

## Improvement of Wheat Genotypes Water Stress Tolerance under Field Conditions by High Throughput Precision Phenotyping

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**Abstract:** Arid and semiarid regions are seriously lacking of fresh water. Wheat is a staple food everywhere which is only moderately tolerant to water stress. Improving water stress tolerance of wheat genotypes is inhibited by lacking of efficient evaluation methods. High throughput precision phenotyping provides an innovative technology to screen for enhanced water stress tolerance from a large of number of genotypes under field conditions and can have immediate value to plant breeding. Therefore, several phenotyping techniques have been tested i.e., canopy temperature (CT), normalized difference vegetation index (NDVI), water soluble carbohydrates (WSC), crop morphological traits and grain wheat yield and yield components. A field experiment was conducted and fifteen wheat genotypes and cultivars were used. The water stressed plants (WS) were irrigated and maintained during the whole growing season up to about 100% of crop evapotranspiration (ET<sub>c</sub>) except the 35 days for imposing water stress. The control plants (WW) received 393 mm for whole wheat growing season, while plants with water stress for 35 days received 313 mm. The experiment was laid out according to an alpha lattice design with incomplete blocks 3 x 5 with two replications, 15 genotypes, 12 blocks and 5 plots per block in each replication. Results revealed that response of genotypes to water stress was varied compared to its response in well-watered conditions. The broad sense heritability of CT was 0.89, NDVI 0.78 and WSC 0.96 and grain yield 0.69. Strong correlation/linear regression/polynomial regression between all used wheat phenotyping techniques (CT, NDVI, WSC) and in-season biomass/grain yield were documented ( $P \leq 0.01$ ). Some genotypes were selected as water stress tolerant. It could be concluded that the presented results confirmed that CT, NDVI and WSC can be used as a rapid and cheap predictor for selecting wheat genotypes with better grain yield and water use efficiency under water stress conditions.

**Key words:** Biomass · Canopy temperature · Grain yield · NDVI · Water soluble carbohydrates · Wheat

### INTRODUCTION

Arid and semiarid regions are solemnly lacking of fresh water. Water shortages in these regions have become common rather than the irregularity. The situation of water shortage is becoming the worst because of immediate population growth and climatic changes. All of these factors will reduce the amount of water assigned to the agricultural sector, which consumes about 75% of the available water supply. Therefore, as the water supply for agronomic purposes becomes inadequate, development of new varieties with higher yield potential becomes more

crucial and it will be one of the major adaptation strategies to sustain crop productions under arid and semiarid conditions.

Food crisis is a major concern in Egypt, where drought soils are ubiquitous. Wheat is a staple food in Egypt, which is only moderately tolerant to drought. Altogether there is nowhere else like Egypt to which the screening technique for drought tolerance of wheat is so important and urgently needed for meeting its rapidly increasing demand for food production by enhancing wheat grain yield under drought conditions. To improve wheat genotypes for these conditions, it is necessary to

evaluate a large number of genotypes using various selection criteria. Some morpho-physiological traits, particularly those related to crop processes, yield characteristics and water stress tolerance mechanisms such as relative water content, canopy water content, canopy temperature, grain yield and harvest index are usually effective as useful supportive selection criteria for screening genotypes under diverse environmental conditions [1]. Straight measurements of those traits using traditional procedures are destructive and time consuming and some of them are hard to do when a large number of genotypes need to be evaluated across various environments. Phenotyping of a large number of genotypes and mapping populations in field trials is still laborious and expensive.

Selection of physiological traits related to water stress tolerance has been shown to be an effective approach in improving wheat performance under abiotic stress [2, 3]. Thermal imaging is an important procedure to evaluate the water stress based on leaf or canopy temperature (CT). CT has been shown by some researchers to be associated with yields of wheat under drought [4, 5]. A number of studies have been conducted to dissect the physiological basis of the association of CT and yield. For example, a study under water stress condition has found that lower CT wheat genotypes tended to partitioning more assimilates to deeper roots [6] and hence improves water extraction from deep soil and consequently improves yield production [5]. As CT is an integrative trait and is affected by photosynthetic potential through feedback on stomatal opening [7], some reports have documented that wheat genotypes with cooler canopies usually had higher photosynthetic rate, stomatal conductance and transpiration [8, 9] and thus were capable to give more photosynthetic assimilates for grain filling. Reynolds [10] summarized a number of hypotheses for the physiological basis of the association of CT and yield, involved (i) low CT reflects an intrinsically higher metabolic capacity, (ii) low CT is denotative of a good vascular system able of meeting evaporative requisition and (iii) low CT reflects a inferior reaction to reduced soil water potential between irrigations. Recently they further ascribed low CT to a higher portion of roots in deeper and wet soil [6]. CT is regulated by a number of environmental elements involving the quantity of solar radiation striking the canopy, soil moisture, wind speed, temperature and relative humidity [11]. Genetic differences in CT consequence from the variation in the ability of the plant to move water through the vascular system, differences in

stomata aperture driving transpiration, root biomass and depth, metabolism and source sink balance [11]. As alike, CT has been shown to correlate with these physiological traits under field conditions [12, 13] and integrates them into a single low cost characteristic measurement that has potential for selection of parental genotypes tolerant or early generation breeding accessions [7].

The GreenSeeker is an integrated optical sensor that uses light emitting diodes (LED) to produce red and near-infrared (NIR) light to measures the normalized difference vegetation index (NDVI) [14]. NDVI was found to be an effective indicator of vegetation response to water stress depending on the relationships between NDVI and a meteorologically based water stress index [15]. NDVI is based on the difference between the maximum absorption of radiation in the Red spectral region (from 620 to 690 nm) as result of chlorophyll pigments and the maximum reflectance in near infrared (NIR, from 760 to 900 nm) light as result of the leaf cellular structure [16]. NDVI value varies with absorption of red light by plant chlorophyll and the reflection of NIR radiation by water-filled leaf cells [17]. Healthy and living canopies absorb most of the Red light by the photosynthetic pigments, while the NIR light is mostly reflected due to light scattering in leaf internal structure and canopy architecture. Therefore, NDVI-value, computed as  $(NIR - Red)/(NIR + Red)$ , integrates biomass (or leaf area) and leaf chlorophyll content [18], thus providing a proxy for grain yield [19]. In wheat, NDVI has been shown to be associated with water stress adaptive traits as well as grain yield under stressed conditions [20, 21, 22]. Information on NDVI is not available for spring wheat genotypes adapted to Egyptian and dry area conditions during growth stages and grain development. Therefore, this study was conducted to examine variation for NDVI at different growth stages, determine its relationship to grain yield and identify wheat genotypes maintaining a higher NDVI value during grain filling stage.

Accumulation of water soluble carbohydrates (WSC) is a function of genetic characteristics specifically the storage capacity of stems as well as environment which will influence the former as well as the subsequent availability of assimilates for storage. The total amount of WSC may reach to 40% or more of the total stems dry weight when WSC levels peak in early grain filling stage [23, 3]. WSC storage may show a compromise with investing in other sinks such as deeper root growth [6], tiller survival or developing spikes. The main proportions of WSC are located in the peduncle and penultimate internode, hence taller genotype with long peduncles tend to has a larger capacity. It has been shown that WSC to

be adaptive for water stress tolerance when the supply of carbohydrates from photosynthesis during grain filling is inhibited and stored WSC may contribute up to 50% of the grain yield. Under terminal water stress, WSC have been shown to buffer biomass production, grain yield and harvest index, associated with increased water uptake and water use efficiency [24]. Traits involved in breeding for genotypes with greater stem storage and remobilization of WSC may lead to improved grain filling and increased grain yield [24].

