Pak. J. Bot., 47(6): 2093-2105, 2015.

CAPABILITY OF MULTIPLE SELECTION CRITERIA TO EVALUATE CONTRASTING SPRING WHEAT GERMPLASMS UNDER ARID CONDITIONS

SALAH E. EL-HENDAWY [1,2*], NASSER A. AL-SUHAIBANI[1], KHALED AL-GAADI[3], AND SHAFIQ UR REHMAN[5*]

[1]*Plant Production Department, College of Food and Agriculture Sciences, King Saud University, 11451 Riyadh, Saudi Arabia*
[2]*Agronomy Department, Faculty of Agriculture, Suez Canal University, 41522 Ismailia, Egypt*
[3]*Department of Agricultural Engineering, Precision Agriculture Research Chair, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia*
[5]*Department of Botany, Kohat University of Science & Technology, Kohat, Pakistan*
[*]*Co-Corresponding authors: mosalah@ksu.edu.sa, drshafiq@yahoo.com*

Abstract

Selection criteria that would evaluate a large number of germplasm in a rapid and non-destructive manner would be considered advantageous in plant breeding programs. Trade-off between traditional and non-destructive screening criteria in evaluating 90 wheat accessions under water shortage was tested using multivariate statistical techniques. Only three irrigations during the growing cycle of germplasm were applied with the amount of water totalling 2550 m^3 ha^{-1}. Sequential path analysis identified one traditional trait (grain weight per plant) and two non-destructive traits (leaf area index and stomatal conductance) as important first-order traits that influenced final grain yield. The three traits, taken together, explained 96.8% of the total variation in grain yield. Total dry weight per plant, green leaf area per plant, harvest index, grain number per plant, leaf water content and canopy temperature were identified as important second-order traits that influenced grain yield. Although canopy temperature was ranked as a second-order trait, it explained 64.4% of the total variation in stomatal conductance. Approximately 78.0% of the total variation in grain weight or leaf area index was explained by the leaf water content (66.2%) and total dry weight (11.5%). The 90 examined spring wheat germplasms were grouped into five clusters based on all agro-physiological traits using the centroid linkage method. The tested wheat germplasm that produce high grain yield under water shortage were characterised by good performance of certain rapid, easy and non-destructive physiological traits such as high leaf area index, high stomatal conductance and low canopy temperature. Therefore, these three traits could be used in combination as quick and easy screening criteria to select suitable genotypes for water-limiting conditions.

Key words: Canopy temperature; Leaf area index; Phenomics; Sequential path analysis; Stomatal conductance.

Introduction

Water shortage has plagued every country in arid and semiarid areas. It is fundamental factor determining the distribution and productivity of many staple food crops in these areas. It is imperative to identify new germplasm adapted to these conditions in such situation. Evaluation of a large number of germplasm using different phenotypic traits at regular time intervals throughout the whole life cycle of plants is the first step to identify high yielding germplasm adapted to water-limiting conditions. In recent years, physiologists are looking for new indirect traits rather than grain yield as screening tools to select superior germplasm. These new indirect traits must be characterised by several features such as being easy, inexpensive, non-destructive and fast to observe or measure. Additionally, responding rapidly and sustainably to the environmental conditions. Moreover, showing a wide range of variability within the germplasm, being genetically associated with grain yield and stable over the measurement period (Araus *et al*., 2008; Winterhalter *et al*., 2011; Monneveux *et al*., 2012). Therefore, any trait that meets the above mentioned features would be a strong possibility as a screening tool in breeding programs for evaluating a large number of germplasm.

Many physiological processes respond rapidly and sustainably to different environment conditions, which, can be used as indirect and reliable indicators for some important direct traits that confer adaptation to water shortage (i.e., final grain yield, biomass accumulation, leaf area and water use efficiency, etc.), but measuring these direct traits are difficult, time-consuming and destructive. For example, canopy temperature (CT) has been considered a reliable predictor of final grain yield of wheat under water shortage (Feng *et al*., 2009; Li *et al*., 2012). Measurements of stomatal conductance (SC) and CT both provide indirect indicators for the water status of plants and agronomic water use efficiency under water stress as well as the capacity of roots to access available soil water (El-Hendawy *et al*., 2005; Pask & Reynolds, 2013; Marenco *et al*., 2014). Measurements of SC also provide important information about the main limitations of photosynthesis and growth under water stress (Munns *et al*., 2010; Khakwani *et al*., 2013). The leaf area index (LAI), which is the ratio of the leaf green area to the area of ground on which the crop is growing, can be used as a reliable indicator for leaf area expansion of a cereal crop, relative water content and dry matter production under water stress (Royo *et al*., 2005). Fortunately, the availability of different devices for high-throughput phenotyping allows measurement of some indirect traits such as CT, SC and LAI in a fast and non-destructive manner.

Drought stress induces stomatal closure to reduce water loss from the canopy by decreasing transpiration rates, with this; the CT tends to increase because in leaf cooling transpiration has a major role. Several studies have demonstrated that germplasms with warmer canopies under drought stress conditions are less tolerant than germplasms with cooler canopies (Jones 1999; Rashid *et al*., 1999; Feng *et al*., 2009; Mutava *et al*., 2011). Furthermore, a close relationship has been found between CT and many physiological traits such as SC, transpiration rate, plant water status, water use efficiency, LAI and crop yield in various crops under different stressed environmental conditions (Fuchs, 1990; Balota *et al*., 2007; Pinto *et al*., 2010; Liu *et al*., 2011). Significant differences between maize germplasm under water stress have been detected using the CT (Winterhalter *et al*., 2011; Zia *et al*., 2013). A positive correlation has been found between a drought susceptibility index and CT under water stress (Li *et al*., 2012). CT has potential as a screening tool for water stress of wheat genotypes (Rashid *et al*., 1999; Hackl *et al*., 2012). Therefore, in the recent years, most breeders have paid attention to the CT trait as a rapid and non-destructive screening tool to evaluate a large numbers of germplasms.

Improvement of biomass production under water stress can be achieved by breeding for early ground cover to reduce water loss by direct evaporation from the soil. Previous studies have demonstrated that the soil grown by wheat may lose up to 40% of total soil water, especially under Mediterranean conditions; however, germplasms are able to reduce this amount and increase water use efficiency if they are able to cover soil early (Zhang *et al*., 1998; Pask & Reynolds, 2013). In general, increasing LAI causes soil temperature and evaporation water losses to decrease as a result of shading of the soil surface by crop canopy. This may lead to the availability of water over a longer period of time particularly during grain filling (Parton *et al*., 1996; Mullan & Reynolds, 2010; Mutava, 2012). Thus, indirect and non-destructive measurements of LAI using Plant Canopy Analysers can also be used as a rapid and non-destructive screening tool to evaluate a large numbers of germplasms.

It is well known that there is always a close and complicated relationship among indirect traits, especially under environmental stress, which may lead to lose the accuracy of these traits as screening criteria. For instance, the stomatal conductance, transpiration rate, photosynthetic rate and leaf temperature are always interrelated physiological processes. A decrease in stomatal conductance due to water stress is often accompanied by a decrease in photosynthesis, transpiration rates and an increase in CT (Flexas *et al*., 2004). There is always a close relationship between LAI, leaf area, leaf senescence and biomass accumulation (Li *et al*., 2012). There is also a close relationship between different yield components. Therefore, determining the relative importance of indirect traits in screening strategies might be useful for helping breeders to make balance in choices between the accuracy of indirect traits and their ability to evaluate a large number of germplasm in a rapid, inexpensive and non-destructive manner.

