

Detection of Yield and Physiological Responses to Water Stress using Leaf Turgor Pressure and Thermal Images in Pepper

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Research Article

Keywords:

Posted Date: June 13th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-3034985/v1>

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Abstract

Today, the use of sensors and imaging techniques to obtain information about plants and soil in smart irrigation systems is rapidly becoming widespread. This study aimed to investigate the usability of leaf turgor pressure and thermal images to detect water stress and the irrigation time of pepper and to determine their relationship with physiological traits (leaf water potential, stomatal conductivity, superoxide dismutase, and catalase activity). For this purpose, the pepper (*Capsicum annuum* L. cv. "California Wonder") experiment was carried out in Canakkale, Turkey in 2017 and 2018. A total of four treatments were applied in the experiment, control (100%) and three different water stress levels (75%, 50%, and 25%). Leaf turgor pressure (Pp), thermal images and physiological measurements were carried out during the growing season. Soil moisture and leaf turgor pressure (Pp) were monitored in real time by remote.

As a result of the study, the average evapotranspiration (ET_c) was 697 mm, and the yield value was 83.7 t ha^{-1} under non-stress conditions. Depending on the decrease in ET_c , yield values also decreased significantly. The physiological traits of pepper were also adversely affected by water stress. Leaf water potential and stomatal conductivity values were statistically different in all irrigation treatments. The change in the activity of catalase (CAT) due to water stress was greater than that of superoxide dismutase (SOD). In this case, it can be said that other physiological traits are more successful than SOD in distinguishing water stress. According to the regression models, significant relationships were determined between both the indices calculated from the thermal images and Pp, yield and physiological traits. The predictive ability of Pp values has been strengthened with the addition of meteorological properties to the model in general. The highest correlation ($R^2 = 0.63$) was between Pp + meteorological properties and the catalase activity. All the regression models between CWSI and I_{Gp} calculated from thermal images were statistically significant. The highest R^2 values were obtained in August. In this month, the highest correlations were between CWSI and leaf water potential and stomatal conductivity ($R^2 = 0.91$), I_{Gp} and stomatal conductivity ($R^2 = 0.80$). The predictive power of $CWSI_p$ was higher than I_{Gp} . The experiment illustrated that Pp and temperature data, which are plant-based monitoring methods, have the potential to detect water stress in peppers.

Introduction

Today, as in all sectors, the use of technology in the modernization process in agriculture is rapidly becoming widespread. Among these, remote sensing and sensor technologies in agricultural fields has gained importance. By using these techniques and technologies, it is now easier to understand the physiological state of the plant. Studies aimed at reducing the possible effects of environmental stress on plants were emphasized. Accordingly, the employment of new technologies has been the subject of studies to prevent the possible effects of water/drought stress on plants.

Numerous studies have been conducted from the past to the present on the effects of water stress on plants. There are many valid indicators measured in the monitoring of plant water status, especially leaf water potential. However, the techniques used in the determination of the said indicators have disadvantages, such as damaging the plant, taking a long time to determine, and not allowing instant monitoring. Jones (2004) also pointed out some of the drawbacks of the various instruments employed in plant-based observations. In

response to these disadvantages, sensors based on turgor pressure that allow continuous monitoring of the water status of the plant have been developed (Zimmermann et al. 2008). The use of these sensors has been researched in various plants (Rüger et al. 2010; Zimmermann et al. 2010; Ehrenberger et al. 2012; Fernandes et al. 2017). In addition, studies on the determination of symptoms caused by water stress with remote sensing techniques are increasing. Remote sensing is used because it is difficult to measure water stress indicators with ground-based techniques. For example, stomatal conductivity, which is an important indicator of the plant's stress level, can be estimated with indices calculated from thermal images (Jones 1999).

Plants under stress use enzymatic mechanisms to protect themselves. In such a case, superoxide dismutase, catalase, peroxidase, ascorbate peroxidase, and glutathione reductase enzymes are active (Arora et al. 2002). These components involved in the enzymatic mechanism are used to inactivate free radicals called reactive oxygen species (ROS), which will occur when plants are exposed to stress. While the synthesis of ROS species is low under normal growing conditions, the amount of these compounds increases when plant growth is stressed (Smirnoff 1993; Mullineaux and Karpinski 2002; Miller et al. 2010). Under stress conditions, the plant defends itself with enzymatic or non-enzymatic mechanisms and tries to inactivate ROS species by synthesizing the above-mentioned components. Water stress, or drought, are both the most important sources of environmental stress for plants. As with other environmental stresses, water stress causes the synthesis of ROS species and creates oxidative stress (Scandalios 1997). Some studies have been conducted on the synthesis of enzymes that inactivate ROS species under conditions of water stress (Yaşar et al. 2013; Murshed et al. 2013). However, in these studies the relations between these enzymes used to determine the onset and level of stress cannot be detected visually and the thermal measurements and turgor level of the leaf were not discussed.

Turkey, particularly the province of Canakkale, plays an important role in pepper production. Pepper production ranks second among vegetables in the province (Anonymous 2015). The fact that pepper has great potential for the region and no research been carried out globally on the determination of water stress in pepper using turgor pressure and thermal images, prompted the necessity of the study.

In order to determine water stress and irrigation time; this study, which is unique because it uses turgor and thermography techniques allowing rapid and instant monitoring without damaging the plant, aims to demonstrate the usability of plant-based monitoring techniques instead of soil-based monitoring techniques, and to create statistical models among them with yield and known physiological stress indicators (leaf water potential, stomatal conductivity, superoxide dismutase, and catalase activity).

Material and methods

Experimental site and design

In 2017 and 2018, the experiment was conducted on Aristocrat F1 pepper (*Capsicum annuum* L. cv. 'California Wonder') in the Crop Production and Research Station of the Faculty of Agriculture at Canakkale Onsekiz Mart University. Some soil features of the experiment area are given in Table 1. Available water capacity (AWC) in the root zone (60 cm) of pepper is 81.4 mm. According to meteorological data, the average air temperature and

total rainfall from May to October were 22.3°C and 143 mm in 2017, and 22.7°C and 187 mm in 2018. The long-term average (1950–2017) is 21.1°C air temperature and 152.2 mm precipitation.

Table 1
Some physical analysis results of the experiment area soils

Soil Depth (cm)	Texture	Bulk Density (gr cm ⁻³)	Field Capacity (Pv, %)	Wilting Point (Pv, %)
0–30	SL	1.49	34.9	22.4
30–60	SL	1.53	33.9	19.3

Each plot had an area of 3.5 × 6.6 m, with three replicates in a randomized block design of each treatment. The peppers were planted at a density of 33 x 70 cm on 26.05.2017 and 25.05.2018. To prevent water from flowing between treatments and the measurements being affected, a 2.1-meter gap was left between each parcel.

In the study, four irrigation regimes were applied to the pepper plants: S100 (full irrigation), S75 (75% of the water consumed in S100), S50 (50% of the water consumed in S100) and S25 (25% of the water consumed in S100). In full irrigation, lack of moisture in the soil was completed to field capacity when 40 ± 5% of the available water capacity in the effective root zone was consumed.

