

Assessment of sunflower water stress using infrared thermometry and computer vision analysis

Atefeh Nouraki, Samira Akhavan, Yousef Rezaei and Sigfredo Fuentes

ABSTRACT

The objectives of the current study were to implement affordable and non-invasive measurements of infrared thermometry, leaf relative water content (RWC), crop water stress index (CWSI), leaf area index (LAI) from computer vision analysis and seed yield of sunflowers. The experiment was designed as split-plot based on randomized complete blocks with three replications. Treatments were four different levels of deficit irrigation as the main plots and three fertilization treatments were applied as sub-plots. Results showed a significant effect ($P \leq 0.01$) of water stress and fertilizer on CWSI during different stages of sunflower growth. Changes in fertilizer amount and type resulted in a change in lower (dT_{LL}) and upper (dT_{UL}) limits of canopy-air temperature difference. A combination of chemical fertilizer with biofertilizer could help to decrease CWSI. From computer vision analysis, the normalized difference red blue index (NDRBI) had a strong linear relationship with RWC and CWSI for sunflowers (R^2 of 0.87 and 0.93, respectively) and the normalized difference green blue index (NDGBI) had a linear relationship with seed yield ($R^2 = 0.79$). Therefore, analysis of digital RGB images and CWSI were efficient, non-destructive and low-cost methods to assess crop water status for sunflowers under different irrigation and fertilizer treatments.

Key words | crop water stress index, digital image processing, leaf area index, seed yield, vegetation indices

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HIGHLIGHTS

- The CWSI values were sensitive not only to different irrigation regimes but also to amount and type of fertilizer.
- The CWSI can be derived from the plant index (NDRBI) and used for appropriate irrigation scheduling.
- A positive correlation was observed between image indices with CWSI and LAI.
- Combination of chemical fertilizer with biofertilizer could help to decrease CWSI.

INTRODUCTION

Water resources are under pressure due to the rapid increase in world population, natural resources pollution and climate change. As water scarcity increases and more

agricultural water is diverted to other sectors, advanced technology, methods and equipment must be used for irrigation systems and scheduling so that the limited water resources will meet the needs of farmland irrigation (Wenting *et al.* 2014). These methods could be classified as destructive (e.g. stem water potential using a pressure bomb, non-destructive (e.g. infrared thermography (Cohen

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et al. 2005)/thermometry (Jackson 1982)), expensive requiring high technology and know-how (e.g. using a large number of sensors for precision irrigation or cameras for thermal infrared/multispectral/hyperspectral as payload of unmanned aerial/unmanned aerial and terrestrial vehicles (UAV, UTV respectively) (Gago et al. 2015), and low-cost (e.g. digital camera (Zakaluk & Sri Ranjan 2008)). Among these methods, infrared thermometry techniques measure canopy temperature non-invasively by measuring reflected long wave radiation from plant canopy surfaces and have high temporal resolution to monitor plant water stress (Cohen et al. 2005). To transform canopy temperature data into information on plant water status, many indices have been proposed. One of the most common and popular indices is the crop water stress index (CWSI) (Cohen et al. 2005; Durigon & van Lier 2013), which is based on the canopy and air temperature differences. Previous researchers have shown that CWSI is a useful indicator for the water stress monitoring of different crops, in arid and semi-arid conditions, such as for soybeans and cotton (O'Shaughnessy et al. 2011), red paper (Sezen et al. 2014) and sunflowers (Orta et al. 2002; Taghvaeian et al. 2014). Moreover, CWSI values are influenced by not only water conditions but also management practices and environmental factors such as fertilizer type and dosage. A few studies have documented the interaction of CWSI and crop nutrient status (Carroll et al. 2017). It should be emphasized that these measurements (canopy measurements (T_c) and leaf relative water content, RWC) were time-consuming and required a large amount of manual sampling to describe a whole field (Jackson 1982). Therefore, these techniques are considered less effective, frequently challenging, and time and resource consuming (Lee & Lee 2013).

Color digital image processing within the visible (400–700 nm) region through computer vision algorithms has been used with some success in the diagnosis of crop growth and water status as a low-cost and efficient tool (Lee & Lee 2013). Many studies have used spectral vegetation indices to establish a correlation with crop water status (Zakaluk & Sri Ranjan 2008; Elazab et al. 2015), leaf area index (LAI) (Liu & Pattey 2010; Wang et al. 2013; Poblete-Echeverría et al. 2015) and yield (Elazab et al. 2015).

Overall, farmers can monitor crop growth parameters (such as LAI and yield), crop water status (such as leaf

RWC and CWSI) using inexpensive equipment (digital camera and infrared thermometer) and computer vision techniques at the canopy level for a sunflower crop under different management. However, more information is required related to the application of the mentioned approach to detect crop growth and water status of sunflowers at the canopy level under different management practices and specifically the effect of type and amount of fertilizer on CWSI. The main objectives of this study were: (1) determine upper and lower baselines for calculating CWSI under fertilizer treatments in semi-arid conditions; (2) evaluate the ability of reflectance-based indices to detect water stress in comparison to plant measurements; (3) determine the relations between image feature parameters and LAI and seed yield; (4) determine the effect of water stress during the growing season on RWC, LAI and sunflower yield.

MATERIALS AND METHODS

Study area and experimental treatments

The field experiment was carried out during the summer from August to October 2015. The pilot farm is located in Aghile Agricultural Research Center, located near the city of Shoushtar in Khuzestan province, Iran (32° 27' N, 48° 53' E, 110 m.a.s.l.) (Figure 1). Gotvand weather station data was used to monitor daily weather variables such as air temperature, pan evaporation, relative humidity, rainfall and wind speed. The maximum, minimum and average temperatures, as well as relative humidity values, for the 2015 growing season (August to October) are presented in Figure 2. The average annual rainfall and annual temperature are 371 mm and 25.3 °C, respectively. Some of the soil physical and chemical characteristics for the experimental site are given in Table 1. The irrigation water had pH of 7.5–8 and the average electrical conductivity (EC) of 3.5 dS m⁻¹.