The objective of the current study was to trade-off between different agro-physiological traits in their ability to evaluate 90 wheat accessions under irrigation water shortage in a rapid, reliable and non-destructive manner using multivariate statistical techniques.

Materials and Methods

Plant material: To create a large genotypic variability, a total of 90-spring wheat germplasms, comprising 22, 34 and 30 $F_{4:6}$ and $F_{4:7}$ recombinant inbred lines (RIL) from the crosses Sids1/Sakha61, Sids 1/Sakha 93 and Sakha 93/Sakha 61, respectively, three parents and one drought-sensitive cultivar (Yecora Rojo) were used and grown under irrigation water shortage in the years 2012/2013 (F_6) and 2013/2014 (F_7). The three parents used in the crosses, Sakha 93, Sakha 61 and Sids 1, were characterized as tolerant, moderately tolerant and sensitive to water stress, respectively (Abd El-Kareem & Saidy, 2011). Yecora Rojo is very sensitive to drought stress, especially at the grain filling and maturity stages (Barakat *et al*., 2010).

Experimental conditions: Field experiments were conducted at the Agricultural Research Station of King Saud University (Dierab, near Riyadh; 24° 25N, 46° 34E, 400 m alt.). The weather of this study area is mostly sunny and dry during the growing cycle of the wheat crop. The soil texture is loamy sand (82.4% sand, 9.5% silt and 8.1% clay) with a plant available water retention capacity of approximately 120 mm m^{-1}. In addition, the soil is low in organic matter and alkaline (pH 7.9). The daily values of mean temperature, mean relative humidity and evapotranspiration rate at the experimental station during the two growing seasons are summarized in Fig. 1.

The field experiment was conducted using a randomised complete block design with three replications. Each germplasm was planted in six-row plots with a plot size of 4 m in length and 1.2 m in width. The plant-to-plant and row-to-row distances were 5 and 20 cm, respectively. The seeding rate for each germplasm was 145 kg ha^{-1}. All essential nutrients, including N, P and K, were adequately supplied based on previous soil nutrient analysis before planting. Plots were kept free from weeds and diseases throughout the growing season.

In arid conditions, farmers generally irrigate winter wheat seven times, with 7500 m^3 ha^{-1} of the total water application for each season. In this study, only three irrigations with the base irrigation were applied during the growing cycle of germplasm. The base, first, second and third irrigation were applied during the seedling (ZS 15), tillering (ZS 25), heading (ZS 59) and complete emergence of florescence (ZS 69) growth stages (Zadoks *et al*., 1974), with the amount of water totaling 2550 m^3 ha^{-1}. Irrigation was provided via the furrow method. The irrigation system had one water-emitting tube for each plot to deliver constant and equal amounts of water to each plot. The amount of water was monitored with a discharge gauge and regulated through manually operated control valves.

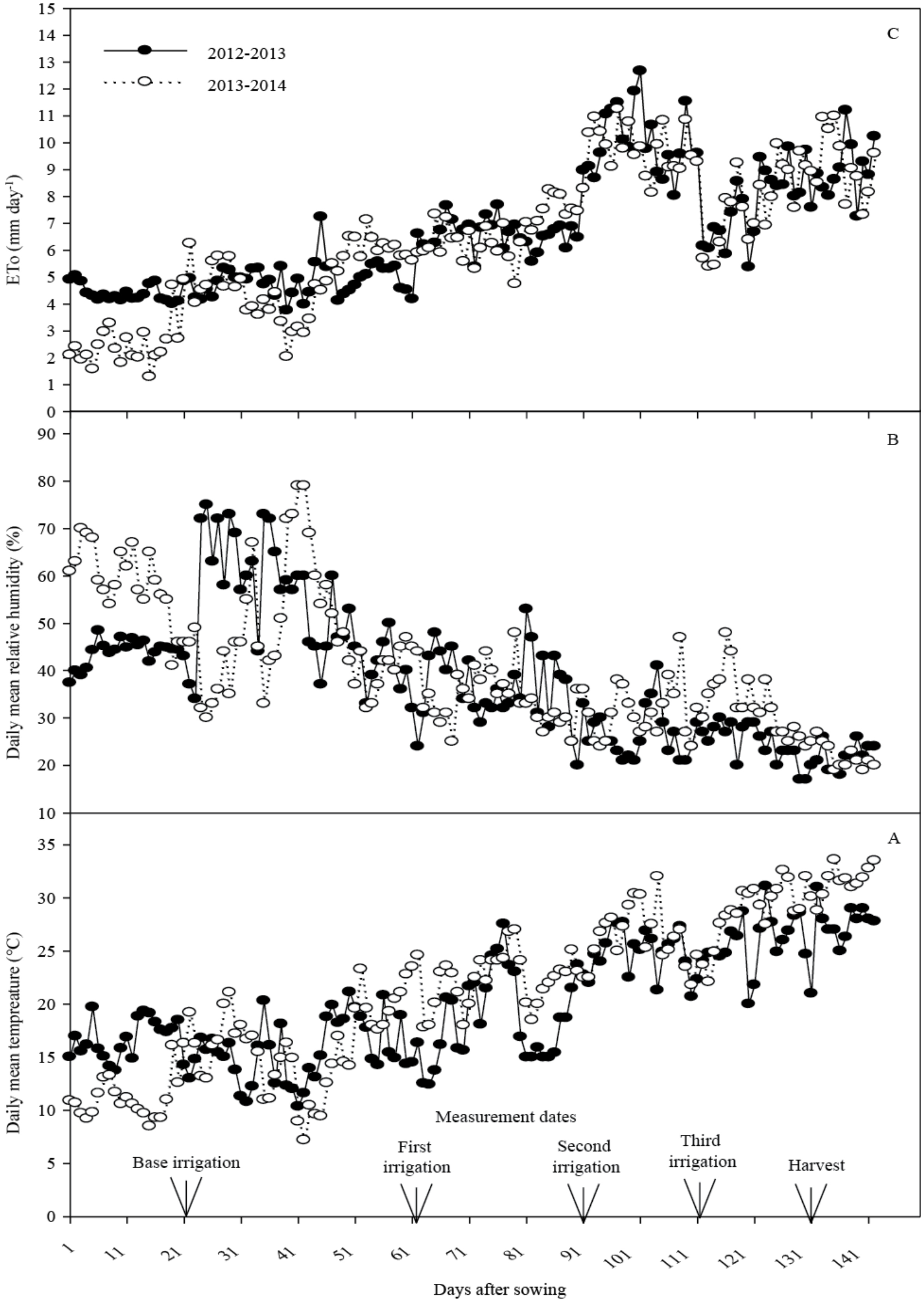

Fig. 1. Daily values of mean temperature (A), mean relative humidity (B) and evapotranspiration rate, ETo (C) at the experimental station during the growing periods of wheat in 2012/13 and 2013/14.

Agronomic traits measurement: Ten plants were randomly selected at heading stage (Zadoks stage 59), from each replicated germplasm set to determine the plant height, the number of tillers per plant, the number of green leaves per plant, the leaf area of green leaves per plant, and the total dry weight per plant. Plant height (PH) was measured from plant base to the tip of main stem spike, including awns. The tiller number per plant (TN) included both the fertile (with spikes) and non-fertile (without spike) tillers. The green leaf number (GLN) included all leaves of plant, excluding leaves that were completely brown. The green leaf area (GLA) was measured as the maximum width × maximum length × 0.75 for green leaves (Francis *et al*., 1969). The total plant dry weight (TDW) was obtained from the weight of oven-dried (80°C for 72 h) plant material.