In both years, each plot received 20 kg ha⁻¹ of 20-20-20 NPK + trace elements before planting and 4 L ha⁻¹ of humic acid and 4 kg ha⁻¹ 12-8-13 NPK + 3 MgO + trace elements at the flowering stage of the pepper. Further fertilization procedures were performed during fruit formation (2 kg ha⁻¹ MAP 12-61-0 and 4 L ha⁻¹ humic acid twice, 0.6 L ha⁻¹ 16% Ca). Herbicides and pesticides were used when necessary.

Irrigation water amount and evapotranspiration

Sensors (GS1 model, Decagon Devices, Inc., WA, USA) were placed to 0–30 cm, 30–60 cm, and 60–90 cm depths of the soil on all parcels for monitoring soil moisture required to determine the irrigation water requirement and evapotranspiration. The data from the sensors were instantly monitored via a remote monitoring system (Devint Informatics, Izmir, Turkey). The sensors were calibrated with actual soil moisture values before the experiment started.

The moisture level in the soil was kept at the level of field capacity continuously until July 10 in the first year and July 11 in the second year. After these dates, irrigation was started according to the treatments. The volume of water applied to each treatment was calculated by multiplying the area by the percentage of cover and was applied to the experimental plots in a controlled manner using a water meter. The percentage of cover was obtained by dividing the cover width of five randomly selected plants between rows before each irrigation. This value was taken as at least 30%.

Calibrated data obtained from the soil moisture sensors placed on 0–30 cm, 30–60 cm and 60–90 cm soil layers for all repetitions of each subject was used to determine evapotranspiration. The effective plant root depth was taken as 60 cm, and the moisture content at 60–90 cm soil depth was monitored for deep infiltration and water table movement.

The evapotranspiration amounts for each experimental treatment were calculated using Eq. 1, according to the water budget method (James 1988):

$$ET = I + P - D \pm R \pm \Delta S \quad (1)$$

where ET: evapotranspiration (mm), I: irrigation water (mm), P: precipitation (mm), D: deep percolation (mm), R: the runoff (mm), ΔS : the change in soil water storage (mm)

During the experiment, deep percolation was ignored since there was no change in the moisture sensors placed in the 60–90 cm soil layer. In addition, since the amount of precipitation occurring in this period was at a level that does not cause runoff, this value was accepted as zero in the equation.

Yield

All plants in each plot, except for edge effects, were regularly harvested and the yield was calculated as tonnes per hectare by weighing the harvested pepper. Harvest started on the 66th and 70th days after planting and ended on the 155th and 154th days, according to 2017 and 2018, respectively.

Plant based measurements

Stomatal conductance

Stomatal conductance was measured on the abaxial surfaces of the leaves using a steady state leaf porometer (SC-1, Decagon Devices, USA). The measurements were made on a sun-exposed and mature leaf of 3 randomly selected plants at each replication, before irrigation (the 45th day after planting), between 11:00 and 14:00.

Leaf water potential

Leaf water potential was measured in leaves from 3 different plants at each replication using a pressure chamber instrument (Model 1000, PMS Instrument, USA) following the standard methodology (Scholander et al. 1965). The fully developed and sun-exposed leaves of the plant at that time were used in the measurements. These measurements were made before irrigation and in the midday.

Superoxide dismutase and catalase activity

Leaf samples required for the determination of enzyme activity were taken five times on the days when other measurements were made, considering the developmental status of the plants. Three plants alike with plants in other measurements were selected from each plot for sampling. Three leaves from these plants were taken, and biochemical analyses were made. Superoxide dismutase (SOD) (Kakkar et al. 1984) and catalase (CAT) activity (Luck 1963) values were determined in these samples.

Leaf turgor pressure and thermal measurements

In the study, leaf patch clamp pressure (LPCP) probes (YARA ZIM Plant Technology GmbH, Hennigsdorf, Germany) were used to determine leaf turgor pressure and a thermal camera (Fluke Ti27 model, Fluke, USA) was used to obtain thermal images. The outputs of LPCP are called patch pressure (Pp). The concepts of this

probe were described in further depth by Zimmermann et al. (2008). Details of the procedures in this study are also described in Camoglu et al. (2021).

The modified crop water stress index (CWSI) and stomatal conductance index (I_G) were calculated using the thermal imaging by means of Eqs. (2) and (3), respectively (Jones 1999). The measurement and calculation details regarding these are also given in detail in Camoglu et al. (2021):

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

2

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

3

Temperature, relative humidity, wind speed and precipitation were recorded hourly with the help of the climate station placed in the experimental area to be used in thermal measurements and leaf pressures.

Statistical analysis

Data obtained from the two-year field experiments were subjected to variance analysis. Duncan's multiple comparison test was applied to compare differences between mean values. Regression analysis was used to determine the relationships between leaf turgor pressure, thermal indices, and the traits examined. In this context, the simple linear regression method was used for the estimation of yield and physiological traits by means of thermal indices. Regression graphs were prepared in the MS Excel program, and the significance levels of the regression models obtained were determined in the SAS package program (SAS Institute, 1999). The multiple linear regression method was used to estimate the yield and physiological traits by employing the leaf turgor pressure and meteorological properties. These models were created using the stargazer package of the R program (R development Core Team, 2012).

Results and discussion

Changes in soil moisture

In the first year of the experiment, all treatments were brought to field capacity on the 38th day after planting (DAP38), and irrigation treatments began to be applied on the 45th day (Fig. 1). Irrigation was finished on DAP125 and soil moisture was monitored until the day (DAP155) of the last harvest. When the soil moisture values of the irrigation treatments are examined, it is seen that the moisture values in the S100 treatment generally vary between the field capacity and the 40% value, which is the allowed portion (R_y) of the available water capacity. Soil moisture decreased below the 40% limit since DAP110 for S75, DAP72 for S50, and DAP57 for S25. However, the moisture content of S25 remained at the wilting point level since DAP86.

In the second year of the experiment, all parcels were brought to field capacity on DAP43, and irrigation treatments began to be applied on DAP47 (Fig. 1). Irrigation was finished on DAP119, and soil moisture was monitored until DAP154, when the last harvest was made. In the second year, it is seen that the moisture changes in S100 are closer to the field capacity, compared to the first year. Although the soil moisture in S75 treatments fell below the Ry value from time to time, it changed above the limit value with irrigation and precipitation. For S50, the moisture value changed between Ry and the wilting point since DAP80. As for S25, although soil moisture values had approached the wilting point for a day or two (DAP98, DAP101-DAP103), they were generally above the wilting point.

When a general evaluation is made, it is seen that the moisture values in all treatments were higher in the second year of the experiment compared to the first year. It can be said that the reason for this is that the plant growth in the second year is weaker than the first year.

Total amount of irrigation water and evapotranspiration

The total irrigation water and seasonal evapotranspiration values were calculated to be 242–684 mm and 313–734 mm in the first year of the experiment, and 209–524 mm and 347–660 mm in the second year, respectively (Fig. 2). The values in the first year were higher than the second year. This may be due to the meteorological differences between the two years as well as the fact that the first-year plant growth was slightly better than the second year.

