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Article

# **Considerations When Deploying Canopy Temperature to Select High Yielding Wheat Breeding Lines under Drought and Heat Stress**

R. Esten Mason<sup>1,\*</sup> and Ravi P. Singh<sup>2</sup>

<sup>1</sup> Department of Crop, Soil, and Environmental Sciences, University of Arkansas, 115 Plant Sciences Building, Fayetteville, AR 72701, USA

- <sup>2</sup> International Maize and Wheat Improvement Center (CIMMYT), Apdo Postal 6-641, C.P. 06600, D.F. Mexico; E-Mail: r.singh@cgiar.org
- \* Author to whom correspondence should be addressed; E-Mail: esten@uark.edu; Tel.: +1-479-575-5725; Fax: +1-479-575-7465.

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Abstract: Developing cultivars with improved adaptation to drought and heat stressed environments is a priority for plant breeders. Canopy temperature (CT) is a useful tool for phenotypic selection of tolerant genotypes, as it integrates many physiological responses into a single low-cost measurement. The objective of this study was to determine the ability of CT to predict grain yield within the flow of a wheat breeding program and assess its utility as a tool for indirect selection. CT was measured in both heat and drought stressed field experiments in northwest Mexico on 18 breeding trials totaling 504 spring wheat lines from the International Maize and Wheat Improvement Center (CIMMYT) Irrigated Bread Wheat program. In the heat treatment, CT was significantly correlated with yield (r = -0.26) across all trials, with a maximum coefficient of determination within the individual trials of  $R^2 = 0.36$ . In the drought treatment, a significant correlation across all trials was only observed when days to heading or plant height was used as a covariate. However, the coefficient of determination within individual trials had a maximum of  $R^2 = 0.54$ , indicating that genetic background may impact the ability of CT to predict yield. Overall a negative slope in the heat treatment indicated that a cooler canopy provided a yield benefit under stress, and implementing selection strategies for CT may have potential for breeding tolerant genotypes.

stress; plant breeding

### 1. Introduction

Canopy temperature (CT) is a useful indicator of crop water status [1] and has potential as a tool for indirect selection of genotypes tolerant to drought and heat stressed environments [2]. For field experiments in wheat, CT data is most commonly measured on a whole plot basis using a handheld infrared thermometer [3], although more rapid assessment using thermal imaging [4] is growing in popularity. CT is influenced by a number of environmental factors including the amount of solar radiation hitting the canopy, soil moisture, wind speed, temperature and relative humidity [5]. Genetic differences in CT result from variation in the plant's ability to move water through the vascular system, differences in stomata aperture driving transpiration, root biomass and depth, metabolism and source sink balance [5]. As such, CT has been shown to correlate with these physiological traits under field conditions [6–9] and integrates them into a single low-cost diagnostic measurement that has potential for selection of tolerant parental genotypes or early generation breeding lines [2,8].

CT has moderate heritability across environments in both diverse sets of germplasm [7,10] and in related material such as recombinant inbred populations [11–13]. Lopes and Reynolds [13] found similar broad sense heritability for a diverse set of 294 spring wheat lines ( $H^2 = 0.38$ ) and a set of 169 sister-lines ( $H^2 = 0.34$ ) across well-watered, drought stressed and heat stressed environments in northwest Mexico. Genetically, CT is a quantitative trait. Saint Pierre, *et al.* [14] determined the gene action for CT to be mainly additive × additive in five wheat populations with some dominant effects. Genetic mapping shows CT to be controlled mostly by small effect loci that are pleiotropic with variation in other traits, such as days to heading and plant height [6,11,12,15].