After physiological maturity at approximately 131 days after sowing, an additional 20 plants from each replicated germplasm were harvested at random to determine the spike length (SL), the number of spikes (SN) and grains (GN) per plant, the weight of grains per plant (GW), and the 1000-grain weight (TGW). A grab sample of 50 fertile stems was taken from each plot for the determination of the harvest index (HI). The grab sample was dried and weighed. The spikes were cut from the grab samples and threshed. In addition, the grain was weighed. The biomass was determined as the difference between the grain weight and dry grab sample weight. The harvest index was calculated as the grain yield to biomass ratio. The total grain yield per hectare was determined by harvesting and threshing an area of 3 internal rows, each 3 m in length (1.8 m^2 in total area), from each plot. The grain yield was adjusted to a water content of 15.5%.

Physiological trait measurements: Physiological traits were measured for all germplasms using standard protocols as described by Pask *et al*. (2012). The leaf water content (LWC), leaf area index (LAI), stomatal conductance (SC), and canopy temperature (CT) were also measured at heading stage (Zadoks stage 59).

The LWC of the flag leaf was determined by taking 7-10 cm^2 from four flag leaves per each plot and immediately determining the fresh weight (FW). Then, the leaf samples were rehydrated in de-ionised water at 25°C until fully turgid, blotted and the turgid weight (TW) was determined. Finally, the leaf samples were dried at 80°C in an oven until no further change in dry weight (DW) was observed. The LWC was calculated using the following equation:

$$\text{LWC (\%)} = ([\text{FW}-\text{DW}]/[\text{TW}-\text{DW}])\times 100$$

Stomatal conductance was measured using an AP4 diffusion porometer (Delta-T Devices, Cambridge, UK) from 11:00 h to 14:00 h as mmol $H_2O\ m^{-2}\ s^{-1}$. Three flag leaves of each plot were screened. The sensor head was placed on the flag leaf top surface, and a measurement was completed after 30 seconds.

Leaf area index was measured using an LAI-2000 Plant Canopy Analyser (LI-COR Inc., Lincoln, Nebraska, USA) between 10:00 h and 14:00 h. The leaf area index for each plot was determined from the average of four sets of LAI measurements. Each set of LAI measurements consisted of one reading above the canopy to measure the total light received by the canopy and four readings below the canopy. The four readings below the canopy were taken at positions approximately 0, 25, 50, and 75% of the distance between the two centre rows of each plot. The LAI measurements were taken with a 45° view cap attached to the lens to restrict the viewing space of the lens and to prevent the operator or frame from being detected. The four sets of LAI measurements that made up the mean LAI for each plot were taken with the view opening facing two directions: two measurements perpendicular to the rows and two measurements parallel to the row. This ensured accurate representation of the actual LAI of the plot.

Canopy temperature was measured using thermal infrared imaging (Therma CAM SC 3000 infrared camera, FLIR System, USA) between 10:00 and 14:00 h. The thermal camera was operated in a wavelength range of 8–9 μm with a thermal resolution of 0.05°C, 320 x 240 pixels and 45° x 34° lens of field view. The emissivity for the canopy and soil was set at 0.95 to simplify the measurements. The external optics transmission was fixed at 1. The temperature span for measurements was assigned to 20°C, and the relative humidity was kept at 50%. The camera was held at a height approximately 1.0 m above the top of the ear. From each plot, four pictures were taken. Canopy temperature analysis of the IR images was performed using FLIR Quick Report 1.2 SP1 (FLIR Systems, Oregon, USA). Because the bare soil and the other non-plant parts are significantly hotter than the canopy itself (Luquet *et al*., 2003), a suitable scale and palette was chosen to result in a quite clear and informative thermal image. The software provides the functionality to measure the spots, lines and area. In this study, a polygon area was drawn to measure the overall canopy temperature.

Data analyses: Data were analysed using SPSS 21.0 (SPSS Inc., 2012) statistical software. The trade-off between the different agro-physiological traits used for the screening strategies was investigated using multiple statistical procedures. A matrix of correlation between all investigated traits was determined by simple correlation analysis. Principle component analysis (PCA) was used to classify a large number of variables into major components. The first two principle components explain as much of the variability in the data as possible (Everitt & Dunn, 1992). The factor analysis reduces a large number of related variables to a small number of clusters of variables called factors. The matrix of factor loading goes through a varimax orthogonal rotation. Cluster analysis was used to arrange a set of variables into clusters, so that the degree of association is strong between members of the same cluster and weak between members of different clusters. Cluster analysis was performed using Pearsons' correlation and the centroid linkage method. Path analysis was used to measure both the direct and indirect effects of traits. Path coefficient analysis was performed based on logical relationships between grain yield and other traits and taking grain yield as a dependent characteristic and the remaining estimated characteristics as causal. A sequential path analysis was used to organise the different agro-physiological traits into first-, second- and third-order paths on the basis of their respective contributions to the total variation of the final grain yield and to eliminate the traits with very low contributions to the model. In the first step of this analysis, all traits were considered as independent variables and grain yield as a dependent variable in the stepwise regression. The traits indicating the closest correlation with grain yield were considered as first-order traits. Consecutively, the first-order traits were considered as dependent variables and the remaining traits as independent variables in the second stepwise regression after eliminating the grain yield from the model to reveal the second-order traits. The second-order traits were considered as dependent variables and the remaining traits as independent variables in the third stepwise regression after eliminating all of the second-order traits from the model to reveal the third-order traits. Combined analyses were performed across two years.

Results

Phenotypic variability under irrigation water shortage: On the basis of average two growing seasons, phenotypic variation was confirmed by the minimum and maximum values, arithmetic mean and standard deviations of each agro-physiological trait (Table 1). A wide range between the minimum and maximum values was observed for all traits. For instance, plant height (PH) ranged from 0.49 to 1.06 m; tiller number per plant (TN) ranged from 2.7 to 5.6; green leaves number per plant (GLN) ranged from 4.9 to 18.1; green leaves area per plant (GLA) ranged from 73.8 to 433.8 cm^2; total dry weight per plant (TDW) ranged from 4.1 to 11.1 g; spike number per plant (SN) ranged from 1. 7 to 5.3; main spike length (SL) ranged from 13.1 to 18.8 cm; grain weight per plant (GW) ranged from 2. 5 to 10.3 g; grain number per plant (GN) ranged from 33.7 to 171.5; thousand grain weight (TGW) ranged from 30.8 to 55.8 g; harvest index (HI) ranged from 0.31 to 0.54; leaf water content (LWC) ranged from 61.9 to 80.6%; leaf area index (LAI) ranged from 0.99 to 3.57; stomatal conductance (SC) ranged from 166.7 to 455.0 mmol/m^2/s; canopy temperature (CT) ranged from 27.1 to 35.7°C, and grain yield (GY) ranged from 2.14 to 7.55 ton ha^{-1} (Table 1).

