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Using an Airborne Platform to Measure Canopy Temperature and NDVI under Heat Stress in Maize

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*In light of anticipated climate change, we assessed the possibility to use an airborne platform to measure canopy temperature (CT) and the normalized differential vegetation index (NDVI) as well as the suitability of both traits for their use in breeding for tolerance to heat stress. We evaluated 71 subtropical maize (Zea mays **L.**) hybrids under heat stress and combined heat and drought stress in an environment with average temperatures of 29.8°C during the growing season and 31.2°C during the flowering period. Grain yield (GY) ranged from 0.33 to 4.19 Mg ha⁻¹ under heat stress and from 0 to 1.37 Mg ha⁻¹ under combined heat and drought stress, going along with increases in CT from 42.5°C to 49.5°C and decreases in NDVI from 0.54 to 0.48. The NDVI explained differences between and within treatments, while CT explained differences in GY among treatments and genotypes within the heat and drought stress treatment, as indicated by genetic correlations with GY. A principal component analysis was used to identify combinations of physiological characteristics associated with genotypic variation in GY. Results showed that selection gains for GY could be improved by 0.486 Mg ha⁻¹ and 0.015 Mg ha⁻¹ under heat and combined heat and drought stress, respectively, if selection is simultaneously carried out for GY, NDVI, and lower CT and shorter*

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anthesis silking interval. We postulate that the use of selection indices, including CT and NDVI in conjunction with GY, will improve selection gains and increase cost efficiency of breeding programs.

INTRODUCTION

Agricultural production and global food security will be detrimentally affected by climate change (Fischer et al. 2005; Schmidhuber and Tubiello 2007; Ainsworth and Ort 2010). The largest negative impacts may occur in the lowland tropics, where high temperatures already limit grain yield in many maize production environments (Easterling et al. 2007). Heat stress in maize is associated with a shortened life cycle (Muchow, Sinclair, and Bennett 1990); and reductions in light interception (Stone et al. 2001), radiation-use efficiency (Cichino, Rattalino Edreira, and Otegui 2010; Rattalino Edreira and Otegui 2012), photosynthesis (Crafts-Brandner and Salvucci 2002), and pollen viability (Schoper et al. 1987). Analysis of more than 20,000 historical maize yield trials in southern Africa showed that each degree-day accumulated above 30ºC reduced the final yield by 1% under optimal rain-fed conditions, and by 1.7% under drought conditions (Lobell et al. 2011). Reductions were even more accentuated under combined drought and heat stress, reducing grain yield by 40% with every degree day above 30°C (Lobell et al. 2011).

The association between heat and drought stress is poorly understood. Drought stress is often a combination of low water availability and increased temperature resulting from reduced transpirational cooling under limited water conditions. However, there is evidence that the response to drought stress at elevated ambient temperatures is unique and cannot be extrapolated from the sum of the effects of both stresses (Rizhsky, Liang, and Mittler 2002, 2004; Barnabas, Jaeger, and Feher 2008; Cairns et al. 2013). Although high temperatures often coincide with low water availability (Tester and Bacic 2005), less effort has been invested in understanding heat stress than drought stress (Cairns et al. 2013; Zia et al. 2013).

Grain yield remains the most important trait for selection in many breeding programs. Heritability for grain yield under stress (e.g., nitrogen deficiency and drought stress) is typically lower than that under optimal conditions, reducing genetic gain from selection under abiotic stress conditions (Weber et al. 2012). Several studies have shown that selection for some physiological traits can increase grain yield under stress conditions. For example, selection for increased flowering synchrony resulted in yield gains of up to 144 kg ha^{-1} per year under drought stress (Edmeades et al. 1999). However, Monneveux,

Sanchez, and Tiessen (2008) suggested that repeated selection for increased synchrony can potentially reduce genetic variation, and that new traits would be required to sustain genetic gain under drought conditions.

Breeding for tolerance to both drought and heat stress would require identification of key physiological traits associated with both stresses. Such traits need to be closely associated with grain yield and show additional genetic gain for grain yield under stress conditions and at the same time not have any detrimental effects on grain yield under optimal conditions.

Two traits that can potentially be used in a breeding program to improve lines and hybrids for their tolerance to high temperatures are canopy temperature and the normalized differential vegetation index (NDVI). The NDVI is the ratio between reflected light in the near-infrared spectrum and light reflected in the visible spectrum (Henik et al. 2012). In maize, the NDVI has been used for site-specific nutrient management (Inman, Khosla, and Mayfield 2005), the evaluation of crop management practices (Verhulst et al. 2011), field variability (Masuka et al. 2012), yield predictions (Mkhabela, Mkhabela, and Mashinini 2005), the assessment of heterosis (Araus et al. 2010), and drought responses in maize hybrids (Lu et al. 2011). To date, several projects have used aerial images to investigate levels of drought stress (Sepulcre-Canto et al. 2009; Suárez et al. 2008) and nitrogen fertilization (Boegh et al. 2002; Chiara et al. 2014; Quemada, Gabriel, and Zarco-Tejada 2014) in maize. We are not aware of any attempt to predict heat and drought tolerance via NDVI.

