Rapid phenotyping of different maize varieties under drought stress by using thermal images

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Abstract: The development of maize genotypes with high yields under drought is of pivotal relevance for the International Maize and Wheat Improvement Centre (CIMMYT). Thermal images of the canopy of different 92 maize genotypes were acquired in the time interval between anthesis and blister stage with each picture containing five plots of different genotypes. Mean temperature differences of more than 2°C between different genotypes under water stress were then detected using thermal images. Genotypes better adapted to drought exhibiting lower temperatures. A canopy thermal image is a potential promising method to accelerate the screening process and thereby enhance phenotyping for drought adaptation in maize.

Keywords: Maize genotypes, water stress, thermal images, canopy temperature,

1. INTRODUCTION

The development of maize genotypes with high and stable yields under water stress is vitally important for CIMMYT (International Maize and Wheat Improvement Center) (Bolanos ***et al.*** 1996). Different techniques such as soil moisture measurements, leaf water potential and stomatal conductance have already been applied to monitor the water status of plants. However, these measurements are often time consuming, labor intensive and require a number of repetitions to achieve reliable results (Rebetzke ***et al.*** 2001). Furthermore, these methods have not yet been automated, something which would allow researchers to quickly distinguish between different crop varieties and treatments. As an alternative, leaf temperature detection by infrared thermometers has been used to detect water stress - which results in stomata closure and an increase in temperature through decreased adiabatic cooling. It is a fast and non-destructive way to identify plant water status; however, this approach is only able to provide information for a small area around each measurement point (Evans ***et al.*** 2000) and the heterogeneity of the maize canopy usually prevents proper measurement of the canopy as a whole. In contrast, infrared thermography allows to study whole canopies in an affordable manner; therefore, by placing a thermal camera at an appropriate distance it is possible to obtain information over a large area, incorporating canopies which contain a variety of genotypes at the same time (Jones and Leinonen, 2003). Recently, a number of studies have investigated the suitability of using thermal imaging to detect stress, both in the field and in greenhouses (Cohen ***et al.*** 2005). For example, (Grant ***et al.*** 2006) found temperature differences in vineyards under two different treatments and recommended the application of thermal imaging for irrigation scheduling. However, further studies need to be carried out in order to assess the potential of using thermal imaging for different crops and for different locations with varying environmental conditions (Alchanatis ***et al.*** 2010). Even though, (Jones ***et al.*** 2009) suggested the use of thermal imaging for selection in plant breeding, so far the use in phenotyping has not been investigated. However, this is not trivial since for a given irrigation regime the range of genotypic variability in canopy temperature is probably lower than differences in canopy temperature due to by contrasting irrigation

regimes. The main objective of the research was to investigate the suitability of infrared thermography to quickly identify differences in the responses of different genotypes to water stress, based on canopy temperature. The suitability of thermal imaging for measuring water stress tolerance in maize was also assessed by correlating canopy temperature with CWSI as well as with yield, NDVI and the chlorophyll content

2. MATERIALS AND METHODS

Location

Experiments were conducted at the maize experimental station of the International Maize and Wheat Improvement Center (CIMMYT) in Tlaltizapán, Morelos in México (18°41'N, 99°10'W, 940 m a.s.l. Ninety-two single cross-hybrids were replicated twice for each treatment, and the sample group was chosen based on a similar date of anthesis. All hybrids had the same tester: CML-312SR. Entries were planted on 25th November 2009 in two row plots (5 m rows with 0.25 m spacing between plants and 0.75 m between rows). For the experiments, two different treatments were applied: full irrigation or well-water conditions (WW), and water stress (WS).

Irrigation scheduling

The stress treatment was imposed by stopping irrigation about 2 weeks before anthesis, in order to ensure water stress during flowering. Stressed plants were irrigated one more time about one week after silking, at the time of the milk stage. During the time of stress monitoring (anthesis to harvesting) ET0 amounted to 276.8 mm. The crop water requirement was calculated as 332.0 mm based on a Kc value of 1.2 of which 34.6 mm were covered by rainfall, resulting in an irrigation requirement of 297.6 mm. The well-watered treatment received 348 mm during this period, while the stress treatment received 87 mm, resulting in a deficit of 183.6 mm. Figure 1 shows the irrigation scheduling and the amount of water for the well-watered (WW) and water-stressed (WS) plants during the whole growing season.