There have been many studies in Turkey and worldwide in which different applications (irrigation, fertilization, salt applications, chemical applications, etc.) were made on different pepper varieties. Despite this, studies on irrigation regarding the "California Wonder" variety in our study are insufficient. In one study of this variety in Canakkale, Erken (2004) used five different pan evaporation coefficients (0.25, 0.50, 0.75, 1.00 and 1.25) and applied irrigation water between 121.8–609.0 mm and 183–915 mm, respectively. Demirel et al. (2014) applied four different irrigation levels (100%, 66%, 33%, and 0% according to soil moisture) on pepper plants. As a result of their study, different amounts of irrigation water (between 72 mm and 801 mm) were applied to the treatments. Yildirim et al. (2017) used five different pan evaporation coefficients (0.00, 0.33, 0.66, 1.00 and 1.25) in their study from which they reported that the total irrigation water amount and seasonal evapotranspiration values varied between 72–951 mm and 72–1047 mm, respectively, in the first year of the experiment and between 106–1115 mm and 106–1085 mm, respectively, in the second year of the experiment. Some differences were observed between studies conducted in the same region and this study. It can be said that these differences may be due to meteorological characteristics that occurred between years and differences in the varieties used, even if it is the same pepper type, and since they consider different methods (such as Pan-Evaporation) in calculating the amount of irrigation water.

In studies conducted in India on the same pepper variety, Paul et al. (2013) applied the amount of irrigation water according to the pan evaporation on mulched and non-mulched treatments. They used three different pan evaporation coefficients (1, 0.8 and 0.6) and stated that the amount of applied irrigation water varied between 199 mm and 319 mm. Antony and Singandhupe (2004) has applied different irrigation methods (surface and drip irrigation) and different irrigation levels according to pan evaporation (1.2, 1.0, 0.8 and 0.6 for surface irrigation and 100%, 80%, 60% and 40% for drip irrigation). As a result of the study, they applied

irrigation water between 200 mm and 440 mm with the surface irrigation method and between 141 mm and 282 mm with the drip irrigation method. It is clear that there are differences between the results obtained from studies conducted in different countries and this study; due to the irrigation methods used, method of application, meteorological characteristics between regions, and use of different varieties.

Yield

Yield values changed between 22.9 and 90.7 t ha⁻¹ and 22.5 and 76.6 t ha⁻¹ according to the irrigation treatments in 2017 and 2018, respectively (Fig. 3). It was seen that the yield differences among the treatments were statistically significant in both years of the experiment. However, although the yield values of the first year for S100 and S50 were different compared to the second year, there was no difference between S75 and S25 treatments.

In studies conducted in the same region (Canakkale) with the same pepper variety, Erken (2004) applied irrigation water using five different pan evaporation coefficients for two years. As a result of the study, it was reported that yield varied between 23.49 and 68.88 t ha⁻¹ in the first year and between 21.39 and 65.64 t ha⁻¹ in the second year. Demirel et al. (2014) obtained yield values between 20.25 and 84.16 t ha⁻¹ as a result of a study in which they applied four different irrigation levels. Yildirim et al. (2017) used five different pan evaporation coefficients. They reported that the yield values varied between 20.25 and 84.16 t ha⁻¹ in the first year of the experiment and between 12.22 and 66.93 t ha⁻¹ in the second year. Similarities were observed between studies conducted in the same region and the findings obtained from the research. It is seen that the yield values, especially among the least irrigated treatments (except for the second year of the experiment by Yildirim et al. (2017), are very close to each other, and the yield values obtained by Demirel et al. (2014) are similar to those of this study. We consider that this similarity exists because the amount of irrigation water applied in the experiments is calculated according to the moisture loss in the soil in both studies. Erken (2004) and Yildirim et al. (2017) used pan evaporation while calculating the amount of irrigation water to be applied in their studies. For this reason, although too much irrigation water was applied, there was not much difference among yields in these studies.

In studies conducted in India on the same pepper variety, Paul et al. (2013) stated that the yield values in mulch and non-mulched treatments varied between 18.2 and 28.7 t ha⁻¹. Antony and Singandhupe (2004) compared surface and drip irrigation methods using pan evaporation, and they obtained yield values between approximately 28 and 45 t ha⁻¹ and 33 and 50 t ha⁻¹ (read from the chart), respectively, according to the methods.

Physiological results

Leaf water potential

Considering the change in leaf water potential according to irrigation treatments in the current study, it was determined that there are statistically significant differences between irrigation treatments for 2017 (Table 2). It was determined that the water constraint increased respectively among the irrigation treatments applied to the peppers and the leaf water potential decreased in the S75, S50 and S25 treatments. While the mean lowest leaf water potential value was -1.84 MPa for S25, it was followed by S50 (-1.56 MPa) and S75 (-1.38 MPa)

treatments, respectively. The average highest leaf water potential value with -1.16 MPa was obtained from the S100 irrigation treatments (control) where the reduced moisture was completed to the field capacity when $40 \pm 5\%$ of the usable moisture in the 0–60 cm soil layer was consumed (Table 2).

According to the leaf water potential values of the experiment in 2018, it was determined that the irrigation treatments had a statistically significant effect on the leaf water potential in the other months except September and in the average values (Table 2). In this context, the lowest average leaf water potential value was measured at S25 (-1.52 MPa), while the highest leaf water potential value was determined at S100 (-1.15 MPa).

Table 2
Change of average leaf water potential values according to treatments and months

Treatments	July	August	September	Mean
2017*				
S100	-1.23 A ns	-1.14 A ns	-1.13 A ns	-1.16 A
S75	-1.38 B ns	-1.39 B ns	-1.36 B ns	-1.38 B
S50	-1.50 C ns	-1.59 C ns	-1.59 C ns	-1.56 C
S25	-1.73 D a	-1.86 D b	-1.88 D b	-1.84 D
Treatments	2018*			
S100	-1.18 A ns	-1.17 A ns	-1.06 NS ns	-1.15 A
S75	-1.34 B b	-1.30 B b	-1.16 NS a	-1.27 B
S50	-1.43 C b	-1.45 C b	-1.12 NS a	-1.38 C
S25	-1.54 D b	-1.65 D c	-1.17 NS a	-1.52 D

*: $p < 0.05$, Note: Different capital letters in each month indicate the difference between irrigation treatments. Different lowercase letters in each topic indicate the difference between months.

When the changes in leaf water potential values of the treatments are examined according to the months, the leaf water potential values of the S100, S75 and S50 treatments did not show a significant difference according to the months during the experiment, which included the vegetative and generative periods in 2017. In the second year, this situation was only realized with respect to S100. The leaf water potential value of S25, where water stress was applied the most in both years, decreased in the following July but increased again with the effect of precipitation in September of the second year. It is thought that the change in leaf water potential, especially in this regard, may be due to the increase in the heat load on the plant and the number of windy days in Canakkale in August and September, compared to July. According to Kaufman (1981), the critical level in the leaf water potential varies depending on the plant type and growth period, as well as the environmental conditions, and the value decreases rapidly with the decrease in the soil water potential.

In a study conducted on dwarf green beans, it was stated that the leaf water potential values differed between irrigation treatments, and the value increased as the irrigation water level increased (Köksal et al. 2010). Similarly, Pıtır (2015) stated that the leaf water potential and the relative water content of the leaves decreased because of the reduction in irrigation during the period from flowering to harvest in Jalepeno peppers.

