Monitoring physiological responses to water stress in two maize varieties by infrared thermography

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Abstract: Water stress is one of the main causes of yield reductions in crops, especially in arid and semi-arid regions where the water supply is limited. Plant water status is frequently assessed by pre-dawn leaf water potential (Ψ_{PD}) or leaf stomata conductance (gL) measurements, in support of advanced irrigation scheduling. However, both methods are time and labour consuming. A non-invasive approach to water status detection is the use of infrared thermography (IRT). This experiment was conducted in a greenhouse on two potted maize varieties under irrigated and non-irrigated conditions, and the measurements began when the crop had reached its twelve leaf stage. In order to establish the IRT measurements for detecting the water status of maize, an IRT-based crop water stress index (CWSI) was calculated and compared with simultaneously measured Ψ_{PD} and gL data. Good correlations were found between CWSI and gL data (r^2 =0.71 & 0.81), as well between CWSI and Ψ_{PD} data (r^2 = 0.53 & 0.81). These results highlight the appropriateness of infrared thermal imagery to detect and differentiate between the crop water statuses of different genotypes.

Keywords: plant water stress, leaf temperature, stomatal conductance, leaf water potential, CWSI, thermal imaging, maize.
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1 Introduction

Maize (*Zea mays*) is one of the most widely grown crops and is predominantly cultivated in arid and semi-arid regions in the world. In these regions it is often subjected to water stress, for which even short term

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exposure might result in yield reductions[1]; for example, a short period of water stress at the silking stage may reduce yields by more than 50%, and in some cases may even cause total crop failure[2,3]. As a result, water stress needs to be quantified in order to develop effective strategies for irrigation scheduling that are accurate both, in terms of timing and volume of water application.

Irrigation scheduling based on soil water content and meteorological data, or by using more advanced measurements like leaf stomata conductance of water vapour (gL) or leaf water potential (Ψ_{PD}), is both labour intensive and time consuming. Using canopy surface temperature measured from infrared thermography (IRT) to determine the water stress status is a non-contact method, and thus fast and practical. This method is capable of monitoring large leaf populations simultaneously, plus providing information on (gL) variations and dynamics, thereby providing information on the physiological status of all plants within a field.

Under the same environmental conditions, difference in leaf temperatures is a function of transpiration and stomata opening[4]. As water becomes limited, stomatal closure occurs, resulting in reduced evaporative cooling and an increase in canopy temperature[5]. The instances of leaf temperature being affected by other physiological processes are very rare[6]; for example, an increase in the respiration rate could theoretically impact on leaf temperature, but the heat generated may be too small to have a noticeable effect[7]. Calculation of a crop water stress index (CWSI) is based on two baselines[8-10]; the lower limit (maximum leaf cooling through maximum transpiration) represents the non-stress baseline and the upper limit (maximum leaf temperature due to fully closed stomata) corresponds to the full-stress baseline.

Although the CWSI can be calculated easily, there are a number of issues that need to be considered, which are related to the environmental conditions within which the measurements are made, such as air temperature, radiation, humidity, and wind speed. Wind speed in particular is a critical factor. A higher wind speed can cause cooler canopy temperature readings when compared to conditions where wind speeds are low[11,12]. Gardner et al.[13] identified other non-environmental factors, such as the location at which the air temperature and relative humidity are measured, for even small changes in distance from the canopy can result in significant changes in readings. As a result, consistency throughout the measurement process is extremely important. In addition, the occurrence of any extraneous surfaces in the measurement aspect like soil layers due to thin canopy stands during the early growth stage should be avoided[14].

A wide range of studies have been carried out to determine the CWSI for a variety of crops in different climatic zones. The CWSI has been used to schedule irrigation for various crops, such as grapevines[15,16], cotton[17,18], potatoes[19], olive trees[20], wheat[21], and maize[22,23]. Also, water stress and CWSI have been linked to soil water availability[24], leaf water potential[25,26], stomata conductance[27], and yield[23,28].

A given crop’s response to water stress may vary under different climatic conditions. Water availability in plant tissues varies by cultivar and genotype; therefore, the critical CWSI value for each crop should be determined, as it is significant variety and location dependent. The main objective of this study was to determine the IRT-based CWSI for two maize varieties, and to evaluate the relationships between CWSI, soil water content, leaf water potential, and stomata conductance.