The correlation between CT and yield is consistently negative in the literature in both drought and heat environments such that a cooler canopy provides a yield benefit under stress [11,16,17]. Exceptions have been shown in both bread wheat [18]—where CT measurements taken in Mexico were positively correlated with yield at international sites-and in durum wheat [19]-where CT was found to increase with date of cultivar release and increasing yield. Experiments investigating CT are often conducted with sets of lines pre-selected for variation in canopy temperature or other tolerance traits [7,8], international trials of elite drought and heat tolerant lines [10,16,18,20], or using historical germplasm [9,19,21] and may not be representative of variation present in the early stages of yield testing in a breeding program. Reynolds et al. [2] did show advanced lines derived from "physiological crosses" targeted at one or more adaptive traits had a yield benefit over "conventional crosses" where physiology was not necessarily a consideration in parent selection. However, there exists a need to investigate the ability of CT to select high yielding lines within the germplasm flow of a breeding program where very little pre-selection for stress tolerance per se has occurred. Therefore the objective of this study was to determine the ability of CT to predict yield under both drought and heat stress conditions and determine the ability of CT to select high yielding breeding lines entering the first year of multi-environment testing.

## 2. Results and Discussion

## 2.1. Analysis of Check Cultivars across Trials

Comparison of the two check cultivars across all trials found RoelfsF2007 to outperform Waxwing, yielding 6.0 tons ha<sup>-1</sup> and 5.4 tons ha<sup>-1</sup> in the drought and heat stress treatments, respectively, compared to 5.2 tons ha<sup>-1</sup> and 5.0 tons ha<sup>-1</sup> for Waxwing (Table 1). Canopy temperature for RoelfsF2007 was 0.9 °C lower than Waxwing under drought but only 0.1 °C cooler under heat. The coefficient of variation (CV) for all traits was low in both treatments, ranging from 1.5% to 4.8%, indicating that experimental error was low. Significant trial variation was detected for all traits in both treatments with the exception of days to heading (Table 2). However, entry by trial interaction was not significant indicating that the check cultivars performed consistently across trials.

**Table 1.** Performance of the check cultivars RoelfsF2007 and Waxwing across 18 breeding yield trials in two treatments, Ciudad Obregon, Mexico.

	Drought Stress				Heat Stress			
Entry	Yield (tons/ha)	Canopy Temp (°C)	Days to Heading (Julian)	Plant Height (cm)	Yield (tons/ha)	Canopy Temp(°C)	Days to Heading (Julian)	Plant Height(cm)
RoelfsF2007	6.0	26.7	85.2	102.4	5.4	26.2	67.9	93.8
Waxwing	5.2	27.6	89.4	88.4	5.0	26.3	67.8	82.2
Mean	5.6	27.1	87.3	95.4	5.2	26.2	67.9	88.0
Error	0.1	0.2	2.1	3.3	0.1	0.2	1.1	3.3
LSD (5%)	0.8	1.3	4.4	5.6	0.7	1.1	3.3	5.6
CV (%)	4.8	1.7	1.7	1.9	4.7	1.5	1.6	2.1

**Table 2.** Analysis of variance *p*-values for check cultivars Roelfs and Waxwing across

 18 breeding yield trials in Ciudad Obregon, Mexico.

Effect	Entry	Rep <sup>a</sup>	Trial	<b>Incomplete Block</b>	Entry by Trial		
Heat Stress							
Yield	< 0.0001	0.1367	< 0.0001	0.0024	0.0922		
СТ	0.1419	< 0.0001	< 0.0001	0.0201	0.3668		
Days to Heading	0.8811		0.3797				
Plant Height	< 0.0001		0.0036				
Drought Stress							
Yield	< 0.0001	0.1956	< 0.0001	0.7963	0.6638		
СТ	< 0.0001	< 0.0001	< 0.0001	0.1145	0.1147		
Days to Heading	< 0.0001		0.6665				
Plant Height	< 0.0001		0.0034				

<sup>a</sup> Data for days to heading and plant height taken on a single replication per trial so all variance components could not be estimated.

