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Maize leaf temperature responses to drought: Thermal imaging and quantitative trait loci (QTL) mapping

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ABSTRACT

Leaf temperature has been shown to vary when plants are subjected to water stress conditions. Recent advances in infrared thermography have increased the probability of recording drought tolerant responses more accurately. The aims of this study were to identify the effects of drought on leaf temperature using infrared thermography. Furthermore, the genomic regions responsible for the expression of leaf temperature variation in maize seedlings (*Zea mays* L.) were explored. The maize inbred lines Zong3 and 87-1 were evaluated using infrared thermography and exhibited notable differences in leaf temperature response to water stress. Correlation analysis indicated that leaf temperature response to water stress played an integral role in maize biomass accumulation. Additionally, a mapping population of 187 recombinant inbred lines (RILs) derived from a cross between Zong3 and 87-1 was constructed to identify quantitative trait loci (QTL) responsible for physiological traits associated with seedling water stress. Leaf temperature differences (LTD) and the drought tolerance index (DTI) of shoot fresh weight (SFW) and shoot dry weight (SDW) were the traits evaluated for QTL analysis in maize seedlings. A total of nine QTL were detected by composite interval mapping (CIM) for the three traits (LTD, RSFW and RSDW). Two co-locations responsible for both RSFW and RSDW were detected on chromosomes 1 and 2, respectively, which showed common signs with their trait correlations. Another co-location was detected on chromosome 9 between LTD and shoot biomass, which provided genetic evidence that leaf temperature affects biomass accumulation. Additionally, the utility of a thermography system for drought tolerance breeding in maize was discussed.

1. Introduction

Drought is presently a serious environmental constraint to crop survival and productivity due to continued reductions in global water resources. Maize yield (and crop survival) in regions of sub-optimal rainfall or limited irrigation availability may be better guaranteed by selecting for genotypes tolerant or resistant to water stress (Landi et al., 1995). However, in order to improve agricultural management practices and breeding efforts under continued climate change, the effects of drought on plant viability must be thoroughly understood (Chaves et al., 2003).

Drought resistance is a complex multi-genetic trait involving many physiological processes. Stomata are leaf and stem epidermal pores that serve a role in drought tolerance, by controlling carbon dioxide assimilation for photosynthesis while limiting water loss via transpiration. During water stress conditions, water uptake rate cannot match the potential transpiration rate and stomata close to maintain the plant water balance. Stomata closure causes evapotranspiration to cease, which in turn leads to an increase in leaf temperature (Lourtie et al., 1995). The increasing availability of sensitive infrared imaging system opens up the possibility of high resolution studies of variation in stomatal conductance over leaf surfaces and their dynamics (Jones et al., 1999). Instrumentation that allows simultaneous thermographic and gas exchange measurements from the same leaf area has been developed that demonstrates the linear relationship between leaf temperature and transpiration (Kummerlen et al., 1999). Jones et al. (1999) also reported that stomatal conductance calculated from thermographic measurements correlated well with estimates obtained from a diffusion porometer. Recently, infrared thermography was successfully used as an effective non-contact, high throughput tool for screening large populations of Arabidopsis to identify mutants exhibiting leaf temperatures that differed from wild-type plants (Merlot et al., 2002; Wang et al., 2003; Song et al., 2006; Zhang et al.,

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2008). Verslues et al. (2006) and Price et al. (2002) reported that the use of infrared thermal imaging to study energy balance and stomatal function provided the opportunity to advance a more holistic understanding of physical and biochemical processes related to water use and drought tolerance. Maize reacts rapidly to developing water stress and its response to drought can be readily visualised, which does not necessary holds for other common crops. Isohydric crops like maize are well suited for thermographical monitoring. To date in maize, most scientists have paid attention to canopy temperature (canopy size, canopy architecture and leaf compensation to water deficits) and soil and atmospheric conditions using infrared thermometer instrumentation. However, this approach is not capable of integrative observations of leaf temperature and eliminates the heterogeneity characteristic of the entire leaf, observations readily obtained using infrared thermography.

The development of advanced molecular techniques and refinement of analytical methods has led to the identification of QTL involved in the expression of important agronomic traits and the discovery and characterization of genes underlying QTL (Kraja and Dudley, 2000; Salvi and Tuberosa, 2005). QTL analysis is not only important in breeding programs, but also a powerful tool for studying the relationships between complex physiological traits (Prioul et al., 1997; Vreugdenhil et al., 2007). The genetic basis of drought tolerance has been studied extensively in maize and other taxa (Tuberosa et al., 2002; Ribaut et al., 2004). Masle et al. (2005) proved the ERECTA Arabidopsis gene to also have a role in transpiration efficiency, acting on the coordination between transpiration and photosynthesis through structural and numerical changes in leaf stomata. However, less is known about the molecular genetic basis of plant leaf temperature variation in response to water stress.