Therefore, the aim of this work was to 1) screen for enhanced water stress tolerance from a large number of wheat genotypes under field conditions, 2) assess the benefits and effectiveness of high throughput phenotyping technology of thermal imaging (canopy temperature), spectral reflectance (NDVI) and water soluble carbohydrates (WSC) in evaluating the water stress tolerance of 15 wheat genotypes as a quick, economic and nondestructive evaluation mechanism, 3) extend our knowledge on the agronomic and economic feasibility of using plant phenotyping technologies to improve wheat production in real time under water stress conditions and 4) understand the limitation of such tests and to improve the water stress tolerance in wheat crop.

## MATERIALS AND METHODS

**Experimental Procedures:** A field experiment was conducted at the Experimental Station of the National Research Centre (NRC), Nubaria region (30°30'.054"N 30°19'.421"E) to study wheat phenotyping at water stress condition. Some characteristics of the sandy soil and irrigation water used are shown (Table 1; Table 2; Table 3; Table 4). Fifteen wheat genotypes and cultivars were sown on November 21, 2013 and harvested on April 20, 2014. Table 5 describes the 15 wheat genotypes and cultivars evaluated for phenotyping under water stress in 2013/14 season. Wheat grains were selected for uniformity by choosing those of equal size and with the same color. The selected grains were washed with distilled water, sterilized with 1% sodium hypochlorite solution for about 2 min. and thoroughly washed again with distilled water. Fertilization practices were 240 kg N ha<sup>-1</sup> (as ammonium sulphate 20% N), 107 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (as calcium super phosphate 15% P<sub>2</sub>O<sub>5</sub>) and 57 kg K<sub>2</sub>O ha<sup>-1</sup> (as potassium sulphate 48% K). All other recommended agricultural practices for wheat crop were done as recommended by the Egyptian Ministry of Agriculture and Land Reclamation. Control plants (well-watered; WW) were

Table 1: Chemical analyses of extract of saturated soil at the site of the experiment

	pH	EC <sub>c</sub>	SP	CO <sub>3</sub>	HCO <sub>3</sub>	Cl <sup>-</sup>	SO <sub>4</sub>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>
Depth	1:2.5	dS/m									
0-20	8.13	1.9	22.1	-	2.00	12	4.30	5.20	4.10	8.27	0.73
20-40	8.1	1.88	21.0	-	1.00	13	2.80	5.08	4.00	7.20	0.52
40-60	8.09	1.96	21.0	-	1.30	12.2	4.65	5.00	3.00	9.61	0.54

Table 2: Elemental concentration of the soil

N	P	K	Fe	Zn	Mn	Cu
mg/kg soil						
32.5	78.1	3.39	0.39	0.49	7.13	0.26

Table 3: Mechanical analyses of the soil

Coarse sand	Fine sand	Silt	Clay	Texture
(%)				
68.9	17.4	8.4	5.3	Sandy

Table 4: Chemical characteristics of irrigation water used

Cations and anions (meq/L)										
Anions					Cations				EC	
SAR%	SO <sub>4</sub> <sup>2-</sup>	Cl <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup>	CO <sub>3</sub> <sup>2-</sup>	K <sup>+</sup>	Na <sup>+</sup>	Mg <sup>2+</sup>	Ca <sup>2+</sup>	(dSm <sup>-1</sup> )	pH
2.8	1.3	2.7	0.1	--	0.2	2.4	0.5	1.0	0.41	7.35

Table 5: Description of wheat lines and cultivars evaluated for phenotyping under drought stress in 2012/13 season at the Experimental Research Station of the National Research Centre (NRC) at Nubaria in sandy soil.

No.	Source	Line	NAME / PEDIGREE	SELECTION HISTORY	SOURCE
1	CIMMYT	1	CBSME4SA-BV05	CMSW96WM00910S-3DNB-010B-4DNB-015B-03DNB-0Y	
2	CIMMYT	2	CBSME4SA-BV05	CMSS98M00735T-040Y-099BYB-010Y-040M-030Y-2M-2Y-0M	
3	CIMMYT	3	CBSME4SA-BV05	CMSS98M00735T-040Y-099BYB-010Y-1BBY-030Y-68M-010Y-0M	
4	CIMMYT	4	CBSME4SA-BV05	CMSS98M00735T-040Y-099BYB-010Y-1BB Y-030Y-68M-010Y-0M-0IND	
5	CIMMYT	5	CBSME4SA-BV05	CMSS97M00316S-0P20M-0P20Y-43M-0Y	
6	ICARDA	25	MISKEET-17	ICW01-00164-0AP-2AP-0AP-0AP-8AP/MOR-0AP/MOR-0AP	Elite HF YT- MOR 2011
7	ICARDA	87	NESMA*2/14-2/2*SAFI-3	ICW00-0818-1AP-0AP-0AP-40AP/MOR-0AP/MOR-0AP	Elite HF YT- MOR 2011
8	ICARDA	92	SEKSAKA-7/3/SHUHA-2//NS732/HER	ICW01-00054-0AP-11AP-0AP-0AP-13AP-3AP-0AP	AWYT-LR-HF 2011
9	ICARDA	132	SEKSAKA-6/QAFZAH-27	ICW03-0185-28AP/0TS-0AP-0AP-8AP-0AP	AWYT-LR-CA 2011
10	ICARDA	138	CROC-1/AE.SQUARROSA (224)// OPATA/3/QAFZAH-21/4/SOMAMA-3	ICW03-20006-1AP-12AP/0TS-0AP-0AP -15AP-0AP	AWYT-LR-HF 2011
11	ICARDA	139	CROC-1/AE.SQUARROSA (224)// OPATA/3/QAFZAH-21/4/SOMAMA-3	ICW03-20006-1AP-12AP/0TS-0AP-0AP -23AP-0AP	AWYT-LR-HF 2011
12	ICARDA	180	CHAM-6 (CHECK)	CM39992-8M-7Y-0M-0AP	BIG INC 2011
13	CIMMYT	215	267398	20	6171454
14	EGYPT	Gemiza-9	ALD'S/HUAC'S//CMH74.630/5X	Egypt (2000) - CGM 4583-5GM-1GM-0GM	Cross made in the country, one CIMMYT parent
15	EGYPT	Sakha-93	SAKHA 92/TR 810328	Egypt (1999) - S8871-1S-2S-1S-0S	Cross made in the country, one CIMMYT parent

irrigated and maintained during the whole growing season up to about 100% of crop evapotranspiration (ET<sub>c</sub>) using drip irrigation system. For imposing water stress, water stress was created for 35 days started from day 52 till day 86, i.e., from 12/1/2014 till 16/2/2014 by withholding irrigation water. The water stress treatment was terminated when wheat plants were not yet reached the wilting point, which were considered as water stressed plants. The water stressed plants (WS) were irrigated and maintained during the whole growing season up to about 100% of crop evapotranspiration (ET<sub>c</sub>) except the 35 days for imposing water stress. The control plants (WW) received 393 mm for whole wheat growing season, while plants with water stress for 35 days received 313 mm. Soil moisture content was checked daily using HydroSense II, Campbell Scientific, Inc. Utah, USA.