The experiment was set up in a split-plot design, based on randomized complete blocks design with three replicates. The main plots consisted of four levels of deficit irrigation (irrigation after 50, 90, 130 and 170 mm of cumulative evaporation from Class A Pan; I₅₀, I₉₀, I₁₃₀, and I₁₇₀). Deficit irrigation treatments were selected based on previous studies in the area (Soleymani et al. 2016). Total

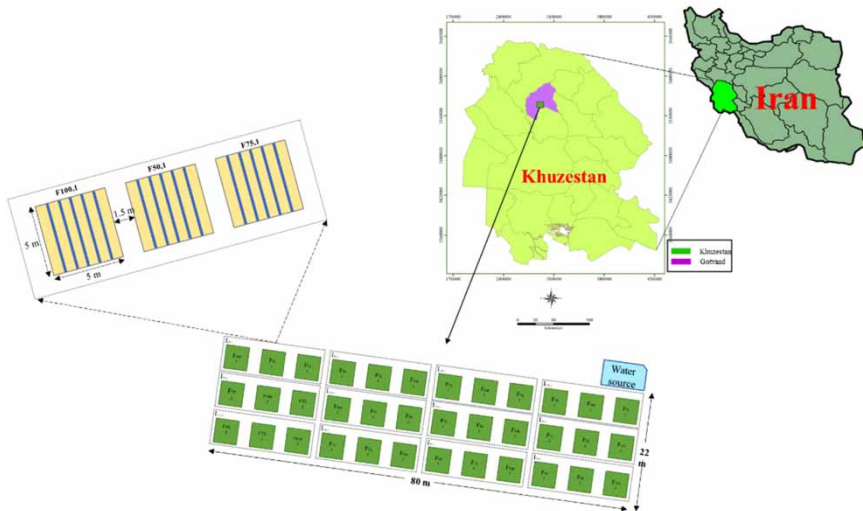


Figure 1 | The location of research farm in northern Khuzestan and plot layout for 2015.

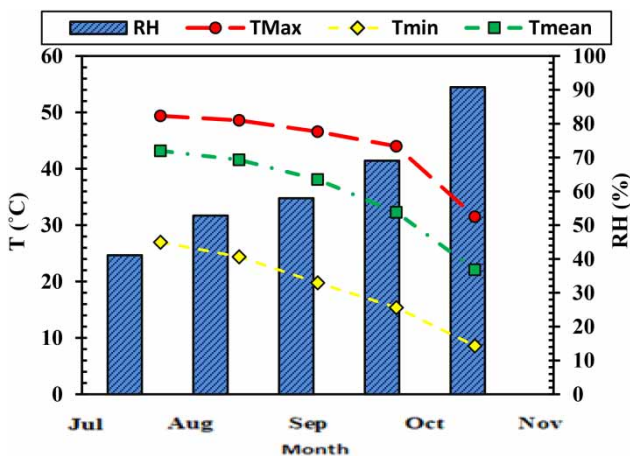


Figure 2 | Meteorological data during the growth period of experimental area (Tmax: maximum air temperature; Tmin: minimum air temperature; Tmean: mean air temperature; RH: relative humidity).

irrigation amounts for I_{50} , I_{90} , I_{130} and I_{170} treatments was 484, 360, 330 and 297 mm, respectively. The irrigation frequency for I_{50} , I_{90} , I_{130} and I_{170} treatments was 15, 11, 10 and 9 times, respectively.

After soil analysis, three fertilization treatments (F_{100} = 100% chemical fertilizer + no biofertilizer; F_{75} = 75%

chemical fertilizer + 100% biofertilizer; F_{50} = 50% chemical fertilizer + 100% biofertilizer) were chosen as subplots. Chemical fertilizer (urea, 46% nitrogen) was applied at planting time and stem elongation stage. Biofertilizer was employed in the form of seed inoculation (before planting) and fertigation, consisting of Nitroxin (*Azospirillum* sp., *Pseudomonas* sp., *Azotobacter* sp.) and bio-super phosphate microbial bio-fertilizer (including *Bacillus coagulans*).

Sunflower Hysun 25 hybrid (*Helianthus annuus* L.) was sown in all plots on August 3, 2015, with a population of 66,700 seeds ha^{-1} and row spacing of 0.75 m. Each plot was 25 m^2 (5 m \times 5 m).

Soil water content (SWC) measurements

Before each irrigation event, soil samples at two depths 0–60 cm (0–30; 30–60 cm) were obtained from the centre of the plots to measure the SWC by the gravimetric method (Black 1965), and the amount of irrigation water was calculated based on the soil moisture deficit (to fill the FC). Also, the irrigation interval was estimated based on the cumulative

Table 1 | Physical and chemical characteristic of experimental field soil

Soil depth (cm)	Field capacity (%)	Soil texture	Wilting point (%)	Bulk density ($g\ cm^{-3}$)	K_2O (ppm)	P_2O_4 (ppm)	$CaCO_3$ (%)	pH	EC ($ms\ cm^{-1}$)
0–30	22.02	Loam	10	1.4	250	9	0.6	8.23	4.54
30–60	23.10	Loam	11.5	1.45	250	8	0.55	8.15	3.44

evaporation from the pan. Equation (1) was applied to calculate irrigation water volume for each treatment, based on the pre-irrigation SWC (Micheal & Ojaha 1987)

$$I = (W_{FC} - W_i) \times \gamma \times D \times A \quad (1)$$

where I is the amount of irrigation water (m^3), W_{FC} is soil water content at field capacity (%), W_i is pre-irrigation soil moisture content (%), γ is soil bulk density ($g\ cm^{-3}$), D is soil depth (0–0.6 m), and A is surface area of the plot (m^2).

Sample collection and plant measurements

The temperature of the sunflower canopy (T_c) was measured with a hand-held infrared thermometer (IRTs, model: Testo 830-T1, KGaA, Inc., Lenzkirch, Germany). Data collection for T_c was initiated from September 3, 2015, when the plant coverage was nearly 75–80%. Foliage temperature measurements were performed from 12:00 to 14:00 h (local standard time and considering clear days). During the growth period, canopy temperature was measured nine times through the season. The VPD was estimated with the standard psychrometer equation (based on dry and wet bulb temperatures) (Monteith & Unsworth 2013).

To calculate CWSI, $T_c - T_a$ is plotted against dT_{LL} and dT_{UL} limits of canopy-air temperature difference which would be obtained under non-water-stressed baseline (NWSB) and non-transpiring baseline (NTB) conditions, respectively (Taghvaeian et al. 2014). Considering empirical equation suggested by Idso et al. (1981), the CWSI was calculated by Equation (2):

$$CWSI = (dT_m - dT_{LL}) / (dT_{UL} - dT_{LL}) \quad (2)$$

where dT_m , dT_{LL} and dT_{UL} are measured, lower limit and upper limit of dT , respectively.

Idso et al. (1981) offered an empirical approach for estimating dT_{LL} and dT_{UL} . According to this method, dT_{LL} for $T_c - T_a$ against the VPD relationship was specified using data collected only after irrigation from the unstressed treatment (irrigation after I_{50}) (Idso et al. 1981). dT_{UL} was computed according to the procedures explained by Idso et al. (1981).

LAI, RWC and seed yield were measured under deficit irrigation and fertilization treatments.

Image acquisition

The imaging procedure was carried out using a 16-Mega-pixel resolution digital camera with 140–280 mm focal length. All the images were taken weekly at Nadir at a fixed distance of 1 m over the sunflower plants. To minimise the variation and shadows, all images were taken at the same time of the day under a similar light condition.