Due to reducing sink capacity and solute mobilization, biomass weight and yield potential of crop plants generally decrease under drought stress. These two traits are usually used as integrative indicators to judge the drought-tolerant potential of plants (Collins et al., 2008). In this study, seedling biomass was regarded as a criterion to identify drought tolerance capability. Advanced infrared thermography was applied to investigate the association between leaf temperature differences and relative biomass accumulation in response to drought at the seedling stage in maize. Additionally, stomatal behavior was observed and QTL analysis was conducted in order to explore the physiological and genetic mechanisms of drought tolerance underlying the inherent variability in leaf temperature.

2. Materials and methods

2.1. Plant material and cultural conditions

The $F_{2:3}$ mapping population from a cross between two elite maize inbred lines, Zong3 and 87-1 was constructed by Yan et al. (2003). A population of 294 F_8 RILs, was created by a single seed descent method. In this study 187 RILs was randomly selected to carry out the genetic map construction and QTL analysis. All RILs and both parents were grown in the greenhouse at the Chinese Academy of Agricultural Science, Beijing in April, 2005. Seeds were selected from good ears and imbibed by soaking for 10 h in a growth chamber. The seeds were subsequently washed three times with tap water and placed into a damp towel to germinate at 28 °C. After 36 h, uniformly germinated seeds were chosen and sown in plastic pots (23 cm × 16 cm) with three holes in the bottom for adequate drainage. 12 seeds were sown in each pot and four uniformly strong plants were retained finally at the 2–3 leaf stage. To obtain uniform development, each pot was filled with 1.8 kg loamy soil, 0.7 kg vermiculite (1:1 (v/v)) and a solution mixed with 3.0 g soluble compound fertilizer (45%).

The experiment was conducted following a split-plot design with two replications. Each RIL (or parent) of each replication was referred to as main plot and water stress treatment as split-plot. Therefore, drought and control treatment plants were spaced close on the arrangement. The relative soil water content (RSWC) for the control plants exceeded to 80%. The drought treatment plants did not receive water from the 13th day post-sowing, until the RSWC decreased to 50% (moderate drought stress), then the soil weighing method was applied every day to keep the RSWC stable.

2.2. Acquisition of infrared thermal images

Thermal images were obtained using a ThermaCAM SC3000 infrared camera (FLIR Systems, USA). The system incorporates a cooled quantum well infrared photodetector (QWIP) with a spectral range of 8–9 μm, 320 × 240 pixels and a built-in 20° lens. In addition, a QWIP sensor delivers ultra high sensitivity of less than 20 mK at 30 °C ensuring low thermal noise. Emissivity was set at 0.95 to view leaves. To ensure thermal images taken rapidly, the instrumentation was set up. The camera was mounted at a height approximately 1.8 m above the ground, pointed downwards, yet flexible enough to move if necessary.

Infrared thermal images were taken between 9:00 and 11:30 a.m. the 20th day after sowing. An adumbral net, 80% light transmission rate, was also applied to the top of the solar greenhouse. The day length of Beijing at that time was approximately 14 h. Photosynthetic active radiation (PAR) was 650–800 $\mu mol\ m^{-2}\ s^{-1}$ and the air temperature was 25 ± 2 °C. The plants for the control and drought treatment of each RIL was next to take the infrared thermal images. The overall of top full-expanded leaf per plant was determined. Each pot was taken pictures four repeats. Images were saved as 14-bit radiometric IR digital image (IMG) on the compatible ATA flash card and were subsequently analyzed for temperature determination using the ThermaCAM Researcher 2002 software on the computer. A suitable scale and palette was chosen to result in a quite clear and informative thermal image. The software provides the function of measuring spot, line and area. In this study, a polygon area was drawn to measure the overall leaf temperature.

2.3. Stomatal bioassay

A small blade section was removed mid-way along the leaf of both parents (87-1 and Zong3) and rapidly placed in H_3PO_4 buffer to remove any dirt on the leaf surface. Cut leaf sections were then fixed overnight in 2.5% glutaraldehyde, dehydrated through an ethanol series and critical point dried in a critical point drier Hitachi HCP-2. Samples were sputter coated with gold particles in an ion coater Giko IB-5 and examined with a Hitachi S-570 scanning electron microscope (SEM) operating at a 12 kV accelerating voltage. Images were digitally recorded and further analyzed by Image-Pro plus 5.0 software. Observations were performed on four randomly selected leaf blade points symmetrical along the main vein from each piece of three leaves after infrared thermography determination.

2.4. Shoot biomass determination

The shoot of each plant was rapidly cut and weighed in the greenhouse the next day following acquisition of infrared thermal images. Shoot was dried for 48 h at approximately 75 °C and dry weight was determined.