#### 2.2. Phenotypic Correlations

Lack of entry by trial interaction (Table 2) allowed for trait means to be adjusted relative to the performance of the check cultivar RoelfsF2007 within each trial, similar to methods used by Graybosch and Peterson [22]. RoelfsF2007 was chosen based on its yield performance compared to Waxwing and its stability across trials. Phenotypic correlations between traits are presented in Table 3. In the heat treatment, yield was significantly correlated with CT (r = -0.26), plant height (r = 0.26) and days to heading (r = -0.18). Canopy temperature was also significantly correlated with days to heading (r = -0.34) but not with plant height. In the drought treatment, yield was significantly correlated with plant height (r = 0.24) and days to heading (r = -0.50) but not with canopy temperature. Canopy temperature was significantly correlated with both plant height (r = -0.35) and days to heading (r = -0.09). The negative correlation between days to heading and yield is in agreement with previous studies showing escape as an adaptive response to drought [17,23]. In addition, Lopes *et al.* [21] showed a strong negative correlation (r = -0.64) between plant height and CT under drought, as did Olivares-Villegas, *et al.* [24], again in agreement with the results presented here.

Relative Trait	Canopy Temperature	Plant Height	Days to Heading				
Heat Stress							
Yield	-0.26 ***	0.26 ***	-0.18 ***				
Canopy Temperature		-0.04	-0.34 ***				
Plant Height			0.07				
Drought Stress							
Yield	-0.01	0.24 ***	-0.50 ***				
Canopy Temperature		-0.35 ***	-0.09 *				
Plant Height			-0.028				

**Table 3.** Relative mean trait correlations for 504 breeding lines in two treatments, Ciudad Obregon, Mexico.

\*\*\* significant at p = 0.001; \* significant at p = 0.05.

## 2.3. Regression Analysis

Regression was used to further investigate the relationship between CT and yield. In the heat treatment, this relationship (slope) was negative and significant ( $R^2 = 0.07$ , p = 0.0001) (Table 4 and Figure 1a) indicating that a cooler canopy provided a yield benefit. The variation in yield explained by CT increased when plant height ( $R^2 = 0.13$ , p = 0.0001), days to heading ( $R^2 = 0.14$ , p = 0.0001) or both plant height and days to heading ( $R^2 = 0.21$ , p = 0.0001) were fit as covariates in the regression (Table 4). When trials were analyzed individually, the relationship between CT and yield was negative in 17 of 18 trials (data not shown), indicating a cooler canopy was beneficial for yield production, with a maximum coefficient of determination observed in EYT8 ( $R^2 = 0.36$ , p = 0.001) (Figure 1b).

In the drought stress treatment, the relationship between CT and yield was not significant ( $R^2 = 0.00$ , p = 0.8952) (Table 4 and Figure 2a). When plant height was fit as a covariate, a significant negative relationship was detected ( $R^2 = 0.07$ , p = 0.0001). When days to heading ( $R^2 = 0.26$ , p = 0.0001) or both

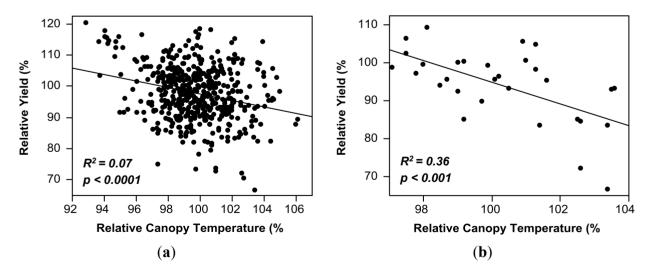
plant height and days to heading ( $R^2 = 0.31$ , p = 0.0001) were fit as covariates, a positive slope was observed, indicating that warmer canopies were associated with higher yield under drought (Table 4). When trials were analyzed individually, the slope was negative in 14 of the 18 trials (data not shown), with a maximum coefficient of determination observed in EYT16 ( $R^2 = 0.54$ , p = 0.001) (Figure 2b). While this indicates an overall benefit associated with a cooler CT, variability in the slope may make selection using CT under drought difficult as some lines which have higher CT also perform very well in terms of yield.