Stomatal conductance

It was determined that different irrigation levels applied to the pepper plant used in the study significantly affected the stomatal conductance of the leaves (Table 3). In both years, the highest stomatal conductance values were determined in the S100. This treatment was followed by S75, S50, and S25, respectively. All irrigation treatments were statistically separated from each other in all months (except for September 2018) of both years. In September 2018, it was seen that S100 and S75 were in a different group from other irrigation treatments. It was observed that stomatal conductance values increased in the same month, similar to the leaf water potential values. This can also be explained by the precipitation in September. As a matter of fact, this increase can also be seen when the changes in the treatments are analyzed according to the months. While stomatal conductance decreased as expected towards September in 2017, the opposite was seen in 2018.

Table 3
Change of average stomatal conductance values according to treatments and months

Treatments	July	August	September	Mean
2017*				
S100	544 A b	627 A a	611 A a	609 A
S75	478 B ns	500 B ns	451 B ns	484 B
S50	380 C a	316 C b	271 C c	316 C
S25	278 D a	199 D b	178 D b	212 D
Treatments	2018*			
S100	556 A b	579 A b	700 A a	582 A
S75	463 B b	481 B b	640 A a	503 B
S50	335 C b	323 C b	448 B a	356 C
S25	268 D b	226 D b	338 C a	276 D

*: $p < 0.05$, Note: Different capital letters in each month indicate the difference between irrigation treatments, and different lowercase letters in each topic indicate the difference between months.

This phenomenon, which occurred among the treatments in both years, is because plants control O_2 and CO_2 diffusion by closing their stomata in the face of abiotic stress. In other words, stomatal permeability decreases in pepper leaves because of decreased transpiration. Costa et al. (2000) also stated that the closure of stomata is one of the drought-avoidance mechanisms that allows plants to keep water in their tissues and that it can reduce the photosynthetic rate and slow down the growth rate of the plant, as it prevents CO_2 from

entering the mesophyll cells. Jones (1992) stated that leaf stomatal permeability may decrease under the influence of environmental factors. Eriş et al. (1998) stated that the stomatal conductance and transpiration rate decreased as water deficiency increased in grapevine varieties under the same stress level conditions. Camoglu et al. (2019) stated that the stomatal conductance of tomato plants decreased significantly due to the increase in water stress. Many researchers have also pointed out that drought can lead to decreases in stomatal conductance and reported that the change in stomatal conductance is one of the important factors affecting the drought resistance performance of the varieties (Mehri et al. 2009; Kuşvuran et al. 2009; Nawaz et al. 2015).

Superoxide dismutase and catalase activity

Superoxide dismutase (SOD) and catalase (CAT) activity, which shows the stress status in the plant, changed significantly depending on the irrigation treatments. When the changes in SOD activity by months were examined, a difference was observed among the treatments only in August, when the stress was highest in 2017, while this difference was not statistically significant in any month in 2018 (Table 4). However, in the second year of the experiment, S25 was in a different group than the others, according to the mean SOD values. In the first year, this did not occur. When the changes in the treatments according to the months were examined, an increase was observed in the SOD values of all treatments after July, and this increase was statistically significant.

Table 4
Change of mean SOD and CAT values according to treatments and months

Treatment	July	August	September	Mean
	SOD			
	2017*			
S100	9.70 NS b	19.15 B a	24.58 NS a	17.81 NS
S75	10.52 NS b	20.84 B a	24.61 NS a	18.65 NS
S50	8.84 NS b	26.93 A a	26.93 NS a	20.90 NS
S25	12.32 NS b	30.71 A a	31.87 NS a	24.97 NS
	2018*			
S100	16.77 NS b	24.15 NS b	36.64 NS a	25.86 B
S75	19.75 NS b	26.75 NS ab	33.87 NS a	26.79 B
S50	18.09 NS b	28.88 NS a	37.97 NS a	28.31 B
S25	25.47 NS b	31.22 NS ab	49.06 NS a	35.25 A
Treatment	CAT			
	2017*			
S100	29.73 C b	47.47 D a	23.45 D c	33.55 D
S75	48.16 B b	65.26 C a	31.73 C c	48.38 C
S50	48.87 B b	83.84 B a	73.87 B a	68.86 B
S25	56.49 A c	101.62 A a	88.48 A b	82.20 A
	2018*			
S100	32.33 C b	49.76 C a	13.33 B c	31.81 C
S75	37.73 BC b	60.93 BC a	29.40 A b	42.69 B
S50	44.09 AB b	67.47 B a	31.43 A c	47.67 B
S25	49.60 A b	87.56 A a	36.50 A c	57.89 A

*: $p < 0.05$, Note: Different capital letters in each month indicate the difference between irrigation treatments, and different lowercase letters in each topic indicate the difference between months.

According to the change of CAT activity by months, the highest enzyme activity values were recorded in August in both years (Table 4). Contrary to SOD values in CAT activity, differences among the treatments in all months and mean values were found to be statistically significant. When the changes in the treatments according to the months are examined, it is seen that the CAT value is the highest in August.

Studies have shown that both SOD and CAT activity can be used as indicators of abiotic stress (Manchandia 1999). CAT has the ability to inhibit H_2O_2 , that occurs as a result of stress (Polidoros and Scandalios 1999). SOD, on the other hand, has the ability to inhibit the first free radicals formed during stress, by removing O_2 accumulated in the cell and reducing the OH^- ions (Mittler 2002). With these aspects, the synthesis of both enzymes increases in order to reduce the effect of free radicals formed in plant cells during stress. Findings from the study also confirm this. However, it has been observed that there may be differences in the time-dependent change of the enzyme activities in question due to the year or environmental conditions.

Relationships between leaf pressure, meteorological parameters, and the examined traits

The results of the regression analyses performed to estimate the yield, leaf water potential, stomatal conductivity, SOD, and CAD values from the instantaneously recorded leaf pressure values and meteorological data are given in Table 5. The model results for each trait in question are explained separately below.

Table 5
Regression analysis between leaf pressure, meteorological parameters, and the examined traits

Yield (t ha ⁻¹)							
	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Leaf turgor pressure (kPa)	-0.901 ^{***}				-0.906 ^{***}	-0.906 ^{***}	-0.906 ^{***}
Air temperature (°C)		-9.194			0.749	0.749	0.749
Relative humidity (%)			-7.669				
Wind velocity (km day ⁻¹)				13.337			
Constant	101.444 ^{***}	330.035	411.174	-15.722	78.907	78.907	78.907
R ²	0.547	0.033	0.033	0.033	0.548	0.548	0.548
Leaf Water Potential (MPa)							
	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Leaf turgor pressure (kPa)	-0.007 ^{***}				-0.007 ^{***}	-0.007 ^{***}	-0.007 ^{***}
Air temperature (°C)		-0.006			0.0002	0.003	-0.002
Relative humidity (%)			0.005			0.004	0.003
Wind velocity (km day ⁻¹)				-0.012			-0.043 ^{**}
Constant	-1.046 ^{***}	-1.232 ^{***}	-1.677 ^{***}	-1.365 ^{***}	-1.053 ^{***}	-1.319 ^{***}	-0.890 [*]
R ²	0.244	0.002	0.017	0.003	0.244	0.253	0.288
Stomatal Conductance (mmol m ⁻² s ⁻¹)							
	(1)	(2)	(3)	(4)	(5)	(6)	(7)