2.5. Data analysis

Ambient temperature varied slightly, therefore differences in leaf temperature between the water-stressed and well-watered

Table 1
Phenotypic analysis of drought tolerance at the early seedling stage in the RIL population and parents lines Zong3 and 87-1.

Trait[a]	Zong3			87-1			Significance level[c]	RILs biomass DTI (LTD)			
	WW (g)	WS (g)	DTI[b]	WW (g)	WS (g)	DTI		Mean	Min	Max	h^2b
SFW	5.79	3.27	0.564	6.18	4.43	0.716	**	0.657	0.387	1.016	0.80
SDW	0.422	0.278	0.658	0.453	0.362	0.799	*	0.826	0.545	1.081	0.78
LT	26.27	26.40	0.13	26.15	26.45	0.30	**	0.19	0	0.47	0.67

[a] SFW: fresh shoot weight; SDW: dry shoot weight; LTD: leaf temperature difference (°C).
[b] WW: well-watered condition; WS: water-stress condition; DTI: drought tolerance index (trait value in WS/trait value in WW).
[c] *,**Represent the difference between Zong3 and 87-1 at the $P<0.05$ and $P<0.01$, level of significance, respectively.

conditions were analyzed rather than measuring the absolute leaf temperature of each individual plant independently. Leaf temperature difference (LTD) in response to water stress was defined as the difference in maize leaf temperature under water-stress and well-watered conditions. In addition, shoot fresh weight (SFW) and shoot dry weight (SDW) (two traits related to drought tolerance) were analyzed using the drought tolerance index (DTI), determined by calculating the ratio of drought to well-watered treatment values, relative SFW(RSFW) and relative SDW(RSDW).

Analysis of variance using ANOVA was conducted to test for significant differences between the two parents and among the RILs for each trait (LTD, RSFW and RSDW) using SAS PROC GLM (SAS 8.2, SAS Institute, 1999–2001). Broad-sense heritability (h^2b) for each trait was estimated as $\sigma^2g/(\sigma^2g+\sigma^2e/r)$, where σ^2g was the genetic variance, σ^2e was the error variance and r was the number of replicates. Two replications of each trait measurements were averaged for simple Pearson correlation coefficients analysis (PROC CORR, SAS) and QTL mapping.

2.6. Linkage map construction and QTL mapping

The genetic linkage map was constructed using Mapmaker/exp ver3.0 software (Lincoln and Lander, 1993). The integrated linkage map consisted of 248 SSR markers, covering all 10 maize chromosomes and spanning 2509.8 cM with an average interval of 10.1 cM between markers. Windows QTL Cartographer Ver2.5 was used to perform QTL analysis (Wang et al., 2005). Composite interval mapping (CIM) fit parameters for a target QTL in one interval while simultaneously fit partial regression coefficients for "background markers" to account for variance caused by non-target QTL. Model 6 was employed for QTL mapping and estimating their effects. The genome was scanned at 2 cM intervals using the forward regression method. The default values of 5 for control markers and 10 for window size were deployed. The threshold for logarithm of odds (LOD) scores was estimated by permutation tests (Churchill and Doerge, 1994) with 1000 replications at a $P<0.05$ level of significance for an experiment wise Type I error.

3. Results

3.1. Performance traits for parent lines Zong3 and 87-1

3.1.1. Seedlings biomass

The seedling biomass was measured as SFW and SDW and subsequently calculated as DTI to compare the drought response performance of Zong3 and 87-1. Results indicated that water stress treatments exhibited enough influence to elicit differential phenotypes in the two maize inbred lines (Table 1). Significant difference was detected between Zong3 and 87-1 ($P<0.05$ and $P<0.01$). The inbred line 87-1 presented a higher RSFW and RSDW than Zong3. The results suggested that in comparison with Zong3, 87-1 maintained an increased growth potential under water stress conditions and 87-1 exhibited increased drought tolerance than Zong3.

3.1.2. Difference in leaf temperature

Leaf temperature variation in maize inbred lines captured by FLIR ThermaCAM SC3000 is shown in Fig. 1. Leaf temperature markedly increased under water stress in both inbred lines Zong3 and 87-1. Analysis results showed that significant differences in LTD were found between the two maize inbred lines. Compared to Zong3, 87-1 exhibited a significantly greater ($P<0.01$) response to