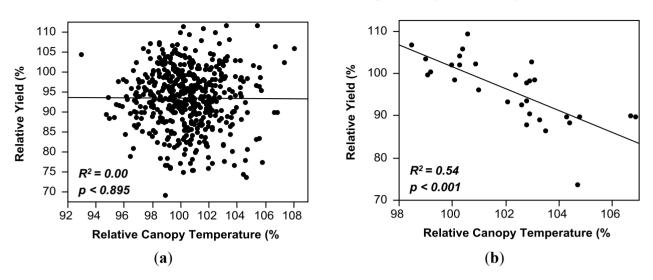
**Table 4.** Regression equations describing the relationships between relative yield and canopy temperature with and without covariates for 504 breeding lines in two treatments, Ciudad Obregon, Mexico.

Variable and Covariate(s)	Variable and Covariate(s) Regression Equation						
Heat Stress							
Canopy temperature (CT)	y = 201.21 - 1.04(CT)	0.07 ***	0.00-0.36				
$CT + CT \times Height$	$y = 197.05 - 1.30(CT) + 0.003(CT \times HGT)$	0.13 ***	0.05-0.56				
$CT + CT \times Heading$	$y = 241.47 - 0.98(CT) - 0.005(CT \times HD)$	0.14 ***	0.02-0.52				
$CT + CT \times Height + CT \times Heading y = 23$	0.21 ***	0.08-0.57					
Drought Stress							
Canopy temperature (CT)	y = 95.47 - 0.02(CT)	0.00	0.00-0.54				
$CT + CT \times Height$	$y = 61.46 - 0.06(CT) + 0.00394(CT \times HGT)$	0.07 ***	0.06-0.60				
$CT + CT \times Heading$	$y = 111.36 + 0.46(CT) - 0.0063(CT \times HD)$	0.26 ***	0.12-0.66				
$CT + CT \times Height + CT \times Heading y = 80$	$84 + 0.42(CT) + 0.0035(CT \times HGT) - 0.0061(CT \times HD)$	0.31 ***	0.31-0.69				

Y, Yield; CT, Canopy temperature; HGT, Plant Height; HD, days to heading; \*\*\* Significant at p = 0.0001; <sup>a</sup> Range of  $R^2$  for individual yield trials.

**Figure 1.** Relationship between relative canopy temperature and yield in the heat treatment for all 504 entries (**a**) and 30 entries from EYT8, the most significantly correlated yield trial (**b**).





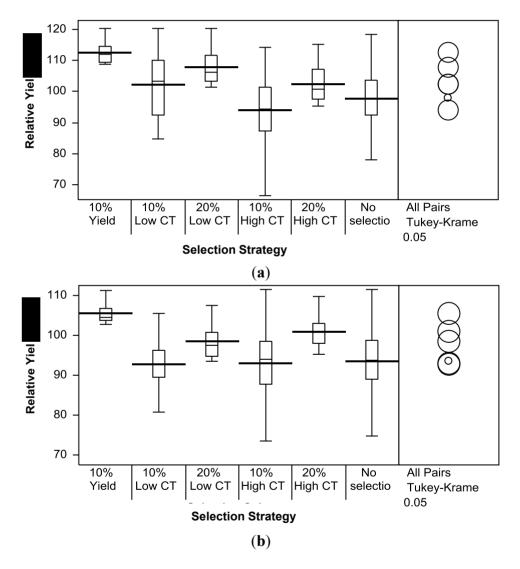
**Figure 2.** Relationship between relative canopy temperature and yield under drought stress for all 504 entries (**a**) and 30 entries from the most significantly correlated yield trial (**b**).