Image analysis

After image acquisition, the pre-processing steps included size reduction, background removal, normalization, noise removal and image binarization to improve image quality and remove unnecessary objects from images.

The first stage was dividing the main image into its three components (Red, Green and Blue) then Equation (3) (Gée et al. 2008) was used to normalize the image bands. The range of new normalized spectral r, g and b components was normalized from 0 to 1.

$$\begin{aligned} r &= R / (R + G + B), \quad g = G / (R + G + B), \quad b \\ &= B / (R + G + B) \end{aligned} \quad (3)$$

The filtering of the sunflower canopy and its background was carried out in the second step. In the visible range of the light spectrum, green vegetation has a reflectance peak in the green wavelength (495–570 nm), whereas there is not any apparent change in soil albedo. Therefore, in this research, the difference between sunflower canopy and the non-canopy area can be enhanced by the greenness index (Liu & Pattey 2010), as defined in Equation (4).

$$Greenness = 2g - b - r \quad (4)$$

where r, g, and b represent the intensity of levels by normalized red, green and blue components in a digital camera. Once a threshold was set, a binary mask image was created. The pixel value = 1 specifies a pixel with a greenness value higher than the threshold indicates a sunflower canopy. A pixel value = 0 represents the background and includes soil and plant residues. Then all the individual masks were applied to the relevant image, so the background was

removed from all images (Figure 3). Also, the color of the sunflower seed head section was sharply different from that of the canopy section and it made noise, so to separate sunflower seed heads, the by threshold method was used.

The digital images were further processed to calculate plant stress indices, which are represented in Table 2. They have been used to estimate the RWC, CWSI, LAI and sunflower yield. Figure 4 shows the basic procedure of the proposed method and image analysis algorithm.

Analysis of variance (ANOVA) was conducted on all observed crop and reflectance data by using SAS 9.2 software (SAS Institute, Cary, NC, USA). In all cases, the coefficient of determination (R^2) and root mean square error (RMSE) were used to assess the most appropriate regression equation.

RESULTS AND DISCUSSION

Baseline equations and CWSI

NWSB is shown in Figure 5 for three growth stages, vegetative, reproductive, and maturation stages as well as the whole of the sunflower season, under different levels of fertilizer. To calculate NWSB and CWSI, regression curves were fitted for mentioned periods to obtain a and b coefficients (Table 3). As shown in Table 3, the range of R^2 for the NWSBs at different stages of growth was observed between

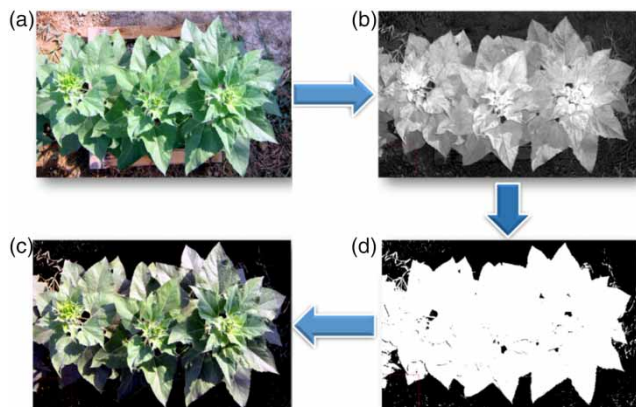


Figure 3 | The RGB image of a sunflower canopy and non-canopy background removal (a) original image, (b) applying the Greenness Index, (c) the binary image mask, (d) the image of sunflower canopy without non-canopy portions (soil and plant residues).

Table 2 | Vegetation indices derived from the RGB images

Index	Name	Equation	Citation
NDGBI	Normalized difference green blue index	$\frac{G - B}{G + B}$	Gitelson et al. (2002)
NDRBI	Normalized difference red blue index	$\frac{R - B}{R + B}$	Gitelson et al. (2002)
NDGRI	Normalized difference green red index	$\frac{G - R}{G + R}$	Gitelson et al. (2002)
GRS	Green red slope transformation	$\frac{G - R}{GPWL + RPWL}$	Zakaluk & Sri Ranjan (2008)
GBS	Green blue slope transformation	$\frac{G - B}{GPWL + BPWL}$	Zakaluk & Sri Ranjan (2008)
RBS	Red blue slope transformation	$\frac{R - B}{RPWL + BPWL}$	Zakaluk & Sri Ranjan (2008)
VARI	Visible atmospherically resistant index	$\frac{G - R}{G + R - B}$	Zakaluk & Sri Ranjan (2008)
SAVI _{Green}	Soil adjusted vegetation index	$(1 + L) \frac{G - R}{G + R + L}$	Li et al. (2010)
CC	Canopy cover	$\frac{g_{ci}(x, y)}{n_{ci}}$	Wang et al. (2013)
GMR	Green minus red	G-R	Wang et al. (2013)

GPWL: peak wavelength (nm) of the green image band, RPWL: peak wavelength (nm) of the red image band and BPWL: peak wavelength (nm) of the blue image band, L: the soil base line, $g_{ci}(x, y)$: percentage of the number of pixels reflecting the canopy, n_{ci} : total of the whole image.

$R^2 = 0.92-0.99$ ($P < 0.01$) and it was about $R^2 = 0.99$ for the whole of the sunflower growing period under different levels of fertilizer. The R^2 values are similar to those reported by previous studies. Idso (1982), Judy (2011) and Taghvaeian et al. (2014) developed NWSB for well watered sunflowers as: $dT_{LL} = 0.66-1.95VPD$, $R^2 = 0.98$ in Kansas, $dT_{LL} = 1.36-0.19VPD$, $R^2 = 0.93$ in South Khuzestan and $dT_{LL} = 1.67-2.00VPD$, $R^2 = 0.99$ in northern Colorado, respectively.

The calculated a and b of NWSB equations for well water sunflowers were partly different from reported values by other researchers (Idso 1982; Judy 2011; Taghvaeian

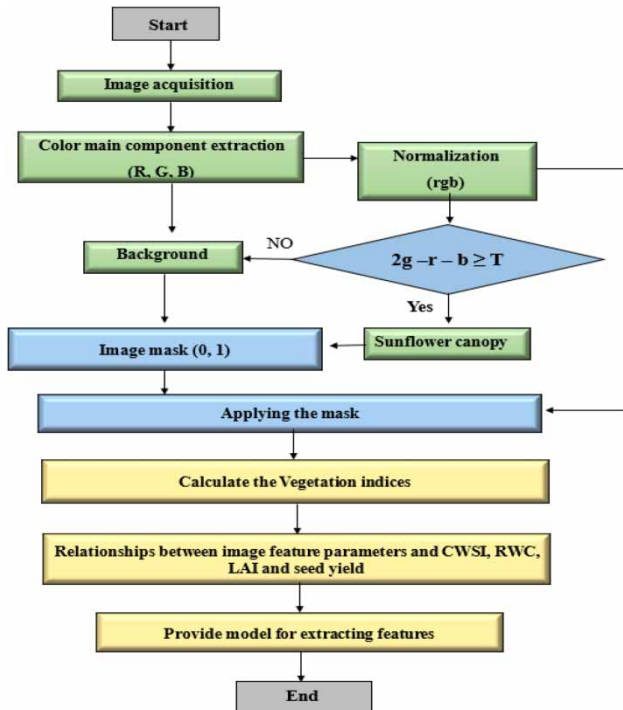


Figure 4 | Flowchart of the proposed image processing method.

et al. 2014). These differences could be related to the different type of climate, crop variety, management, soil and water conditions. Also, these differences confirm that this equation needs to be validated for region and crop type.