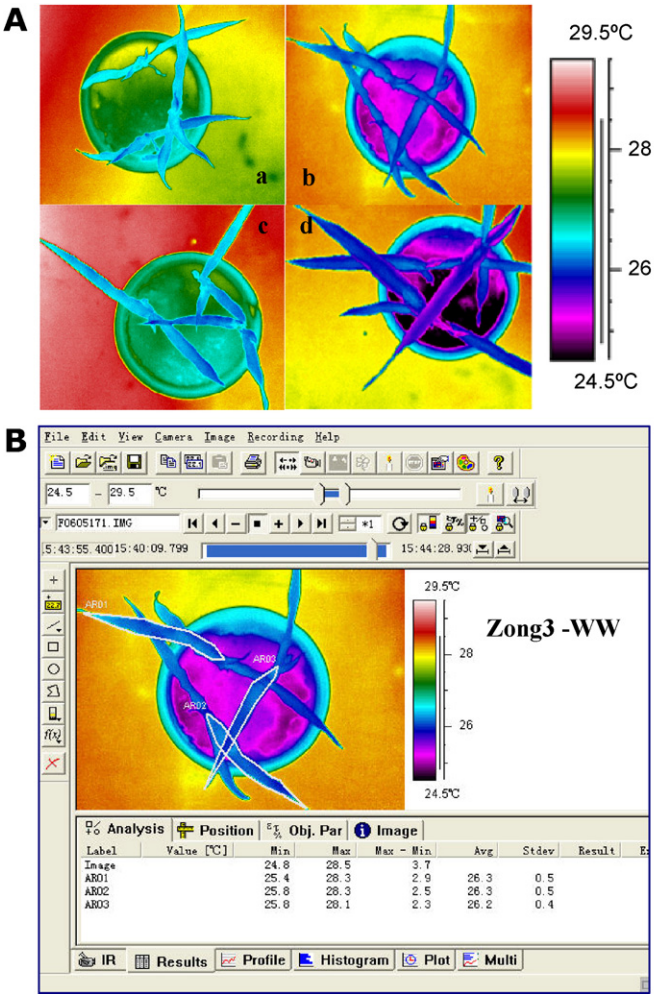

Fig. 1. (A) Temperatures are visualised according to the shown color palette. The different color points in the palette represent different temperature values. The overall of top full-expanded leaf per plant was determined. (a) and (c) represent Zong3 and 87-1 under water-stress conditions, respectively. (b) and (d) represent Zong3 and 87-1 under well-watered conditions, respectively. Leaf temperature varied obviously under water-stress (aand c) and well-watered (band d) conditions. Leaf temperature was higher (light blue) under water stress as compared to well-watered conditions (dark blue). (Soil appears respectively warmer and colder than the plant leaves.) (B) The taken thermal images were analyzed by the ThermaCAM Researcher 2002 software, using the software's function of measuring spot, line and area a polygon area was drawn to measure the overall leaf temperature.

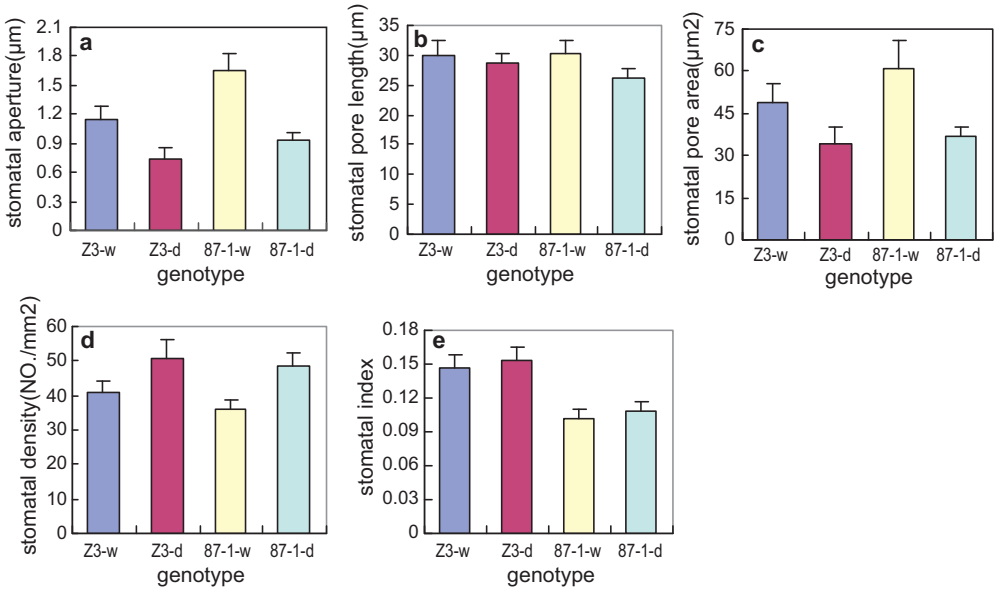

Fig. 2. Leaf stomatal features response to water stress between Zong3 and 87-1. *w* and *d* on the *x* axis represent those under well-watered and water stress conditions, respectively. Zong3 was abbreviated to Z3. (a) Stomatal aperture (μm), (b) stomatal pore length (μm^2), (c) stomatal pore area (μm^2), (d) stomatal density (stomatal number per mm^2) and (e) stomatal index (100 × stomatal density/(stomatal density + epidermal cell density)).

drought. LTD in 87-1 reached 0.30 °C while LTD in Zong3 reached 0.13 °C (Table 1).