2.4. Comparison of Theoretical Selection Strategies

Six theoretical selection strategies were used to determine the practical application of CT as a tool to select high yielding lines (Figure 3). In the heat treatment, direct selection of the top 10% yielding entries (10% Yield) had a relative mean of 113% compared to a mean of 98% for all entries (No Selection). The most efficient indirect selection strategy using CT was to select 20% of the entries based on coolest canopy temperature and then keep the top 50 entries based on yield. This strategy resulted in a mean relative yield of 108% which was statistically equal to the 10% Yield strategy and significantly higher than the No Selection scheme at 98%. From a practical standpoint, this strategy would require a larger number of entries to be kept for yield testing the year following selection with CT (20% *versus* 10%) but could result in significant allele enrichment if implemented in the early stages of selection or yield testing. It should also be noted that no significant difference was observed between the 20% Low CT and 10% Yield schemes for either days to heading or plant height (data not shown) so this selection could be done without modification of phenology. Using high CT to discard low performing entries would also be beneficial as selection of the 10% highest CT lines (10% High CT) had a relative mean of 94%, significantly lower than all other strategies (Figure 3).

Under drought stress, the results were much less clear. Direct selection of the top 10% yielding lines gave a relative mean of 106%, significantly higher than all other strategies. The 20% High CT and 20% Low CT strategies gave statistically equal means of 101% and 98%, respectively, indicating both opening and closing of stomata as adaptive mechanisms to drought stress. It is likely that some entries with high relative CT also escaped stress through early heading and maturity, in agreement with the negative correlation between yield and days to heading and the positive relationship observed between CT and yield in the regression analysis when days to heading was fit as a covariate (Table 4). Both the 20% High CT and 20% Low CT strategies resulted in means that were significantly higher than the No Selection strategy (93%), so again some benefit could be seen from CT selection. Selection for high CT also resulted in significantly shorter plant height compared to direct selection of yield (data not shown) whereas selection for low CT did not significantly change plant height or days to heading.

**Figure 3.** Box-plots representing the yield performance of 10% of the 504 entries based on direct selection of yield or indirect selection of canopy temperature (CT) under heat stress (a) or drought stress (b). The selection schemes included: selecting 10% of the entries (50 entries) based on yield (10% Yield); selecting 10% of the entries based on lowest CT (10% Low CT); selecting 20% of the entries based on lowest CT and keeping the 50 highest yielding entries (20% Low CT); selecting 10% of the entries based on highest CT (10% High CT); selecting 20% of the entries based on highest CT and keeping the 50 highest yielding entries (20% High CT), and; the mean of all entries (No selection). The means of each selection scheme are represented by bold lines through the box-plot. Significant differences between means were tested using the Tukey-Kramer test at p = 0.05 (right panel).



### 3. Experimental Section

#### 3.1. Wheat Germplasm and Experimental Design

The germplasm for this study consisted of 504 advanced spring wheat breeding lines derived from 245 unique crosses from the Irrigated Bread Wheat breeding program at the International Maize and Wheat Improvement Center (CIMMYT). The number of sister-lines tested per cross varied from one

to twelve. The entries were divided into 18 Elite Yield Trials (EYT1-EYT18) with two checks ("RoelfsF2007" and "Waxwing") included in each of the 18 trials for a total of 30 entries per trial. Experiments were conducted at the Norman E. Borlaug Experimental Station (CENEB) in Ciudad Obregon, Sonora, in northwest Mexico (27°20' N, 109°54' W, 38 meters above sea level) in the 2009–2010 growing season on a Aridosol soil (USDA classification) with a pH = 7.7. Trials were sown in an alpha lattice design with three replications. Plots were 3 m long and 1.6 m wide that consisted of two raised beds with three rows per bed at a seeding rate of 300 seeds  $m^{-2}$ . For the reduced irrigation (moderate drought) treatment, trials were sown in the last week of November and received two applications of irrigation to field capacity (~300 mm total). In addition to irrigation, the field site received an additional 16 mm of precipitation over the growing season [21]. The late-sown (heat) treatment trials were planted at a delayed sowing date in the last week of February and received five applications of irrigation to field capacity (~500 mm total). No additional precipitation was recorded in the heat environment. Weather data associated with this location and year has previously been reported [21]. The average maximum temperature of the heat stress treatment was 30.7 °C compared to 27.4 °C for the drought stress, both above the optimal growing temperature for wheat ( $\sim$ 15–20 °C), but not uncommon for most wheat growing regions. Standard trial management practices were followed for nitrogen and also included fungicide and herbicide applications for pest control.

