

Agro-physiological traits for enhancing grain yield in rainfed durum wheat

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Abstract

Understanding the physiological traits enhancing grain yield is important for wheat breeding under rainfed conditions. Nineteen durum wheat genotypes differing in origin were evaluated for their grain yield and agro-physiological characteristics under rainfed conditions for two consecutive cropping seasons,2017-18 and 2018-19. The main effects due to year, genotype and their interactions were significant for most of the investigated traits. The genotype by trait biplots (known as GT biplot) indicated that the relationships among the studied traits were not consistent over the years but they facilitated visual genotype comparison and selection at each year. The 1000-kernel weight (TKW) and normalized difference vegetative index (NDVI) consistently associated with grain yield suggesting the importance of these traits to be used as selection criteria for enhancing grain yield under rainfed conditions. Based on the cluster heat map, the genotypes classified into five groups with different levels of combined grain yield and physiological and agronomic attributes. The traits heading date, NDVI, Fv/Fm, TKW and SPAD-reading had contributed to grain yield. The study also identified potential lines with high yield and drought tolerance for subsequent varietal development for water limited areas.

Key words: Durum wheat, physiological traits, GT biplot, cluster heat map, rainfed condition

Introduction

Breeding high yielding genotypes of durum wheat (*Triticum turgidum* L. ssp. durum) in Mediterranean conditions is difficult due to high genotype x environment (GE) interaction (Annicchiarico 2002). Thus selection of genotypes in the Mediterranean dryland conditions may be improved by selection of traits related to yield with higher heritability than yield (Reynolds et al. 2004; McIntyre et al. 2010). Further,

the increase in yield potential and stress adaptation of wheat is mainly achieved through experimental selection for grain yield. There are some indications that phenotyping using physiological traits, as a complement to agronomic traits, can help select suitable traits that improve yield under drought stress conditions (Fischer 2007; Foulkes et al. 2007; Fluery et al. 2010 Chen et al. 2012; Liu et al. 2015). Thus, to accelerate increasing grain yield in wheat, there is a need for trait-based breeding that is complemented by genetically and high-yielding genotypes (Chen et al. 2012; Liu et al. 2015; Reynolds et al. 2017).

Wheat breeding for physiological traits can improve genetic gains for grain yield by approximately 50% (Reynolds et al. 2012). Phenological traits such as heading date, agronomic traits such as plant height and 1000-kernel weight (TKW) and physiological traits such as canopy temperature (CT), chlorophyll content (SPAD-reading), normalized difference vegetative index (NDVI), maximum guantum yield of PSII (Fv/ Fm) and stomatal conductance (SC) have contributed to improve grain yield in wheat (Chen and Hao 2015; Zhang et al. 2016; Gao et al. 2017). Some physiological traits such as relative leaf water loss (RWL) and leaf relative water content (RWC) have been suggested as important indicators of water status under drought stress conditions (Pellegrino et al. 2007; Mir et al. 2012). The Fv/Fm also may provide a sensitive indicator of stress conditions in plants, to estimate thermal energy dissipation activity in PSII, which protects photosynthetic systems from the negative effects of light and heat stress (Murchie and Lawson 2013). Stay-green expression in wheat has been shown

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to have a significant yield advantage during grain-filling under drought conditions compared with genotypes not possessing this characteristic (Lopes and Reynolds 2012).

Genotype evaluation basis on multiple traits is another key issue in crop improvment and there are numerous ways to understand the causes of interaction between genotype, trait and environment. The genotype by trait (GT) biplot analysis allows visualization of genetic correlation between traits (Yan and Frégeau-Reid 2008) and it provides information on the usefulness of genotypes for production and redundant traits, as well as the ability to identify suitable traits for indirect selection for grain yield (Yan and Rajcan 2002; Gonzalez et al. 2006; Mohammadi and Amri 2011). The main objectives of this study were to (i) characterize the different sources of durum wheat genotypes for agro-physiological traits, (ii) determine the efficiency of selection criteria to classify the genotypes, and (iii) investigate the traits enhancing grain yield in rainfed conditions.