3.1.3. Stomatal behavior of maize response to water stress

The following stomatal characteristics related to stomatal behavior were analyzed: stomatal aperture, stomatal pore length, stomatal pore area, stomatal density, and stomatal index were analyzed (Fig. 2). Significantly smaller apertures and decreased pore area were observed in the two maize inbred lines with significantly increased stomatal density in response to water stress ($P < 0.05$ or $P < 0.01$). Stomatal pore length in 87-1 showed a significant ($P < 0.05$) response to water stress, which was not observed in Zong3. Stomatal index of Zong3 was significantly higher than that of 87-1 under both conditions. Stomatal index exhibited little variation for two inbred lines. The discrimination, Δ, between stomatal pore traits (aperture and area) under water-stress and well-watered conditions was significantly different ($P < 0.05$ and $P < 0.01$, respectively). The inbred line 87-1 (0.73 μm for stomatal aperture Δ and 24.20 μm^2 for stomatal pore area Δ) showed a significant ($P < 0.01$) increase in stomatal response to drought compared to Zong3 (0.40 μm for stomatal aperture $\Delta\Delta$ and 14.6 μm^2 for stomatal open area Δ). No significant difference was found for stomatal density Δ and stomatal index Δ between the two maize lines.

3.2. The RIL population performance

A two-way ANOVA revealed there were significant differences between well-watered and water-stressed treatments for all traits among the RIL population. From SFW and SDW determined, we calculated the water content of the seedling. 90.3% plant water content averaged across the RILs population under water-stressed was significantly lower than 93.2% of that under well watered conditions, which showed 50% RSWC has already made the plants encountered the certain severe drought stress. One-way ANOVA results using LTD for leaf temperature or DTI for SFW and SDW as a single factor indicated highly differential genotypes associated with water stress for all three traits (data not shown). Broad-sense heritability (h^2b) for the three traits calculated over the two water regimes varied ranging from 0.67 (LTD) to 0.80 (RSFW). These results suggested that in maize, drought-related traits are moderately heritable. LTD heritability could be subject to the influence of ambient conditions. Tremendous continuous variation and large transgressive segregation was present for LTD, RSFW and RSDW measured among the RILs (Table 1). The RIL population exhibited normal distribution for all traits, indicating the population was suitable for QTL mapping for these specific traits.

3.3. The causal relationship between LTD and seedling biomass

Genetic correlation coefficients were obtained for LTD and relative shoot biomass (RSFW and RSDW) using SAS Pearson correlation coefficients analysis. RSFW exhibited the highest significant positive correlation with RSDW (0.85**). The correlation of RSFW (0.160*) showed a significant ($P < 0.05$) positive correlation with LTD, consistent with that of RSDW (0.163*). These results suggested the variation in maize leaf temperature response to water stress was to some extent associated with whole plant biomass accumulation.

3.4. QTL analysis

After permutation tests with 1000 replications at a $P < 0.05$ level, the threshold LODs at genome-wide significance levels ($P < 0.05$) were set at 3.0, 3.0 and 3.1, respectively, for RSFW, RSDW and LTD. A total of nine putative QTL were detected for RSFW, RSDW and LTD (Table 2). These QTL were mapped on chromosomes 1, 2, 9 and 10, respectively (Fig. 3) and each explained from 6.2% to 13.1% of the total phenotypic variation. The alleles for increasing trait values were derived from the high-value parent 87-1 contributing eight QTL and the low-value parent Zong3 contributed only one QTL from LTD.

Three QTL were resolved for RSFW among the RILs populations and were located on chromosome 1, 2 and 9, respectively. The three detected QTL contributed 25.9% to the total phenotypic variation. Among them, qRSFW2 and qRSFW9 explained 6–8% of the relative shoot fresh weight phenotypic variation, while qRSFW1 accounted for a relatively high 11.0% of the phenotypic variation. The three RSFW QTL alleles exhibited the same additive effects as the parental inbred line 87-1.

Table 2
Putative QTL for drought tolerance in three traits at the early seedling stage of the Zong3/87-1 RIL population.

