

Application of infrared thermal imagery for monitoring salt tolerant of wheat genotypes

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Abstract: Salinity is an international problem causing soil degradation and desertification; thereby threaten sustainable crop production in agriculture. Traditional methods of measuring wheat growth have been time-consuming and have often involved in the destructive harvest of plants. Thermal infrared imaging (IR) is now an established technology for the study of stomatal responses and for phenotyping the stomatal behavior under various environmental stresses. Two Experiments were carried out at the experimental farm of El Quantara Shark, Suez Canal University, Ismailia, Egypt to test whether thermal imaging can be used to distinguish between tolerant and sensitive wheat genotypes. Ten bread wheat genotypes differing in salt tolerance were evaluated under two soil salinity levels ($EC = 3.78 \text{ dS m}^{-1}$ and $EC = 8.24 \text{ dS m}^{-1}$). The salinity stress created a wide range in leaf temperature, stomatal conductance (I_G) and Crop Water Stress Index (CWSI) between tested genotypes. Proline content increased by 13 fold in landraces 1, 2, 3 and 10 folds in genotypes Sakha 8 and Kharchia due to salinity stress. There were inverse correlation between canopy temperature and CWSI ($r = -0.31$) and I_G ($r = 0.53$). Thermal imaging can distinguish between salinity stress and non-stress canopies. Therefore, the application of these technologies and methodologies for efficient salinity managements in wheat open new opportunities and challenges in future precision farming.

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1. Introduction

Soil salinity is a widespread problem, restricting plant growth and biomass production especially in arid and semiarid areas. Over 6% of the world's land area is affected by salt, either by salinity or sodicity (FAO, 2010). Salinity is one of the most significant environmental issues facing Egypt agriculture today. Salt accumulation in irrigated soils is one of the main factors that diminish crop productivity. Egypt is one of the countries that suffer from severe salinity problems. For example, 33% of the cultivated land, which comprises only 3% of total land area in Egypt, is already salinized due to low precipitation (<25 mm annual rainfall) and irrigation with saline water (Ghassemi *et al.*, 1995).

Wheat is the most important and widely adapted food cereal in Egypt. However, Egypt supplies only 40% of its annual domestic demand for wheat (Salam, 2002). Therefore, it is necessary to increase wheat production in Egypt by raising the wheat grain yield. Obviously, the most efficient way to increase wheat yield in Egypt is to improve the salt tolerance of wheat genotypes (Epstein *et al.*, 1980; Shannon, 1997; Pervaiz *et al.*, 2002) because this way is much less expensive for poor farmers in developing countries comparing with other management practices (e.g. leaching salt from the soil surface etc., (Qureshi and Barrett-Lennard, 1998)

Estimation of canopy temperature is an important aspect of plant water status monitoring. Infrared thermography is widely used to acquire temperature data by observing the radiation signal of an object and representing the canopy temperature by different color on a camera screen. This approach can rapidly measure canopy temperature over large areas and is a nondestructive, rapid and reliable method for monitoring of whole plant response to water stress. Measured of canopy temperature (T_c) by infrared thermal camera as an indicator of plant water status is based on the principle that plant stomata closure take place during water stress (Christoph *et al.*, 2002).

Leaf temperature of plants is the result of external and internal (physiological) factors. Measurement of leaf temperature using thermal infrared (IR) sensing is primarily used to study plant water relations, and specifically stomatal conductance, because a major determinant of leaf temperature is the rate of evaporation or transpiration from the leaf. The cooling effect of transpiration arises because a substantial amount of energy is required to convert each mole of liquid water to water vapor, and this energy is then taken away from the leaf in the evaporating water and, thus, cools it. The environmental factors solar radiation, air temperature, and relative humidity (RH), and the water status of the shoot tissue determine the temperature of plants via

stomatal transpiration. There is a correlation between leaf temperature and water status, as water is the primary source of infrared absorption in plant tissue (Serraj *et al.*, 2009). Thermal imaging is, therefore, particularly well suited for screening plants for differences in stomatal conductance. Also, one of the best known indices for evaluating crop water stress is the Crop Water Stress Index (CWSI), which expresses the difference between ‘well-watered baseline’ and ‘total stress’ temperatures and is normalized against vapor pressure deficit (Jones *et al.*, 2002; Cohen *et al.*, 2005).