Note: * p < 0.1; ** p < 0.05; *** p < 0.01, n = 24 for yield, n = 96 for leaf water potential and stomatal conductance and n = 40 for SOD, CAT

Yield (t ha ⁻¹)							
	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Leaf turgor pressure (kPa)	-5.850 ^{***}				-5.815 ^{***}	-5.744 ^{***}	-6.081 ^{***}
Air temperature (°C)		-9.592			-3.823	-1.541	-3.970
Relative humidity (%)			5.334 ^{**}			4.063 ^{**}	3.718 [*]
Wind velocity (km day ⁻¹)				4.316			-21.419 ^{**}
Constant	742.641 ^{***}	701.933 ^{**}	159.090	388.723 ^{***}	856.928 ^{***}	592.317 ^{**}	807.653 ^{***}
R ²	0.456	0.012	0.041	0.001	0.458	0.481	0.502
SOD (U g ⁻¹)							
	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Leaf turgor pressure (kPa)	0.236 ^{***}				0.228 ^{***}	0.225 ^{***}	0.220 ^{***}
Air temperature (°C)		-1.152 [*]			-0.982 [*]	-1.095 [*]	-0.46
Relative humidity (%)			-0.058			-0.112	0.086
Wind velocity (km day ⁻¹)				4.514 ^{**}			3.830 [*]
Constant	12.161 ^{***}	59.559 ^{***}	28.069 ^{***}	4.198	41.789 ^{**}	50.566 ^{**}	4.710
R ²	0.296	0.074	0.002	0.135	0.349	0.358	0.418
CAT (U g ⁻¹)							
	(1)	(2)	(3)	(4)	(5)	(6)	(7)

Note: * p < 0.1; ** p < 0.05; *** p < 0.01, n = 24 for yield, n = 96 for leaf water potential and stomatal conductance and n = 40 for SOD, CAT

Yield (t ha ⁻¹)							
	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Leaf turgor pressure (kPa)	0.610 ^{***}				0.647 ^{***}	0.599 ^{***}	0.592 ^{***}
Air temperature (°C)		4.322 ^{**}			4.802 ^{***}	2.925 ^{**}	3.913 ^{**}
Relative humidity (%)			-2.159 ^{***}			-1.858 ^{***}	-1.550 ^{***}
Wind velocity (km day ⁻¹)				10.462 [*]			5.959
Constant	25.112 ^{**}	-69.300	160.798 ^{***}	10.213	-119.719 ^{**}	26.229	-45.109
R ²	0.219	0.114	0.373	0.08	0.359	0.613	0.629
Note: *p < 0.1; **p < 0.05; ***p < 0.01, n = 24 for yield, n = 96 for leaf water potential and stomatal conductance and n = 40 for SOD, CAT							

Evaluation for yield

According to the results of the model (1) created by assigning leaf pressure as the sole independent variable, it is seen that the change in leaf pressure can explain a 54.7% variation in yield (Table 5). This model was found to be statistically significant. It has been determined that it is not possible to explain the change in efficiency if the measurements of temperature, relative humidity, and wind speed, which are meteorological characteristics, are used separately as estimators. There is no change in the predictive power of the model ($R^2 = 54.8$) in terms of explaining the existing change in yield if meteorological properties are included in the model, as well as leaf pressure.

Evaluation for leaf water potential

Model (1) created by assigning the leaf pressure as the independent variable in the prediction models created for the leaf water potential can explain 24.4% of the change in the leaf water potential (Table 5). It is understood from the meteorological features that it is insufficient to explain the change in leaf water potential as a predictor by itself. We determined that approximately 4% of the change in leaf water potential can be explained if all meteorological characteristics in addition to leaf pressure are assigned to the model as predictors for the prediction of leaf water potential.

Evaluation for stomatal conductance

According to the regression coefficient of the model in which only leaf pressure is used as an estimator among the regression models created for the estimation of stomatal variability, it is seen that 45.6% of the variation in

stomatal conductivity can be explained by leaf pressure (Table 5). If the temperature, humidity, and wind values are added to this model (7), 50.2% of the variation in stomatal conductivity can be explained by the model. In other words, the success of the model can be increased by 5% if meteorological features are used together with leaf pressure. The linear regression equation of the model in question was found as stomatal conductivity = $807.653 - 6.081 \times \text{leaf pressure} - 3.970 \times \text{temperature} + 3.718 \times \text{relative humidity} - 21.419 \times \text{wind speed}$.

In the literature, the relationships between leaf water potential and stomatal conductivity were investigated using the data obtained with leaf pressure sensors. Bramley et al. (2013) found statistically significant relationships between these physiological traits and leaf pressure in wheat. Martinez-Gimeno et al. (2017) stated that there was a good correlation between leaf pressure and shoot water potential in persimmon.

Evaluation for superoxide dismutase

The regression coefficient ($R^2 = 0.29$) of the model (1), in which leaf pressure was used alone as an estimator among the regression models created for SOD activity, was found to be low (Table 5). Therefore, the models including temperature and wind speed data can be effective in explaining the change in SOD activity, albeit at a low level. In addition to the leaf pressure values, the regression coefficient of the model in which all meteorological features were used was found to be significantly higher than the single models; so much so that when the model in which only leaf pressure is used (1) is compared with this model (7), it is seen that there is an 11% difference in the regression coefficient.

Evaluation for catalase activity

In models where leaf pressure and meteorological features are used separately as predictors of CAT activity, regression models appear to be statistically significant when relative humidity and temperature are used alone. It was determined that the change in relative humidity alone could explain 37.7% of the change in CAT activity. Thereby, 62.9% of the change in CAT enzyme activity can be predicted with the model (7), in which all variables are used. The linear regression equation of the model in question was obtained as: $\text{CAT} = -45.109 + 0.592 \times \text{leaf pressure} + 3.913 \times \text{temperature} - 1.550 \times \text{humidity} + 5.959 \times \text{wind speed}$.

Relationships between thermal indices and the examined traits

Regression models between thermal indices and yield

According to the results of the regression analyses between the yield obtained at the end of the growing period and $\text{CWSI}_p - I_{Gp}$, a negative linear relationship was found between yield and CWSI_p , and a positive linear relationship with I_{Gp} (Fig. 4). The R^2 values between yield and CWSI_p varied between 0.43 and 0.91, and 0.33 and 0.85 with I_{Gp} , depending on the months. In both indices, the lowest value was in September, while the highest value was in August. This means that yield estimation can be made with higher accuracy by using thermal measurements made in August. Camoglu et al. (2018) found the R^2 values between yield and CWSI_p varied between 0.52 and 0.75, depending on the measurement days, in their study on a different pepper cultivar (Demre). It was stated that the relationship between them increased in further measurements after planting. In studies on different plants, O'Shaughnessy et al. (2011) obtained the R^2 value between soybean yield and

empirical CWSI ($CWSI_e$) as 0.88 in 2004 and 0.83 in 2005, and the value between cotton yield and $CWSI_e$ as 0.78. Camoglu and Genc (2013) also found the R^2 value between green bean yield and $CWSI_p$ to be 0.34.