2 Materials and methods

2.1 Experiment design

The experiment was conducted in a greenhouse at the University of Hohenheim in Stuttgart, Germany (48°42'41.04″N, 9°12′34.2″E) between December 17th, 2009 and January 2nd, 2010 – a time period of 17 days (days of experiment, DOE 1-17). Two maize varieties, Amadeo and Sileno, were used for the experiment, and altogether 48 potted maize plants were investigated. Before the experiment, the substrate of all the pots was saturated with water, with the 24 Amadeo maize plants – 12 pots with two in each, and the 24 Sileno maize plants – also 12 pots with two plants in each, divided into four groups. Twelve of the pots (6 Amadeo and 6 Sileno) were allowed to dry out, with no irrigation, for which the soil water content (θ) in 3 Amadeo and 3 Sileno was measured with a TDR-probe (Trime-IT, Imko Germany) at two-hour intervals. The remaining twelve pots (6 Amadeo and 6 Sileno) served as a reference and were placed in a steadily irrigated catchment tray to ensure the availability of sufficient water. Among the reference pots, soil water content was measured with a TDR-probe in one Amadeo pot and one Sileno pot at two-hour intervals. The soil of all 24 pots was covered with tinfoil to prevent soil evaporation and heating.

2.2 Thermal imaging

Pictures were taken daily at 10:00 a.m., 12:00 p.m., and 3:00 p.m., and a VarioCAM (InfraTec GmbH) infrared camera was used to take the thermal and true-color images simultaneously. The IR-lens of the camera displayed the object on a micro-bolometer array, at a resolution of 384 × 288 pixels. Irbis-professional-3 software allowed corrections to take place for object emissivity, object distance, temperature, and relative humidity. The distance between the camera and the plants was set at 3.7 m and the selected emissivity value was 0.95. A leaf sprayed with water was used as the wet reference (approximating maximum adiabatic cooling of the leaves) and another leaf coated with petroleum jelly was used as the dry reference (approximating maximum heating of the leaves due to completely closed stomata).

CWSI was calculated according to Jones[29] as follows:

$$CWSI = \frac{(T_{canopy} - T_{wet})}{(T_{dry} - T_{wet})}$$

Where, T_{canopy} is the mean canopy temperature; T_{wet} and T_{dry} are the temperatures of the leaves sprayed with water and coated with petroleum jelly, respectively.

Note that T_{wet} and T_{dry} are equivalent to T_{base} and T_{max} in the original formulation of *CWSI* developed by Idso et al. in 1981[8]. The value of *CWSI* varies between 0 and 1, where 1 represents full stress when plants display no transpiration and gL is the lowest, while 0 represents absence of stress when plants transpire at a maximum rate and gL is the highest.

2.3 Ambient and plant water status monitoring

The temperature and relative humidity of the air in the greenhouse were logged at five-minute intervals (Hobo U12-011, Hobo USA). Predawn leaf water potential (Ψ_{PD}) was measured, one leaf per pot, by using a Scholander pressure chamber. Because the maize leaves were too long to fit in the chamber, only the top one-third of each leaf was used in these measurements. The laminar alongside the leaf vein was torn a little to ensure the exposed leaf vein could be inserted through the sealing ring of the chamber. Leaf stomata conductance (gL) were measured using a porometer (SC-1, Decagon devices USA), and were made simultaneously with the IRT shots for every pot on two pre-selected leaves. All the non-irrigated pots were weighed each day in the morning to determine the water loss by transpiration, while the daily pan evaporation value (E_{pan}) from an open water surface (pan diameter = 21 cm) was determined gravimetrically.

3 Results

The meteorological conditions prevailing throughout the period of the experiment are shown in Figure 1, revealing the diurnal fluctuations in weather variables. During the experiment, the daytime temperature was around 25℃ and the night-time temperature around 17℃. According to temperature and humidity trends, the average vapour pressure deficit (VPD) values were higher at the beginning of the experiment and distinctly lower during the last three days. During the days of the experiment, a reduction in soil water content resulted in a decrease in transpiration, with a resulting decrease in the ratio of daily transpiration to pan evaporation (E_{pan}), as shown in Figure 2. At the start of the experiment, the θ of the two maize genotypes - Amadeo and Sileno, ranged from 33% to 35%, while at the end of the experiment, θ values for the non-irrigated (dry) treatments were between 15.4% (Amadeo) and 10.6% (Sileno). The averaged Ψ_{PD} values are shown in Figure 3. It has to be noted that Amadeo showed earlier signs of stress such as leaf rolling, and therefore the measurements were stopped at DOE 10, while Sileno measurements continued for another seven days until signs of water stress were visible.