For determination of the crop water requirements (CWR), crop evapotranspiration was calculated under standard conditions (ET<sub>c</sub>) as follows:

$$ET_c = ET_o \times K_c \quad \text{(Equation 1)}$$

where:

- ET<sub>c</sub> = Crop evapotranspiration [mm/day]
- ET<sub>o</sub> = Reference crop evapotranspiration [mm/day]
- K<sub>c</sub> = Crop coefficient

The values of ET<sub>c</sub> and CWR are identical, whereby ET<sub>c</sub> refers to the amount of water lost through evapotranspiration and CWR refers to the amount of water that is needed to compensate for the loss. ET<sub>c</sub> calculated from climatic data by directly integrating the

effect of crop characteristics into ET<sub>o</sub>. The Food and Agriculture Organization of the United Nations (FAO) Penman-Monteith method is now the sole recommended as the sole standard method for calculating ET<sub>o</sub>. The Penman-Monteith equation is given by the following equation [25].

$$ET_o = \frac{0.408 \Delta (R_n - G) + \gamma \frac{900}{T + 273} u_2 (e_s - e_a)}{\Delta + \gamma (1 + 0.34 u_2)} \quad \text{(Equation 1)}$$

where:

- ET<sub>o</sub> = Reference evapotranspiration [mm/day]
- R<sub>n</sub> = Net radiation at the crop surface ([MJ/m<sup>2</sup>] per day)
- G = Soil heat flux density ([MJ/m<sup>2</sup>] per day)
- T = Mean daily air temperature at 2 m height [°C]
- u<sub>2</sub> = Wind speed at 2 m height [m/sec]
- e<sub>s</sub> = Saturation vapour pressure [kPa]
- e<sub>a</sub> = Actual vapour pressure [kPa]
- e<sub>s</sub>-e<sub>a</sub> = Saturation vapour pressure deficit [kPa]
- Δ = Slope of saturation vapour pressure curve at temperature T [kPa/°C]
- γ = Psychrometric constant [kPa/°C]

The equation used the standard climatological records of solar radiation (sunshine), air temperature, humidity and wind speed for daily calculations. Amount of irrigation water was calculated according to the following equation for the drip irrigation systems:

$$AW = \frac{ET_c}{E_a \times (1 - LR)} \quad \text{(Equation 3)}$$

where:

AW = Applied irrigation water depth [mm/day]

E<sub>a</sub> = Application efficiency equals 90% for drip irrigation system

LR = Leaching requirements equals 10% for drip irrigation system.

The seasonal irrigation water applied [mm/ha/season] for control plants (WW) was 393 mm for whole wheat growing season, while plants with water stress for 35 days received 313 mm.

**Layout and Experimental Design:** The experiment was laid out according to an alpha lattice design with incomplete blocks 3 x 5 with two replications, 15 genotypes, 12 blocks and 5 plots per block in each replication. This arrangement of experimental units and blocks has been found to minimize the variation within the block while maximizing variation among blocks. The genotypes were planted in plots with six rows of 2.0 m length and 0.2 m apart and the distance between plants was 0.05 m for each genotype in each replication. At the end of water stress cycle, one fourth square meter of wheat plants were taken from each wheat genotype for each treatment (either well-watered or water stressed) for in-season biomass and flag leaf area (cm<sup>2</sup>) and plant height (cm) measurements. Dry weight of wheat plants was estimated after drying in a forced draft oven at 70 °C for 72 hr. At final harvest all wheat above ground plant parts were harvested. Ten representative individual tillers were selected for measuring plant height (cm) and thousand grain weight (TGW) in g, so, each mean for those traits represents the mean of 10 replicates. All above ground plants for each genotype were collected and biological yield (g m<sup>-2</sup>) and grain yield (g m<sup>-2</sup>) were recorded.

**Canopy Temperature (CT) Measurements:** Canopy temperature measurements were obtained using a near infrared temperature sensor Fluke Thermal Imager Ti32; Fluke Corporation, Everett, WA, USA. Images were analyzed by SmartView Software version 3.12, Fluke thermography, Plymouth, MN, USA. Measurements were taken when the sky is clear and there is little or no wind and when the plant surfaces are dry and not wet from dew, irrigation or rain. Measurements were taken from one hour before to two hours after solar noon; typically from 11:00 h to 14:00 h (when the plant is most water stressed). Two measurements per plot were taken. Always measurements are taken from the part of the plot which is most exposed to the sun and ensure to avoid the shadow of the operator and/or shadows from the neighboring

plots. For getting an idea of the upper and lower CT thresholds between which the crop CT readings should lie. This was done by spraying: (i) a transpiration inhibitor and (ii) water onto two different areas of the border plot of the trial we are testing, waiting for three minutes and then measuring their CT. The two readings serve as 'reference readings' for no transpiration (transpiration inhibitor; upper CT) and maximum transpiration (water; lower CT). CT readings depend on the environment in which the measurements were taken there are as many responses in CT as there are environments. It is therefore a relative measurement. Generally, the 'good' genotypes are those which have relatively cooler canopies than genotypes with warmer canopies (typically by 1-2°C).

#### **Normalized Difference Vegetation Index (NDVI)**

**Measurements:** Measurements of normalized difference vegetation index (NDVI) were done by a field-portable NDVI sensor (GreenSeeker<sup>®</sup> Handheld Crop Sensor; Trimble Navigation Limited, Westminster, CO, USA), which provides rapid ground level measurement of crops at a resolution to characterize the canopy for leaf area index (LAI) and green area index (GAI), biomass and nutrient content (e.g., nitrogen). NDVI is calculated from measurements of light reflectance in the red and near infrared (NIR) regions of the spectrum. A healthy green canopy will absorb most of the red light and reflect most of the NIR light as chlorophyll absorbs mainly blue and red light and the mesophyll reflects NIR light:

$$NDVI = (R_{NIR} - R_{Red}) / (R_{NIR} + R_{Red})$$

The sensor displays the measured value in terms of an NDVI reading (ranging from 0.00 to 0.99) on its LCD display screen. Two measurements were taken per plot of a fixed duration. Measurements were taken after imposing the water stress event/period. Effects on NDVI (e.g., for estimation of green biomass) will allow discrimination of sensitive and stress tolerant/resistant genotypes. Whilst taking measurements, the sensor head was held to be 1) leveled horizontally so that the field of view is directly over the crop, 2) consistently aligned over the plot, typically centered over the middle row; ideally the field of view should cover two or more rows and 3) a distance of 60-120 cm above the crop – within the optimal distance range the readings are not affected by height variance.

#### **Water Soluble Carbohydrates (WSC) Measurements:**

Water soluble carbohydrates (WSC) were extracted by overnight submersion of dry tissue in 10 ml of 80% (v/v) ethanol at 25°C with periodic shaking and centrifuged at

600g. The supernatant was evaporated till completely dried then dissolved in a known volume of distilled water to be ready for determination of soluble carbohydrates [26]. The total sugars were determined colorimetrically according to the method of Dubois *et al.* [27] as follows: An aliquot of 1ml of sugar solution was transferred into test tube and treated with 1ml of 5% aqueous phenol solution followed by 5 ml of concentrated sulphuric acid. The tubes were thoroughly shaken for ten minutes then placed in a water bath at 23-30 °C for 20 min. The optical density of the developed color was measured at 490 nm using Shimadzu spectrophotometer model UV 1201.