More researches have been developed in attempts to improve the sensitivity of infrared estimation of crop stress indices by the use of either dry (Qiu *et al.*, 1996) or wet and dry (Jones *et al.*, 1997; Jones 1999) reference surfaces. Among a number of indices derived for stress was (I_G), which is proportional to the leaf conductance to water vapor transfer: $I_G = (T_{dry} \pm T_1) / (T_1 \pm T_{wet})$ where T_1 is the temperature of the transpiring surface, T_{wet} is the temperature of a corresponding wet surface, T_{dry} is the

temperature of a similar but non-transpiring surface (Meron *et al.*, 2003; Moller *et al.*, 2007). The thermal camera and its software are able to automatically compensate for the reflected radiation and the emitted radiation from the atmosphere, which can be used for calculation stress index, stomatal conductance and temperature reflected from the surrounding. Therefore, the objective of this study was to assess the potential of infrared thermal images for detection salinity tolerance in wheat and to characterize the practical issues to be addressed in evaluating crop water status based on leaf temperature measurements

2. Material and Methods

Two field experiments were conducted at the experimental farm at El Qantra Shark, Ismailia governorate, Egypt in two sites differed in salinity levels during 2012/2013 and 2013/2014 to investigate the behavior of ten wheat genotypes under field condition. The first site had EC about 3.78 dSm⁻¹ (non salinity) and the second site had EC= 8.24 dSm⁻¹ (salinity stress) as shown in Table (1).

Table 1. Soil chemical properties of the selected three sites.

Location	CaCO ₃ %	EC dSm ⁻¹	pH 1:2.5	Cations meq/l				Anions meq/l			
				Ca ²⁺	Mg ²⁺	Na ⁺	K ⁺	Cl ⁻	HCO ₃ ⁻	SO ₄ ²⁻	CO ₃ ²⁻
El Qantra Shark 1	0.411	3.78	7.59	5.00	3.50	18.7	0.60	17.00	2.50	8.30	0.00
El Qantra Shark 2	1.510	8.24	7.95	30.0	38.5	76.9	0.95	105.0	3.50	37.8	0.00

Plant Materials

Ten bread wheat genotypes (*Triticum aestivum* L.) six local varieties Giza 168, Giza 163, Maser 1, Gemmeza9, Sakha 69, Sakha 8 with three landraces were collected from saline soil in Sahel El Tinna south Port Said governorate and one introduced genotype from India (Kharchia) which were used in the two sites to find speed and ease technique for screening wheat genotypes for salinity tolerance.

Growth Measurements

Ten guarded plants were randomly chosen from each plot to determine shoot length, days to 50% heading, spike length, 1000-kernel weight, grain yield per square meter and harvest index.

Chlorophyll content measurements

Leaf chlorophyll content was determined using a hand-held SPAD 502 meter (Minolta, Osaka, Japan). Average SPAD chlorophyll readings were calculated from 10 measurements from the leaf tip to the leaf base. The measurements were made before anthesis

Proline determination

Proline was determined in fully expanded leaves before anthesis according to Pesci and Beffagna (1984). The samples (50 mg dry weight) were extracted with 10 ml of 3% sulphosalicylic acid

solution for 1 hour at room temperature and filtered on Whatman fiber glass paper. A part of extract was added to 4 ml of ninhydrin reactive (1.25% w/v ninhydrin, 2.4 M phosphoric acid in acetic acid) and 4 ml of acetic acid and incubated in boiling water for 1 hour. After fast cooling in ice, the samples were added to 5 ml of toluene and strongly shaken. The toluene phase, containing the colored complex was used to measure the absorbance at 515nm versus toluene. From obtained absorbance values it has been calculated the proline amount of each sample by means of a calibration curve, made by starting from known amounts of proline.