Trait	Chromosome	QTL[a]	Marker intervals[b]	Position (cM)	LOD score	Additive[c]	R^2 (%)[d]
RSFW	1	qRSFW1	bnlg1556–umc2029	271	3.5	−0.040	11.0
	2	qRSFW2	umc1042–bnlg2144	151	3.6	−0.034	7.7
	9	qRSFW9	umc1310–bnlg1525	144	3.2	−0.030	6.2
RSDW	1	qRSDW1	bnlg1556–umc2029	275	3.3	−0.045	13.1
	2	qRSDW2	umc1042–bnlg2144	151	3.1	−0.032	7.2
LTD	2	qLTD2	umc1042–bnlg2144	147	3.3	−0.029	7.5
	9	qLTD9-1	phi027–bnlg127	74	3.6	0.035	9.0
	9	qLTD9-2	bnlg1209–umc2119	96	4.6	−0.036	9.5
	10	qLTD10	umc2122–phi323152	165	3.6	−0.028	7.3

[a] QTL are denoted by trait abbreviations plus chromosome number.
[b] Markers that are underlined represents the nearest marker to the QTL.
[c] Additive effect are associated with the contribution of favorable alleles from two parental lines, a positive value represents the contribution of 87-1 alleles whereas negative values show the contribution of Zong3.
[d] R^2 value indicate the phenotypic variation explained by each putative QTL.

Two QTL for RSDW, qRSDW1 and qRSDW2, were, respectively, detected on chromosomes 1 and 2, respectively, and accounted for 13.1% and 7.2% of the phenotypic variation. qRSDW1 explained the most phenotypic variation of all mapped QTL. The inbred line 87-1 provided all favorable alleles for increasing RSDW trait values.

Four QTL were detected for LTD on chromosome 2, 9 and 10 and explained 6.9–9.6% of the phenotypic variation. Together, this accounted for 33.3% of all the phenotypic variation in this trait. 87-1 provided alleles at three of the four QTL, which increased LTD. On chromosome 9 two QTL (qLTD9-1 and qLTD9-2) exhibited opposite direction of additive effect.

For all mapped QTL of the three maize traits, common alleles existed that demonstrated pleiotropic effects at the seedling stage. Two QTL co-location regions between RSFW and RSDW were detected. QTL qRSFW1 and qRSDW1 were located in the common region bnlg1556–umc2029 on chromosome 1. Another co-location region was located in the interval umc1042–bnlg2144 on chromosome 2, with a QTL allele qLTD2 underlying LTD, which accounted for 7.5% of the phenotypic variation.

4. Discussion

4.1. Experimental environmental conditions

The surface temperature of plant leaves depends on environmental factors and transpirational cooling (the outward latent heat flux) (Nobel, 1991). Variation in leaf temperature would be primarily determined by a small set of factors if other influences remained relatively constant. In the present study, our experimental design enabled leaf temperatures for the two treatments to be measured almost simultaneously, which ensured that genetic factors would be responsible for the differences observed for each genotype of RILs. Approximately 50% of the relative soil water content (RSWC) under moderate water stress could distinguish stomatal performance of each genotype, which referred to the study of Ray and Sinclair (1997). In addition, the plants were grown in a greenhouse to avoid the influence of air currents (wind) in field conditions, which can carry heat away from leaves resulting in leaf cooling. Furthermore, the greenhouse environment can keep humidity and CO_2 concentration stable. An ambient temperature of in general maximises stomatal aperture and promotes plant development and growth. The application of an adumbral net ensured a uniform amount of sunlight to delivery the leaf surface of the plants. Therefore, the advanced infrared thermography apparatus FLIR ThermaCAM SC3000 exhibited optimal performance, far superior to conventional infrared thermometer methodology. The measurements obtained in this study were very reliable for comparing leaf temperature variation between cultivars upon drought treatment.

4.2. LTD response to water stress in relationship to drought tolerance

The larger LTD in 87-1 as compared to Zong3, which demonstrated that water stress increased leaf temperature in 87-1 more than that in Zong3. In addition, the SEM results showed higher variability in stomatal features, particularly stomatal aperture Δ and stomatal pore area Δ in 87-1 as compared to Zong3. The main differences in stomata response to water stress were changes in stomatal pore traits such as stomatal aperture and stomatal pore area, which is congruent with previous studies (Reymond et al., 2003; Masle et al., 2005). Stomatal density showed an increasing trend under stress condition, while stomatal index remained nearly constant, which could be caused by a marked reduction in leaf elongation rate under moderate water deficits. A close relationship between leaf temperature increases and stomatal closure due to water stress would be responsible for this response. From the correlation analysis results of LTD and relative shoot biomass among the RILs, LTD showed significant correlation with RSFW (RSDW). It indicated that genotypes with a higher leaf temperature difference exhibited a better biomass increase. Some studies on stomatal response (Hashimoto et al., 1982; Hashimoto et al., 1984) have also confirmed that thermal imaging can provide adequate resolution to detect local variation in stomatal aperture and demonstrated a relationship between leaf temperature variation and stomatal aperture using electron microscopy (Jones, 1999).