As seen from Table 3, under the fertilizer control treatment (F_{100}), the average dT_{UL} during the growing season was reported at 3.56°C for the whole sunflower crop season. Judy (2011) obtained dT_{UL} of 4.60°C and 4.42°C estimated for April and May, respectively, in South Khuzestan, Iran. The differences in reported NTB values by previous studies come from different climates, management and crop varieties.

According to Table 3, the variation in the a is greater than the b under different fertilizer treatments. Based on the findings reported in Table 3, NTB values increased from 3.14°C at F_{50} treatment to 4.55°C at F_{100} treatment in the vegetative stage, from 2.83°C at F_{50} treatment to 3.81°C at F_{100} treatment in the reproductive stage, and from 1.57°C at F_{50} treatment to 2.56°C at F_{100} treatment in the maturation stage. These results show that changes in fertilizer dosage and type (management) resulted in a change in dT_{LL} and dT_{UL} estimates, these changes might

be due to the effect of nitrogen chlorophyll content in leaves, leading to more green reflectance and higher transpiration rate (lower leaf temperature and higher difference in air temperature), especially in periods with higher atmospheric demand.

Sunflower CWSI was estimated for the 12:00–14:00 hourly period, considered as the highest CWSI values (Sezen *et al.* 2014). Table 4 indicates variations of the CWSI for each irrigation treatment during each of the vegetative, reproductive, and maturation stages and for the whole sunflower season. The results of analyzing data variance showed a significant effect of drought stress on CWSI ($P < 0.01$) during different stages of growth (Table 4). It can be seen from Table 4 that sunflower CWSI values increased with increase in water stress level in all three growth stages. Previous studies also found that reduction of water application amount caused CWSI to increase (Sezen *et al.* 2014). Furthermore, in the maturation period the values of CWSI were higher than those of the vegetative and reproductive stages under all water treatments. It seems that during the maturation stage, the treatment effects became more significant. At this stage, sunflowers experienced more water stress, since the irrigation frequency decreased.

The fertilizer treatments also had a significant effect on CWSI values in the vegetative stage and through the whole sunflower growing period ($P < 0.01$). By contrast, a non-significant effect of fertilizer treatments on CWSI values was observed in the reproductive and maturation stages. Biological and chemical fertilizer application were associated with a decrease of CWSI during the sunflower phenological stages compared with F_{100} (Table 4). As seen from Table 4, F_{75} treatment recorded lower significant values for the CWSI compared to F_{100} . The reduction of CWSI values under combined chemical fertilizer and bio-fertilizers might also be related to changes in root morphology such as root dry matter and root distribution (Ordoorkhani *et al.* 2011). The results confirmed that the CWSI values are sensitive to different types of management such as irrigation regime, dosage and type of fertilizer, so it could be effectively applied to detect water stress and irrigation scheduling of sunflowers. Generally, bio-fertilizers could improve the uptake of nitrogen and influence canopy temperature due to changes in chlorophyll content, leaf color, or

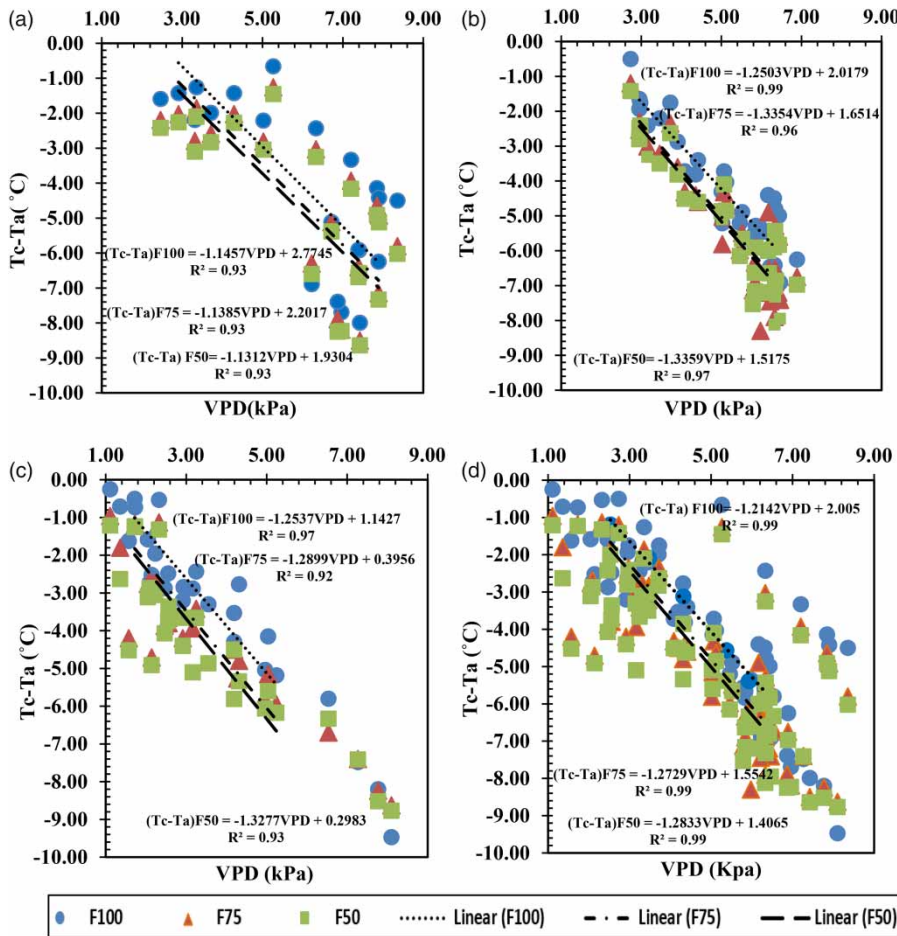


Figure 5 | Canopy-air temperature differential ($T_c - T_a$) and vapor pressure deficit (VPD) for well-watered sunflowers to develop a non-water-stressed baseline (NWSB) under fertilizer treatments during (a) vegetative stage, (b) reproductive stage, (c) maturity stage and (d) whole sunflower season.