Thermal indices and physiological characteristics regression models

Regression analyses between thermal images and physiological traits (leaf water potential, stomatal conductivity, SOD, and CAT) measured or calculated at the same time during the growing period in both experiment years are given in Fig. 5. According to the analysis results, the R^2 values between leaf water potential and $CWSI_p$ varied between 0.59 and 0.91 depending on the months (Fig. 5). The values were between 0.33 and 0.76 for I_{Gp} . The R^2 values between stomatal conductivity and $CWSI_p$ and I_{Gp} were also between 0.68 and 0.91 and 0.43 and 0.80, respectively (Fig. 5). The highest values between both leaf water potential and stomatal conductivity and the indices were realized in August, as in the yield. In addition, the predictive power of the $CWSI_p$ index for both physiological traits were found to be higher than I_{Gp} . Accordingly, these can be predicted more accurately by using $CWSI_p$ in August. This could be explained by the fact that the irrigation was started in July, when the plants were not yet fully stressed, and the leaves of the plants entered the aging process due to the harvest in September.

Camoglu and Genc (2013) and Garcia-Tejero et al. (2017) reported that thermal and spectral data are useful tools to predict physiological characteristics such as plant water status, stomatal conductivity, and chlorophyll. Camoglu et al. (2018) found the R^2 value between leaf proportional water content and $CWSI_p$ to be 0.23, which they determined as an indicator of plant water status in the Demre pepper variety. Relationships between leaf water potential and CWSI were also investigated in studies on other plants. Cohen et al. (2005) found that the R^2 values between leaf water potential and CWSI in cotton were between 0.79 and 0.90, according to the measurement time. Moller et al. (2007) obtained the R^2 values between CWSI and leaf water potential calculated from thermal images to be between 0.52 and 0.91. Ben-Gal et al. (2009) stated in their study on olives that CWSI values calculated with the help of thermal images can be used to determine both leaf water potential and stomatal conductivity. Zia et al. (2013) obtained the R^2 value between stomatal conductivity and CWSI as 0.62 in maize. Camoglu et al. (2019) found the R^2 value to be between 0.53 and 0.91, and stomatal conductivity between 0.55 and 0.96 between CWSI and leaf water potential, which they calculated empirically from thermal images in tomato. They also stated that the strength of the relationship increased after the first measurement.

Among the enzyme activities, the R^2 values between SOD and CAT values and $CWSI_p$ were 0.02–0.31 and 0.68–0.80, respectively, and the values between I_{Gp} were 0.005–0.23 and 0.34–0.66, respectively (Fig. 5). The highest R^2 values were also found in August. The correlation between the indices and SOD was found to be lower than that of the CAT enzyme. In particular, the R^2 value between CAT and $CWSI_p$ was higher than the others. In this case, it can be said that the CAT enzyme can be predicted more successfully with the $CWSI_p$ index.

Conclusions

According to the results of the study, it was determined that the pepper plant is very sensitive to water stress. It was concluded that the evapotranspiration and amount of irrigation water should be calculated correctly so that the pepper plant is not exposed to water stress; for this, the soil moisture needs to be constantly monitored. To accomplish this, soil moisture sensors and remote monitoring systems that allow instant and continuous monitoring of moisture are very advantageous tools. The total irrigation water amount, seasonal evapotranspiration, and yield values under non-water stress conditions were obtained as 604 mm, 697 mm, and 83.7 t ha⁻¹, respectively, according to the average of two years. The highest monthly evapotranspiration in these non-stress plants was in August.

Water stress had a negative impact on all the physiological properties of pepper plants. Leaf water potential and stomatal conductance values differed significantly according to irrigation treatments. In order to prevent the pepper from being exposed to water stress and to determine the irrigation time, these physiological traits can be taken as a threshold value for the results on the S100, separately for each month. While superoxide dismutase values from enzyme activities were not successful in distinguishing the treatments from each other, catalase activity was successful in determining the stress level. In this case, it can be said that catalase activity is more useful than superoxide dismutase in determining stress.

Statistically significant relationships were obtained in the estimation of investigated traits using leaf pressure + meteorological properties. According to the multivariate regression models, the coefficients of determination between leaf pressure + meteorological properties and yield, leaf water potential, stomatal conductance, superoxide dismutase, and catalase activity were determined as 0.55, 0.29, 0.50, 0.42, and 0.63, respectively. In this case, the catalase enzyme can be predicted more accurately than the other traits.

All the models obtained according to the simple linear regression analysis between the thermal indices and the traits examined in the study were found to be statistically significant. In the analyses made by months, the best relations were found in August. In addition, the predictive power of CWSI_p was higher than I_{GP}. Accordingly, the mentioned index can be suggested for predicting yield and physiological characteristics.

This study has revealed the extensive potential to identify water stress in peppers using leaf patch clamp pressure probes and thermal cameras, which are plant-based monitoring tools.

Declarations

Acknowledgment: This study was funded by the Scientific and Technological Research Council of Turkey under Research Project No: 116O264.

Availability of data and materials: Requests for data and materials should be addressed to Gokhan Camoglu.

Conflict of interest: On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Figures