Simple correlations between agro-physiological traits: All agro-physiological traits that evaluated across the 90 germplasms and two growing seasons, except for thousand grain weight, were all significantly correlated with final grain yield ($p<0.05$) (Table 2). LAI, SC and CT traits were highly significantly correlated with GY, with coefficients of 0.94, 0.71 and −0.68, respectively ($p<0.001$). Some destructive screening criteria, such as GLN (r = 0.73, $p<0.001$), GLA (r = 0.70, $p<0.001$), TDW (r = 0.79, $p<0.001$), GW (r = 0.99, $p<0.001$), GN (r = 0.77, $p<0.001$), HI (r = 0.79, $p<0.001$), and LWC (r = 0.82, $p<0.001$), had the same level of strong correlations with GY. Although PH, TN and SN were correlated with final grain yield, but the correlation coefficients between them were weak (Table 2). The highest correlation for CT, as a rapid screening criterion, was found with SC (r = −0.80, $p<0.001$), the second highest with GW (r = −0.69, $p<0.001$), and third highest with HI (r = −0.56, $p<0.05$) and LWC (r = −0.54, $p<0.05$) (Table 2).

Classifying the agro-physiological traits into major components: The principal component analysis (PCA) grouped all agro-physiological traits into four components that all together explained 81.2% of the total observed variability among the 90 germplasms (Table 3). All agro-physiological traits except PH, TN, SN and TGW were the main contributors to PCA1 (59.18% of total variability). PCA2 (10.36% of total variability) was mainly related to TN and SN, whereas PH and TGW were significant contributors to PCA3 (6.61% of total variability) and PCA4 (5.01% of total variability), respectively (Table 3).

Table 1. Basic statistics (minimum and maximum values, arithmetic mean and standard deviation (SD)) showing variation in agro-physiological traits of 90 spring wheat germplams grown under water stressed conditions. Data is the average of two seasons.

Traits	Minimum	Maximum	Mean	SD
Plant height (m) (PH)	0.49	1.06	0.76	0.107
Tiller number (no.) (TN)	2.7	5.6	4.2	0.62
Green leaf number (no.) (GLN)	4.9	18.1	10.3	2.49
Green leaf area (cm^2) (GLA)	73.8	433.8	191.7	56.87
Total dry weight (g) (TDW)	4.1	11.1	6.6	1.29
Spike number (no.) (SN)	1.7	5.3	3.3	0.69
Spike length (cm) (SL)	13.1	18.8	16.0	1.09
Grain weight (g) (GW)	2.5	10.3	6.5	1.51
Grain number (no.) (GN)	33.7	171.5	98.8	31.64
Thousand grain weight (g) (TGW)	30.8	55.8	42.8	4.91
Harvest index (%) (HI)	0.31	0.54	0.42	0.04
Leaf water content (%) (LWC)	61.9	80.6	71.4	4.44
Leaf area index (LAI)	0.99	3.57	1.74	0.46
Stomatal conductance (mmol m^{-2} s^{-1}) (SC)	166.7	445.0	263.3	60.84
Canopy temperature (°C) (CT)	27.1	35.7	31.7	2.09
Grain yield (ton ha^{-1}) (GY)	2.14	7.55	4.93	1.39

Table 2. A matrix of simple correlation coefficients for different agro-physiological traits evaluated across the two seasons and 90 spring wheat germplasms under irrigation water shortage condition

Traits*	PH	TN	GLN	GLA	TDW	SN	SL	GW	GN	TGW	HI	LWC	LAI	SC	CT	GY
PH																
TN	0.22^{*}															
GLN	0.31^{*}	0.61^{**}														
GLA	0.50**	0.55^{**}	0.78^{***}													
TDW	0.39^{*}	0.47^{*}	0.78^{***}	0.75^{***}												
SN	0.13^{ns}	0.70^{***}	0.60^{**}	0.47^{*}	0.57^{**}											
SL	0.29^{*}	0.31^{*}	0.51^{**}	0.61^{**}	0.63^{**}	0.30^{*}										
GW	0.24^{*}	0.44^{*}	0.75^{***}	0.73^{***}	0.80^{***}	0.44^{*}	0.65^{***}									
GN	0.20^{ns}	0.36^{*}	0.63^{**}	0.61^{**}	0.66^{***}	0.41^{*}	0.51^{**}	0.77^{***}								
TGW	-0.14^{ns}	-0.17^{ns}	-0.20^{ns}	-0.09^{ns}	-0.10^{ns}	-0.16^{ns}	0.10^{ns}	0.06^{ns}	0.04^{ns}							
HI	-0.13^{ns}	0.27^{*}	0.59^{**}	0.50^{**}	0.66^{***}	0.38^{*}	0.48^{*}	0.78^{***}	0.66^{***}	0.08^{ns}						
LWC	0.24^{*}	0.31^{*}	0.62^{**}	0.65^{***}	0.67^{***}	0.27^{*}	0.56^{**}	0.81^{***}	0.64^{**}	0.07^{ns}	0.65^{***}					
LAI	0.20^{ns}	0.36^{*}	0.70^{***}	0.64^{**}	0.78^{***}	0.40^{*}	0.61^{**}	0.93^{***}	0.74^{***}	0.07^{ns}	0.77^{***}	0.79^{***}				
SC	0.08^{ns}	0.23^{*}	0.56^{**}	0.45^{*}	0.56^{**}	0.30^{*}	0.50^{**}	0.70^{***}	0.58^{**}	0.06^{ns}	0.61^{**}	0.56^{**}	0.64^{**}			
CT	-0.11^{ns}	-0.20^{ns}	-0.48^{*}	-0.42^{*}	-0.52^{**}	-0.15^{ns}	-0.46^{*}	-0.68^{***}	-0.46^{*}	-0.07^{ns}	-0.56^{**}	-0.54^{**}	-0.62^{**}	-0.80^{***}		
GY	0.21^{*}	0.40^{*}	0.73^{***}	0.70^{***}	0.79^{***}	0.42^{*}	0.65^{***}	0.99^{***}	0.77^{***}	0.08^{ns}	0.79^{***}	0.82^{***}	0.94^{***}	0.71^{***}	-0.68^{***}	1.00

* The codes of traits are presented in Table 1

*,**,*** Indicate significance at p<0.05, p<0.01 and p<0.001, respectively, and ns: not significant

Table 3. Eigen value of the correlation matrix for different agro-physiological traits using the principle component procedure for the overage two seasons and the 90 spring wheat germplasms investigated under irrigation water shortage condition.

Traits	PC1	PC2	PC3	PC4
Plant height (m) (PH)	0.309	0.485	**0.749***	-0.024
Tiller number (no.) (TN)	0.522	**0.597**	-0.296	0.226
Green leaf number (no.) (GLN)	**0.831**	0.322	-0.114	-0.087
Green leaf area (cm^2) (GLA)	**0.803**	0.331	0.200	0.073
Total dry weight (g) (TDW)	**0.871**	0.194	0.024	-0.011
Spike number (no.) (SN)	0.530	**0.535**	-0.458	0.227
Spike length (cm) (SL)	**0.703**	-0.024	0.225	0.154
Grain weight (g) (GW)	**0.971**	-0.119	0.006	0.010
Grain number (no.) (GN)	**0.801**	-0.054	-0.064	0.069
Thousand grain weight (g) (TGW)	0.009	-0.562	0.143	**0.759**
Harvest index (%) (HI)	**0.776**	-0.291	-0.361	-0.014
Leaf water content (%) (LWC)	**0.828**	-0.143	0.131	0.007
Leaf area index (LAI)	**0.919**	-0.160	-0.011	0.013
Stomatal conductance (mmol m^{-2} s^{-1}) (SC)	**0.740**	-0.302	-0.094	-0.267
Canopy temperature (°C) (CT)	**-0.693**	0.356	-0.030	0.329
Grain yield (kg/ha) (GY)	**0.967**	-0.163	0.004	0.006
Eigenvalue	10.652	1.865	1.189	0.901
Variability (%)	59.175	10.362	6.608	5.005
Cumulative (%)	59.175	69.537	76.146	81.151