Salt tolerance index (STI)

Salt tolerance index (STI) was calculated for the grain yield of each genotype as: $STI = Y_s / Y_c$. Where, Y_s is the mean of the genotype under salt stress and Y_c the mean of genotype under control condition

Thermal Image Acquisition

Thermal images of the plots were taken with infrared thermal camera Ti-32 (Fluke Thermography, Germany) equipped with a 320 x 240 pixel microbolometer sensor, sensitive in the spectral range of 7.5–13 μm. The canopy height was about 1 m

Images were analyzed in Ti-32 Pro software (Infrared Solutions); Emissivity for measurements of leaves and plant canopies was set at 0.96 while transmission correction was 85 %. For more accuracy, the span of auto adjusted thermal image is manually set, in addition to level of the displayed as an important camera feature in order to detect maximum and minimum temperature of the entire display (Wilcox and Makowski, 2014).

Thermal Indices and Stomatal Conductance

Where individual leaves were imaged in 2013/2014, dry and wet references were used to mimic leaves with fully closed and fully open stomata, respectively (Jones *et al.*, 2002). These references were determined for wheat leaves, cut from the canopy prior to measurements and placed close to the leaves of interest. Wet reference leaves were sprayed with water on both sides, regularly, to maintain their moisture. Dry reference leaves were covered in petroleum jelly (Vaseline) on both sides. The temperatures of these references were obtained (T_{dry} and T_{wet}) and used in conjunction with leaf temperatures to obtain thermal indices

The index IG was proportional to the leaf conductance to water vapor transfer which was calculated from leaf temperatures as follow:

$IG = (T_{dry} - T_{leaf}) / (T_{leaf} - T_{wet})$. This index is theoretically proportional to stomatal conductance (gs) (Cohen *et al.*, 2005). An index analogous to Idso's (1982) crop water stress index (CWSI) was also calculated, where in this case

$$CWSI = (T_{dry} - T_{leaf}) / (T_{dry} - T_{wet}).$$

Statistical analysis

The experiment was conducted in a randomized complete block design with three replicates. Each field trial was analyzed separately, because of differences in salinity level. Data were statistically analyzed using the appropriate analysis of variance according to Steel *et al.* (1997). Combined analysis of variance over the two seasons was applied after using the homogeneity test. A computer program Genstat 8 Rel.PL16 was used for analyzing data.

3. Results and Discussion

Ten wheat genotypes chosen on the basis of their different salinity tolerance were grown in two field experiments which had EC about 3.78 ds m^{-1} (non salinity) and $EC = 8.24 \text{ ds m}^{-1}$ (salinity stress). Salinity stress had significant effects on plant height, number of effective tillers, spike length, 1000 kernel weight, grain yield/plant, harvest index, SPAD value and concentration of Na^+ , K^+ , K^+/Na^+ ratio, total (Table 2). The ten wheat genotypes showed various behaviors for salinity index of different traits. A significant depressive effects of salinity stress on behaviors of wheat genotypes was detected. Reduction of heading

date is an escaping important adaptive mechanism and is usually the first strategy for a plant adopts when water becomes limiting. The result indicated that Na^+ concentration ranged from 60.2 to 116.7 mg g^{-1} , where, K^+ ranged from 52.5 to 62.2 mg-1/g (Table 2). The Results could be indicated that both salt-sensitive and salt-tolerant genotypes had a higher Na^+ accumulation in whole plant and organs than control when Na^+ accumulation was investigated in genotypes under salt stress. Colmer *et al.* (2006) with wheat, reported similar results and indicated that the distribution of Na^+ ions varies among organs and tissues of green parts of plants that tolerate salt well. They also noted that transportations of Na^+ ions into young leaves were limited because the plants keep them in old leaves, which is known as the most desirable characteristics of salt-tolerant plant

Confirming what has already been observed under early salinity stress and non-stress conditions, it could be concluded that C.V (%) was higher under stress salinity than non-stress for all the studied traits. These results indicated that salinity condition raising the non-uniformity in testing conditions (Table 2).

Proline content (Pro) increased due to salinity stress by 13 fold in landraces 1, 2, 3 while it increased by 10 folds in genotypes Sakha 8 and Kharchia due to salinity stress (Fig. 1). These wheat genotypes had the ability to synthesize osmoticants such as proline to prevent the damage due to soil water deficits and salinity (Grant *et al.*, 2007).