In our study, water cessation for several days imposed moderate water stress on maize seedling and resulted in an increase in leaf temperature and decrease in biomass. Biomass accumulation is intrinsically linked to transpiration, because stomatal aperture and leaf area determined the rate of both photosynthesis and transpiration (Collins et al., 2008). Stomatal regulation is considered highly related to plant drought-tolerance. Therefore, we speculated that a rise in leaf temperature primarily caused by stomatal closure is only a surface phenomenon, in fact water stress resulted in the closure of stomata which reduced water loss thus enhanced biomass accumulation. Plant biomass accumulation in response to water stress is presumably related to leaf temperature variation, leaf temperature variation could reflect the biomass performance of genotype in advance.

4.3. Genetic mechanisms underlying leaf temperature response to drought

Drought tolerance in plants is a complex phenomenon which is strongly influenced by morphological, physiological and biochemical traits as well as environmental factors and their interactions. Different crops or crop varieties adapt to water stress in various ways and have different drought tolerance mechanisms. Therefore,

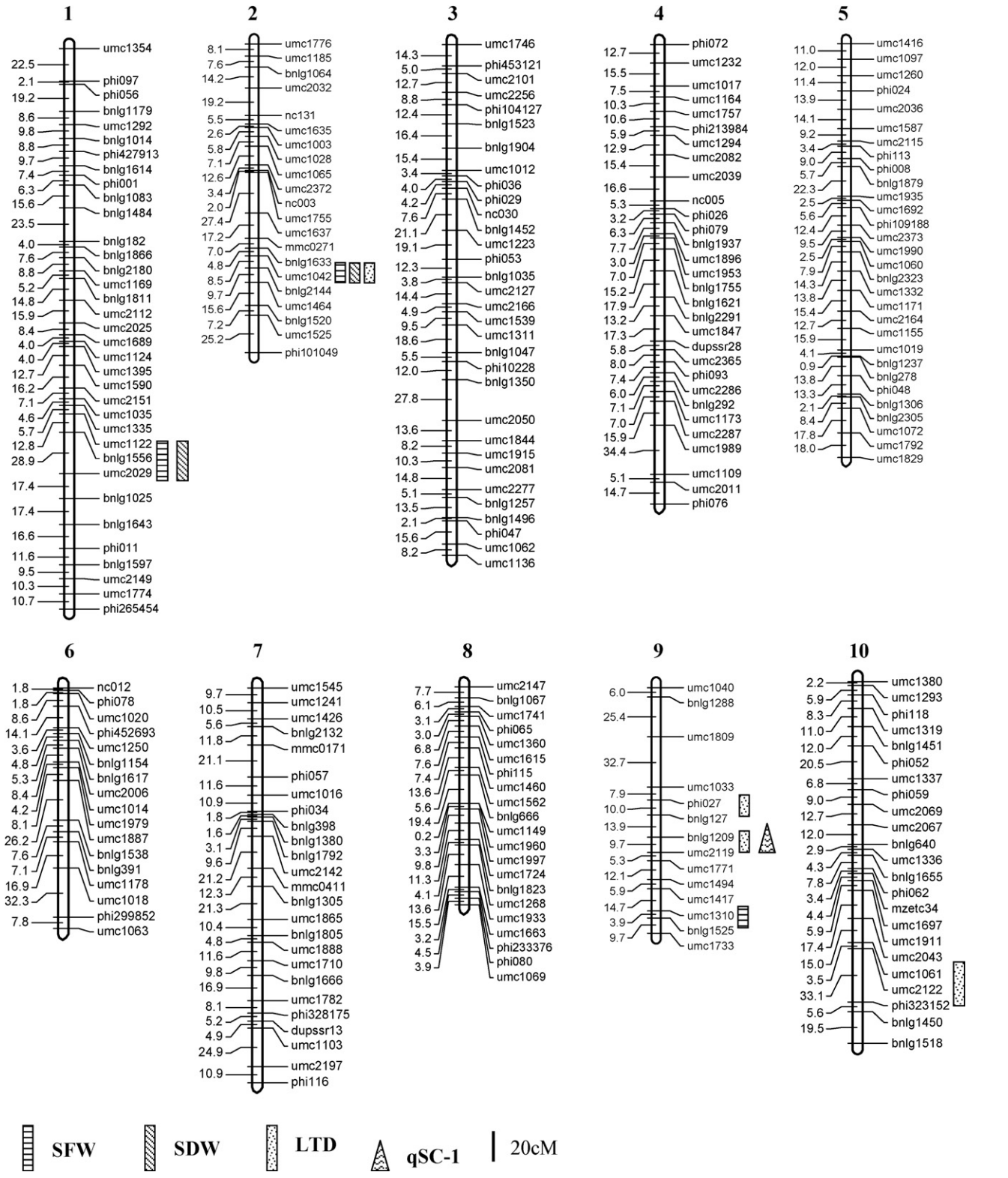

Fig. 3. The distribution of QTL on the linkage map.

there can be tremendous quantitative trait loci to participate in the process of drought tolerance of plants, most of which have only a minor effect and contribute very little to phenotype variation.