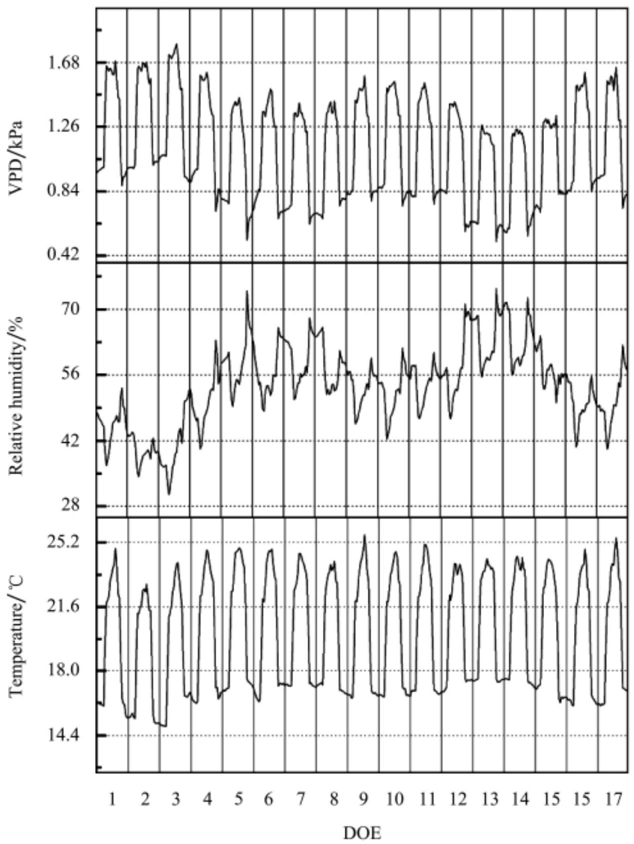


Figure 1 Daily course of temperature, humidity and vapour pressure deficit (VPD) during the days of experiment (DOE)

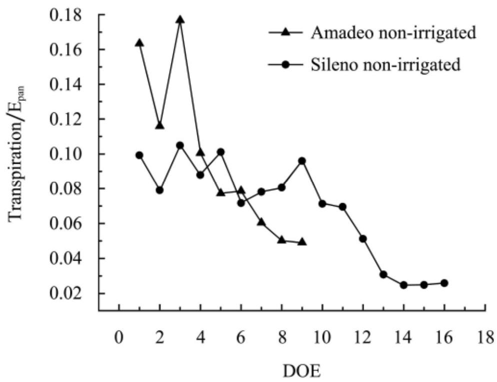


Figure 2 Ratio of daily transpiration and pan evaporation (E_{pan}) during the days of experiment (DOE)

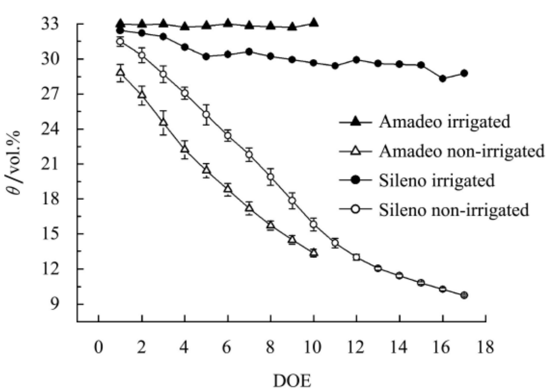


Figure 3 Average volumetric moisture content (θ) of the soil during the days of experiment (DOE)

On the first few days after the treatments started, the maize canopies were uniform, that is, no physiological, thermal or visual differences could be found, but during the course of the experiment the stressed parts of the canopy changed greatly in terms of physiological and visual status. Also, the transpiration rate decreased with increasing stress, as shown by the increasing (gL) values (Figure 4) and higher CWSI values.