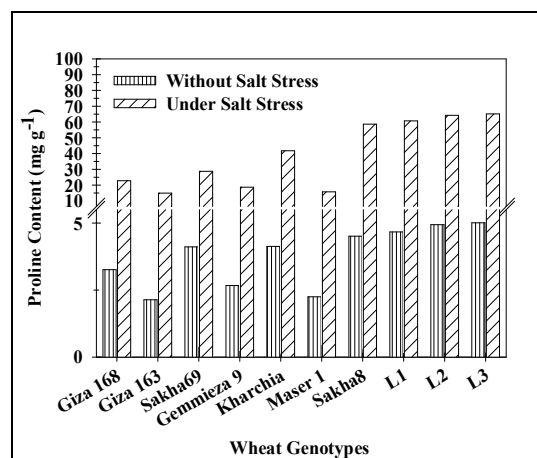


Figure (1) Changes in proline accumulation mg/g in wheat genotypes exposed to salinity stress

Furthermore, proline may play a role as an enzyme-stabilizing agent and has the ability to mediate osmotic adjustment, stabilized sub-cellular structure and scavenge free radicals (Hassanien, 2004). An important component of the plant response to salinity is the accumulation of solutes compatible with cellular metabolism, which are thought to play a

central role in the osmotic adjustment. Proline is, with glycine betaine, the most common osmolyte accumulated in water stress conditions and since many years the accumulation of these compounds is thought to represent an important adaptive response to

salinity stress (Mattioni *et al.*, 1997). The accumulation of osmolytes was also investigated during salinity stress in durum wheat. Proline was strongly up-regulated by water stress, the proline content increased about 20times in plants stressed

Table (2): Mean, range and coefficient of variability over the two years of wheat genotypes under two levels of salinity for the studied characters

Traits	Salt treatments	Mean	Range	CV (%)	Salinity index
Heading date (days)	Non Saline	100.7	98.7 -110.7	8.17	0.95
	Saline	95.9	81.0 -109.0	9.15	
Plant height(cm)	Non Saline	77.7	117.8- 129	15.19	0.85
	Saline	66.2	92.3-110.1	18.8	
No. of effective tillers	Non Saline	2.2	3.4 – 5.5	24.3	0.63
	Saline	1.4	2.3 -13.3	27.6	
Spike length (cm)	Non Saline	12.6	9.3 -13.2	10.31	0.76
	Saline	9.6	8.2 – 11.2	10.7	
1000-kernel weight(g)	Non Saline	38.5	33.1 -45	11.83	0.83
	Saline	32.2	29.5 -36.1	9.2	
Grain yield (g m ⁻²)	Non Saline	564.2	550.6 -756.4	14.23	0.77
	Saline	436.9	401.2 -555.3	24.4	
Na ⁺ (mg g ⁻¹)	Non Saline	50.9	41.3-78.5	21.3	0.57
	Saline	89.1	60.2-116.7	24.9	
K ⁺	Non Saline	44.5	44.1-48.0	9.06	0.86
	Saline	51.6	52.5-64.2	12.8	
K ⁺ / Na ⁺	Non Saline	0.79	0.74- 0.84	19.81	0.73
	Saline	0.58	0.61- 0.67	20.98	
SPAD value	Non Saline	51.1	67.1-75.9	12.1	0.65
	Saline	38.1	22.5 -48.9	19.5	
Harvest index	Non Saline	51.9	39.1 - 60.6	10.7	0.81
	Saline	42.4	- 45.433.2	22.3	

Estimation of stomatal conductance (I_G) and crop water stress index (CWSI)