For a single cross, typically only a small number of QTL underlying the drought tolerance can be detected because favorable alleles governing the expression of traits related to drought tolerance are likely to be dispersed in different combinations each crop accession (Tuberosa et al., 2002). In the present study, LTD from the RIL population exhibited a normal distribution with moderate broad-heritability (0.61), LTD is therefore a quantitative trait and controlled by multiple minor genes. Four QTL were detected for the trait and additive effects were derived from the two parents 87-1 and Zong3. A few QTL were identified for seedling biomass, which explained a small amount of the total phenotypic variation.

Despite this, co-locations of QTL were detected on some intervals of the genetic map, providing us with useful information to elucidate the causal pathways linking two or more traits (Simko et al., 1997), such as morpho-physiological traits and biomass under drought (Lebreton et al., 1995; Sanguineti et al., 1999; Tuberosa et al., 2002).

In this investigation, three co-location alleles were located in identical or nearby positions on the linkage map with one or two other related QTL. The direction of genetic effects for the QTL was consistent with the sign of their phenotypic correlations. The results suggested that pleiotropic effects operate in some QTL for maize response to drought at the seedling stage. Two co-location QTL regions between RSFW and RSDW exhibited rather common signs with their trait correlations. Among them, one near bnlg1556 on chromosome 1 was also associated with maize grain yield, kernels per row and row numbers found on the $F_{2:3}$ population derived from the same two inbred lines by Yan et al. (2006).

A QTL co-location region between LTD and seedling relative biomass was consistent with the significant correlation of related traits, and should be considered a common QTL for both traits. The results indicated that to some extent, the variation in leaf temperature response to water stress is genetically associated with accumulation of shoot biomass.

4.4. Comparison with previously reported maize QTL

In this investigation we mapped two QTL for RSDW, which appeared to have similar chromosome locations to those located on bin 1.08 near umc83 and bin 2.07 near umc4, previously associated with dry biomass yield (forage stage) by Lübberstedt et al. (1998). Presterl et al. (2007) detected one QTL anchored near the marker umc1497 on bin 2.07 for fresh matter yield (six to eight fully developed leaves). These results suggested that some QTL influencing biomass accumulation at different maize developmental stages are stable across different populations. Additional QTL for shoot fresh (dry) weight have been mapped (Fracheboud et al., 2004; Jompuk et al., 2005; Leipner et al., 2008), however, none of those co-localized with the two major QTL for relative biomass were identified in the present study. The different QTL detected in other reports indicate that biomass accumulation in maize is a complex quantitative trait, depending on the population, developmental stage and environment.

Several QTL for leaf temperature differences have been mapped with related stomatal behavior traits. Pelleschi et al. (2006) identified two out of three QTL for stomatal conductance, including Gsc.f (nearest marker umc22 on bin 2.07) and Gss.i (nearest marker bnl5.10 on bin 9.03), which overlapped with QTL intervals for RSFW, RSDW and LTD. Fracheboud et al. (2002) mapped a QTL for stomatal conductance under normal environmental conditions on bin 9.03 near umc114. Lebreton et al. (1995) also detected a QTL for leaf ABA concentration located in the vicinity of npi454 on bin 9.03. Stomatal sensitivity to ABA concentration has shown significant variation among maize lines (Conti et al., 1994). Compared to these studies, we located the locus qLTD9-2 on a common chromosome region according to IBM2 Neighbor's consensus genetic map. This region would be considered a universal drought tolerance QTL, according to the consensus map relevant to drought tolerance and markers adjacent to each QTL (Li et al., 2005). The comparison indicated that these QTL are involved in stomatal regulation processes important in maize drought tolerance.

We speculated that a common QTL region might be located on bin 9.03 in maize, which regulates stomatal movement in response to water stress. This result not only provides further evidence for the existence of positional convergent QTL for biomass relative to drought tolerance, but also suggested that these QTL may be associated with stomatal movement. Therefore, further study should address the homologous genes underlying the QTL involved in stomatal movement and biomass accumulation, which will provide further insights into drought tolerance in maize.

5. Conclusion

This study provided evidence for leaf temperature response to water stress at the maize seedling stage. Further study at more developmental stages is necessary to validate the presence of common plant response mechanisms to water stress. Compared to previous studies, advanced infrared thermography generates high definition thermal maps representing an entire plant leaf temperature and provides a very useful tool to evaluate leaf temperature variation in response to drought. In this study, phenotypic, physiological and genetic analyses of leaf temperature response to water stress showed significant positive association to biomass accumulation, which suggested the probability of using an infrared thermography system in drought tolerance breeding. However, considering the complexity of drought tolerance, we suggested it can only act as an accessory means in an active breeding program for drought tolerance in maize.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.envexpbot.2010.11.010.

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