*Values in bold denote traits for the suggested factor name

The reduction of agro-physiological traits to factors: The factor analysis reduced the 18 agro-physiological traits to three common factors that accounted for 71.3% of the total variability (Table 4). The first factor included all agro-physiological traits except PH, TN, SN and TGW and accounted for 57.4% of the total variability. Interestingly, the first factor included all rapid and non-destructive traits, such as LAI, SC and CT. This factor had high positive loadings for LAI (0.950) and SC (0.786), and high negative loadings for CT (−0.720). This factor had also high positive loadings for GLN (0.808), GLA (0.782), TDW (0.819), GW (0.978), GN (0.758), HI (0.729), LWC (0.768) and GY (0.970) (Table 4). Thus, this factor can be considered as the most important. The second factor possessed high negative loadings of PH (−0.643) and TN (−0.456) and high positive loadings of TGW (0.296). Because the third factor included only a high negative loading of SN (−0.554), it can be considered as less important.

Arranging agro-physiological traits into cluster groups: The different agro-physiological traits were grouped into five clusters with similarity values above 0.90 (Fig. 2). The results of the cluster analysis (CA) revealed that cluster 3 included a large numbers of traits (13 traits). In addition, this cluster was divided into five subgroups. The traits of SL and SC were separated in subgroups 1 and 2, respectively, whereas the traits related to yield such as GY and GW, and the traits related with growth such as GLN, GLA and TDW were separated together in subgroups 3 and 4, respectively. Subgroup 5 includes HI and GN. Interestingly, the CT, as a fast and non-destructive screening criterion, was distanced from other traits in cluster 5. The traits TN and SN were grouped together in cluster 2, whereas clusters 1 and 4 included PH and TGW, respectively (Fig. 2).

Table 4. Rotated (Varimax rotation) factor loadings and commonalities for different agro-physiological traits of the overage two seasons and the 90 spring wheat germplasms investigated under irrigation water shortage condition.

Traits	Factor 1	Factor 2	Factor 3	Communality
Plant height (m) (PH)	0.322	**-0.643***	0.520	0.788
Tiller number (no.) (TN)	0.439	**-0.456**	-0.312	0.498
Green leaf number (no.) (GLN)	**0.808**	-0.256	-0.116	0.732
Green leaf area (cm^2) (GLA)	**0.782**	-0.305	0.161	0.730
Total dry weight (g) (TDW)	**0.819**	-0.239	-0.026	0.729
Spike number (no.) (SN)	0.490	-0.518	**-0.554**	0.815
Spike length (cm) (SL)	**0.672**	-0.060	0.142	0.475
Grain weight (g) (GW)	**0.978**	0.133	0.054	0.976
Grain number (no.) (GN)	**0.758**	0.085	-0.057	0.585
Thousand grain weight (g) (TGW)	-0.050	**0.296**	0.126	0.106
Harvest index (%) (HI)	**0.729**	0.382	-0.312	0.774
Leaf water content (%) (LWC)	**0.768**	0.100	0.131	0.617
Leaf area index (LAI)	**0.950**	0.173	0.057	0.936
Stomatal conductance (mmol m^{-2} s^{-1}) (SC)	**0.786**	0.239	-0.082	0.682
Canopy temperature (°C) (CT)	**-0.720**	-0.211	-0.005	0.563
Grain yield (kg/ha) (GY)	**0.971**	0.190	0.043	0.981
Variance	10.325	1.586	0.926	12.837
Factor variance (%)	57.360	8.808	5.145	71.313

*Values in bold denote traits for the suggested factor name

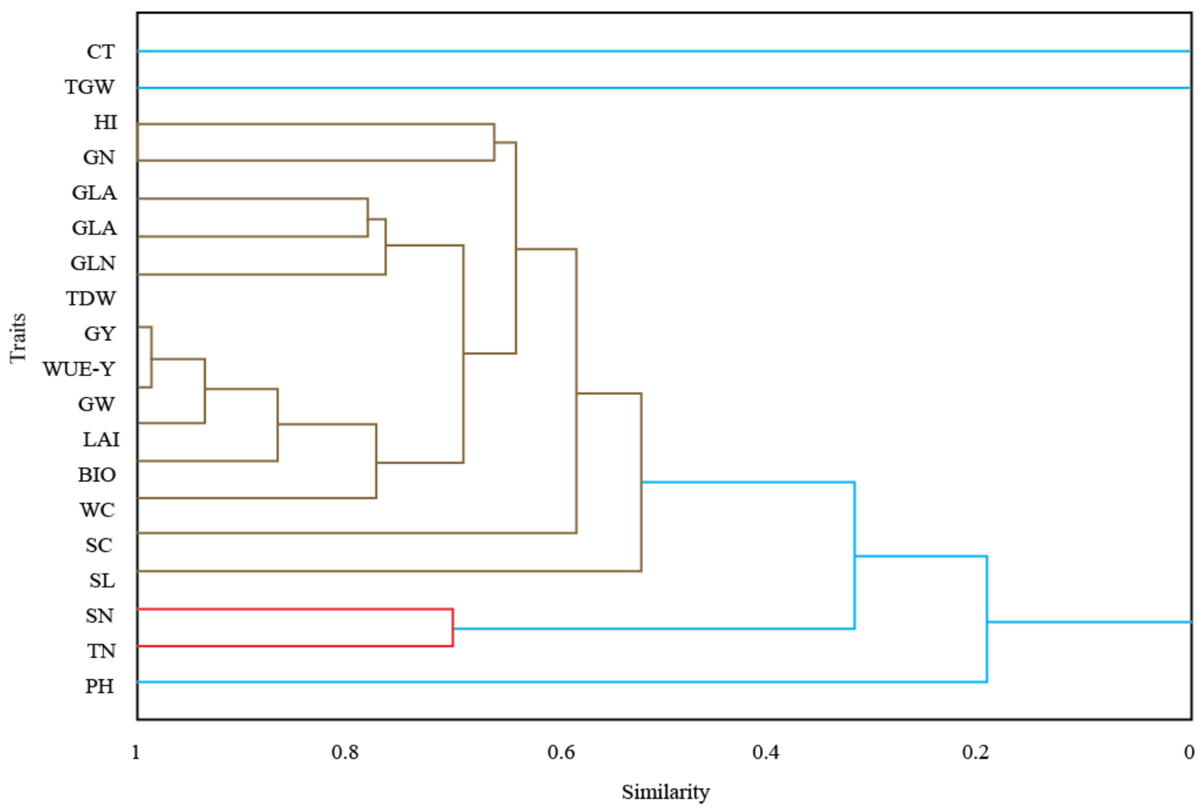

Fig. 2. Clusters and similarity levels of the estimated different agro-physiological traits of the 90 spring wheat germplasms investigated under irrigation water shortage condition using the hierarchical cluster analysis (centroid linkages). PH, plant height; TN, tiller number; GLN, green leaf number; GLA, green leaf area; TDW, total dry weight; SN, spike number; SL, spike length; GW, grain weight; GN, grain number; TGW, thousand grain weight; HI, harvest index; LWC, leaf water content; LAI, leaf area index; SC, stomatal conductance; CT, canopy temperature; GY, grain yield.