Canopy temperature strongly depends on stomatal conductance and the plant's access to water. This makes canopy temperature a potential trait for indirect selection of maize germplasm for improved drought resistance (Garrity and O'Toole 1995; Saint Pierre et al. 2010). Canopy temperature can be measured using infrared thermometry, which has been used in research on heat-stress resistance (Reynolds et al. 1994; Badaruddin, Reynolds, and Ageeb 1999), drought tolerance (Aston and Van Bavel 1972; Clawson and Blad 1982; Sadler, Bauer, and Busscher 2000), and combined heat and drought stress (Trethowan and Reynolds 2007). To best capture genetic variation in canopy temperature, it is important that measurements are taken within a short period of time of the day to exclude environmental factors (e.g., clouds, changes in radiation intensity) varying across the course of the day, which can limit large-scale application of this method when applied in a breeding program.

Various methods are available to measure canopy temperature and NDVI, each with its advantages and disadvantages. Satellite-based remote sensing has successfully been used in supporting large-scale field tests to quantify nitrogen and water requirements of a crop, but the spatial resolution is not high enough to measure small plots as typically used for yield trials by breeders (Nebiker et al. 2008). Unmanned Aerial Vehicles (UAVs) are promising because of their ease of use and immediate availability of data after landing, allowing the use by researchers at experimental stations. However,

commercially available UAVs are often payload restricted, limiting the weight and type of cameras that can be mounted onto a UAV (Nebiker et al. 2008).

Alternatively, an actual plane can be used for image acquisition. Images could be taken at the same speed as with an UAV but at higher payloads, thus allowing them to carry combinations of several cameras at the same time. Images with a thermal camera and a hyperspectral camera could be taken simultaneously at a spatial resolution high enough to allow the identification of individual small trial plots in the field. Despite increased cost, this seems to be the method of choice.

The aim of this study was to assess: i) the possibility to use thermal and multispectral cameras mounted to a small airplane to quantify effects of high temperature, and combined drought and high temperature stress on grain yield, and ii) the suitability of canopy temperature and NDVI for use in breeding programs as determined by heritability, correlation with grain yield, and predicted genetic gains.

MATERIALS AND METHODS

Crop Environment and Germplasm Features

Experiments were carried out at the CIMMYT experimental station in Ciudad Obregon, Sonora, Mexico (27°20′ N, 109°54′ W, 38 masl) during the 2013 summer season in an environment with average temperatures of 29.8° C during the growing season and 31.2° C during the flowering period, with temperatures ranging from 25° C at night to 50° C at midday during flowering. Temperature and precipitation during the growing season are shown in Figure 1. The trial consisted of 71 hybrids that were planted on the 23rd of May 2013 in two-row,
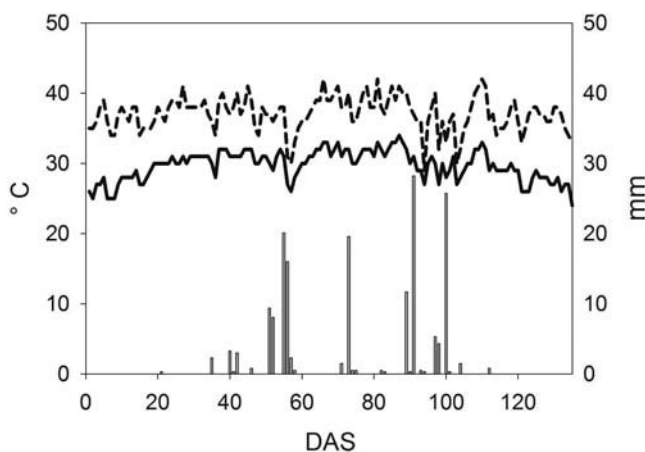

FIGURE 1 Daily mean (full line) and maximum temperature (dotted line) and precipitation (bars) measured throughout the maize growing period displayed in days after sowing (DAS).

5 m long plots, with 0.19 m spacing between plants within a row, and 0.8 m row-to-row spacing. We used a randomized incomplete-block design replicated twice. The experiment was carried out under well-watered and water-deficit conditions. In the well-watered treatment (subsequently referred to as heat-stress treatment), plants were continuously irrigated throughout the cropping season. In the drought-deficit treatment (subsequently referred to as combined heat and drought stress treatment), irrigation was reduced by 50% relative to the well-watered treatment starting two weeks before anticipated flowering. Irrigation treatments were assigned to main plots while genotypes were randomly assigned to sub-plots within the main plots. Two seeds were sown per hill, and then thinned to one plant per hill at the V_3 stage, resulting in a final plant density of 6.68 plants m^{-2}. All plots received an initial application of 100 kg ha^{-1} of mono-ammonium phosphate (NH_4) H_2PO_4 and 500 kg ha^{-1} of ammonium sulfate $((NH_4)_2$ $SO_4)$ at sowing. A second application of 250 kg ha^{-1} of ammonium sulfate was applied at V_5. Weeds, insects, and diseases were controlled as needed.

Irrigation was applied once every seven days using drip irrigation at a rate of 5 mm/h for 6–14 hours, depending on potential evapotranspiration.

Agronomic Traits

Anthesis and silking dates were recorded when 50% of the plants within a sub-plot had shed pollen and 50% of the plants had silked, respectively. The anthesis-silking interval (ASI) was calculated as the difference between days to silking and days to anthesis. At physiological maturity, all plants were hand-harvested, and grain yield was measured. All grain weights are expressed on a dry weight basis.