**Statistical Analysis:** Data were satisfying the Shapiro-Wilk normality test using R version 3.5.1 software [28]. Statistical analysis was done by META-R (MULTI ENVIRONMENT TRAIL ANALYSIS WITH R FOR WINDOWS) VERSION 4.1 [29], produced and distributed by International Maize and Wheat Improvement Center (CIMMYT). META-R is a set of R programs that performs statistical analyses to calculate BLUEs, BLUPs, genetic correlations among locations and genetic correlations between variables, broad-sense heritability and other statistics for breeding trials are given too, in order to make boxplots and histograms. Analyses may be performed by location, across management conditions or across all locations. META-R contains a graphical JAVA interface that helps the user to easily choose input files, which analysis to implement and which variables to analyze. The arrangement of treatments in alpha lattice into groups gave possibility for analysis the data as a randomized complete block design (RCBD) experiment. The error mean squares from each analysis (alpha lattice & RCBD) were used to estimate the relative efficiency of an alpha lattice design compared to RCBD according to the following equation:

$$\text{Relative efficiency} = \frac{\text{Error mean squares in RCBD}}{\text{Error mean squares in alpha lattice design}} \times 100$$

An estimated relative efficiency (ERE) less than 100 indicates that RCBD is more efficient, while values nearly equal to 100 suggest that the two designs yield similar results. Value of ERE greater than 100 suggests that the alpha lattice design is more efficient design than RCBD. The ERE value (data not shown) were more than 100 in all studied traits, so, it can be concluded that the alpha lattice analysis was more efficient than the randomized complete block design on analyzing all traits of interest in this study. Figures, correlation analysis and regression analysis were done in Microsoft Excel 2007.

## RESULTS

**Best Linear Unbiased Prediction (BLUP):** BLUPs (adjusted means) of the studied traits of 15 wheat genotypes at combining two water management conditions (well-watered; WW and water stressed; WS) are presented in Table 6. Genotypes No. 10, 8, 14, 6, 12 and 2 scored the highest values of grain yield across water management conditions compared to all other genotypes. Similar trends were found for the other studied traits. Response of genotypes to water stress was varied compared to its response in well-watered conditions.

**The Broad Sense Heritability:** The broad sense heritability of 15 wheat genotypes for these 11 investigated traits was estimated in combining two water management conditions (well-watered; WW and water stressed; WS) are presented in Table 6. The broad sense heritability at day 35 from imposing water stress of flag leaf area is 0.80, CT 0.89, NDVI 0.78, WSC 0.96. and at harvest the thousand grain weight was 0.79 and grain yield 0.69.

**The Genetic Correlations Between Grain Yield and Other Traits:** The genetic correlations of 15 wheat genotypes among all the studied variables at combined locations are illustrated in Fig. 1. A cluster and PCA analysis based on the trait distance matrix (1 - Genetic Correlation matrix) is also presented. There was strong evidence between grain yield and FLA, grain yield and TGW, grain yield and NDVI at 14 d, grain yield and NDVI at 21 d, grain yield and NDVI at 28 d, grain yield and NDVI at 35 d, grain yield and WSC are highly positively associated ( $r = 1.000$ ;  $P \leq 0.01$ ), with one another in a linear way at combined two water management conditions (well-watered; WW and water stressed; WS). There was strong evidence between grain yield and CT ( $r = -1.000$ ;  $P \leq 0.01$ ) and between TDW and CT ( $r = -1.000$ ;  $P \leq 0.01$ ), are highly negative associated with one another in a linear way at combined two water management conditions (well-watered; WW and water stressed; WS). There was strong evidence between grain yield and TDW ( $r = 1.000$ ;  $P \leq 0.01$ ) are highly positively associated with one another in a linear way at combined two water management conditions (well-watered; WW and water stressed; WS).

**Canopy Temperature and Relation of In-season Biomass and Grain Yield to Canopy Temperature:** Genotypes with 'cooler' canopy temperatures can be used to indicate a better hydration status. Fig. 3. Shows full visible image of

Table 6: BLUPs (adjusted means) of the studied traits of 15 wheat genotypes at combined two water management conditions (well-watered; WW and water stressed; WS)

Genotype	Plant H (cm)	Biomass (g m <sup>-2</sup> )	FLA (cm <sup>2</sup> )	TGW (g)	Grain Y (g m <sup>-2</sup> )	WUE (kg ha <sup>-1</sup> mm <sup>-1</sup> )	CT (d 35)	NDVI (d 14)	NDVI (d 21)	NDVI (d 28)	NDVI (d 35)	WSC (mg g <sup>-1</sup> )
1	59.3	1044	32.1	43.2	437	12.4	20.0	0.78	0.74	0.73	0.70	271
2	63.3	1049	32.3	43.0	445	12.6	19.4	0.78	0.76	0.74	0.71	280
3	59.3	1035	31.7	42.6	438	12.4	20.1	0.78	0.72	0.73	0.67	269
4	68.2	1032	31.8	47.5	423	12.0	20.6	0.77	0.70	0.73	0.67	263
5	65.6	1036	32.2	46.9	434	12.3	20.0	0.78	0.73	0.73	0.67	268
6	58.1	1049	31.0	41.8	449	12.8	19.3	0.79	0.76	0.74	0.73	278
7	59.1	1044	32.4	38.1	446	12.7	19.4	0.79	0.75	0.74	0.73	281
8	58.8	1038	31.3	47.8	454	12.9	18.9	0.75	0.72	0.73	0.71	296
9	61.4	1041	32.5	45.1	447	12.7	19.4	0.78	0.73	0.73	0.72	285
10	60.6	1038	31.6	43.8	468	13.3	18.6	0.78	0.71	0.73	0.70	309
11	65.0	1049	31.4	45.9	432	12.2	19.6	0.79	0.75	0.73	0.72	267
12	56.1	1050	32.7	41.8	447	12.6	19.4	0.77	0.76	0.74	0.73	288
13	54.0	1035	29.8	39.0	417	11.8	20.6	0.78	0.74	0.73	0.71	258
14	59.0	1048	30.5	42.7	452	12.7	19.0	0.78	0.72	0.73	0.70	292
15	59.8	1020	29.8	44.6	360	10.2	21.1	0.75	0.68	0.72	0.65	245

Parametric test:												
Heritability	0.60	0.12	0.80	0.79	0.69	0.66	0.89	0.33	0.00	0.21	0.78	0.96
Genotype Variance	22.80	588.01	1.08	10.20	885.60	0.73	0.54	0.00	0.00	0.00	0.00	274.36
GenxLoc Variance	26.04	7873.22	0.37	5.39	761.76	0.73	0.11	0.00	0.00	0.00	0.00	17.05
Residual Variance	7.92	1570.52	0.35	0.08	86.03	0.07	0.06	0.00	0.00	0.00	0.00	9.32
Grand Mean	60.51	1040.67	31.53	43.59	436.60	12.4	19.69	0.78	0.78	0.73	0.70	276.62
LSD (0.05)	4.4	23	1.0	0.4	14	0.4	0.4	0.01	0.01	0.02	0.01	5
Genotype significance	NS	**	**	**	*	*	**	NS	NS	NS	**	**
GenxLoc significance	**	**	*	**	**	**	**	**	**	**	**	**