Table 3 | NWSBs and NTBs of sunflower estimated for vegetative, reproductive, maturation stages and the whole sunflower season in current research

Growth stage	Treatment	VPD range (kpa)	NESB Slope(b)	NWSB Intercept(a)	R^2	NTB	n
Vegetative	F ₅₀	2.91–7.89	-1.1312	1.9304	0.93	3.14	14
	F ₇₅		-1.1385	2.2017	0.93	3.59	14
	F ₁₀₀		-1.1457	2.7745	0.93	4.55	14
Reproductive	F ₅₀	2.95–6.34	-1.3359	1.5175	0.97	2.83	28
	F ₇₅		-1.3354	1.6514	0.96	3.41	28
	F ₁₀₀		-1.2503	2.0179	0.99	3.81	28
Maturation	F ₅₀	1.72–5.24	-1.3277	0.2983	0.93	1.57	21
	F ₇₅		-1.2899	0.3956	0.92	2.57	21
	F ₁₀₀		-1.1427	1.2537	0.97	2.56	21
Whole sunflower season	F ₅₀	1.72–7.89	-1.2833	1.4065	0.99	2.48	63
	F ₇₅		-1.2729	1.5542	0.99	3.17	63
	F ₁₀₀		-1.2142	2.005	0.99	3.56	63

Fertilizer treatments (F₅₀- 50% chemical fertilizer+ 100% bio-fertilizer, F₇₅-75% chemical fertilizer +100% bio-fertilizer, F₁₀₀- 100% chemical fertilizer).

Table 4 | The effect of water stress and fertilizer treatments on the relative water content (RWC), CWSI (during vegetative, reproductive, maturation stages and the whole sunflower season), leaf area index (LAI) and seed yield

Treatment	CWSI (vegetative)	CWSI (reproductive)	CWSI (maturation)	CWSI (whole sunflower season)	RWC (%)	LAI (m ² m ⁻²)	Seed yield (t ha ⁻¹)
I ₅₀	0.23	0.26d	0.33	0.27	74.79a	4.20a	3.16
I ₉₀	0.34	0.38c	0.42	0.37	67.87b	4.16a	2.68
I ₁₃₀	0.45	0.53b	0.55	0.50	63.84c	3.33b	2.30
I ₁₇₀	0.51	0.59a	0.76	0.61	60.10d	2.68c	1.85
F ₅₀	0.38	0.44	0.51	0.44	69.48a	3.90a	2.79
F ₇₅	0.36	0.42	0.49	0.42	66.54b	3.60b	2.44
F ₁₀₀	0.39	0.45	0.52	0.45	63.93c	3.29c	2.26
ANOVA							
B	**	*	**	**	NS	NS	NS
IRR	**	**	**	**	**	**	**
F	**	NS	NS	**	**	**	**
IRR × F	*	NS	*	*	NS	NS	**

B, block; IRR, irrigation treatments (I₅₀- irrigation after 50 mm evaporation, I₉₀- irrigation after 90 mm evaporation, I₁₃₀- irrigation after 130 mm evaporation, I₁₇₀- irrigation after 170 mm evaporation); F, fertilizer treatments (F₅₀- 50% chemical fertilizer+ 100% bio-fertilizer, F₇₅-75% chemical fertilizer +100% bio-fertilizer, F₁₀₀- 100% chemical fertilizer); NS, not significant, ** and * indicate significant difference at $P < 0.01$ and < 0.05 , respectively.

stomatal conductance (Carroll et al. 2017). Previous studies showed that this significant effect could be related to a good balance of nutrients and water in the root zone (Chen et al. 2010; Mon et al. 2016).

To check the reliability of the measurements, the degree of similarity between samples was calculated by computing the correlation coefficient. The results showed that the correlation coefficients between different measurements for each parameter were 0.91, 0.84, 0.91 and 0.92 for CWSI, RWC, LAI and yield, respectively. The higher correlation coefficient shows greater reliability. So, the high correlation coefficients show the reliability of the samples.

RWC, LAI and seed yield of sunflower

An analysis of variance showed that the effect of drought stress and fertilizer treatments on RWC were significant ($P < 0.01$) (Table 4). In Table 4, results of sunflower leaf RWC show that the I₅₀ treatment had the highest RWC (75%), due to its ability to uptake more water from the soil and higher transpiration from plant leaves, which is in accordance with results obtained by Siddique et al. (2000). Also, RWC values from stressed plants (I₉₀, I₁₃₀, I₁₇₀ treatments) were significantly lower compared to the I₅₀ treatment. Similar results were also obtained by

Gholamhoseini et al. (2013). Biological and chemical fertilizer application significantly resulted in RWC increases compared to chemical fertilizer application (Table 4). As seen in Table 4, plants under F₅₀ treatment recorded the highest significant values for the RWC with 69%. Also, compared with F₁₀₀, the RWC was significantly higher by 9 and 4% under F₅₀ and F₇₅, respectively (Table 4). This increase in RWC value of F₅₀ and F₇₅ treatments may be attributed to the improvement of soil physical and biological characteristics. In addition, the soil chemical properties are enhanced because of readily available nutrients. This nutrient uptake by plant roots influences photosynthesis activity (Hanafy Ahmed et al. 2002). This finding has been confirmed by Shirkhani & Nasrolahzadeh (2016).

Both irrigation and fertilizer treatments had significant effects on LAI values ($P < 0.01$) (Table 4). As water stress increased, the LAI values declined from 4.20 to 2.68 under I₅₀ and I₁₇₀ irrigation treatments, respectively. So, water stress is expected to decrease stomatal conductance and prevent leaf expansion and development. It also leads to a reduction in the amount of light absorption consumed in plant photosynthesis, and it could affect dry matter production amount. Similar results were reported in earlier studies (Orta et al. 2002; Gholamhoseini et al. 2013). Based on the fertilizer treatments, the highest LAI = 3.90 was

observed in F₅₀ treatment. In other words, for the F₅₀ and F₇₅ treatments, LAI increased about 19 and 9% compared to the control treatment (F₁₀₀), respectively. This leaf expansion is related to both N availability and water availability. Moreover, LAI was enhanced by greater water and N uptake due to Azotobacter and Azesprilium bacteria activity in the F₅₀ and F₇₅ treatments. The same findings are documented for crops such as maize (Shirkhani & Nasrolahzadeh 2016) and sunflowers (Gholamhoseini et al. 2013).

Based on the analysis of variance results, the differences between the irrigation and fertilizer treatments for both seed yield were statistically significant ($P < 0.01$) (Table 4). With increasing water stress, the seed yield values declined from 3.16 t ha⁻¹ to 1.85 t ha⁻¹ under I₅₀ and I₁₇₀ irrigation treatments, respectively (Table 4). This is mainly due to the fact that head diameter and seed number per head reduction cause a decline in sunflower seed yield under water stress (Gholamhoseini et al. 2013).