Canopy Temperature and NDVI Measurements Using an Unmanned Aerial Vehicle

In order to contrast the treatments, the flight campaign was conducted in August 2013, two weeks after mean anthesis date, between 12:30 pm and 1:00 pm. The flight plan was designed to overfly the site in an east to west and west to east flight path. The flying altitude above ground was 270 m, at a ground speed of 33.33 m s^{-1}. The flight campaign was carried out with a multispectral imager (Tetracam, Chatsworth, CA) and a thermal camera (A600 infrared camera, Flir, Wilsonville, OR) on-board of a Piper PA-16 Clipper (Piper, Vero Beach, FL). The high-resolution thermal imagery made it possible to identify pure vegetation pixels from each experimental block and to extract the canopy temperature (Figure 2). The multispectral camera had a radiometric resolution of 10 bits, configured at six bands wavelength; 550, 670, 700, 710, 750, 800 nm. The NDVI was calculated based on wavelengths measured with the multispectral sensor.
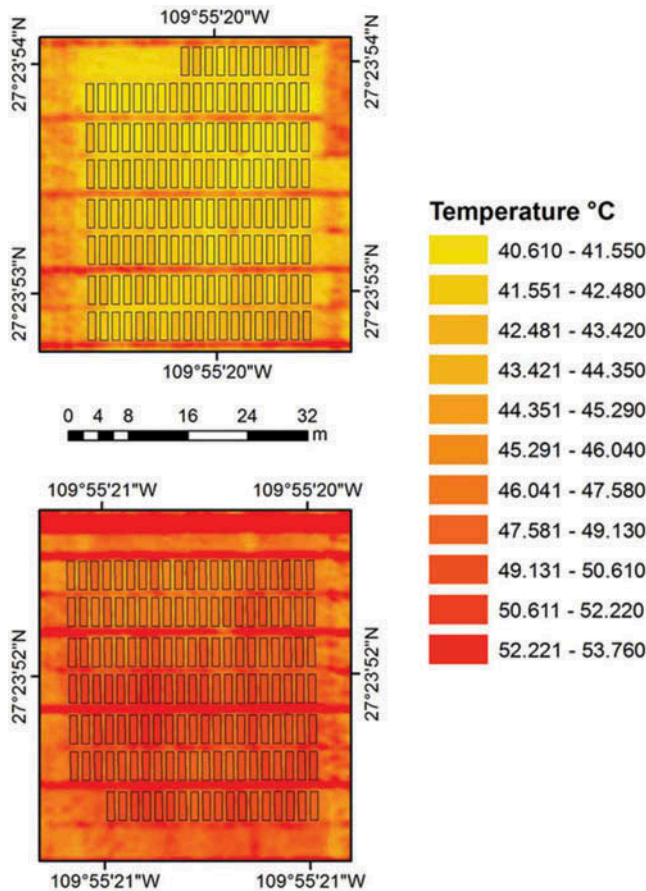
FIGURE 2 Infrared thermal image of all plots evaluated under heat (HS) and combined heat and drought stress (HS+DS).

Thermal Images

A fixed radiometric calibration line, based on previous field and camera measurements, was applied to all the images using imap (Quantalab, Cordoba, Spain). The original image data were stored in kelvin units x 100. Images were automatically mosaicked from individual frames in the post-processing with Autopano software (Version 3.0, Kolor, Francin, France) and manually georeferenced using an image-to-image registration. The image used as reference was a 30 cm world imagery basemap (Esri, DigitalGlobe, GeoEye, i-cubed, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community). Geo-referencing allows knowing the coordinates of each pixel of the image, which makes it possible to overlay images of a trial from different flight days and compare other geo-referenced features.

Multispectral Images

Imagery was atmospherically corrected with irradiance information produced on the basis of the data collected with the sun photometer on the flight day. The processing results in a pair of reflectance data images (range 0–100000) for each frame captured, with three bands stored in each image, summing the six bands acquired. The first image of the pairs was mosaicked with the software Autopano to create a single image for the whole area. The second image of the pairs was mosaicked with the exact same parameters used for the first image of the pair. Both 3-band images were merged into one 6-band image with ENVI software (Version 5.0, Exelis Visual Information Solutions, Boulder, CO). Images were then manually geo-referenced using an image-to-image registration. The image used as reference was a 30 cm world imagery basemap.

Data Extraction

After image correction, image pixel information was extracted from the plots seen on it, using the QGIS software (QGIS Development Team, 2013, QGIS Geographic Information System; Open Source Geospatial Foundation Project). To transform the pixel values to tabular data, the mean value was obtained from the pixels whose center was inside each individual plot. The plots were represented as 4.5 m × 0.8 m polygons (in shape file format) with individual unique attributes of the trial and plot they represent. They were as well geo-referenced. In cases where plot polygons did not correctly match the image, polygons were manually aligned to the plots with the "affine transformations" plugin (QGIS, Newcastle, UK). Once the plots matched the images, a buffer was created to get an area that is smaller on each side than the trial plots, keeping the central area of each plot to avoid borders. Mean values of temperature and reflectance were calculated using the "ZonalStats" plugin (QGIS, Newcastle, UK), generating a table with columns, indicating plot-ID and the mean value obtained from the pixels of the image file. In case of the multispectral image, the tool was run for each 6 bands, or the 2nd and 6th, which are used in the NDVI calculations. The next formula was applied to convert the temperature values to Celsius degrees: (Pixel value)/(100–273.15).