Materials and methods

Plant materials and experimental layout

Nineteen durum wheat genotypes including 17 advanced lines and two new cultivars with a wide range of genetic backgrounds (ICARDA, CIMMYT, IDGB and Iran) adapted to Mediterranean conditions were sown in a randomized complete blocks design with three replications under rainfed conditions for two cropping seasons (2017/2018 and 2018/2019). The experimental unit was a plot of 7.2 m² (6 rows, 6 m long and 0.2 m row spacing). The study was carried out at the Sararood Agricultural Research Station of the Dryland Agricultural Research Institute (DARI), Iran; that is specified as a moderately cold region for rainfed crop breeding in Iran. The soil texture was silty-clay-loam at the research site. Weeds were controlled by hand and fertilizer was used at amount of 50 kg N ha⁻¹ and 50 kg P_2O_5 ha⁻¹ at the time of planting.

Data collection

The climatic data were collected from a weather station situated at approximately 200-500 m from the experiments. During the growing seasons, 11 agro-physiological traits were recorded for each genotype at each plot. Data on physiological traits measured included: initial growth rate (GR%), maximum quantum yield of PSII (Fv/Fm), canopy temperature, chlorophyll content (SPAD reading), canopy temperature (CT) and

normalized difference vegetation index (NDVI). The trait measurements were taken as follow:

The Fv/Fm was measured for three randomly flag-leaves chosen at anthesis stage by a Chlorophyll Fluorimeter (Hansatech instrument, JH BIO, India). The measurements were taken between 11:00 and 14:00 hours. The clips were placed on the samples to prevent the light for at least 30 min. Following dark adaptation, readings were taken for the samples of each plot (Ristic et al. 2007).

Canopy temperature (CT) was measured at early grain filling stage using an infrared thermometer (Kimo KIRAY 100, UK) on all plots between 12:00 to 14:00 hours and from a distance of about 0.5 m in the front of canopy, on a clear and sunny day with minimal wind (Pask et al. 2012).

NDVI was measured at early grain filling stage using the Green seeker hand-held active sensor (Trimble Greenseeker, USA) close to noon, when the plant canopy and soil surface are dry, at about 0.5 m horizontally above the canopy such that the tool is directly above the plot and centered over the middle row (Pask et al. 2012).

The SPAD index was measured for three randomly flag leaves for each genotype at anthesis stage by the SPAD chlorophyll meter (Minolta Co. Ltd., Tokyo, Japan).

To measure the relative water loss (RWL), five leaves were randomly chosen at early heading stage, then weighed (W1), wilted for 2 h (t1) at 30 °C and reweighed (W2) and oven-dried for 48 h (t2) at 72 °C to obtain the dry weight (W3). The rate of water loss for each genotype was calculated as follow (Yang et al. 1991).

$$RWL = \frac{(W1 - W2)/W3}{(t2 - t1)/60}$$

To measure of relative water content (RWC) five leaves were randomly chosen from each plot at anthesis stage. Then 10 leaf discs provided from the samples and weighted (FW), after that the leaf discs floated in water for an over-night to obtain turgid weight (TW), and then oven-dried for 48 h at 72 °C to obtain the dry weight (DW). Finally the RWC was calculated using the following equation (Barrs 1968):

$$RWC(\%) = \frac{(FW - DW)}{(TW - DW)} * 100$$

The rate of initial growth rate (GR) at stem elongation stage was quantified following equation for each plot at each replication according to Hoffmann and Poorter (2002):

$$GR(\%) = \frac{\ln(PH2) - \ln(PH1)}{(t2 - t1)} * 100$$

where In(PH1) and In(PH2) stand for natural logarithms of plant height measurements in initial stem-elongation (t1) and after two weeks (t2), respectively.