Plant height in cm (Plant H); In-season biomass (Biomass (g m<sup>-2</sup>); Flag leaf area in cm<sup>2</sup> (FLA; cm<sup>2</sup>); Thousand grain weight (TGW; g); Grain yield (Grain Y; g m<sup>-2</sup>); Water use efficiency (WUE) in kg ha<sup>-1</sup>mm<sup>-1</sup>, Canopy temperature measured at day 35 from imposing water stress in °C (CT; d 35); Normalized Difference Vegetation Index measured at day 14 from imposing water stress (NDVI; d 14); Normalized Difference Vegetation Index measured at day 21 from imposing water stress (NDVI; d 21); Normalized Difference Vegetation Index measured at day 28 from imposing water stress (NDVI; d 28); Normalized Difference Vegetation Index measured at day 35 from imposing water stress (NDVI; d 35); Water soluble carbohydrates in mg g<sup>-1</sup> (WSC; mg g<sup>-1</sup>). Broad-sense heritability, Least Significance Difference (LSD), grand mean, variance components for all variables is computed.

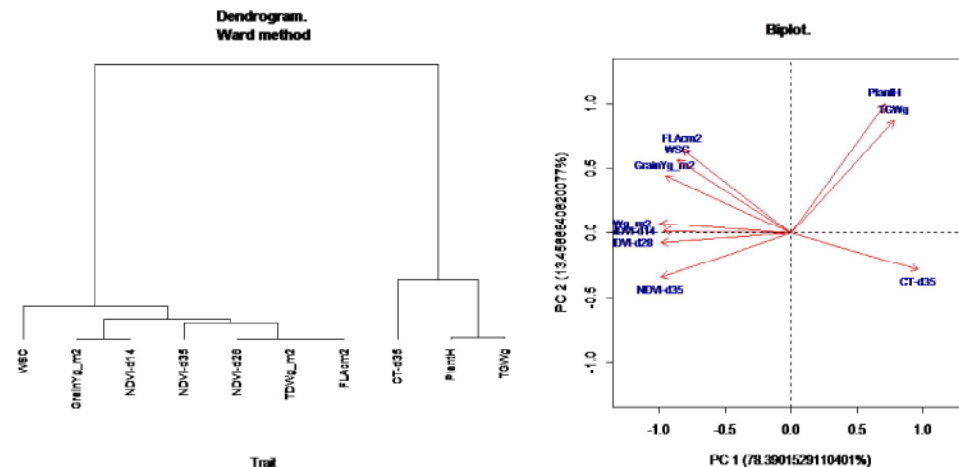


Fig. 1: The genetic correlations of 15 wheat genotypes among all the studied variables within combined locations (water stress; WS and well-watered; WW) is illustrated. A cluster and PCA analysis based on the trait distance matrix (1 - Genetic Correlation matrix) is presented.

Plant height in cm (Plant H); In-season biomass (Biomass (g m<sup>-2</sup>); Flag leaf area in cm<sup>2</sup> (FLA; cm<sup>2</sup>); Thousand grain weight (TGW; g); Grain yield (Grain Y; g m<sup>-2</sup>); Canopy temperature measured at day 35 from imposing water stress in °C (CT; d 35); Normalized Difference Vegetation Index measured at day 14 from imposing water stress (NDVI; d 14); Normalized Difference Vegetation Index measured at day 21 from imposing water stress (NDVI; d 21); Normalized Difference Vegetation Index measured at day 28 from imposing water stress (NDVI; d 28); Normalized Difference Vegetation Index measured at day 35 from imposing water stress (NDVI; d 35); Water soluble carbohydrates in mg g<sup>-1</sup> (WSC; mg g<sup>-1</sup>).

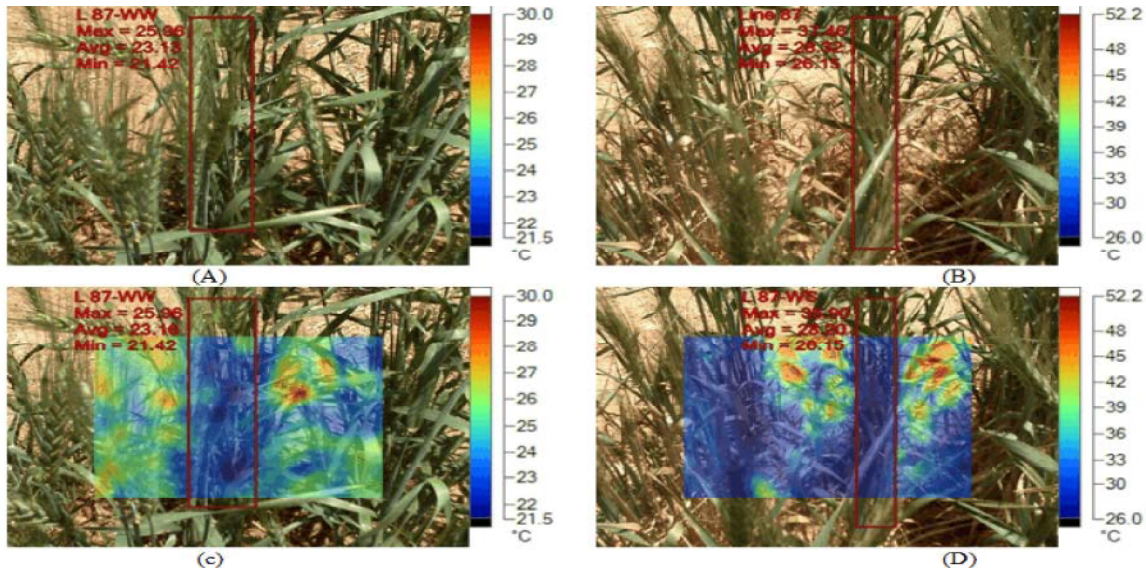


Fig. 2: Full visible image of wheat line 87 under well-watered well-watered (WW)(A) and water stress (B) and blending level infrared image of same wheat line 87 under well-watered (C) and water stressed (D) conditions.

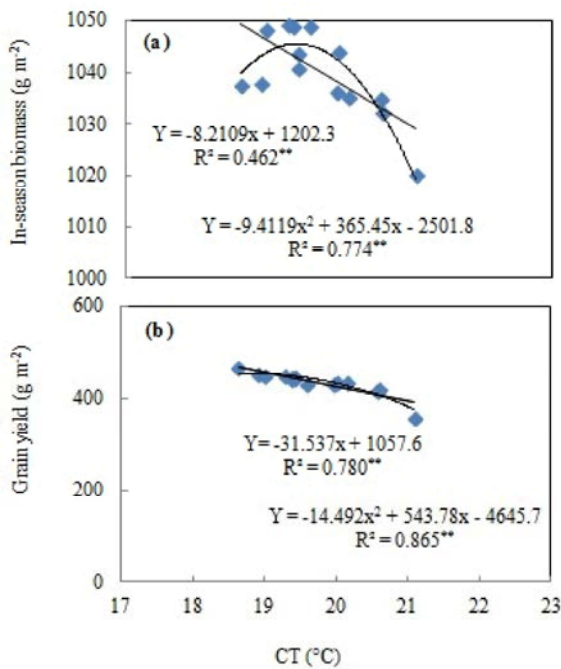


Fig. 3: Linear and quadratic response of in-season biomass (a) and grain yield (b) to canopy temperature (CT) of 15 wheat genotypes at combined two water management conditions (well-watered; WW and water stressed; WS).

