percentage of plants showing heat-sensitive phenotypes. A summary of the end of season results is listed in table 1. As shown in figure 2, during the V9 to tasseling developmental stages heat wave events in 2009 lasted longer and were more severe than those in 2010. As a result, heat induced tissue injuries in sensitive plant lines were more severe in the 2009 season than that in 2010. In general, B76, Tx205, and all six inbred lines developed at Texas A&M (C273A, BR1, B105C, C32B, S1W, and C2A554-4) showed tolerance to high temperature stress (table 1). Plants of tolerant inbred lines showed neither observable leaf injury nor tassel blast phenotypes during field growth in either 2009 or 2010 (table 1). In contrast, severe leaf firing and tassel blasting were observed in both B106 and NC350 plants. Photos in figures 4b and 4c show field phenotype of B106, B76, and B73 in 2009 under WW (figure 4b) and severe drought

**Figure 6**

Effects of moderate drought (MD), severe drought (SD), and permanent drought (PD) on plant growth. Data were expressed as percentage of plant height grown after onset of treatment in drought-stressed plots relative to that in well-watered (WW) plots. Height data were collected on the day of onset treatment and at the end of growing season.



**Table 1**

Plant height and heat-sensitive leaf firing and tassel blasting phenotypes observed in plants grown under well-watered field conditions in the 2009 and 2010 seasons.

Genotype	Plant height (cm)		Leaf firing plant (% of total)		Tassel blast plant (% of total)	
	2009	2010	2009	2010	2009	2010
A&M lines	na	na	0	0	0	0
Tx205	147	155	0	0	0	0
B76	164	175	0	0	0	0
B73	149	190	25**	12*	0	0
B106	139	160	100**	100**	87**	68**
NC350	139	164	100**	100**	30**	18*

\* $p < 0.05$

\*\* $p < 0.01$

(figure 4c) conditions. It appeared that B106 was more sensitive to heat stress than NC350 when comparing the phenotypic impact of a heat event. B73, a popular inbred line in breeding programs, proved to be moderately sensitive to heat stress. Heat events occurring at early and middle growing season induced severe leaf blotching on developing leaves of B73 plants in both 2009 and 2010 seasons. Leaf firing of the flag leaf and one or two adjacent leaves was observed after severe heat waves that occurred when the tassel tissue was about to emerge from the leaf whorl. B73 plants also showed reduced pollen production and shortened pollen shed duration when a heat stress occurred within a few days preceding tasseling in 2009. No tassel blast was observed for B73 plants. However, our greenhouse study showed that tassel development of B73 plants was severely affected by high temperature treatment when applied at the eight- to ten-leaf stages. The observed tassel phenotypes of heat-stressed B73 plants ranged from tiny tassels with no spikelets to small tassels with a fewer branches and reduced number of spikelets (figure 7b).

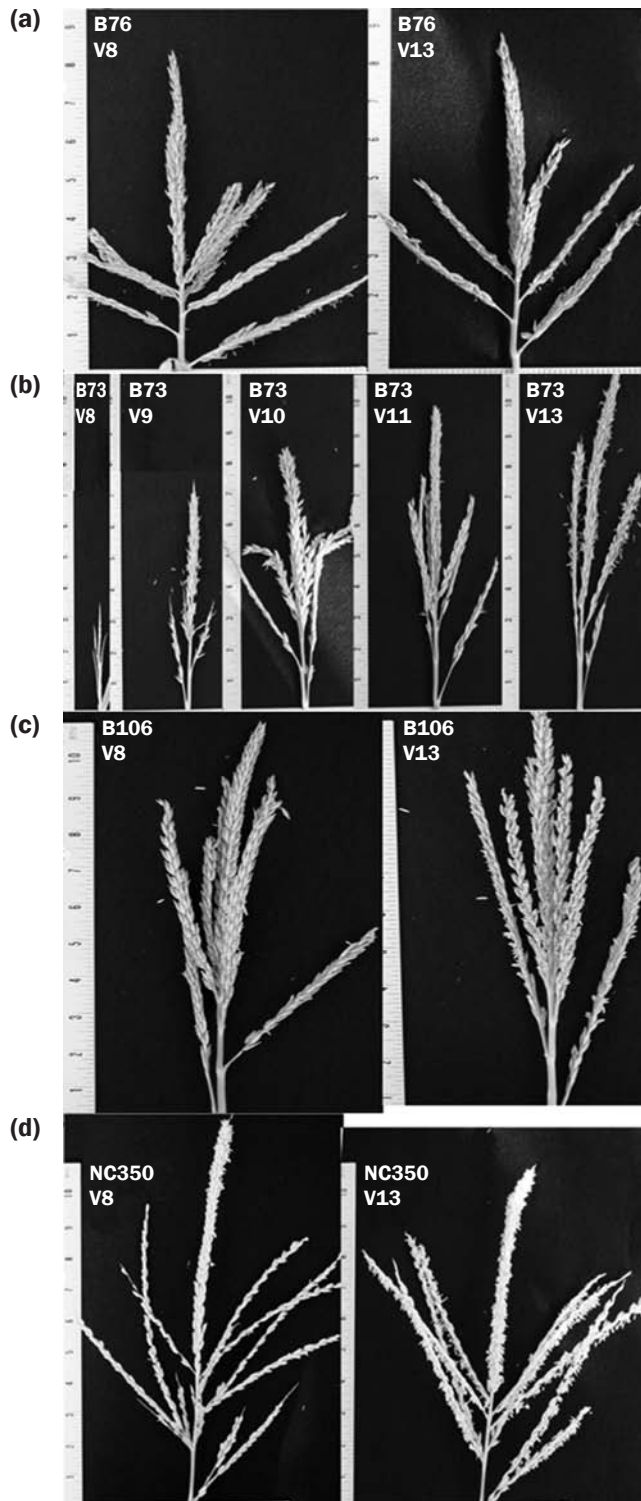
A greenhouse study also showed that tassel development of B76 plants was very tolerant to high temperature treatment (figure 7a). Heat stress at early tassel developmental stages did not affect tassel development and spikelet formation in B76 plants. Surprisingly, tassel development of two heat-sensitive inbred lines, B106 and NC350, was also barely affected by the same treatment (figures 7c and 7d). The differences in heat tolerance/sensitivity observed at different developmental stages of B106, NC350, and B73 suggest that genetic control of heat tolerance in maize is complex. Genetic dissection of different heat-tolerant traits will enhance the effectiveness of incorporating these traits into breeding lines.