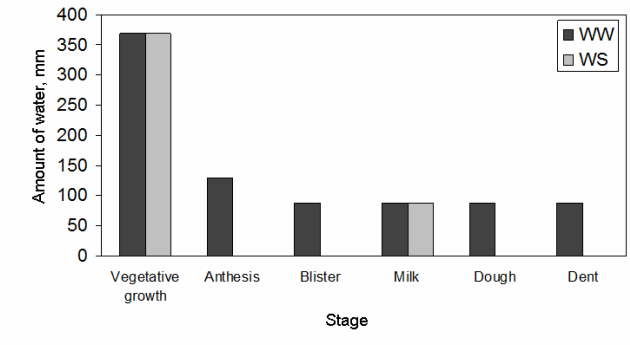

Fig. 1. Irrigation day vs. amount of water

Measurements of yield

Grain yield (t ha-1) per plot was assessed at physiological maturity, when the black layer is visible at the base of the grain. This was calculated from the number of plants harvested per plot (plant density 6.67 plants m-2) and the corresponding grain weight.

Thermal images acquisition

Thermal images were acquired using the Midas 320L infrared camera (Dias Infrared Gmbh, Germany), which has a resolution of 320x240 pixels. Image acquisition took place in February and March 2010 - the period between anthesis and the blister stage (grain filling stage I). The blister stage is a time when kernels are filled with clear fluid and the embryo can be seen, and during this period, maize is more vulnerable to water stress. A platform was mounted on a tractor about eight meters above the canopy, and then moved between the rows to enable top view images to be taken between 10:30 hrs to 15:30 hrs. Measurements started on 22nd February and were taken at ten day intervals, which accounted for a total of 2 times. Measurements were performed on sunny days without wind, and during the image acquisition process the emissivity value was set at 0.94. To identify the plots in the thermo picture, blue paper sheets were used as boundary markers. Zenithal pictures were taken, each comprising five plots and a total of ten rows. A total of different ninety-two genotypes (from the total set trial of 150) with two replications per treatment were photographed. The photos were analyzed using the professional Pyrosoft software (Dias infrared Gmbh, Germany).

Measurements of biomass formation

In order to determine the water status of the maize plants, readings for the Normalized Difference Vegetation Index (NDVI) and chlorophyll content were acquired across all genotypes. NDVI data was collected using a portable spectroradiometer (GreenSeeker, Hand-Held Data Collection and Mapping Unit, NTech Industries, USA). Measurements were taken for each plot at a distance of one meter, for both treatments, and average values calculated for the five readings taken per genotype. Data was acquired at two different intervals between the pre-anthesis and dough stages (grain filling III). In addition, chlorophyll content was measured using a portable SPAD 502-Plus Chlorophyll Meter (Minolta, Japan), with readings from the five different leaves per plot selected randomly and averaged. SPAD data was collected during the anthesis and dough stages (grain filling III). The dough stage representing a time when the

embryo is about half as wide as the kernel and the top part of each kernel is filled with solid starch.

3. RESULTS AND DISCUSSION

NDVI, chlorophyll index

From Figures 2 and 3, differences in the NDVI and chlorophyll indexes can be seen at different stages, for both the water-stressed (WS) and well-watered (WW) plants.

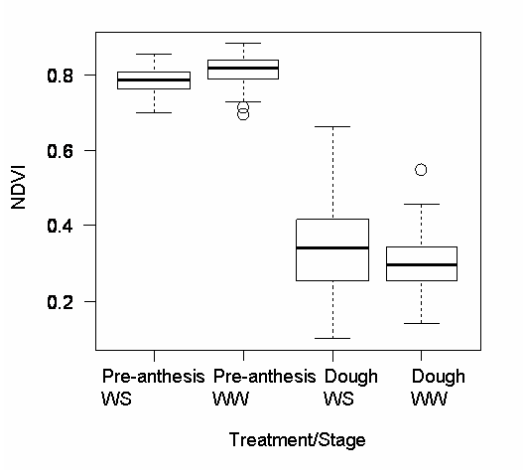

Fig. 2. NDVI data at different stages in water stress (WS) and well-watered (WW) plants