wheat line 87 under well-watered (WW)(A) and water stressed (B) and blending level infrared image of same wheat line 87 under WW (C) and WS (D) conditions. CT

varied with genotypes and scored lowest value of 18.6°C for genotype No.10 and highest value of 21.1°C for genotype No.15 (Table 6). For every Celsius (°C) increase of CT, there was a corresponding in-season biomass decrease of 8.2 g m<sup>-2</sup>. The R<sup>2</sup> value is the regression sum of squares divided by the total sum of squares. This has increased from 46.2 % (linear) to 77.4 % (quadratic). This shows that 77.4 % of the variation in in-season biomass is explained by the quadratic regression model. In other words, the quadratic model is significantly better fit than the linear model. It can be shown that for a quadratic curve, the maximum in-season biomass (Y) is expected when CT (X) is 19.4°C, the corresponding Y is 1046 g m<sup>-2</sup> (Fig.3.a). For every °C increase of CT, there was a corresponding grain yield decrease of 31.5 g m<sup>-2</sup>. The R<sup>2</sup> value has increased from 78.0 % (linear) to 86.5 % (quadratic). In other words, the quadratic model is significantly better fit than the linear model. The estimated polynomial regression equation is significant ( $P \leq 0.01$ ) and about 86.5% of the total variation in grain yield is explained by this equation. It can be shown that for a quadratic curve, the maximum grain yield (Y) is expected when CT (X) is 18.8°C, the corresponding Y is 455 g m<sup>-2</sup> (Fig. 3. b).

**NDVI and Relation of In-season Biomass and Grain Yield to NDVI:** NDVI measurements can reach to 1, with higher values indicating greater plant health. The lowest NDVI value was 0.65 for genotype No. 15 (Table 6). Linear and quadratic response of in-season biomass and grain yield



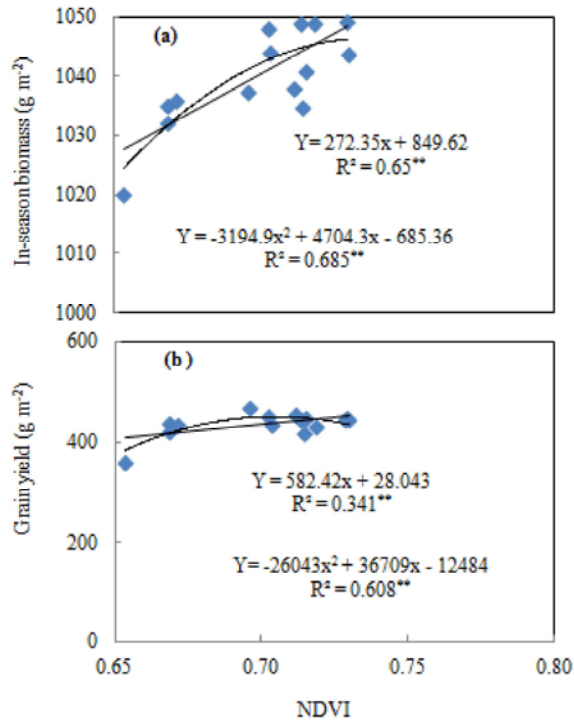


Fig. 4: Linear and quadratic response of in-season biomass (a) and grain yield (b) to Normalized difference vegetation index (NDVI) of 15 wheat genotypes at combined two water management conditions (well-watered; WW and water stressed; WS).

to NDVI at 35 days (d) starting from imposing water stress of 15 wheat genotypes at combined two water management conditions (well-watered; WW and water stress; WS) are shown in Fig. 4a, 4b. Every unit increase of NDVI, there was a corresponding in-season biomass increase of 272 g m<sup>-2</sup>. The R<sup>2</sup> value has increased from 65.0 % (linear) to 68.5 % (quadratic). In other words, the quadratic model is significantly better fit than the linear model. The estimated polynomial regression equation is significant ( $P \leq 0.01$ ) and about 68.5% of the total variation in in-season biomass is explained by this equation. It can be shown that for a quadratic curve, the maximum in-season biomass (Y) is expected when NDVI (X) is 0.74, the corresponding Y is 1046 g m<sup>-2</sup> (Fig. 4a). For every unit increase of NDVI, there was a corresponding grain yield increase of 582 g m<sup>-2</sup>. The R<sup>2</sup> value has increased from 34.1 % (linear) to 60.8 % (quadratic). In other words, the quadratic model is significantly better fit than the linear model. The estimated polynomial regression equation is significant ( $P \leq 0.01$ ) and about 60.8% of the total variation in grain yield is explained by

this equation. It can be shown that for a quadratic curve, the maximum grain yield (Y) is expected when NDVI (X) is 0.70, the corresponding Y is 452 g m<sup>-2</sup> (Fig. 4b).

**Water Soluble Carbohydrates (WSC) and Relation of In-season Biomass and Grain Yield to WSC:** WSC may be expressed as a concentration in dry mass (either as a percentage (%WSC) or as mg g<sup>-1</sup> to demonstrate the potential stems storage capacity of the genotype or as the content per stem (g stem<sup>-1</sup>) or per unit area (g m<sup>-2</sup>) to give an absolute measurement of the carbohydrates available to the grain. WSC varied with genotypes and scored lowest value of 245 mg g<sup>-1</sup> for genotype No.15 and highest value of 309 mg g<sup>-1</sup> for genotype No.10 (Table 6). For every milligram (mg) increase of WSC, there was a corresponding in-season biomass increase of 0.27 g m<sup>-2</sup>. The R<sup>2</sup> value has increased from 26.8 % (linear) to 69.8 % (quadratic). In other words, the quadratic model is significantly better fit than the linear model. The estimated polynomial regression equation is significant ( $P \leq 0.01$ ) and about 69.8% of the total variation in in-season biomass is explained by this equation. It can be shown that for a quadratic curve, the maximum in-season biomass (Y) is expected when WSC (X) is 286 mg g<sup>-1</sup>, the corresponding Y is 1050 g m<sup>-2</sup> (Fig. 5a). Every mg increase of WSC, there was a corresponding grain yield increase of 1.3 g m<sup>-2</sup>. The R<sup>2</sup> value has increased from 77.3 % (linear) to 93.3 % (quadratic). In other words, the quadratic model is significantly better fit than the linear model. The estimated polynomial regression equation is significant ( $P \leq 0.01$ ) and about 93.3% of the total variation in grain yield is explained by this equation. It can be shown that for the quadratic curve, the maximum grain yield (Y) (459 g m<sup>-2</sup>) is expected when WSC (X) is 300 mg g<sup>-1</sup> (Fig. 5b).