Table 5. Summary of sequential path analysis results and direct effect of different agro-physiological traits on grain yield of the overage two seasons and the 90 spring wheat germplasms investigated under irrigation water shortage condition.

Grade	Response variables	Predicator variables	Partial R^2	Model R^2
1	GY*	GW	0.660	0.660
		LAI	0.231	0.891
		SC	0.077	**0.968**
2	GW	LWC	0.662	0.662
		TDW	0.115	0.777
		HI	0.048	0.825
		CT	0.023	0.848
		GN	0.022	0.870
		GLA	0.007	**0.877**
2	LAI	LWC	0.662	0.662
		TDW	0.115	0.777
		HI	0.048	0.825
		GN	0.015	**0.840**
2	SC	CT	0.644	0.644
		GN	0.059	**0.703**
3	LWC	GLN	0.382	0.382
		SL	0.083	**0.465**
3	TDW	GLN	0.606	0.606
		SL	0.071	0.677
		SN	0.018	0.695
		PH	0.015	**0.710**
3	HI	GLN	0.342	0.342
		PH	0.106	0.448
		SL	0.070	**0.518**
3	CT	GLN	0.234	0.234
		SL	0.061	**0.295**
3	GN	GLN	0.398	0.398
		SL	0.051	**0.449**
3	GLA	GLN	0.612	0.612
		PH	0.074	0.686
		SL	0.040	**0.726**

* The codes of traits are presented in Table 1

Relative importance of agro-physiological traits in evaluating water stress tolerance: To determine the relative importance of the measured agro-physiological traits on grain yield, the data were subjected to sequential path analysis (Table 5). According to this analysis, GW, LAI and SC were considered as first-order traits and accounted for 96.8% of the variation in grain yield when the GY was used as the response variable and the remaining traits as predictor variables (Table 5). When the first-order traits were used as response variables and the remaining traits as predictor variables, the results indicated that 77.7% of the total variation in GW or LAI was explained by LWC (66.2%) and TDW (11.5%), whereas 64.4% of the variation in SC was attributed to CT (Table 5). When the predictor variables of second-order being the response variable in the third-order of sequential path analysis, the results indicated that GLN explained alone 38.2, 60.6, 34.2, 23.4, 39.8 and 61.2%, whereas the other remaining traits explained only 8.3, 10.4, 17.6, 6.1, 5.1 and 11.4% of the variation in LWC, TDW, HI, CT, GN and GLA, respectively (Table 5).

The data were also subjected to conventional path analysis which partitioned the simple correlation coefficient into direct and indirect effects. The results of this analysis revealed that GW had the highest positive direct effect (0.799) on GY, followed by LAI (0.184) and SC (0.052) (Table 6 and Fig. 3). The indirect effect of GW on GY via LAI was greater than via SC, whereas the indirect effect of LAI and SC on GY was much stronger via GW (Table 6). At the second-order level, LWC, TDW, HI and GN had a considerable significant positive direct effects on GW and LAI, whereas CT was the single factor most strongly influencing SC (-0.678) (Table 6 and Fig. 3). At the third-order level, GLN had considerable significant positive direct effects on LWC (0.448), TDW (0.485), HI (0.547), GN (0.498) and GLA (0.583), whereas it had negative direct effects on CT (-0.339). The direct effect of PH on HI was negative, but the direct effect on TDW and GLA was positive. The indirect effect of any trait through other traits appeared to be significant in most cases (Table 6 and Fig. 3).

Table 6. Path coefficient analysis showing direct and indirect effects of different agro-physiological traits on grain yield of the overage two seasons and the 90 spring wheat germplasms investigated under irrigation water shortage condition.

Grade	Response variables	Predicator variables	Direct and indirect effects						Total indirect effects effect
			GW	LAI	SC				
1	GY*	GW	**0.779***	0.171	0.036				**0.207**
		LAI	0.725	**0.184**	0.033				**0.758**
		SC	0.545	0.118	**0.052**				**0.663**
			LWC	TDW	HI	CT	GN	GLA	
2	GW	LWC	**0.249**	0.107	0.135	0.107	0.126	0.090	**0.565**
		TDW	0.168	**0.158**	0.136	0.103	0.131	0.103	**0.641**
		HI	0.162	0.104	**0.207**	0.110	0.131	0.069	**0.576**
		CT	-0.136	-0.083	-0.116	**-0.196**	-0.090	-0.057	**-0.482**
		GN	0.159	0.105	0.136	0.089	**0.198**	0.084	**0.573**
		GLA	0.163	0.119	0.105	0.082	0.121	**0.137**	**0.590**
			LWC	TDW	HI	GN			
2	LAI	LWC	**0.315**	0.190	0.168	0.116			**0.474**
		TDW	0.213	**0.281**	0.168	0.121			**0.502**
		HI	0.205	0.184	**0.257**	0.120			**0.509**
		GN	0.201	0.186	0.169	**0.182**			**0.556**
			CT	GN					
2	SC	CT	**-0.678**	-0.124					**-0.124**
		GN	0.309	**0.272**					**0.309**
			GLN	SL					
3	LWC	GLN	**0.448**	0.170					**0.170**
		SL	0.228	**0.335**					**0.228**
			GLN	SL	SN	PH			
3	TDW	GLN	**0.485**	0.146	0.106	0.041			**0.293**
		SL	0.247	**0.287**	0.053	0.038			**0.338**
		SN	0.291	0.087	**0.177**	0.018			**0.396**
		PH	0.151	0.083	0.024	**0.132**			**0.258**
			GLN	PH	SL				
3	HI	GLN	**0.547**	-0.121	0.158				**0.037**
		PH	0.171	**-0.387**	0.090				**0.261**
		SL	0.278	-0.112	**0.311**				**0.166**
			GLN	SL					
3	CT	GLN	**-0.339**	-0.146					**-0.146**
		SL	-0.173	**-0.286**					**-0.173**
			GLN	SL					
3	GN	GLN	**0.498**	0.133					**0.133**
		SL	0.253	**0.261**					**0.253**
			GLN	PH	SL				
3	GLA	GLN	**0.583**	0.079	0.121				**0.200**
		PH	0.182	**0.253**	0.069				**0.251**
		SL	0.297	0.074	**0.237**				**0.371**

* Values in bold underlined denote direct effects
* The codes of traits are presented in Table 1

Response of spring wheat germplasms to irrigation water shortage: To examine the response of different germplasms to irrigation water shortage, the results of cluster analysis were compared with the PCA results (Fig. 4). The 90 germplasms were grouped into five clusters based on all agro-physiological traits using centroid linkage method. The cluster means of different traits of each cluster group are summarised in Table 7. The data in this table shows that the germplasms in cluster 3 were dominated by higher values of CT and lower values for the remaining traits; the opposite held true for the germplasms that formed cluster 5. Therefore, the germplasms of cluster 5 were plotted in the positive coordinates of the two axes of PCA, whereas the germplasms of cluster 3 were plotted in the opposite site of cluster 5 in the negative coordinates of the two axes of PCA (Fig. 4). The averaged values of the traits for most germplasms that formed cluster 1 or 2 were occasionally comparable to those in cluster 5, whereas the values of the traits for the germplasms in cluster 4 were intermediate between clusters 1 and 3 (Table 7). Therefore, most germplasms of cluster 1 and 2 were plotted in the positive coordinates of only one PCA axis, but the germplasms of cluster 4 were plotted between clusters 1 and 3 (Fig. 4).