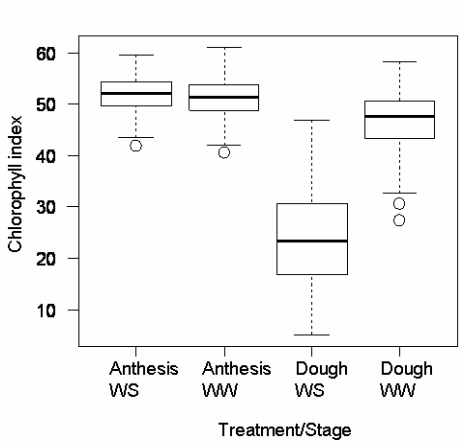

Fig. 3. Chlorophyll data at different stages in water stress (WS) and well-watered (WW) plants

ANOVA revealed the minimal effect ($p<0.1$) of the treatment on the NDVI data, whereas the measurement stages and the cross-interaction between the stages and treatments had a highly significant effect ($p<0.001$) on the NDVI readings. Moreover, the measurement stages and treatments had a highly significant influence ($p<0.001$) on the chlorophyll content (SPAD) values. Furthermore, a low variability of NDVI and SPAD readings within the genotypes was found for both treatments during the pre-anthesis and anthesis stages respectively, indicating that water stress was not yet prevalent during this stage. At the dough stage, as water stress increased, lower values with a larger standard deviation were observed in all treatments, indicating differences in senescence between the genotypes. The correlation coefficients for canopy temperature versus the NDVI and SPAD values in water-stressed and well-watered plants for all genotypes are shown in Table 1.

Table 1. Pearson coefficient of canopy temperature and CWSI vs. NDVI, SPAD and yield in water-stressed (WS) and well-watered (WW) plants.

Anthesis	WS	WW
SPAD (Anthesis)	Ns	Ns
NDVI (Pre-anthesis	-0.40***	Ns
Blister	WS	WW
SPAD (Dough)	-0.35**	Ns
NDVI (Dough)	-0.40***	Ns

Canopy temperature obtained during the blister stage in water stress genotypes were significantly negative correlated ($p<0.01$-0.001) with SPAD and NDVI readings acquired during the dough stage. These results show that blister stage, represents the most suitable time after pre-anthesis to acquire thermal images in order to indicate different levels of stress in maize varieties. Correlations were not observed between canopy temperatures with SPAD, NDVI values for the well-watered treatment.

Thermal images analysis and yield data

In each thermo image the analyzed areas include both sunlit and shaded leaves, with soil in the background showing much lower temperatures which could be easily separated during the analysis from those leaves showing higher temperatures. The temperature difference found between the soil and the upper leaves was 6°C to 10°C. The selected area of interest was outlined by vertical dotted lines. The mean values of the two rows were calculated in terms of the maximum, minimum and mean temperatures, as well as the standard deviation. Clear differences in maximum temperature within genotypes were identified, with values ranging from 30.1°C to 32.7°C. Figure 4 shows significant differences in yields between the stressed and well-watered plants.

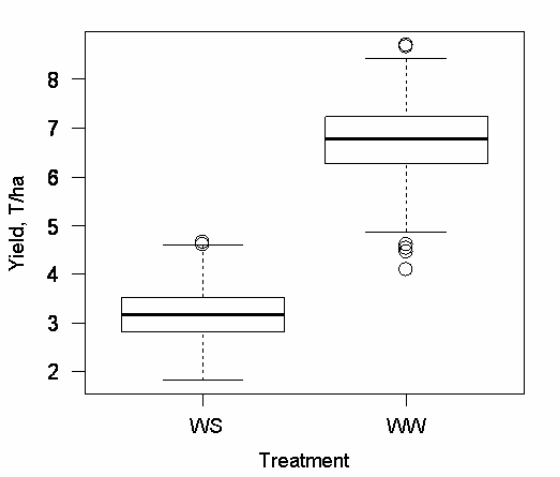

Fig. 4. Yield data in water stress (WS) and well-watered (WW) plants

Difference between treatments

The overall mean canopy temperature values for all genotypes, for both water-stressed and well-watered treatments, are shown in Figures 5. An increase in canopy temperature of between 1 and 2°C was detected using thermal images during the different stages, taking into account the water-stressed and well-watered plants separately. Moreover, canopy temperatures in the water-stressed plants reached mean values above 32 °C close to the end of the blister stage, which was 2.3°C higher than the well-watered plants. ANOVA revealed significant differences ($p<0.01$) in canopy temperatures between water-stressed and well-watered plants during the different stages of image acquisition.