Leaf canopy temperature was used to calculate stress index (CWSI) and stomatal conductance (I_G) for wheat genotypes (Table 3). The genotypic differences in canopy temperature increased under high salt concentrations. The salinity created a wide range of water statuses in wheat genotypes as reflected by leaf temperature, stomatal conductance and Crop Water Stress Index (CWSI). Our study had highlighted the link between higher stomatal conductance, cooler canopies, and increased photosynthesis as SPAD value which leading to higher yields in wheat under salinity stress environments. Leaf temperature is an indicator of stomatal conductance, automating the analysis of thermal images acquired with long-wave infrared (IR) sensors. The I_G ranged from 0.32 (L3) to 0.71 (Giza 168), while the CWSI ranged from 0.242 (L3) to 0.416 (Gemmeiza 9). The landraces 2 and 3 showed the lower values of I_G (0.35 and 0.32) along with lower CWSI (0.258 and 0.242), respectively. However, Giza 168 and Gemmeiza 9 had higher I_G

and CWSI (Table 2). Stomatal conductance can be a reliable indicator of growth rate response to stress, and thermal imaging is a possible screening method for both the laboratory and field. Higher stomatal conductance accompanied with higher transpiration, evaporation, CWSI and lower photosynthetic pigments in the most sensitive genotypes to salinity. Tolerant (small stomatal response) genotypes could be useful for planting in salinity conditions. Sensitive (large stomatal response) genotypes could be useful for non stress environments.

Use of remote imagery, when combined with effective image analysis techniques, provides a powerful approach to the comparison of large numbers of genotypes in typical field situations. It has also been widely suggested that thermal imaging can be used as a component of a remote sensing system for diagnosing plant stresses. In the case of thermal imaging, it was used for providing information on changes in stomatal opening. In its turn, stomatal aperture can be affected by a wide range of primary stresses, including water deficit, flooding, salinity,

pollutants as well as biotic stresses such as pests and diseases. It follows that use of thermal sensing for stress diagnosis, as opposed to simple monitoring where the stress is well defined, ideally requires the

combined application of one or more other imaging techniques in a multi-sensor approach where thermal sensing is combined with reflectance, fluorescence and other sensing techniques (Chaerle *et al.*, 2007).

Table 3. Average temperatures (°C) and stress indices for wheat genotypes under salinity

Genotypes	T _{leaf}	T _{wet}	T _{drv}	I _G	CWSI	SPAD value
Giza 168	25.6	19.1	30.2	0.71	0.414	30.1
Giza 163	26.5	22.1	29.4	0.66	0.397	29.5
Sakha69	28.4	24.1	31.1	0.63	0.386	35.6
Gemmieza 9	23.2	18.7	26.4	0.71	0.416	25.4
Kharchia	29.3	22.3	32.9	0.51	0.340	36.4
Maser 1	26.7	21.4	30.1	0.64	0.391	34.3
Sakha8	30.5	25.2	33.4	0.55	0.354	44.1
L1	22.2	18.4	24.2	0.53	0.345	41.5
L2	22.3	5.7	24.6	0.35	0.258	42.9
L3	26.9	20.0	29.1	0.36	0.242	44.8
Mean	26.2	20.7	29.1	0.56	0.354	36.0

Table 4. correlation matrix among the studied traits

Traits	Yield	SI	K ⁺ /Na ⁺	LAI	SPAD value	CWSI	I _G	T _{leaf}
Yield	-	0.70**	0.88**	0.73**	0.98**	-0.85**	-0.87**	0.55*
SI		-	0.96**	0.45	0.66*	-0.3	-0.34	0.50*
K⁺/Na⁺			-	0.67*	0.87**	-0.93**	-0.91**	0.45
LAI				-	0.73**	-0.49*	-0.51*	0.60*
SPAD value					-	-0.82**	-0.85**	0.47
CWSI						-	0.99**	-0.31
I_G							-	-0.53*
T_{leaf}								-

The association between leaf temperature and other indices

The effect of the association of stomatal conductance (IG), crop water stress index (CWSI) and canopy temperature on wheat yield was shown in Table (4). There were inverse correlation between canopy temperature and CWSI ($r = -0.31$) and IG ($r = 0.53$), suggested that temperature differences between canopies might relate to canopy density rather than stomatal conductance alone cannot be ruled out. A reduced leaf area would result in a reduced area of transpiring surface per area of canopy in a thermal image, which may lead to a lower estimate of conductance ($r = -0.51$), even if the conductance per leaf is the same. Decreased leaf density in non-irrigated vines means that the average canopy temperature could be higher than the average of a similarly transpiring but denser canopy. The correlation coefficients between salinity index (SI) and leaf temperature were positive and significant (0.50). The previous results indicated that thermal sensing could be used efficiently to study plant water relations, and specifically stomatal conductance, because a major determinant of leaf temperature is the rate of evaporation or transpiration from the leaf. The cooling effect of transpiration arises because a substantial amount of energy is required to convert liquid water to water vapour, and

this energy is then taken away from the leaf in the evaporating water and therefore cools it.