## 3.2. Trait Measurement

Grain yield (tons/hectare) was determined on whole plots by combine harvesting when grains were dry at about 4%–5% moisture, and weighing the grain. Days to heading was recorded at 50% emergence of heads from the leaf sheath on a single replication of each entry (Zadok growth stage 59) *et al.* Plant height was measured from the ground to the top of the average plant type, excluding awns, on a single replication of each entry. Canopy temperature (CT) was taken with an infrared thermometer on all replications (Mikron M90, Mikron Infrared Instrument Company Inc., Oakland, NJ, USA) when all entries in a trial were fully headed. Two CT measurements were taken per plot, one on each bed, by scanning the plot from front to back to obtain an average CT reading of each bed. The two measurements were then averaged to obtain one final reading per plot. All CT measurements were taken at midday (12 pm–2 pm) on clear, sunny days with minimal wind.

#### 3.3. Statistical Analysis

All statistical analyses were done using procedures in SAS v9.3 (SAS Institute Inc., Cary, NC, USA) with each treatment (heat and drought) analyzed separately. Analysis of variance using PROC MIXED was carried on the two check cultivars to determine sources of variation within and across trials and determine if genotype by trial interaction was present for the traits measured. All effects in the model (rep, incomplete block, trial, entry x trial) with the exception of entries were treated as random. Data from all trials was then normalized relative to the performance of RoelfsF2007 and used for further analysis of all entries across trials, similar to methods used to analyze historical trial data over years with a recurrent check [22]. PROC CORR was used to determine the phenotypic correlation between relative means of traits. Regression was performed using PROC REG to determine the relationship between CT and yield within each trial individually and across all trials. Initially, regression was used to determine

the ability of CT to predict yield. The interactions of days to heading and plant height with CT were then fit as covariates (PROC ANCOVA) in the regression model to determine their effect on the yield predictability of CT.

### 3.4. Theoretical Selection Strategies

The ability of CT to select high yielding lines was investigated by applying theoretical selection strategies to the data, either through indirect selection using CT or direct selection based on yield performance. The selection strategies included: (1) Selecting 10% of the entries (50 entries) based on yield (10% Yield); (2) selecting 10% of the entries based on lowest CT (10% Low CT); (3) selecting 20% of the entries based on lowest CT and keeping the 50 highest yielding entries (20% Low CT); (4) selecting 10% of the entries based on highest CT (10% High CT); (5) selecting 20% of the entries based on highest CT and keeping the 50 highest yielding entries (20% High CT), and; (6) the mean of all entries (No selection). A Tukey-Kramer test was used to determine significant differences between selection schemes. Box-plot diagrams were drawn using JMP<sup>®</sup> v10 (SAS Institute Inc., Cary, NC, USA).

## 4. Conclusions

Canopy temperature is an efficient low-cost measurement of crop water status that has potential for phenotypic selection of stress tolerant lines. Overall, cooler CT was favorable for higher yield although this association was stronger and more consistent under heat stress. Variation in the coefficient of determination and the slope across individual trials indicates that genetic background plays a definite role in the ability of CT to predict yield and thus background information on the parents of a breeding line may help in making decisions on interpreting the results of CT measurements. While no indirect selection scheme will be as successful as directly selecting for yield, the major applications of CT, based on the results presented here, are as follows (1) Selection for low CT under heat stress; and (3) the strong association of days to heading (escape) and yield under drought makes selection with CT very difficult. The most useful implementation of CT for genetic improvement would be in the early generations of breeding, such as on progeny rows where yield testing is not performed, although considerations would need to be made for an experimental design that utilizes a repeated check cultivar to adjust for spatial variation. In the future, high throughput tools for measuring CT that increase speed and accuracy will also allow breeders to maximize potential genetic gain from CT.

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# **Conflicts of Interest**

The authors declare no conflict of interest.

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