Statistical Analysis

Data were analyzed with a linear mixed model containing the overall mean (μ), block effect (β_i), genotype effect (α_j), which were considered random effects, and experimental error (ε_{ij}) to explain the response variable (y_{ij}) in the below model:

$$y_{ij} = \mu + \beta_i + \alpha_j + \varepsilon_{ij}$$

Best linear unbiased predictors (BLUP) of genotypes, variance components, and broadsense heritability were obtained. Variance components were estimated by restricted maximum likelihood and heritability as the relationship between genetic and phenotypic variances, according to the below formula:

$$b^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2/r}$$

The BLUPs for genotype effects are shrinkage predictors that were obtained as:

$$\tilde{\alpha} = \hat{\mathbf{G}}\mathbf{Z}'\hat{\mathbf{V}}^{-1}(\mathbf{y} - \mathbf{1}\mu)$$

where, using matrix notation, $\mathbf{y}$ is the vector of the response variable, $\hat{\mathbf{G}}$ is the matrix of variance covariance of the random effects, $\mathbf{Z}$ is the design matrix for random effects in the model, $\hat{\mathbf{V}}$ is estimated variance of $\mathbf{y}$, $\mathbf{1}$ is a vector of ones, and μ is the overall mean, the only fixed parameter in the model. For yield per hectare, anthesis date was included as covariate in the model. All results of grain yield were corrected for flowering date. Correlations were calculated using BLUPs of the different genotypes obtained from a linear fixed model.

Genetic correlations between traits were estimated with the method described in Cooper, DeLacy, and Basford (1996). Expected genetic gain (EG) in a trait (1) selected by an indirect measurement (2) is a function of the square root of the heritability of the two traits (h_1 and h_2), the genetic correlation between them (r_{12}), the phenotypic variability of the trait of interest (s_1), standard deviation and the selection pressure (i):

$$EG = is_2h_1h_2r_{12}$$

If selection is made directly for the trait of interest, then r_{12} equals 1 and h_1 by h_2 is the heritability of the trait:

$$EG = is_1 b_1^2$$

Because we are interested in yield and i, s_2 and h_1 remain constants. Irrespective of the indirect measurement used for selection, we calculated a relative expected gain to compare different criteria of selection:

$$REG = h_2r_{12}/h_1$$

Note that for direct selection, REG is equal to 1.

A principal component analysis (PCA), based on the matrix of genetic correlations, was used to identify combinations of physiological characteristics

associated with genotypic variation in grain yield under heat and combined heat and drought stress as function of the genetic correlations.

Selection Indices

We explored the effect of selection with three different indices. The first index (S1) includes grain yield, NDVI, the anthesis silking interval, and canopy temperature; for the second one (S2) grain yield was excluded and the third index (S3) consisted of canopy temperature and NDVI only. For these different indices, we calculated the relative expected genetic gain for yield. Since indices not including grain yield generally showed lower AUC, indices including other trait combinations were not included. Smith selection index (Smith 1936) was used and it was obtained with our own code written in SAS (Alvarado et al. 2015).

In order to evaluate the performance of the different selection indices, receiving operating characteristic (ROC) curves were calculated. In a first step to construct the ROC curve, a selection differential only using grain yield was used, randomly classifying all genotypes as either "selected" or "not selected." Selection was then carried out using either a selection index or a secondary trait to construct a 2 × 2 contingency table in which rows represented selected and not selected genotypes based on the different indices, whereas columns reflected genotypes selected or not selected based on grain yield only. When an indirect measurement is used instead of a direct measurement a higher selection pressure is typically applied because any indirect measurement (not even indices) will not necessarily select the highest yielding genotypes. The contingency table described above is therefore generated for different levels of selection pressure. For each selection pressure, the sensitivity and specificity were calculated, indicating the percentage of entries selected by the indirect measurement relative to selection based on grain yield (sensitivity) and the percentage of entries not selected by the indirect measurement relative to the selection based on grain yield (specificity), respectively. Results are displayed as sensitivity graphed against specificity-1 in ROC curves. A global measurement for the quality of the method is the area under the curve (AUC) where 1 and 0.5 indicate a perfect and random selection, respectively. The ROC curves were calculated using PROC LOGISTIC statement of SAS. Tests comparing different indices and comparing each index with a random selection of genotypes were performed.

RESULTS

Effects of High Temperatures in Well Watered and Drought Conditions

We grew 71 subtropical maize hybrids in an environment with high temperatures under well-watered (heat stress only) and reduced water availability during flowering to induce combined heat and drought stress. Grain yield

TABLE 1 Average, minimum, maximum, as well as first and third quartile for grain yield (GY), canopy temperature (CT), normalized differential vegetation index (NDVI), silking date (SD), and the anthesis silking interval (ASI) measured under heat (HS) and combined heatand drought stress (HS + DS)