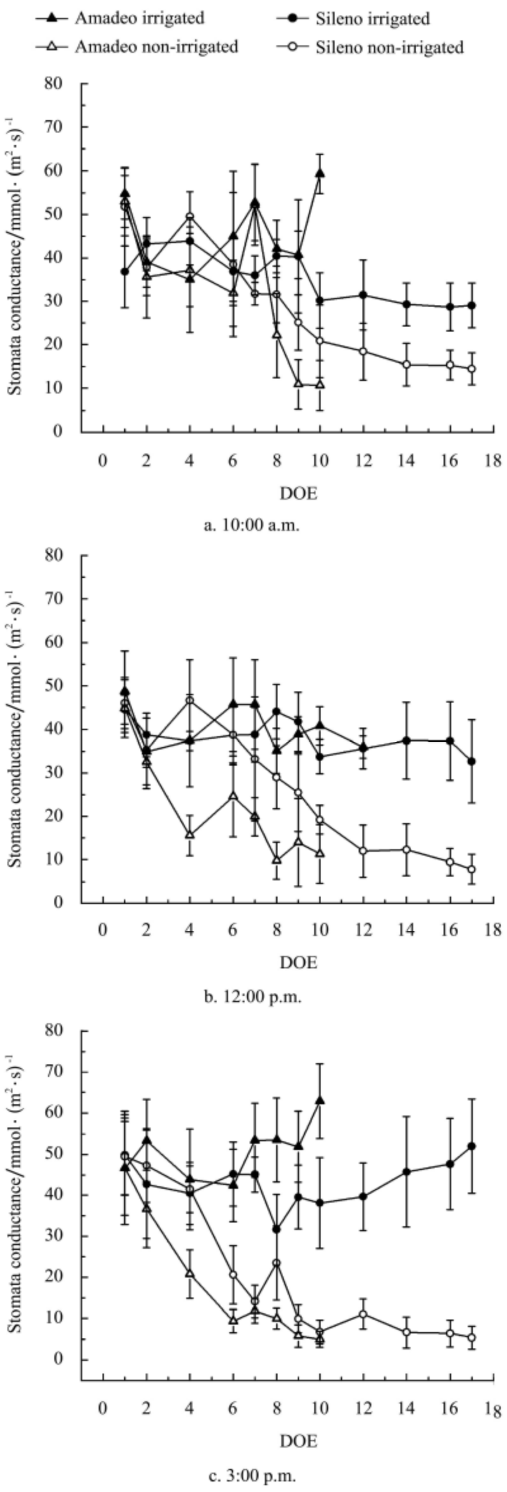


Figure 4 Stomata conductance (gL) measured at different times during the days of experiment (DOE)

The predawn leaf water potential of the irrigated pots was always below 2 bar(1 bar = 10^5 Pa) (Figure 5), but was higher for the non-irrigated pots, reaching up to 9 bar for Amadeo. The canopy temperature of the stressed treatments was consistently higher than the non-stressed treatments, and, as can be seen from the thermal images taken of the irrigated and non-irrigated Sileno at 12:00 p.m. towards the end of the experiment (Figure 6), the maximum difference in the mean canopy temperature between the stressed and non-stressed was 2.2℃ for Amadeo treatments and around 2.9℃ for the Sileno treatments.