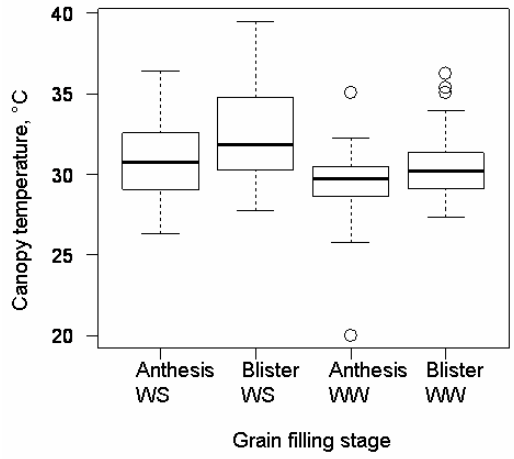

Fig. 5. Canopy temperature at different grain filling stages in water stress (WS) and well-watered (WW) plants

Using thermal imaging, clear differences between water-stressed and well-watered plants were detected. This implies that thermal imaging can be used for the early detection of water stress, as well as for the quantification of that stress. It should be pointed out that the increase in canopy temperature the anthesis and blister stages, for the well-watered and water-stressed trials, might be explained by the increases in air temperature and solar radiation. In addition, this study found that differences between genotypes in terms of their response to water stress can be detected by thermal imagery. Genotypes with a comparatively high yield under water stress showed a lower canopy temperature, which may indicate that these varieties are more efficient at exploiting the soil water available; for example, by having a more efficient root system or a higher root density (Kaman ***et al.*** 2011). In addition, canopy temperature variations within plots can be used to identify tolerant and sensitive genotypes. It was also shown that since stomata closure has an effect on water stress levels, this necessarily leads to a consequently reduction in yields after the end of the vegetative growth period, a finding which aligns with previously published studies on the effects of water deficits during different development stages (Payero ***et al.*** 2009). As a consequence, variety selection is of crucial importance when deficit irrigation strategies are being applied (Kaman ***et al.*** 2011). The influence of plant geometry on measurements was not studied within the framework of this experiment, since leaf angles and the ratio between leaf surface and biomass affect both transpiration and temperature measurements. It is suggested that more emphasis be placed on this issue in future studies. However, it was demonstrated that averaging leaf temperatures reduces the error imposed by the canopy temperature variation (Zia ***et al.*** 2009), and this becomes more important when one attempts to estimate the absolute stomatal conductance (Jones ***et al.*** 2009). In this study each thermal images only captured ten rows (i.e. 5 genotypes) each time which is a limitation in terms of time with the subsequent problems associated with taking series of images exposed to sudden or even daily changes in environmental conditions during measurement. The ideal option, and providing a camera with enough resolution, would be to take the entire trial in one or few images. To this end several alternatives may be accounted, including the use of wide angle lens or placing the camera in remote controlled aerial platforms. Overall genotypic screening for drought adaptation seems possible using thermal imaging, since differences in canopy temperature between genotypes under stress are more visible when compared to other tested methods. Applying stress resulted in a reduction in leaf chlorophyll during the flowering stage, as well as an increase in leaf senescence and biomass reduction, as calculated by the NDVI method, and these effects were observed for all genotypes. Therefore, the use of SPAD and

NDVI measurements in phenotyping for drought resistance is not appropriate

4. CONCLUSIONS

Thermal imaging is suggested as the method to use for screening water stress tolerant maize varieties. This could be important for the creation of a new phenotyping platform to speed-up the selection process for drought stress breeding programs, and to assess genotypic variability in terms of drought adaptation and water use efficiency. Further investigations should include a greater number of maize varieties using images with smaller time intervals, in order to avoid the influence of changing environmental conditions during measurement.

5. ACKNOWLEDGEMENT

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