		GY	CT	NDVI	SD	ASI
HS	Average	2.35	42.15	0.54	71.1	3.0
	Mín.	0.33	40.61	0.49	64.0	0.0
	Máx.	4.19	45.29	0.59	76.0	9.0
	Q1	1.69	41.56	0.52	69.0	2.0
	Q3	3.05	42.48	0.55	73.0	4.0
HS+DS	Average	0.32	49.47	0.48	74.2	6.6
	Min.	0	46.04	0.45	62.0	−1.0
	Max.	1.37	53.76	0.52	88.0	19.0
	Q1	0.07	48.37	0.48	69.0	2.0
	Q3	0.49	50.68	0.48	81.0	12.0

ranged from 0.33 to 4.19 Mg ha^{-1} under heat stress and 0 to 1.37 Mg ha^{-1} under combined heat and drought stress (Table 1). The 71 hybrids on average yielded >10 Mg ha^{-1} under optimal conditions in their adapted environments (data not shown). We did not observe any major disease incidence in Obregon during the growing season, suggesting that high temperatures resulted in decreases in grain yield compared to the same genotypes planted at other locations. Averaged across all genotypes, combined heat and drought stress reduced grain yield by 83.4% relative to the heat stress treatment. Reductions in grain yield under combined heat and drought stress went along with increases in canopy temperature from 42.5°C under heat stress to 49.5°C under combined heat and drought stress, reductions in NDVI (−0.06) and a later silking (+3 d). A longer anthesis silking interval (+4 d) caused by reduced water availability may have contributed to reductions in grain yield under combined heat and drought stress relative to the heat stress treatment. The genotypes showed wide variation for number of days to silking, with differences of 12 and 26 d between first and last female flowering under heat stress and combined heat and drought stress, respectively.

Negative correlations were found between canopy temperature and grain yield (Figure 3a) for both experiments (r = −0.56 for heat stress and r = −0.75 for combined heat and drought stress, respectively). Genotypic variance for canopy temperature was only significant under combined heat and drought stress, whereas genotypic variance for NDVI was only significant under heat stress (Table 2). A positive correlation (r = 0.45) between NDVI and grain yield was also detected under heat stress (Figure 3).

Heritability, Genetic Correlations, and Genetic Gains

Trait heritability was generally higher under heat stress than under combined heat and drought stress. The strongest reduction in heritability was observed for
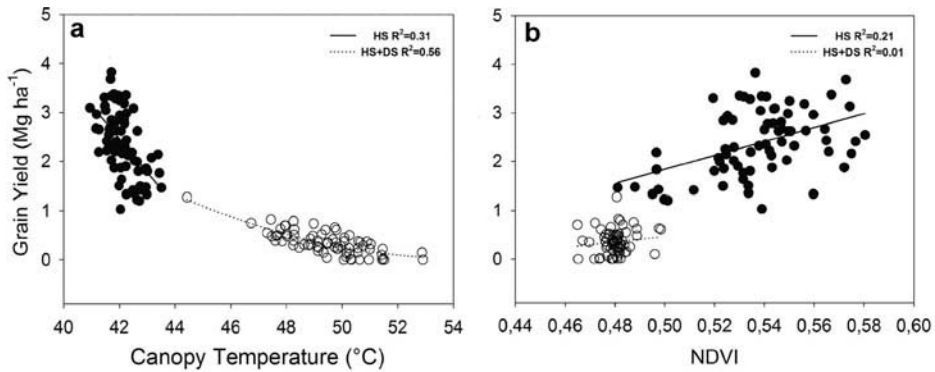
FIGURE 3 Grain yield as affected by canopy temperature (a) and the normalized differential vegetation index (b; NDVI) under heat (HS; full symbols) and combined heat and drought stress (HS+DS; open symbols).

TABLE 2 Variance components and heritability measured for grain yield (GY), canopy temperature (CT), normalized differential vegetation index (NDVI), silking date (SD) and the anthesis silking interval (ASI) measured under heat (upper half of the table) and combined heat and drought stress (lower half of the table)

	GY		CT		NDVI		SD		ASI	
	Est.	p-value	Est.	p-value	Est.	p-value	Est.	p-value	Est.	p-value
Block	0.003	0.134	0.31	0.003	0	0.015	1.1	0.037	0.162	0.429
Entry	0.024	<0.001	0.04	0.187	0	<0.001	3.62	<0.001	0.727	0.064
Residual	0.013		0.17		0		2.13		2.332	
H	79		32		78.5		77.3		38.4	
Block	0.005	0.037	1.18	0.002	0	0.025	10.1	0.019	2.823	0.204
Entry	0.019	<0.001	0.21	0.015	0	0.101	11.9	0.004	4.451	0.194
Residual	0.01		0.39		0					
H	79.8		52.2		36		58.3		29.2	

NDVI, which was $h^2 = 0.79$ under heat stress and $h^2 = 0.36$ under combined heat and drought stress, whereas differences in heritability between treatments were generally smaller for flowering traits (Table 2). Canopy temperature was the only trait that had higher heritability values under combined heat and drought stress relative to heat stress ($h^2 = 0.32$ and HS $h^2 = 0.52$).