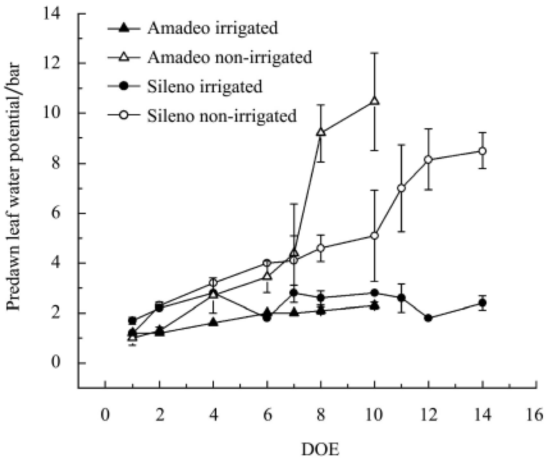
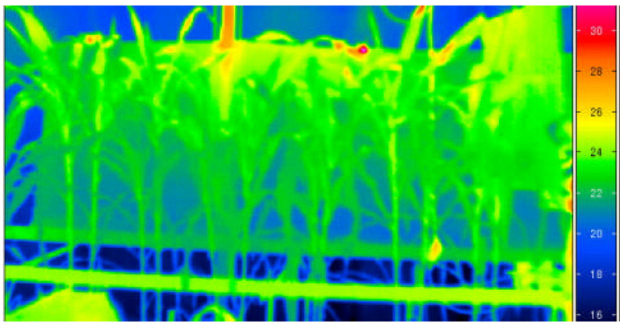
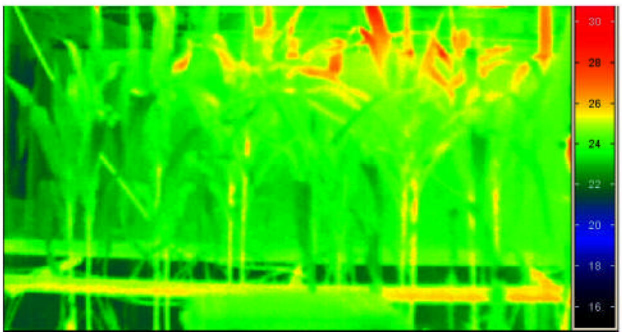


Figure 5 Predawn leaf water potential (Ψ_{PD}) during the days of experiment (DOE)



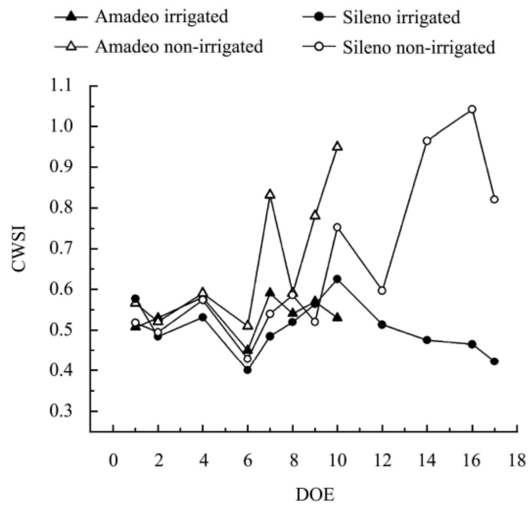
a



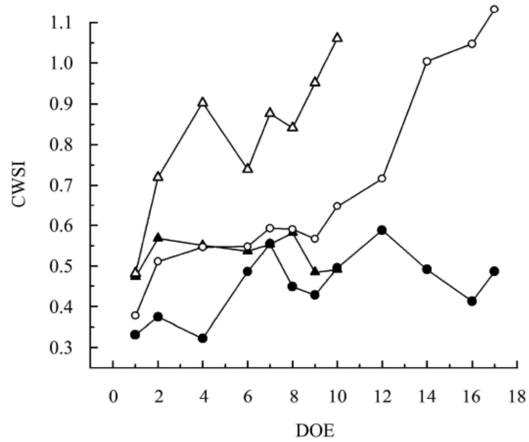
b

Figure 6 Thermal image of Sileno irrigated (a) and non-irrigated (b) at the end of the experiment

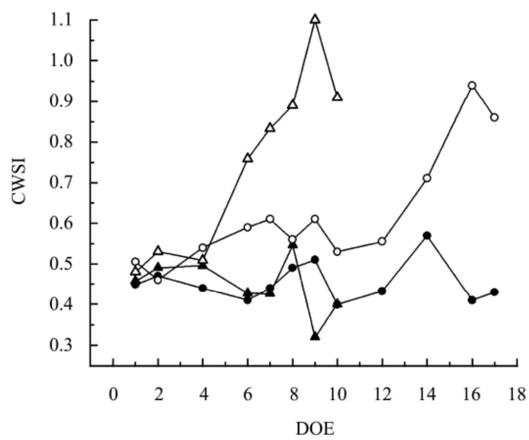
Each morning the CWSI value was less than when measured at 12:00 p.m. and 3:00 p.m. (Figure 7), and similarly, the (gL) values (Figure 4) were also higher in the morning than in the afternoon. The variation in CWSI values across the three different times of the day reveals when the plants experience the most water stress. Before using the remotely sensed CWSI as a field



a. 10:00 a.m.



b. 12:00 p.m.



c. 3:00 p.m.