Infrared thermal image

Thermal image allows the visualization of differences in surface temperature by detecting emitted infrared radiation [long-wave infrared (8–14 μm)]. Computer software transforms these radiation data into thermal images in which temperature levels are indicated by a false-color gradient. Infrared thermography can give frequency distributions of leaf temperature over the target area. A one-shot thermograph has more than 70,000 pixels, each pixel with visual temperature information of sensitivity < 0.1°C. The canopy temperature difference of approximately 8°C in this study is large enough to visually detect transpiration changes in foliage of the genotypes and instantly monitor the soil water stress of plant growth Figs. 1 and 2. The frequency distributions ranged from 18°C to 26°C which were clearly different with the some genotypes under salinity to have a greater variance of temperature (Fig. 2). Thus, infrared thermography has great potential as a tool to instantly monitor water stress in fields.

Exposed wheat plants to salinity and non salinity under field conditions could be recognized by thermal image. The results of canopy temperature for the most tolerant genotypes (L3 and Sakha 8) indicated that mean temperature of thermal image for both genotypes

under salinity conditions was higher than those non salinity experiment by about 5 °C (Fig. 3 A and B, and Fig. 4C and D). These examples illustrated the different distributions of canopy temperature between stresses and non stressed conditions, with salinity stress displaying a far wider range of canopy temperature variation, whether viewed down the normal.

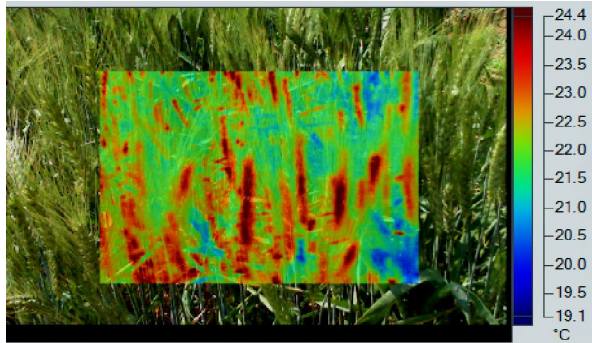


Fig. (2) A visible image together with a corresponding thermal image for canopy temperature of wheat genotypes under salinity stress

Modifications in the water status of a plant caused by adverse conditions lead to changes in leaf transpiration as a result of active regulation of stomatal aperture. The associated changes in patterns of leaf cooling can be monitored instantly and remotely by thermographic imaging.

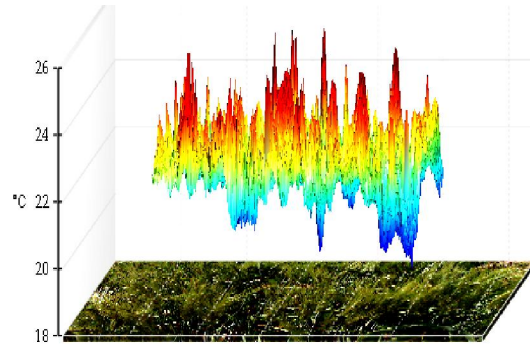


Fig. (3). Thermal image of frequency distribution over the selected area of wheat genotypes