The PCA allowed us to differentiate between genotypes based on canopy temperature and NDVI. PC1 and PC2 explained 77% and 82% of the total genetic variation under heat and combined heat and drought stress, respectively. PC1 allowed differentiating between genotypes with a good vigor measured as NDVI and high grain yield from genotypes with high canopy temperature, late flowering, and long anthesis silking interval in both experiments (Figure 4). Canopy temperature was negatively associated with grain yield and NDVI under both heat and combined heat and drought stress.
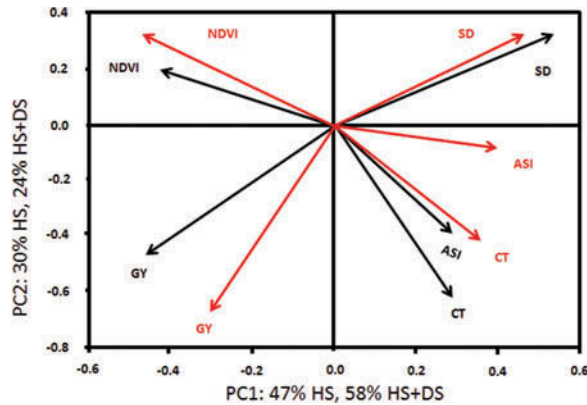
FIGURE 4 Column metric preserving bi-plot based on standardized variables showing the principal components 1 and 2. Variables used are grain yield (GY), canopy temperature (CT), normalized differential vegetation index (NDVI), silking date (SD), and the anthesis silking interval (ASI) measured for 71 sub-tropical hybrids under heat (black arrows) and combined heat and drought stress (red arrows). Length of arrows indicates loadings for different variables.

The genetic correlations varied with treatment and the traits measured (Table 3). Under heat stress, the genetic correlation between canopy temperature and grain yield was non-existent, since the genetic variance was too low as a result of ample water availability. A low and negative genetic correlation (r = −0.14) was observed between canopy temperature and grain yield under combined heat and drought stress. We speculate that this could be attributable to higher absolute temperatures relative to the heat stress treatment, resulting in greater variation among genotypes and a resulting broader canopy temperature range under combined heat and drought stress. A weak positive genetic correlation was detected between NDVI and grain yield in both treatments (r = 0.37 for heat stress and r = 0.16 for combined heat and drought stress). Number of days to female flowering had a strong negative genetic correlation with grain yield under both treatments (r = −0.90 for heat stress and r = −0.84 for combined heat and drought stress), indicating the advantage of an earlier flowering genotype under heat and under combined heat and drought stress. This comes as a surprise, as grain yield was corrected for time needed till male flowering. Genetic variance for

TABLE 3 Genetic correlations between canopy temperature (CT), normalized differential vegetation index (NDVI), silking date (SD), the anthesis silking interval (ASI), and grain yield measured under heat (HS) and combined heat and drought stress (HS + DS)

Trait	HS	HS+DS
CT	0.023	−0.142
NDVI	0.365	0.156
SD	−0.899	−0.835
ASI	0.041	−0.225

TABLE 4 Expectedgenetic gains between traits measured underheat (HS; upper half of the table) and combined heat and drought stress (HS+DS; lower half of the table). Traits evaluated were: grain yield (GY), canopy temperature (CT), normalized differential vegetation index (NDVI), silking date (SD), and the anthesis silking interval (ASI). Columns represent the selected trait; row represent the genetic gain obtained in the referenced trait

		GY	CT	NDVI	SD	ASI
HS	GY		0.015	0.364	−0.889	0.029
	CT	0.036		−0.930	0.033	0.492
	NDVI	0.366	−0.379		−0.427	−0.124
	SD	−0.909	0.014	−0.434		0.286
	ASI	0.059	0.409	−0.253	0.576	
HS+DS	GY		−0.115	0.105	−0.714	0.136
	CT	−0.175		−0.724	0.450	0.235
	NDVI	0.232	−0.707			−0.651
	SD	−0.977	0.403	−0.573	−0.927	0.431
	ASI	−0.372	0.419	−0.802	−0.860	

TABLE 5 Expected genetic gains for indices S1 (grain yield, canopy temperature, NDVI, anthesis silking interval), S2 (anthesis silking interval, canopy temperature, and NDVI) and S3 (canopy temperature and NDVI) on grain yield (GY) under heat (HS) and combined heat and drought stress (HS + DS)

Index	GY
HS	
S1	0.486
S2	0.322
S3	0.237
HS+DS	
S1	0.015
S2	0.002
S3	−0.027

the anthesis silking interval was neither significant under heat nor under combined heat and drought stress.

A pattern similar to genetic correlations was observed for genetic gains, as genetic gain is a function of heritability and genetic correlation (Table 4). While NDVI showed expected genetic gains for grain yield of 0.364 Mg ha^{-1}cycle^{-1} of selection under heat stress and 0.11 Mg ha^{-1}cycle^{-1}under combined heat and drought stress, canopy temperature showed genetic gains for grain yield of −0.115 Mg ha^{-1} cycle^{-1} under combined heat and drought stress.