Figure 7 Crop water stress index (CWSI) measured at different times of day of experiment (DOE) management tool, it is important to verify its correlation with accepted and commonly used methods for estimating water status, such as (gL) and Ψ_{PD}. The data reported here showed a significant correlation between gL, Ψ_{PD} and CWSI.

High coefficients of determination ($r^2 = 0.81$ for Amadeo and $r^2 = 0.71$ for Sileno) have been found between CWSI and (gL) (Figure 9). However, the r-square between CWSI and Ψ_{PD} (Figure 8) for Amadeo was not as high ($r^2 = 0.53$) as it was for Sileno ($r^2 = 0.81$), which might have been due to the fewer measurement days used. However, no correlation was found between CWSI, (gL) and Ψ_{PD} for the irrigated treatments, due to less variation in the day to day measurements.

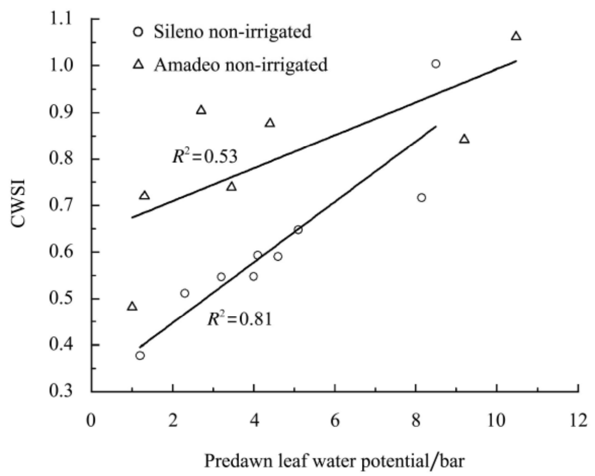


Figure 8 Regression analysis between predawn leaf water potential (Ψ_{PD}) and crop water stress index (CWSI) of Amadeo and Sileno maize genotypes

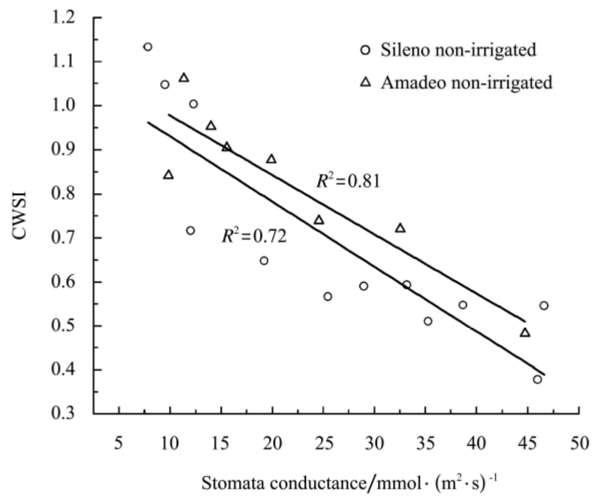


Figure 9 Regression analysis between stomata conductance to water vapour (g_L) and crop water stress index (CWSI) of Amadeo and Sileno maize genotypes

4 Discussion

Thermography as a non-contact technique agreed well with the soil and plant-based measures of water status, showing a clear response to different irrigation amounts. High correlations were achieved ($r^2 = 0.71$ & 0.81) between CWSI and Ψ_{PD}, and between CWSI and (gL) ($r^2 = 0.53$ & 0.81) - for both the Amadeo and Sileno plant varieties. The correlations presented are in accordance with the findings of Alchanatis el al.[30] (where r^2 ranged from 0.79 to 0.90) and Möller et al.[16] (where r^2 was 0.91), for which CWSI was calculated using ambient air temperature +5℃ as T_{dry}, and T_{wet} was measured using an artificial wet reference for cotton plants and grapevines.