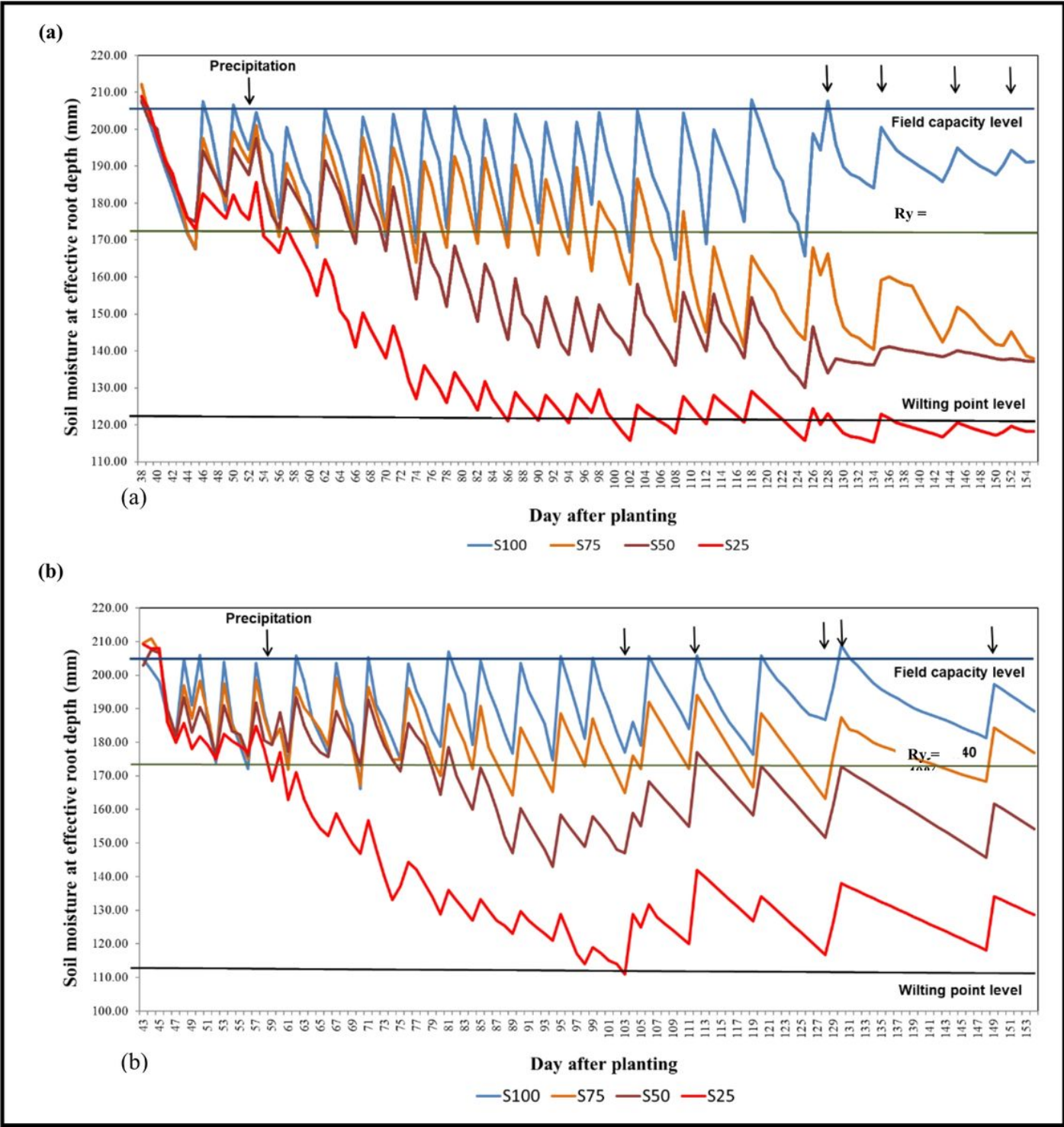


Figure 1

Changes in soil moisture at effective root depth of treatments in (a) 2017, (b) 2018

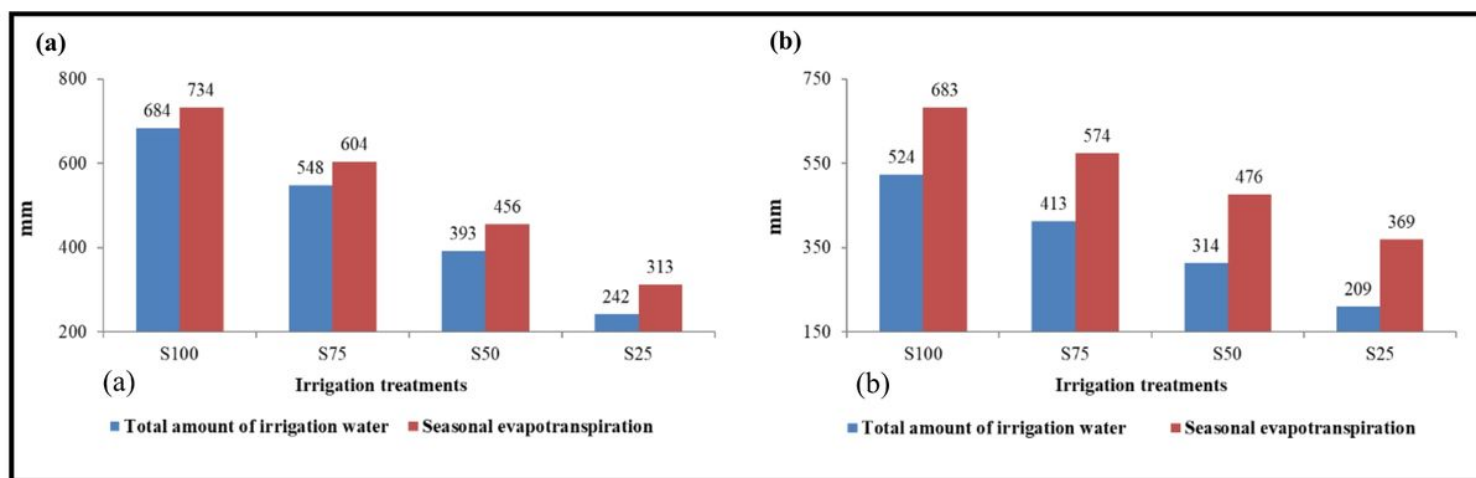


Figure 2

Total amount of irrigation water and seasonal evapotranspiration values of treatments in (a) 2017, (b) 2018