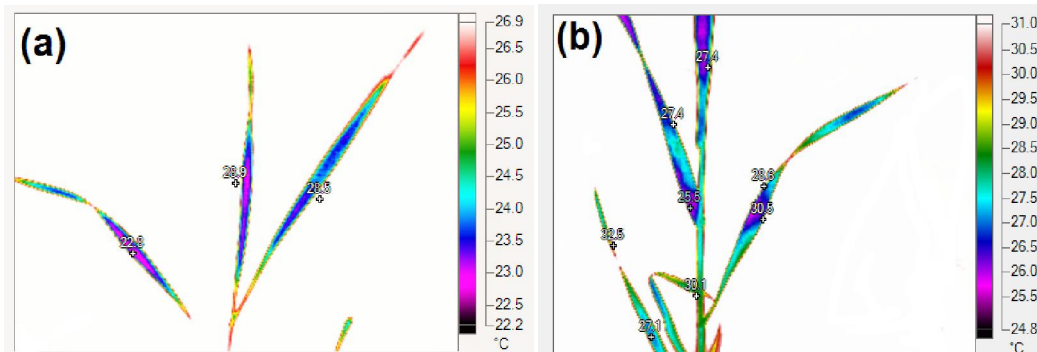


Fig.(4). Infrared thermal leaves image for landrace 3 under non salinity (A) and salinity stress (B)

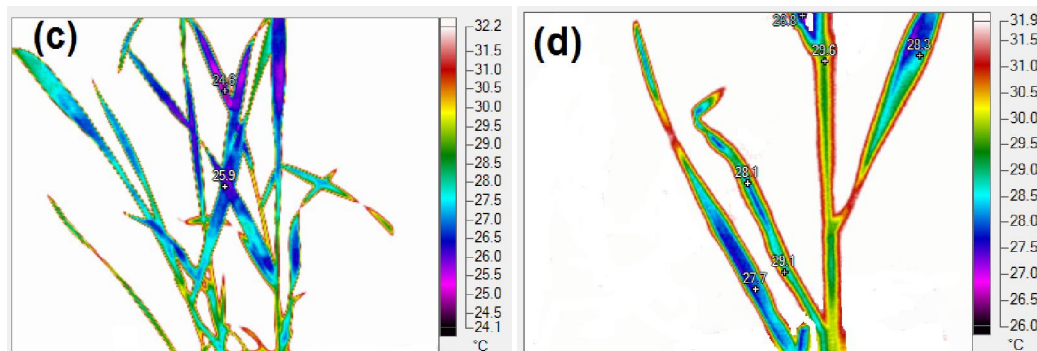


Fig.(5). Infrared thermal leaves image for Sakha 8 under non-salinity (C) and salinity stress (D)

For genetic improvement of salinity tolerance in wheat, canopy temperature has been expected to be a useful physiological parameter to screen genotypes for tolerance to water stress and for yield potential, but it is strongly influenced by environmental conditions

(Leinonen *et al.*, 2006 and Chaerle *et al.*, 2007). Canopy temperature is also phenotypically and genetically associated with grain yield under salinity stress (Olivares-Villegas *et al.*, 2007, Reynolds *et al.*, 2007), with lower canopy temperature expected to

indicate higher grain yield under a given water stress. However, water stress frequently fluctuates with genotypic variation of root water uptake. The ability to take up water from the soil under increasing water deficit may also be a critical factor for remobilizing assimilates to the grain in the latter half of plant growth. The use of canopy temperature for screening requires uniform water stress conditions because canopy temperature responds directly to fluctuating water stress.

Conclusion

A handheld thermal camera was used to detect the temperature of wheat canopies at El Qantra Shark, Ismailia governorate, Egypt in two sites differed in salinity levels. The results showed that the various salinity treatments had significantly different temperatures, suggesting that crop thermography can be an effective tool in practicing canopy temperature-based salinity level. Although the commonest approach to the use of leaf temperature is the calculation of a stress index or the direct estimation of stomatal conductance, there has been some interest in making use of the variability in leaf temperature as a measure of stomatal opening. This approach depends on the increasing contribution of the radiative component to the heat balance as stomata close and evaporation decreases. In our experiments with wheat genotypes, differences between saline and non saline (control) plants in leaf temperature, CWSI and I_G were detectable at the same trend of photosynthetic pigments and proline accumulation, indicating that thermal imaging can be used as useful as more traditional methods for detecting the initiation of stress. The application of these technologies and methodologies for efficient salinity managements in wheat yard open new opportunities and challenges in future precision farming. Thermal and chlorophyll fluorescence imaging can reduce the time needed for screening-based estimation of growth performance. Adapting wheat genotypes to salt stress is a promising breeding technology for mitigating salinity conditions and increasing crop productivity.

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