**In-season Biomass and Grain Yield and Relation of Grain Yield to In-season Biomass:** Identifying genotypes which are able to maintain biomass production during stress conditions is an important means of identifying better adapted lines. The lowest in-season biomass and grain yield values were 1020 g m<sup>-2</sup> and 360 g m<sup>-2</sup> scored for genotype No. 15 (Table 6). Linear and quadratic response of grain yield to in-season biomass of 15 wheat genotypes at individual well-watered (WW) and water stressed (WS) conditions and at combined two water management conditions (WW & WS) are shown in Fig. 6a and Fig. 6b, respectively. It can be shown from the linear curves, for every gram increase of in-season biomass, there was a corresponding grain yield increase of 0.18 g m<sup>-2</sup> and 0.28 g m<sup>-2</sup>, under WS and WW, respectively (Fig. 6a). At WS

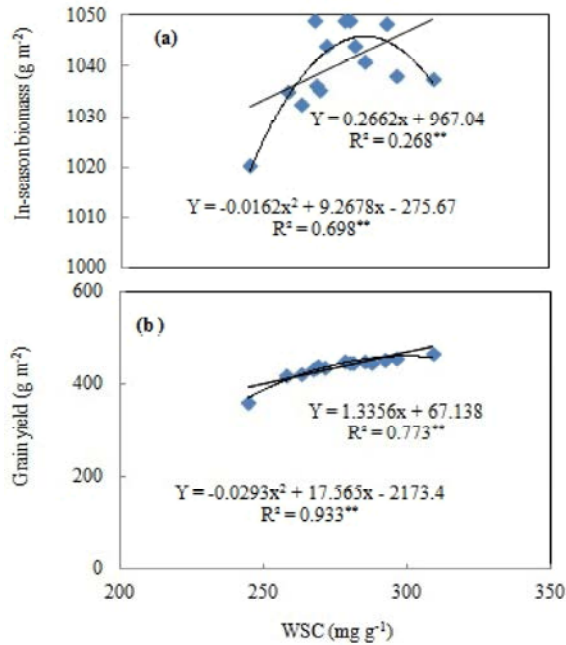


Fig. 5: Linear and quadratic response of in-season biomass (a) and grain yield (b) to water soluble carbohydrates (WSC) of 15 wheat genotypes at combined two water management conditions (well-watered; WW and water stressed; WS).

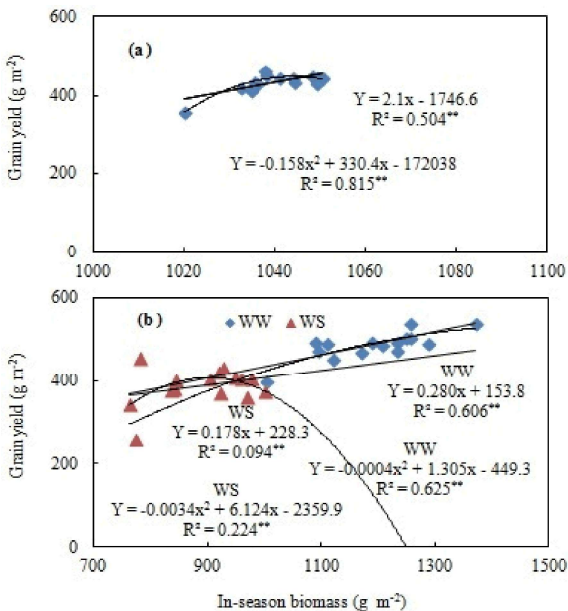


Fig. 6: Linear and quadratic response of grain yield to in-season biomass of 15 wheat genotypes at combined two water management conditions (well-watered; WW and water stress; WS) (a) and at individual well-watered and water stressed conditions (b).

condition, the computed  $R^2$  value of 0.224 indicates that 22.4% of the total variation in the mean grain yields was explained by the quadratic regression equation estimated, while at WW condition the computed  $R^2$  value of 0.625 indicates that 62.5% of the total variation in the mean grain yields was explained by the quadratic regression equation estimated (Fig. 6a). The maximum grain yield (Y) is expected when in-season biomass (X) is 901 g m<sup>-2</sup>, the corresponding Y is 398 g m<sup>-2</sup> at WS, while at WW, Y = 616 g m<sup>-2</sup> when X = 1632 g m<sup>-2</sup> (Fig. 6a). For every gram increase of in-season biomass, there was a corresponding grain yield increase of 2.1 g m<sup>-2</sup> (Fig. 6b). The  $R^2$  value has increased from 50.4 % (linear) to 81.5% (quadratic). In other words, the quadratic model is significantly better fit than the linear model. The estimated polynomial regression equation is significant ( $P \leq 0.01$ ) and about 81.5% of the total variation in grain yield is explained by this equation (Fig. 6b).

## DISCUSSION

Drought stress seems likely to become the most important environmental limiting element for the production of sown cereals in the Mediterranean [30]. Nearly all of the processes associated with plant growth are affected with the presence of water stress. The effects may vary with the degree and the duration of water stress as well as with the growth stage of the plant [31, 32, 33]. Water stress due to low soil water availability and high potential evapotranspiration immediately affects wheat crops and reduces their long term growth and yield potential [34, 35, 36].

Classical measurements for estimating water stress in plants by measuring the plant water status by oven-drying or pressure chambers are irritating and time overwhelming. The frequent changes in the environmental conditions at the field may further affect the measurements and hence require quick measurements [37, 38]. Thus, it is very crucial to develop an effective evaluation approach for noticing water stress in plants that should be reliable, fast, easy, practical and economic. In this study, different high throughput sensing methods such as canopy temperature (CT), the normalized difference vegetation index (NDVI) and water soluble carbohydrates (WSC) were used to detect water stress of various genotypes of wheat under field conditions.

The procedure of BLUP for selection in plant breeding is being used progressively [39], where BLUP analysis allows the exploitation of pedigree information, particularly if information from related genotypes is

available [40]. The BLUP methodology is also extensively used to implement genomic selection programs in plant breeding [41]. Likewise, BLUP may be used to adopt information across zones or mega environments [42]. Genotypes #10, 8, 14, 6, 12 and 2 scored the highest values of grain yield across water management conditions compared to all other genotypes. The genotype #10 with the higher grain yield had the higher water use efficiency, while the genotype #15 with the lower grain yield had the lower water use efficiency (Table 6).

In-season biomass sampling delivers information on crop growth and growth rate, organ size, leaf area and dry mass partitioning between canopy constituents for the calculation of radiation use efficiency and is also a starting point for morphology measurements and nutrients or metabolite analysis (e.g., N, P, protein, etc.). Contrary environmental conditions as water stress can greatly reduce biomass production that in turn decreases the ability of the crop to intercept solar radiation and consequently slows photosynthesis and/or radiation use efficiency [43]. Decreased biomass production also reduces the amount of photosynthates (as WSC) available to be remobilized during grain filling stage (Table 6).