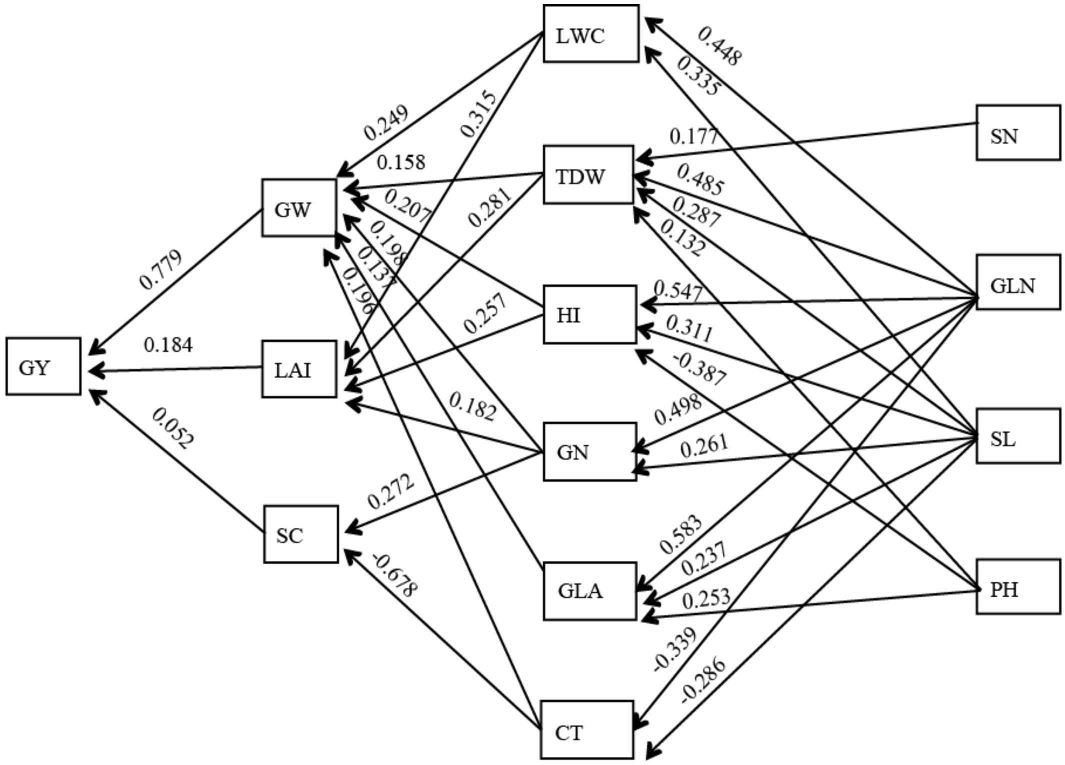

Fig. 3. Sequential path analysis diagram showing the interrelationships among various traits contributing to grain yield under irrigation water shortage condition. PH, plant height; TN, tiller number; GLN, green leaf number; GLA, green leaf area; TDW, total dry weight; SN, spike number; SL, spike length; GW, grain weight; GN, grain number; TGW, thousand grain weight; HI, harvest index; LWC, leaf water content; LAI, leaf area index; SC, stomatal conductance; CT, canopy temperature; GY, grain yield.

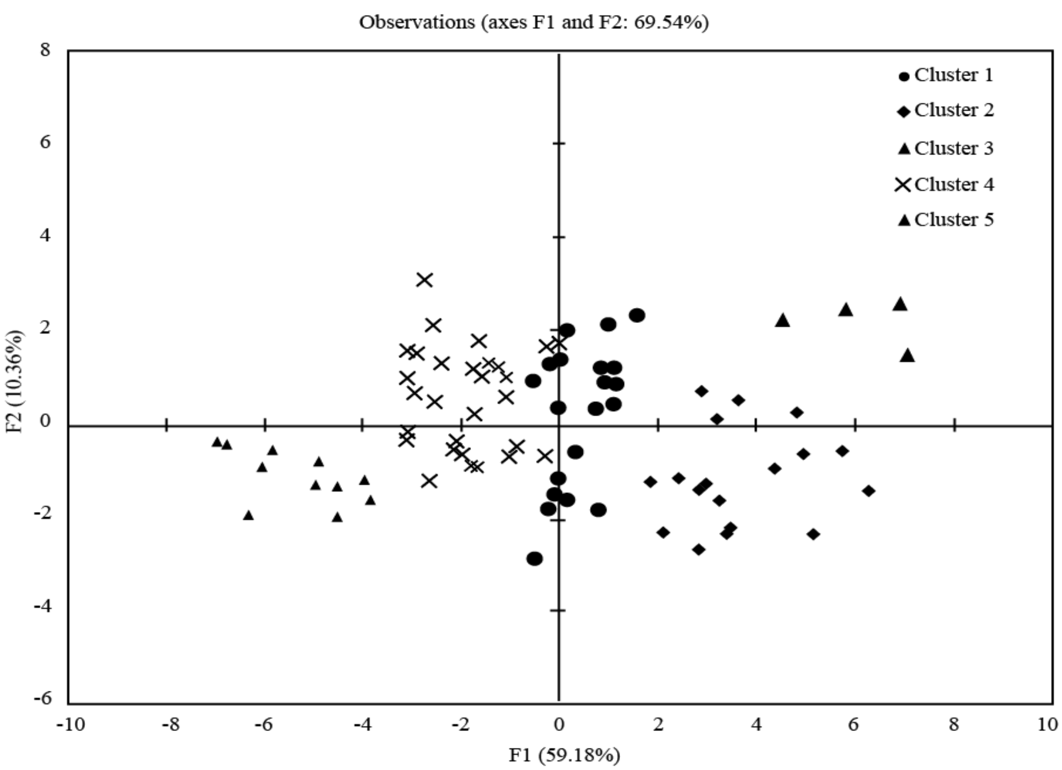

Fig. 4. Biplot of the first two axes of the principal component analysis for the 90 spring wheat germplasms. Symbols show the five groups of cluster (data presented across two years).

Table 7. Mean values of each individual cluster for different agro-physiological traits of the overage two seasons and the 90 spring wheat germplasms investigated under irrigation water shortage condition.

Traits	Clusters means				
	1	2	3	4	5
Plant height (m) (PH)	0.68	0.78	0.68	0.74	0.87
Tiller number (no.) (TN)	4.3	4.2	3.2	4.3	5.3
Green leaf number (no.) (GLN)	10.6	11.9	6.8	9.4	16.8
Green leaf area (cm^2) (GLA)	194.4	225.6	112.7	175.7	353.8
Total dry weight (g) (TDW)	6.6	7.8	4.7	6.1	9.3
Spike number (no.) (SN)	3.5	3.3	2.4	3.2	4.4
Spike length (cm) (SL)	16.1	17.2	14.9	15.5	17.1
Grain weight (g) (GW)	7.0	8.3	4.1	5.6	9.0
Grain number (no.) (GN)	105.6	125.8	65.2	79.5	162.3
Thousand grain weight (g) (TGW)	41.7	45.4	44.6	42.2	38.823
Harvest index (%) (HI)	0.43	0.45	0.37	0.40	0.48
Leaf water content (%) (LWC)	71.1	76.6	65.2	70.0	77.8
Leaf area index (LAI)	1.81	2.28	1.18	1.44	2.53
Stomatal conductance (mmol m^{-2} s^{-1}) (SC)	275.0	331.0	193.7	228.4	320.7
Canopy temperature (°C) (CT)	31.1	29.2	34.2	32.8	31.1
Grain yield (ton ha^{-1}) (GY)	5.45	6.69	2.98	4.12	7.36