Selection Indices

The AUC for all selection indices was higher under heat stress than under combined heat and drought stress (Figure 5; Table 5). Indices with more input variables (e.g., S1) generally had better predictions (AUC of 0.64 under heat
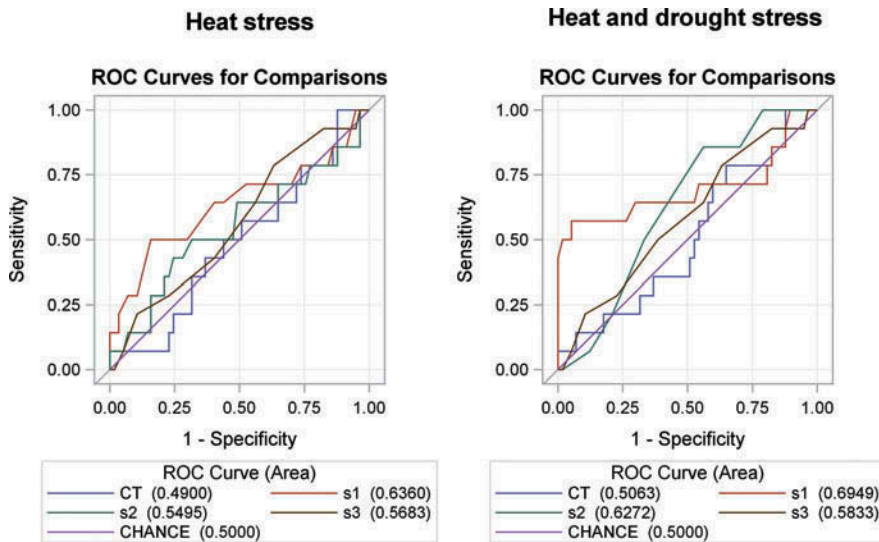
FIGURE 5 Receiver operating characteristics (ROC) curves obtained for three selection indices and canopy temperature (CT). Selection index 1 (S1) included grain yield, canopy temperature, NDVI, and the anthesis silking interval; S2 included the anthesis silking interval, canopy temperature, and NDVI; S3 included canopy temperature and NDVI. The purple line (CHANCE) shows an area under the curve equal to 0.5 which characterizes a non-discriminating indicator, random selection.

stress and 0.69 under combined heat and drought stress) than those with fewer variables, i.e., selections based only on canopy temperature had AUC values (0.49 and 0.50 for heat stress and combined heat and drought, respectively) similar to random selection (0.5 AUC).

Highest selection gains for grain yield were measured for S1 under both heat stress (+0.486 Mg ha^{-1}) and combined heat and drought stress (+0.015 Mg ha^{-1}). While both S2 and S3 equally showed improved genetic gains under heat stress (S2: +0.322 Mg ha^{-1}; S3: +0.237 Mg ha^{-1}), gains under combined heat and drought stress were negligible (S2: 0.002 Mg ha^{-1}) or even negative (S3: −0.027 Mg ha^{-1}).

Irrespective of the selection index used, genotypes that performed better under heat stress did not always coincide with those with better performance under heat and drought stress. Out of 14 genotypes with higher yield under heat stress, only three hybrids ((CML321/CML384//CL106595; CML384/CML444//CL106583 and CML384/CML444//CL106655)) coincided with those genotypes that achieved higher yields under heat and drought stress.

DISCUSSION

We present a novel method to evaluate maize germplasm under high temperatures and combined heat and drought stress using an airborne platform. This

high-throughput technique allows the evaluation of large populations in a short time period. The use of an airborne platform reduces the time needed for the measurement of NDVI using a Greenseeker (Cairns et al. 2012) or visual scores (White et al. 2012) from 30 seconds per plot to the fraction of a second per plot. For canopy temperature measurements, the high throughput is important as the airborne platform allows the measurement of all plots contained in large experiments within a few minutes. By this means environmental variation that would otherwise influence the outcome of a study can be excluded.

Effects of Heat under Well-Watered Conditions vs. Restricted Water Access during Flowering on Grain Yield

Relative to multi-location trials (data not shown) carried out under 'normal' ambient temperatures, grain yield was reduced by 76.5% under high temperatures and by 96.8% under combined heat and drought stress. These results are in agreement with results obtained by Lobell et al. (2011), showing that each degree-day accumulated above 30ºC reduced the final yield by 1% under optimal rain-fed conditions, and by 40% under drought-stress conditions. Optimal temperature for tropical maize is around 35**°**C (Cichino, Rattalino Edreira, and Otegui 2010), whereas average temperatures measured in our study reached up to 45ºC under heat stress and 54°C under combined heat and drought stress. Under well-watered conditions, increases in temperature above 45°C have been shown to reduce photosynthesis by 50% (Crafts-Brandner and Salvucci 2002), potentially explaining the strong reductions in grain yield under high temperatures observed here.

NDVI and Canopy Temperature Allow Identification of Tolerant Hybrids

In addition to well-known traits used in breeding (e.g., days to silking, anthesis silking interval), NDVI explained differences between and within treatments, whereas canopy temperature explained differences in grain yield among treatments and genotypes within the combined heat and drought stress treatment, as indicated by genetic correlations with grain yield. Phenotypic and genetic correlations for most of the traits with grain yield generally showed the same trend. However, in some cases, the low genetic variance led to non-significant genetic correlations, e.g., under heat stress, all genotypes had similar leaf temperatures as a result of ample water availability, allowing for leaf cooling by transpiration.

Under combined heat and drought stress, our results showed negative genetic correlations between canopy temperature and grain yield, indicating that genotypes that maintained a cool canopy under combined high temperatures and drought stress yielded higher. Transpiration is responsible for

keeping the temperature of fully sunlit leaves within physiological limits (Gates 1968). Canopy temperature has been used to identify germplasm with increased tolerance to drought in maize (Romano et al. 2011; Zia et al. 2013) and tolerance to high temperatures in wheat (Cossani and Reynolds 2012). The genetic correlation measured in our study between canopy temperature and grain yield under combined heat and drought stress is of special interest, since the majority of research on abiotic stress tolerance has focused on temperature and drought stress separately, while stresses usually occur conjointly in farmers' fields (Tester and Bacic 2005). The method presented here would therefore allow the identification of germplasm tolerant to conditions encountered in the farmers' fields.