In this study, high-throughput thermal image sensing was found to present some major advantages. Canopy temperature (CT) is an consolidative measurement (i.e., marking the entire canopy of many plants within a plot) and so has preference over other methodology used for stress detection such as stomatal conductance and water potential since it incorporates a larger area of plant measurement, non-destructive, does not intervene with stomata, quicker and not industrious [44]. Some reports [45, 8, 9], documented that wheat genotypes with lower canopy temperature have elite physiological and metabolism perspectives to that of wheat genotypes with higher canopy temperature under normal weather conditions. The results from this study revealed similar phenomenon in wheat with different canopy temperatures grown at combined two water management conditions (WW; WS) (Table 6). As has been shown in previous studies [46, 47], genotypic differences in CT were also observed in wheat experiment (Fig. 2; Fig.3a, b; Table 6). In more detailed, genotype #10 with lower average CT (18.6°C) achieved higher grain yield (468 g m<sup>-2</sup>), while #15 with higher average CT (21.1°C) gave the lower grain yield (360 g m<sup>-2</sup>) at combined two water management conditions (WW; WS) (Table 6). Genotypic ranking among wheat genotypes in CT is generally consistent across environments, with large broad-sense heritability,

our field experiments reported large broad-sense heritability with H= 0.89 (Table 6). The significant correlation between CT and in-season biomass and grain yield was mainly observed under drought stress conditions [4, 46, 10]. However, the obvious and consistent relationship was not detected between yield and CT under irrigation for wheat [48, 49], or sometimes it was detected [50, 51]. In this study the significant correlation between CT and in-season biomass and CT and grain yield has been reported, i.e.,  $r = 0.679$  ( $P < 0.01$ ) and  $r = 0.883$  ( $P < 0.01$ ), respectively (Fig. 3a, b). In addition, the quadratic model was significantly better fit than the linear model for relations, CT and in-season biomass and CT and grain yield (Fig. 3a, b). Our results suggested that lower canopy temperature could be an index of adaptability for those wheat grown under combined two water management conditions (WW; WS). Reynolds *et al.* [52] reported that there was significant association between CT and phenotypic performance and hypothesized that CT could be applied in early and middle offspring selection of wheat genotypes for water stress tolerance. Hence, the classification of wheat genotypes with low or high CT could be introduced in evaluating procedures of wheat variety improvement, especially facilitating the breeding of wheat varieties for water stress tolerance. In other words, our results suggested that lower CT could be an indicator of adaptability for those wheat grown under water stress conditions.

The capacity of the NDVI was tested where measured using GreenSeeker to estimate plant growth in contrasting genotypes of wheat grown under a well watered and water stressed conditions. In water stress resistance, the strength of wheat leaves green colors can be good index for plant water status as being capable to offer an evidence on the capacity of osmotic adjustment [53]. Using a GreenSeeker instrument can offer fast measurements for vegetative green biomass, canopy photosynthetic capacity, LAI, GAI, biomass, nutrient concentration and the results can grant predictions of yield, biomass accumulation, growth rate, soil coverage, early vigor and abiotic stress detection [11]. A previous study has suggested the use of NDVI as a selection criterion to improve grain yield in wheat under optimal conditions [54]. A proxy trait that can be used as an indirect selection criterion must show high association with the trait of interest and should be suitable to be used in large populations for making measurements quickly. Moreover, genotypic variation should be present among germplasm for such an alternative trait. Our findings show

that NDVI meets these criteria to be considered as an alternative trait to be used in indirect selection. Genotypic variation existed for NDVI at each of the fifteen genotypes used in this study. For instance, some genotypes #15 with lower average NDVI achieved lower grain yield at combined two water management conditions (WW; WS) (Table 6). This is particularly important as stress may occur at different growth stages of plants and screening for tolerance to a given stress must be performed in the presence of the stress. Genotypic ranking among wheat genotypes in NDVI is generally consistent across environments, with large broad-sense heritability, our field experiments reported large broad-sense heritability with  $H=0.78$  (Table 6). Strong correlations were observed between NDVI measurements and dry aboveground biomass [20, 55]. Our results reported that NDVI showed a positive correlation with in-season biomass and grain yield and is a rapid method when using a GreenSeeker (Fig. 1) Moreover, Marti *et al.* [20], working with bread wheat in field plots, also found with a similar spectroradiometer increase in the correlations between growth and NDVI measurements performed around anthesis period. NDVI also showed lower coefficient of variation compared with grain yield and hence could be measured precisely. In addition, the improved correlations between growth traits and NDVI measurements at late growth stage can be explained by that maximum biomass in cereals, which is reached around anthesis, correlates strongly with grain yield and is, therefore, a well accepted trait for plant phenotyping in breeding programs [56]. Linear regressions were found to be the simplest adjustment to fit the relationships between NDVI and dry above ground biomass for wheat genotypes [20, 55]. However, exponential rather than linear relationships between NDVI and growth traits have been extensively reported [57, 58]. In our study, we reported that the quadratic model is significantly better fit than the linear model for the response of in-season biomass and grain yield to NDVI at 35 days (d) starting from imposing water stress of 15 wheat genotypes at combined two water management conditions (well-watered; WW and water stress; WS) (Fig. 4a, 4b). Lastly, the use of a portable active sensor spectroradiometer like the GreenSeeker was a useful equipment for prophetic NDVI in fifteen wheat genotypes.

Water soluble carbohydrates (WSC) can be accumulated in wheat stems to more than 40% of total stem dry weight [59]. WSC mobilizes from the stem during the later phase of grain filling and thus hence become an crucial source of assimilate for grain yield in wheat under

terminal water stress condition [60]. Stem WSC accumulation is affected by water stress [60] and WSC have been known to increase in a different species of plants grown at low moisture level [61]. The current study indicated that water stress increased WSC in all genotypes. There is generally an association between grain yield and high stem WSC at anthesis under terminal water stress [62, 63], our field experiment demonstrated that high stem WSC levels were necessarily associated with high grain yield. However, considerable genotypic variation in stem WSC concentration has been observed in wheat [64, 63], our field experiments reported genotypic variation in stem WSC concentration. For instance, some genotypes #10 with higher average stem WSC achieved higher grain yield under water stress conditions (Table 6). Genotypic ranking among wheat genotypes in stem WSC concentration is generally consistent across environments, with large broad sense heritability ( $H=0.90$ ) [64], our field experiment reported large broad sense heritability with  $H=0.96$ . Positive relationships between stem WSC concentration at anthesis and wheat grain yield under certain water limited environmental conditions, have been observed in several studies [64, 63]. Our field experiment reported significant linear and quadratic models for the relations between WSC and in-season biomass and between WSC and grain yield and in both traits the quadratic model was significantly better fit than the linear model (Fig. 5a, b). Trait based breeding for genotypes with greater stem storage and remobilization of WSC may lead to improved grain filling and increased yield [24]. Therefore, high WSC concentration is considered to be a potentially useful trait for improving grain yield in water limited wheat production environments [60, 64, 2]. The results showed that the greater grain yield in tolerant lines under water deficit was due to remobilization of unstructured carbohydrates from shoot to grain. Thus, it seems that selection of genotypes with higher translocated dry matter and contribution of pre anthesis assimilate to grain filling under water stress, may be a suitable way for achieving genotypes with high grain yield under water stress condition.

## CONCLUSIONS

The results under field conditions confirmed that canopy temperature of plants was to the highest degree suitable to detect water stress by proximal sensing method using thermal image camera. Therefore, time consuming destructive methods could be replaced by fast

and nondestructive methods. The results suggested that lower CT could be an indicator of adaptability for those wheat grown under water stress conditions. We reported that the quadratic model is significantly better fit than the linear model for the response of in-season biomass and grain yield to NDVI at combined two water management conditions. The elite set of wheat genotypes used in this study confirms the presence of genotypic variation for NDVI, which can be utilized in breeding programs. The use of a portable active sensor spectroradiometer like the GreenSeeker was a useful equipment for prophetic NDVI in fifteen wheat genotypes. Water stress tolerant genotypes showed an elevated water soluble carbohydrates (WSC), while those susceptible had decline WSC. Reducing WSC concentration might therefore be a useful marker in the selection of stress tolerant genotypes under water stress. We wish these result will be useful for breeding programs of bread wheat.

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