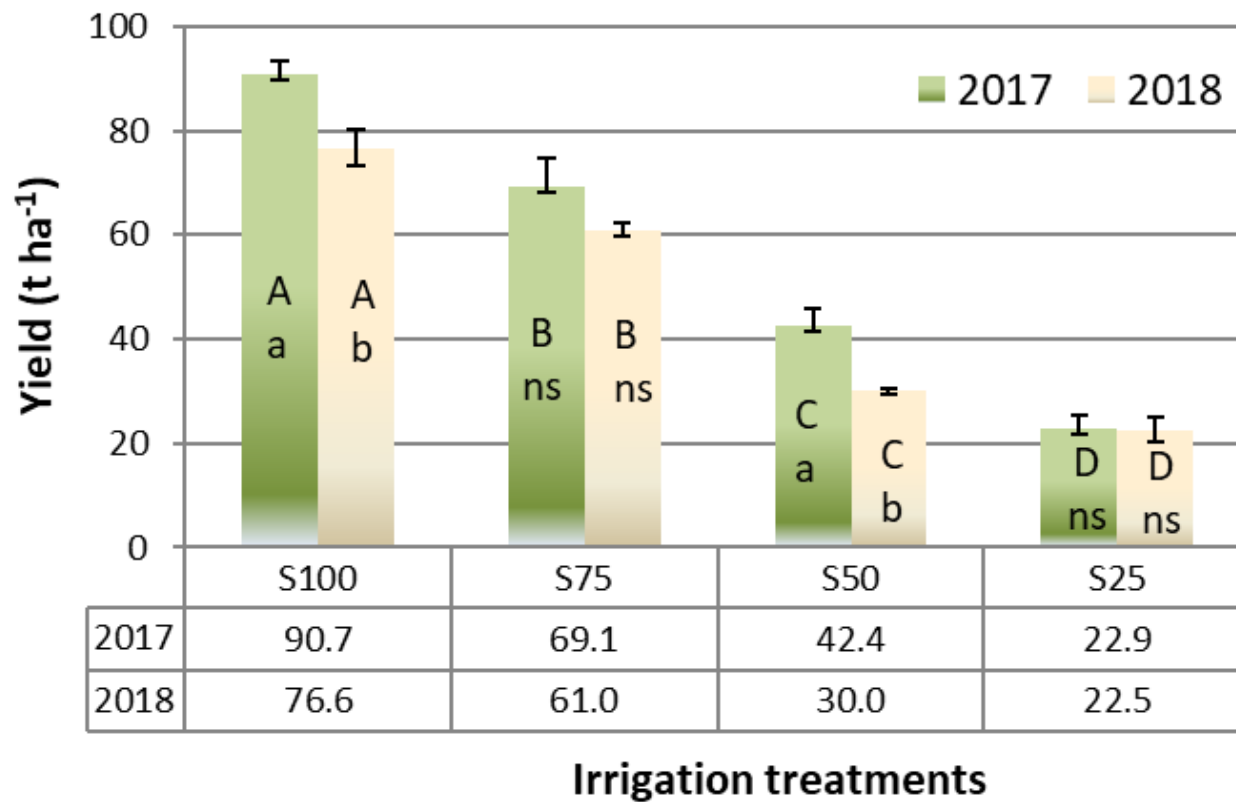


Figure 3

Pepper yields according to irrigation treatments

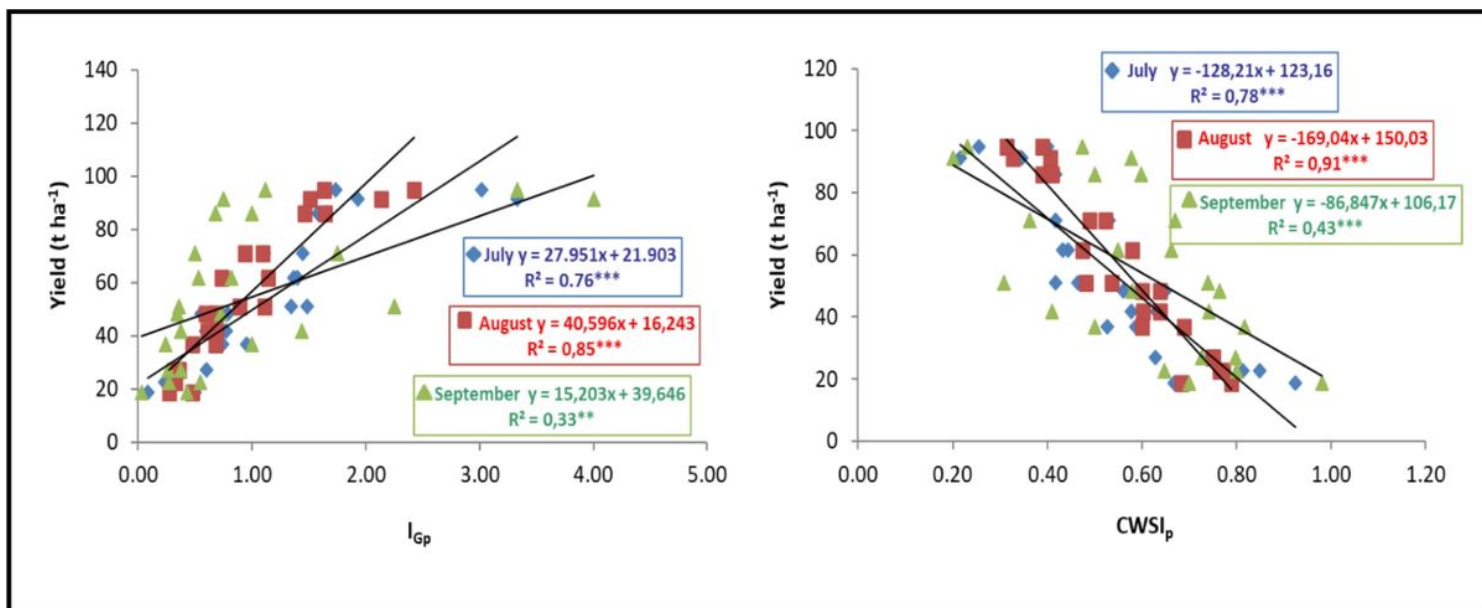


Figure 4

Regression analysis between yield and thermal indices

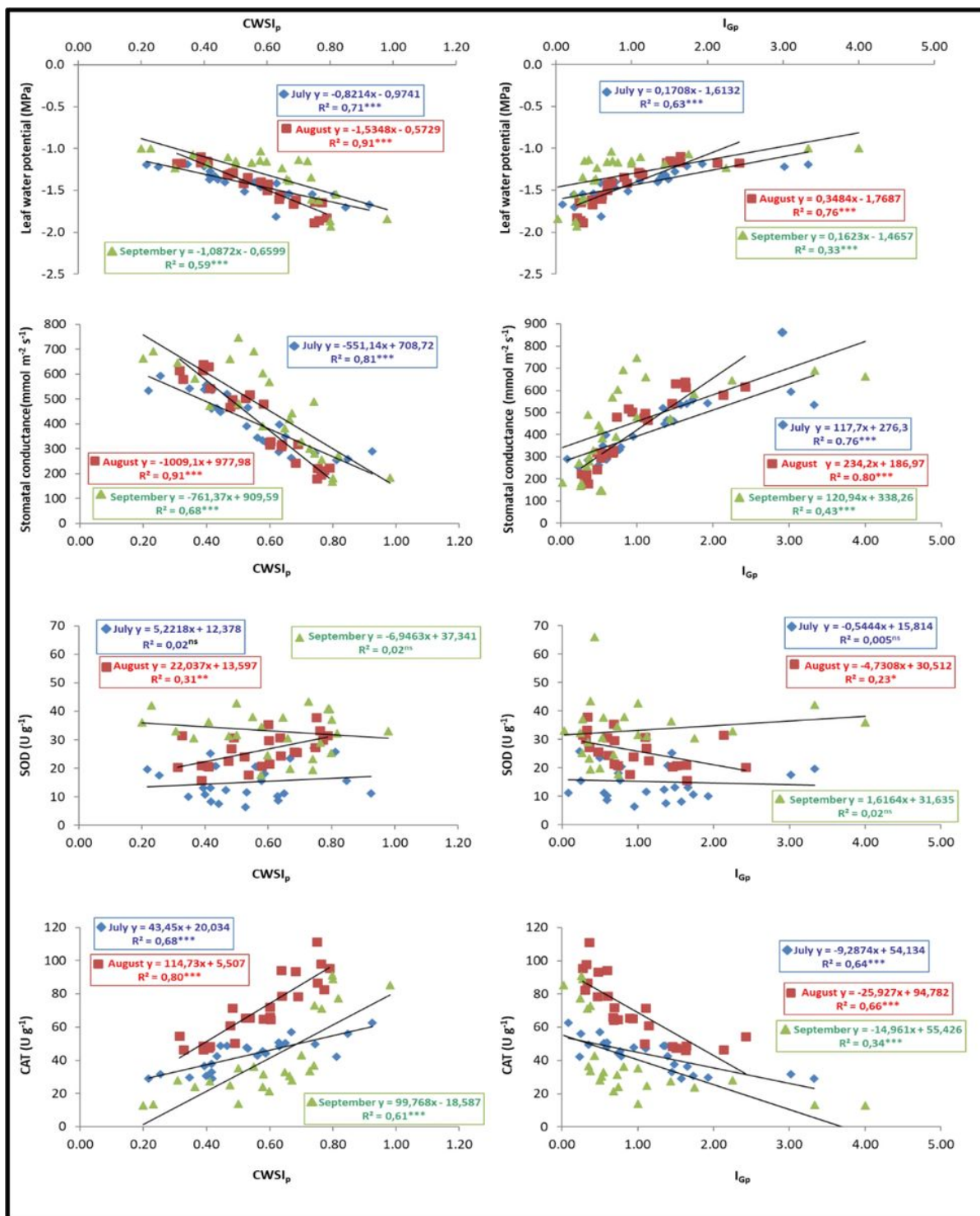


Figure 5

Regression analysis between physiological traits and thermal indices