Discussion

One important goal of recent innovative breeding strategies is to look for new indirect traits rather than grain yield as screening tools to select superior germplasm. This goal can be achieved through established various effective secondary selection traits and making the measurement of these traits easier, faster and cheaper than the primary trait grain yield. To establish effective secondary selection traits, it is necessary to compare these traits using a more diverse germplasm (Liu *et al*., 2011). The 90 bread wheat germplasms used in this study represent exceptionally wide genetic diversity in different agro-physiological traits. A wide range between the minimum and maximum values for all traits was recorded (Table 1). In addition, except for thousand grain weight, significant correlations were observed between grain yield and all agro-physiological traits evaluated across the 90 germplasms and growing seasons (Table 2). These results indicate that several traits measured in this study could be strongly nominated as screening criteria instead of grain yield for evaluating wheat genotypes under water shortage.

To identify which of these traits are effective and reliable as screening traits for evaluating wheat genotypes under irrigation water shortage, multiple statistical procedures were applied. The results of principle component analysis (PCA) and factor analysis (FA) showed that the first PCA and FA had all measured agro-physiological traits except plant height (PH), tiller number (TN), spike number (SN) and thousand grain weight (TGW) and accounted for 59.2 and 57.4% of the total variability among the 90 germplasms, respectively (Tables 3 and 4). The results of cluster analysis (CA) also revealed that most traits of the first PCA and FA were located in the same cluster (group 3) (Fig. 2). This suggests that those traits that clustered together and contributed the most of the total variability of community based on PCA and FA, such as green leaf number (GLN), green leaf area (GLA), total dry weight (TDW), spike length (SL), grain weight (GW), grain number (GN), harvest index (HI), leaf water content (LWC), leaf area index (LAI), stomatal conductance (SC) and canopy temperature (CT) could be nominated as screening criteria for evaluating wheat under irrigation water shortage. Similar results were also reported by previous studies (Chen *et al*., 2012; Li *et al*., 2012; Pask & Reynolds, 2013; Yildirim *et al*., 2013), which indicated a significant contribution for most of these traits to evaluate wheat under different environment conditions.

Although previous statistical procedures (PCA, FA and CA) succeeded at defining the most important agro-physiological traits that can be used as screening criteria in wheat, these procedures failed to produce a complete picture about the relative importance for each trait in screening strategies. Therefore, the measured traits were subjected to sequential and conventional path analysis (Tables 5 and 6, and Fig. 3). The advantages of both analyses are that they break down the simple correlation coefficient into direct and indirect effects, measure indirect effects of each trait through other traits, identify the complicated interrelationships between traits, and array the independent traits in different levels on the basis of their maximum direct effects (Sabaghnia *et al*., 2010; Hussain *et al*., 2014). On the whole, the results of sequential and conventional path analysis revealed that GW, LAI and SC were considered as first-order traits and accounted for most of the total variation in GY (96.8%). Furthermore, GW had the highest positive direct effect on GY followed by LAI and SC. Among the second-order traits, LWC, TDW and HI explained the most variation in (82.5%) and had considerable significant positive direct effects on GW and LAI, whereas most variation in SC was attributed to CT (64.4%). Among third-order traits, GLN explained the greatest variation in LWC, TDW, HI, CT, GN and GLA (Tables 5 and 6, Fig. 3). These results indicate that the relative importance and close interrelationship between traits can be successfully determined using both sequential and conventional path analysis. The relative importance of some traits in this study such as GW, LAI, SC, LWC, and HI, as determined using both path analysis, has also been reported in other previous studies as indicators for evaluating germplasms in wheat and other crops (Munns *et al*., 2010; Lu *et al*., 2011; Chen *et al*., 2012).

Most importantly, the traits that are easy and fast to measure will continue to be favoured as indicators for large-scale evaluating. In the current study, LAI and SC were positively and significantly correlated with GY (Table 2). Furthermore, both traits were considered as first-order important traits, and together with GW, explained most of the total variation in GY using sequential path analysis (Table 5 and Fig. 3). The positive results for LAI and SC suggest that both traits could be strongly nominated for large-scale evaluation in wheat under irrigation water shortage. The effectiveness and reliability of both traits in evaluating strategies may be related to the fact that (1) SC responds rapidly and sustainably to moisture stress, provides the main limitation to photosynthesis and growth under moisture stress, and provides indirect indicators of increased root water use capacity due to the active regulation of stomatal aperture (Reynolds *et al*., 2007; Mullan and Reynolds 2010; Munns *et al*., 2010; Pask & Reynolds 2013), and (2) LAI provides an indirect indicator for rapid ground cover and leaf expansion rate under moisture stress, which thus increases LAI, causing soil temperature and evaporation water losses to decrease as a result of shading of the soil surface by the crop canopy (Liu *et al*., 2011). Loss & Siddique (1994) reported that LAI is a very important trait, especially in Mediterranean-type drought environments, where up to 40% of the total soil water may be lost by evaporation in wheat.

Although the SC trait was considered a first-order important trait in screening strategies in this study, its direct effect on grain yield was weak (0.052) and accounted for only 7.7% of the total variation in GY (Table 5). These results indicate that there might be a high heterogeneity in stomatal properties among germplasms, such as stomatal aperture, stomatal pore area and stomatal density, which provide a high variability in SC within the germplasms (Munns *et al*., 2010; Liu *et al*., 2011). This variability in SC makes it a less reliable screening criterion in moisture stress tolerance evaluation. Therefore, many studies have reported that developments in infrared thermography provide adequate resolution to detect genetic variation in the stomatal response to shortage water and have demonstrated the complicated relationship between integrative traits (Jones 1999; Munns *et al*., 2010; Liu *et al*., 2011). In this study, the CT explained 64.4% of the total variation in SC, and it exerted a relatively strong direct effect (−0.68) on SC (Tables 5 and 6). In addition, a close negative relationship between the CT and SC (−0.80) also indicated that a cooler canopy is an indicator for stomatal aperture, which resulted in an increase in the gas exchange and transpiration rate but, conversely, resulted in an increase in the capacity of the roots to supply water under high evaporative demand and thus is likely to account for the higher yield and water use efficiency under moisture stress.

Conclusions

The results of sequential and conventional path analysis revealed that grain weight per plant, leaf area index and stomatal conductance were considered as first-order traits and accounted for most of the total variation in grain yield (96.8%). In order to evaluate large number of genotypes in a relative short time and non-destructive manner leaf area index and stomatal conductance could be strongly nominated in evaluation strategy. The direct effect of stomatal conductance on grain yield was weak and canopy temperature explained 64.4% of the total variation in stomatal conductance, thermal imaging can be used to detect genetic variation in the stomatal response to water shortage and can be used in combination with leaf area index and stomatal conductance to evaluate a large number of wheat germplasms in a relatively short time. Furthermore, germplasms producing higher grain yield under drought stress were characterised by high leaf area index, stomatal conductance and low canopy temperature.

Acknowledgement

This project was supported by King Saud University, Deanship of Scientific Research, College of Food & Agriculture Sciences and Agriculture Research Center.

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(Received for publication 22 January 2015)