Among the fertilizer treatments, the lowest and highest values of seed yield were reported as 2.26 t ha⁻¹ and 2.79 t ha⁻¹ under F₁₀₀ and F₅₀ treatment, respectively (Table 4). It seems that an appropriate equilibrium between available soil nitrogen and plant nitrogen requirements causes seed yield to increase in F₅₀ and F₇₅ treatments. This is mainly due to the fact that bio fertilizer and chemical fertilizer application improve soil biological activity and nutrient mobilization from different sources (chemical and organic sources) and adjust organic matter decomposition dynamics and the plant nutrient availabilities (Reddy et al. 2014). Also, the increase of microbial biomass is directly related to soil health and thus enhances the balance of nutrient elements and nutrient availability in the root zone that promotes growth and ultimately affects a higher yield (Biari et al. 2008).

Relationships between image feature parameters and sunflower water status, LAI and seed yield

To determine the plant water status, the correlation between the indices extracted from color images (NDGBI, NDRBI, GRS, GBS, RBS, NDGRI, VARI, SAVI_{Green}, CC and GMR) and the indicators of sunflower water stress (RWC and CWSI) were investigated and the best relationship between them was determined (Table 5 and Figure 6(a)–6(d)). Based on the results of Table 5, Pearson correlation coefficient between RWC and CWSI and NDGBI, NDRBI, RBS, VARI, and CC indices were seen to be significant ($P < 0.01$) under different irrigation and fertilizer treatments. Also, a significant relationship was found between NGRDI, SAVI_{Green} and GMR indices with CWSI, but these indices did not have a significant relationship with RWC. In addition, no significant relationship was observed between GRS, GBS and RWC and CWSI indices.

The NDRBI index showed the highest correlation for RWC and CWSI estimates, with a Pearson correlation coefficient of 0.93 and 0.97, respectively (Table 5). This high correlation indicates that water stress has a noticeable effect on the color features of sunflowers, justifying the feasibility of using this technique. The reason for this can be attributed to the stomatal response to visible light. Carter (1991) reported that RWC has a response to both red and blue visible wavelengths. Previous studies indicate that the chloroplasts of the stomatal guard cells are dependent on blue wavelength and carbon dioxide (Zeiger et al. 2002). The other advantages of the RWC index would be its relation with the CWSI (Soleymani et al. 2016). CWSI variations are explained due to water transpiration through the stomata, so CWSI prediction could be acceptable based on the blue and red components. In Figure 6(a)–

Table 5 | Pearson correlation coefficients between sunflower water content indices (RWC and CWSI), crop growth parameters (LAI and seed yield) and the vegetative indices extracted from images

	NDGBI	NDRBI	GRS	GBS	RBS	NDGRI	VARI	SAVI	CC	GMR
RWC	-0.87**	-0.93**	-0.31	-0.43	-0.77**	0.49	0.79**	0.32	0.82**	0.57
CWSI	0.85**	0.97**	0.22	0.34	0.76**	-0.76**	-0.89**	-0.65*	-0.88**	-0.68*
LAI	-0.68*	-0.74**	-0.39	-0.35	-0.76**	0.59*	0.65*	0.49	0.81**	0.44
Seed yield	-0.89**	-0.77**	-0.29	-0.39	-0.75**	0.56	0.70*	0.40	0.76**	0.34

Note: ** and * indicate significant difference at $P < 0.01$ and $P < 0.05$, respectively.

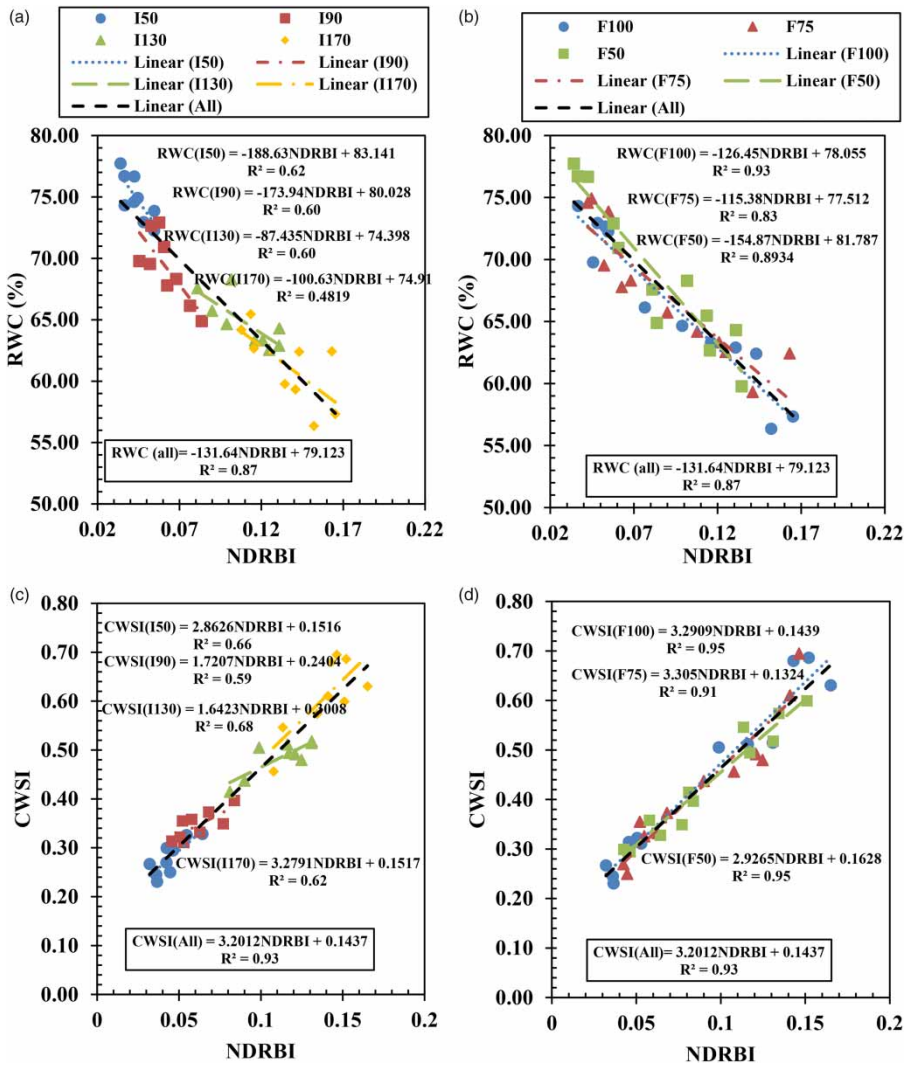


Figure 6 | Relationships between RWC and NDRBI (a and b); CWSI and NDRBI (c and d); LAI and CC (e and f); and seed yield and NDGBI (g and h) for the sunflower under water and fertilizer treatments, respectively. (Continued.)