The correlation measured between NDVI (indicative of source capacity) and grain yield is reflective of the importance of sufficient assimilate availability for yield formation under heat stress ($r = 0.365$). Lower genetic (nonsignificant) correlation measured under combined heat and drought stress, relative to heat stress, is indicative of the tradeoff between a large biomass providing assimilates while at the same time losing water through transpiration under water-limited conditions. In our study, beneficial effects of a high NDVI on grain yield may have been offset under combined heat and drought stress by excessive water losses, resulting in the lower genetic correlation measured relative to heat stress. Positive correlations measured between NDVI and grain yield here are in agreement with a study carried out by Cairns et al. (2012) using tropical maize hybrids.

Surprisingly, the correlation between the anthesis silking interval and grain yield was not significant (under heat stress) or was only weak (under combined heat and drought stress). Since the anthesis silking interval has traditionally been used at CIMMYT to identify germplasm tolerant to drought (Edmeades et al. 2000), it is conceivable that the genetic variation for this trait has been reduced in elite germplasm as postulated by Monneveux, Sanchez, and Tiessen (2008). This finding further emphasizes the need to use additional or new secondary traits in selection to identify germplasm tolerant to high temperatures and drought.

Canopy Temperature and NDVI Can Be Used in Breeding Programs

A principal component analysis (PCA) was used to identify combinations of physiological characteristics associated with genotypic variation in grain yield under heat and combined heat and drought stress as function of the genetic correlations. A PCA carried out as function of the genetic correlation allowed the distinction between genotypes with high grain yield and strong vigor (as indicated by NDVI) and genotypes with a late flowering, longer anthesis silking interval, and high canopy temperature under both heat stress and combined heat and drought stress. The correlation between NDVI and canopy

temperature with grain yield and high heritability for both traits resulted in high expected genetic gains. Therefore NDVI and canopy temperature can be used to reliably identify tolerant germplasm under heat stress. Using canopy temperature or NDVI under combined heat and drought stress produces a relative genetic gain in yield lower than 11%. In order to evaluate the utility of different trait combinations for breeding, several indices were formed: S1 included grain yield, the anthesis silking interval, NDVI and canopy temperature; S2 included NDVI, the anthesis silking interval, and canopy temperature, while S3 only included NDVI and canopy temperature. The receiver operating characteristics (ROC) methodology was used to compare the different indices. Both NDVI and canopy temperature had AUC values above 0.5 (as to be expected by pure chance) indicative of the utility of both traits for selection. The AUC values generally increased with increasing number of traits included in an index, reaching a maximum of 0.636 under heat stress and 0.695 under combined heat and drought stress for S1. Selection could therefore reliably be carried out under both heat stress and combined heat and drought stress using S1. As a result, genetic gains for grain yield could be increased by using selection indices relative to the use of grain yield as stand-alone trait under both heat stress and combined heat and drought stress: Highest genetic gains were achieved by S1 under heat stress, increasing grain yield by 0.486 Mg ha^{-1}. Under combined heat and drought stress highest absolute genetic gains can be obtained by negative selection for canopy temperature, reaching 0.115 Mg ha^{-1}, while genetic gains of 0.015 Mg ha^{-1} could be achieved using S1. While selection gains would be higher using canopy temperature as the only trait in addition to grain yield, selection using S1 would allow moderate desirable selection gains, while at the same time maintaining greater genetic variability in our germplasm.

Selection using S1 would allow for higher selection intensity across environmental conditions. A higher selection intensity at an early stage based on data acquired at one to two phenotyping hubs, would allow discarding of non-desired germplasm and reduce the number of entries to be evaluated at later stages in multilocation trials. Since the evaluation of a plot costs ~$25 USD, reducing the number of entries to be evaluated will significantly reduce rising cost for germplasm evaluation.

It is noteworthy that out of the 14 best hybrids selected under heat stress or combined heat and drought stress, only three hybrids (CML321/CML384//CL106595, CML384/CML444//CL106583, and CML384/CML444//CL106655) of potential interest to breeders were picked up under both environmental conditions. This pattern has been observed previously and has been explained by differences in the genetic basis of mechanisms conveying tolerance to heat stress or combined heat and drought stress (Rizhsky, Liang, and Mittler 2002, 2004; Barnabas, Jaeger, and Feher 2008; Cairns et al. 2013). Nevertheless, these three hybrids represent ideal genotypes for release and production in agro-ecologies prone to both heat and drought stress.

CONCLUSION

We present a novel high-throughput method to evaluate maize germplasm under heat and combined heat and drought stress using an airborne platform measuring canopy temperature and NDVI. The NDVI explained differences between and within treatments while canopy temperature explained differences in grain yield among treatments and among genotypes within the treatment combining heat and drought stress as indicated by genetic correlations with grain yield. Canopy temperature and NDVI showed potential but limited value as an indirect measurement of selection in these two analyzed trials. However, the use of both traits in selection index S1 (including grain yield, anthesis silking interval, canopy temperature, and NDVI) can improve selection, achieving additional genetic gains of 0.486 Mg ha^{-1} under heat stress and 0.015 Mg ha^{-1} under combined heat and drought stress. We postulate that selection using index S1 will allow higher selection gains and higher selection efficiency, ultimately increasing cost efficiency of individual breeding programs as undesired germplasm can be eliminated at a very early stage.

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