When only one picture per day shall be taken in practical CWSI monitoring, a full day experiment should be run in order to determine the optimum time of image acquisition[20]. CWSI values for the morning measurements revealed no differences between the irrigated and non-irrigated plants at the beginning of the experiment, but differences did show up by early and late afternoon, implying that in the morning the stomata of the non-irrigated treatments were open to some extent, but when soil water content decreased, the plants were not able to recover even in the night. The best time to acquire images is when plants are experiencing the maximum amount of stress; therefore, measurements between 12:00 p.m. and 3:00 p.m. should be taken, as late afternoon measurements may overestimate plant stress. In addition, it is important to take all the thermal images at the same time of day, as CWSI values change throughout the day.

A large increase in canopy temperature on the last day of the experiment was not observed, in spite of decreased (gL) and increased Ψ_{PD} values. This may be due to changes in leaf angle and leaf curling in the stressed canopies, something which prevented an increase in leaf temperature despite the increased water stress and variability in both leaf conductance and soil moisture content[16]. CWSI values were sometimes higher than 1, which might have been caused by the reference surface, as the time taken between wetting the leaves and taking the picture could have influenced the temperature of the

T_{wet}. In addition, applying petroleum jelly to the leaves may have changed the surface roughness and as a result the level of absorption and transmission of light, plus the thermal properties of the leaves[31].

A decrease in temperature can affect not only plant photosynthesis but also plant phenological progression and solar radiation interception by the relevant crop[32] . Since the experiment was started at the twelve leaf stage, the stress at the vegetative stage led to a decrease in overall plant growth. A decrease in the photosynthesis and carbon assimilation rates occurred because the plants could not transpire at the rate imposed by the atmospheric conditions, causing a decrease in plant height. Thus, it can be seen that infrared thermography is successful for detecting physiological depression and evaluating different canopy types, at least when the micro-meteorological conditions over the canopy are approximately the same as in this greenhouse study.

A previous field experiment on maize in a Mediterranean climate produced an average seasonal mean CWSI value of 0.52 for non-irrigated and 0.19 for well-irrigated treatments[23]; however, the CWSI value for the irrigated Amadeo and Sileno in this current greenhouse experiment remained around 0.45 throughout the period, and 0.65 for the non-irrigated treatments. This might have been due to the different crop varieties and climatic conditions used, as the response of a crop to water stress is expected to vary with climatic conditions as well as soil and crop types. In addition, the CWSI values in this study were calculated using the reference surfaces as suggested by Jones et al.[15], while in the study mentioned above, the Idso[9] method was used, which does not take into account radiation.

It has been suggested that an increase in canopy temperature variations might be used as an indicator of stress[4]; however, no evidence was found in this study to support this hypothesis, as there was neither a large variation within the canopies, nor was it possible to attribute variations to different stages of drought stress. Furthermore, the standard deviation for canopy temperatures within the thermal images ranged from 0.45℃ to 1.5℃ per treatment, and this aligns with the conclusions of previous work[14,15,33]. In addition, at a time when the plants were under maximum stress in the late afternoon, (gL) values showed less variation in terms of standard deviations when compared to the 10:00 a.m. measurements. Added to this, averaging the canopy temperature reduced the mistakes that might have occurred due to variance in leaf angles and other secondary effects[34].

At the end of the experiment, leaf conductance values were very low because most of the leaves had already lost pigment and become dry; therefore, conductance for the whole canopy might have been lower than that of the leaves selected for measurement with the porometer.

5 Conclusions

This study was conducted in order to assess the potential of IRT as an alternative to the traditional and laborious methods used for estimating the water status of maize and identifying differences between genotypes of the crop. The clear genotype differences found offered another potential application for thermography, that is, phenotypic screening in breeding programs where time-consuming and laborious methods are still used.

In addition, the results presented show that CWSI is an efficient technique to quantify stress under greenhouse conditions, and can be used for irrigation scheduling when taking pictures throughout the day. Based on the data presented here, a threshold value of 0.6 may be used to decide whether to irrigate or not. However, as the absolute value of CWSI depends on the maize variety and reference surfaces used, as well as the environmental conditions, the threshold value must be adapted accordingly. Since in this study CWSI was not tested in irrigation scheduling, it cannot yet be concluded that this method is useful in practical irrigation management. Future studies are needed in order to assess the use of CWSI at different stages of crop development and under different climatic conditions, to establish recommendations for irrigation scheduling.

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