6(d), the relationship between NDRBI index and water stress indices (RWC and CWSI) has been illustrated under water and fertilizer treatments. As seen in Figure 6(c) and 6(d), an increase in the NDRBI index was found as CWSI increases, or in other words, water stress increases.

Therefore, it can be concluded that the reduction of RWC and the increase of CWSI have been observed as a result of water stress in this study, consistent with those in the study of Mirik et al. (2012), who stated that water stresses influence concentrations of chemical substances of the leaf pigment and cell structure of the plant tissues by altering the properties of the relationship between air spaces and

cell walls, cell wall composition, or the size and shape of the cell.

On the other hand, it has been proven that the canopy temperature in various moisture conditions is strongly influenced by changes in the state of nitrogen and the management of this important element, so nitrogen affects the correlation between the CWSI and the crop yield (Chen et al. 2010). Therefore, the results of the image analysis showed that the variation of CWSI is significant for leaves with varying degree of fertilization, so that the leaves low in nitrogen content have a higher difference of red component than blue, and in the leaves richer in

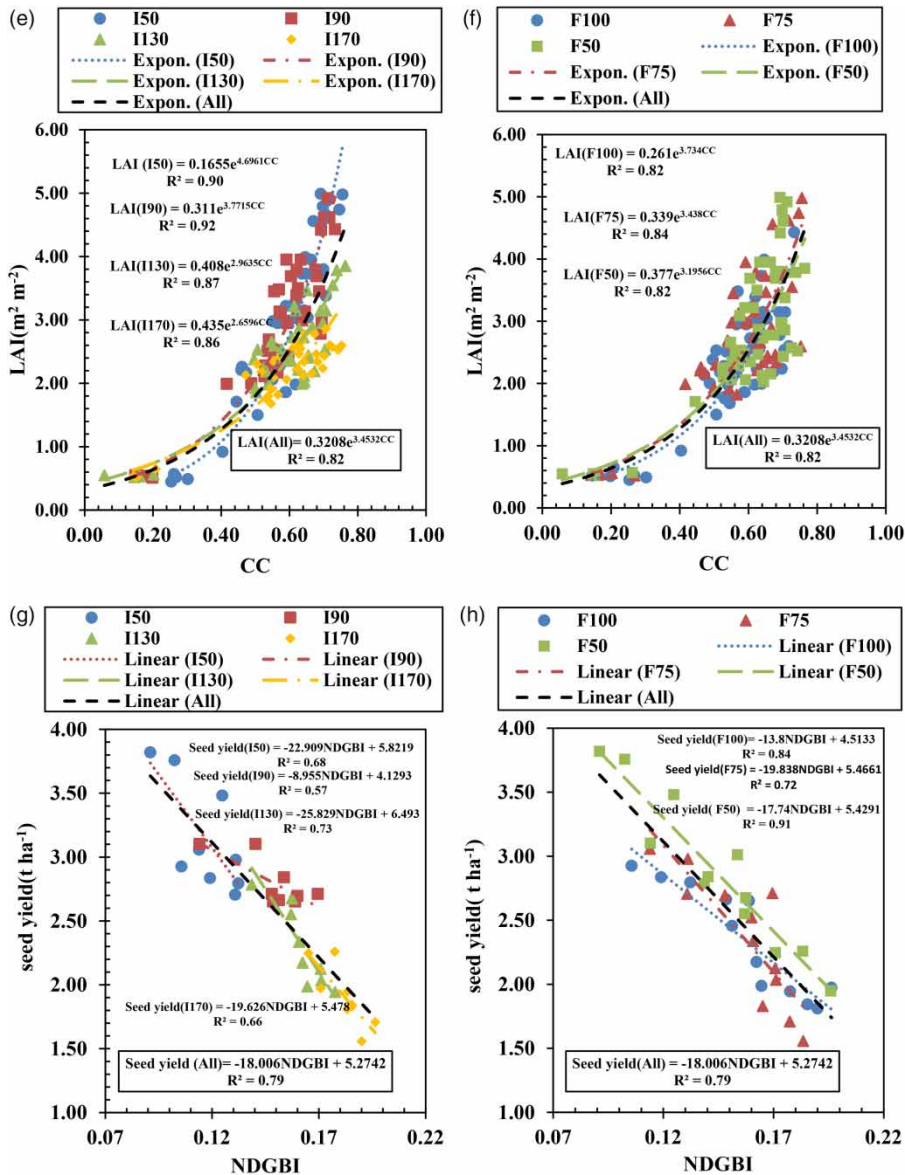


Figure 6 | Continued.

nitrogen, less difference is observed. Accordingly, the findings of this study are similar to those of Carter (1991). Based on Figure 6(a)–6(d), the strong linear relationship between sunflower moisture indicators (RWC, CWSI) and extracted index from the image, NDRBI, indicates that this index can be used to detect sunflower water stress. The linear relationship is presented in Equation (5):

$$y = a + bx \quad (5)$$

where y represents RWC or CWSI, and x represents the NDRBI. Parameters a and b are experimental parameters obtained by the least squares method. Based on these results, the image processing method can be an appropriate alternative to the RWC and CWSI method to estimate water stress and plant responses.

During the growth period only, the LAI were analyzed. The results of investigating LAI and image parameters showed that LAI had a significant correlation with CC (canopy cover) ($P < 0.01$) and with other vegetation indices

(RBS, NDRBI, NDGBI, VARI and NDGRI), but it had no significant correlation with GRS, GBS, SAVI_{Green} and GMR. The CC was estimated by dividing the number of plant canopy pixels by the total number of pixels in the image (Wang et al. 2013) (Figure 3(b) and 3(c)) and the CC derived from the image analysis had a high positive correlation ($r = 0.81$) with LAI (Table 5) and it can be used as a general index to estimate LAI before the physiological maturity stage in sunflower (before leaf yellowing) (Wang et al. 2013). Figures 6(e) and (f) show the LAI of sunflower from emergence to full bloom ($R^2 = 0.82$, $RMSE = 0.70 \text{ m}^2 \text{ m}^{-2}$). According to figures 6(e) and (f), increasing the water stress level leads to reduce the leaf area. Therefore, it is expected that with the increase of water stress, the canopy cover percentage will decrease, which the same trend is observed in the figures 6(e) and (f). So CC is a determinant factor in the crop growth and can be used suitably to determine the plant condition and compute the LAI. The results of this study are consistent with the finding of Wang et al. (2013), showing a strong correlation between LAI and CC. According to the results of this study, the best relationship between CC and LAI shown by the exponential function is obtained as Equation (6).

$$Y = k \exp(d \times CC) \quad (6)$$

where Y is LAI, k is the initial value of the curve function, and d is the curve shaping parameter, and CC is canopy cover.