The heat tolerance of hybrids made from selected heat-tolerant and sensitive inbred lines was also evaluated. No heat-induced leaf firing and tassel blast phenotypes were observed in hybrid plants made from a heat-tolerant parent and a heat-sensitive parent, suggesting heat tolerance is a dominant trait in maize. On the other hand, hybrid plants of two heat-sensitive parents (B106 × NC350 and NC350 × B106) showed tassel blasting and leaf firing phenotypes in WW fields during both 2009 and 2010 (figure 4d). The results imply the importance of



## Figure 7

Effects of a three-day high temperature treatment (38°C/30°C, 14 hours/10 hours) at V8, V9, V10, V11, V12, and V13 leaf stages on tassel development of (a) B76, (b) B73, (c) B106, and (d) NC350 plants. The treated plants were allowed to grow under normal temperature conditions before and after high temperature treatment. The photos were taken at the end of pollination stage. Photos show tassel development of B73 is very sensitive to heat stress at early developmental stages.



selecting heat-tolerant inbred lines in maize breeding programs.

It is well documented that global temperature is steadily rising (Giorgi et al. 2001; IPCC 2007a). This trend of increasing temperature is predicted to continue for the next century and beyond (Keane et al. 2009; IPCC 2007a; Sanderson et al. 2011). The long-term impacts of global temperature rise on crop production have been studied by various simulation models and by analyzing the relationship between crop productivity and temperature in the past (Peng et al. 2004; UNFCC 2007; Lobell et al. 2011). The direct and drastic effect of high temperature events (heat waves) on crop yields has become more and more evident locally and/or regionally in recent years (IPCC 2007b). In nature, unfortunately, heat stress often occurs concurrently with drought stress. Their negative impacts on plant growth and crop productions are synergistic (Mittler 2006; Barnabas et al. 2008; Lobell et al. 2011), causing significant economic loss and threatening food security (UNEP GRID-Arendal 2009; Parry and Hawkesford 2010; Robinson 2012b). While drought stress can be relieved through irrigation management (Roth 1999; Heiniger 2000), little can be done through crop management to ameliorate heat stress. Therefore, the most feasible way to cope with such problem in agriculture is through development of high temperature-tolerant crop varieties, especially as the trend of global warming escalates (Tester and Langridge 2010; Auffhammer 2011). Our study showed that the heat tolerance of maize hybrids could be improved through using inbred lines with superior heat stress tolerance.

## Summary and Conclusions

Integrated management practices to improve agricultural water use efficiency and the ability of plants to alleviate the negative impacts of environmental stresses are two key factors that can be combined to sustain and increase agricultural production under unfavorable environmental conditions. Breeding for drought- and/or heat-tolerant crop cultivars/hybrids is a feasible strategy to help increase and sustain crop production under future challenging environments. Identification and characterization of plant germplasm/inbred lines with superior stress-tolerant trait(s) are prerequisites for the success of such a breeding program. This study evaluated a set of maize inbred lines for drought and heat

tolerance under field conditions and identified inbred lines with superior drought tolerance traits for use as genetic materials for the breeding of drought-tolerant hybrids. In addition, maize inbred lines with superior heat tolerance traits were also identified. Characterization of the hybrids made from heat-tolerant inbred lines demonstrated the potential of these inbred lines for creating heat-tolerant hybrids and the feasibility of combining heat and drought tolerance traits in a single breeding program.

## Disclaimer

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