According to Table 5, the NDGBI indice extracted from digital images had the highest negative correlation of 0.89 sunflower yield. The results of the image analysis showed that the green and red components alone are not suitable for yield estimation. Therefore, the best models are obtained when the blue component is subtracted from the other components. It should be noted that the blue component in color images is strongly influenced by the environmental light conditions. Therefore, subtracting the blue component reduces the noise caused by light changes (Ahmadi Moghaddam et al. 2010).

The relationship between the NDGBI index extracted from the images and seed yield are shown in Figure 6(g) and 6(h). As observed in Figure 6(g) and 6(h), the lowest

index of NDGBI was observed under non-water stress treatment. By increasing the water stress, the value of this index increases, while grain yield decreases. The combination of chemical and bio fertilizers (F_{50}) showed also a better correlation between the NDGBI index and grain yield.

The NDGBI index has linear relationships with seed yield, so that the linear equation with the highest coefficient of determination (0.79), in Equation (7), was known to be the best regression for determining seed yield.

$$y = a + bx \quad (7)$$

in which y represents the seed yield and x represents NDGBI. Both parameters a and b were obtained by the least squares method.

In a study by Wang et al. (2013), the CC index was reported to be the best model for estimating biomass with a coefficient of 90% for rice. Elazab et al. (2015) showed that NDGRI index is the best model for the wheat yield estimation under water stress condition. The close relationship between NDGBI with seed yield is an important tool for evaluating seed yield in sunflower (Figure 6(g) and 6(h)). Although field studies are the most common method to determine yield, field surveys can be time-consuming, difficult and costly. So, image indicators could present an appropriate estimate of the sunflower seed yield under different management conditions.

Error analysis results can only obtain the overall performance of the indices extracted. Mean absolute error in predicted CWSI, RWC, LAI and seed yield were 0.03, 1.65%, $0.55 \text{ m}^2 \text{ m}^{-2}$ and 0.21 t ha^{-1} , respectively. The distribution of percentage error in predicted CWSI, LAI, RWC and seed yield is shown in Figure 7. Reasons for the different errors may be due to differences in input surface reflectances, and the effect of water stress and fertilizer on the stomatal response to visible light.

CONCLUSION

The current study evaluated the ability of infrared thermometer and digital camera (RGB images) to monitor sunflower crop water status (CWSI and RWC) and crop growth indices (LAI and seed yield) in a sunflower field,

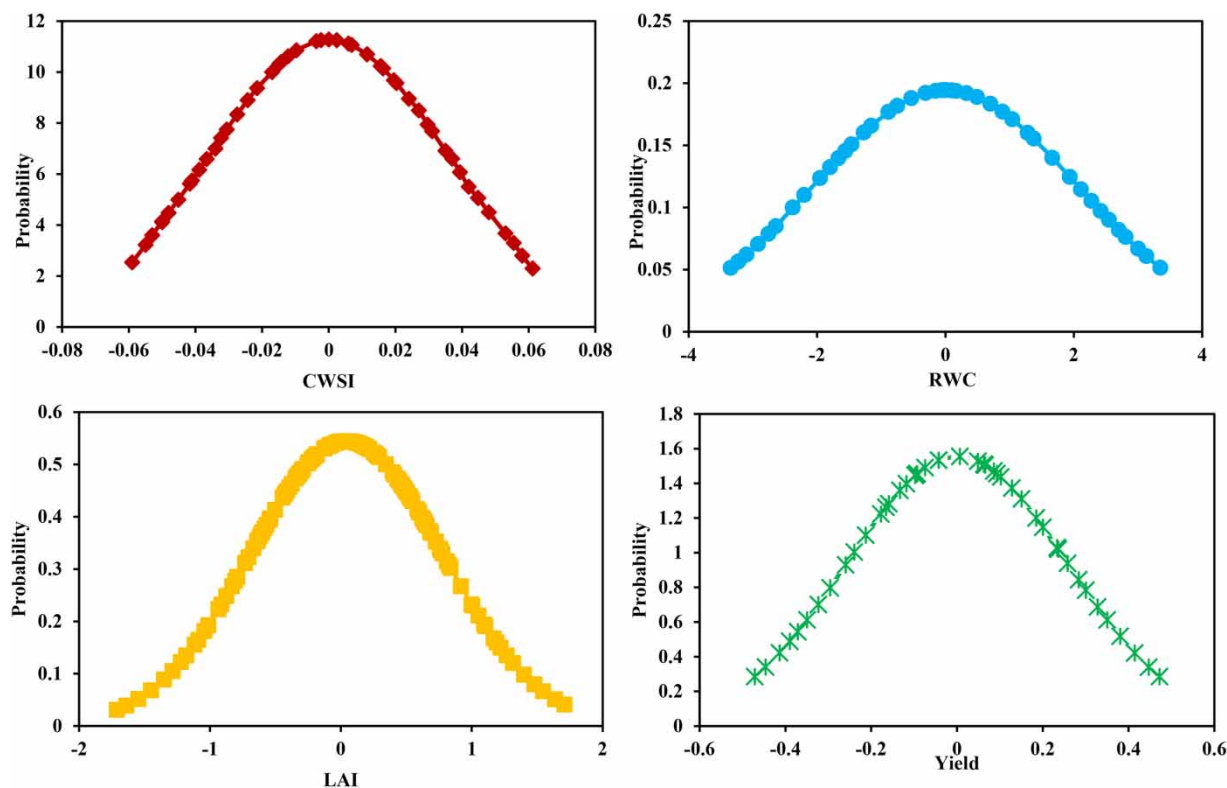


Figure 7 | Error distribution of predicted values of CWSI, RWC, LAI and yield by image feature parameters under different irrigation and fertilizer treatments.

northern Khuzestan, Iran. The findings demonstrated that the CWSI values were sensitive to different irrigation regimes and amount and type of fertilizer, so it would be appropriate for irrigation scheduling of sunflowers and detection of water stress. The application of bio-fertilizer contributed to the reduction of CWSI values in all water stress treatments. Overall, a CWSI value of 0.29 or smaller would be introduced as a threshold value to initiate irrigation to produce maximum seed yield of sunflowers in the present study. The results of image indices showed that the RGB images could be applied to monitor the effects of water and fertilizer treatments on sunflower growth parameters such as LAI and seed yield. The NDRBI index with RWC and CWSI (linear function), the CC index with LAI (exponential function) and the NDGBI index with seed yield (linear function) had the highest correlation. The strong correlation between these indices and crop growth parameters indicated their potential suitability to develop strategies and make a decision by farmers and managers to track crop growth and water stress using digital

cameras and image processing methods as a reliable, fast, less expensive approach. However, future studies should aim at verifying and/or modifying relationships between CWSI and image indices presented in this paper for other crops and under different climatic conditions.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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