Heading date (DH) was scored as the date when 50% of the plants had fully spikes, and days to

physiological maturity (DM) was recorded when 50% of the spikes in a plot showed a total loss of green color. The plant height (PH) was measured for each genotype from the soil level to tip of spike (excluding the awns). The TKW was calculated based on the weight of 1000 grains for each genotype. The grain yields were taken from 1 meter square of each plot area and recorded as g/m2 for each plot.

Statistical analyses

The recorded data on the traits were subjected to combined analysis of variance (ANOVA) following the method described by Steel et al. (1997) to determine

Table 1.	The name, pedigi	ee, type and origir	of 19 durum wheat	genotypes in the study

Code	Genotype	Туре	origin
G1	Saji	Modern cultivar	Iran
G2	Zahab	Modern cultivar	Iran
G3	Icasyr1/3/Gcn//Stj/Mrb3	Breeding line	ICARDA
G4	IcamorTA042/4/Bcr/Lks4/3/Altar84/Stn//Lahn/5/Beltagy2/6/OssI1/Stj5/ 5/Bicrederaa1/4/	Breeding line	ICARDA
	BezaizSHF//SD19539/Waha/3/Stj/Mrb3		
G5	Mgnl3/Ainzen1/3/Bcr/Gro1//Mgnl1	Breeding line	ICARDA
G6	Azeghar2	Breeding line	ICARDA
G7	BERKMEN//68.111/WARD	Breeding line	IDGB
G8	RICCYA/BERKMEN//GDOVZ381/KOBAK4636-1	Breeding line	IDGB
G9	RICCYA/BERKMEN//GDOVZ381/KOBAK4636-2	Breeding line	IDGB
G10	COCORIT C71/BERKMEN	Breeding line	IDGB
G11	237.4.13.3	Breeding line	IDGB
G12	URRACA	Breeding line	IDGB
G13	ALTAR 84	Breeding line	CIMMYT
G14	GUAYACAN INIA/2*SNITAN/3/SOMAT_3/GREEN_22//2*RASCON_ 37/2*TARRO_2	Breeding line	CIMMYT
G15	ALTAR 84/STINT//SILVER_45/3/ GUANAY/4/ GREEN_14//YAV_ 10/AUK/5/SOMAT_	Breeding line	CIMMYT
	4/INTER_8/6/SOMAT_3/GREEN_22//2*RASCON_37/2*TARRO_2-1		
G16	ALTAR 84/STINT//SILVER_45/3/GUANAY/4/GREEN_14//YAV_ 10/ AUK/5/SOMAT_4/	Breeding line	CIMMYT
	INTER_8/6/SOMAT_3/GREEN_22//2*RASCON_37/2*TARRO_2-2		
G17	BCRIS/BICUM//LLARETA INIA/3/DUKEM_12/2*RASCON_21/5/ SILK_3/DIPPER_6	Breeding line	CIMMYT
	/3/ACO89/DUKEM_4//5*ACO89/4/ PLATA_7/ILBOR_1//SOMAT_3		
G18	SINCHI/3/PF70354/ALD//MES/4/PATKA_7/YAZI_1/5/2*PATKA_7/ YAZI_ 1/6/ADAMAR_15//ALBIA_1/ALTAR 84/3/ SNITAN/7/ALBIA_1/ALTAR 84	Breeding line	CIMMYT
	//RCOL/3/PLATA_6/GREEN_17/8/ SILK_3/DIPPER_6/3/ACO89/DUKEM_ 4//5*ACO89/4/ PLATA_7/ILBOR_1//SOMAT_3		
G19	ATIL/BAIRDS	Breeding line	CIMMYT

et al. 2001).

Broad sense heritability (H²b) was estimated for each trait individually in year and across years as:

(PCA) (Lever et al. 2017), and cluster analysis (Everitt

$$H_b^2 = \sigma_g^2 / (\sigma_g^2 + \frac{\sigma_{ge}^2}{e} + \frac{\sigma_e^2}{re})$$

where σ_g^2 = genotypic variance, σ_{ge}^2 = GE interaction variance, σ_e^2 = environmental variance r= number of replications, and e= number of environments.

To calculate the phenotypic correlation coefficients for all possible trait combinations the formula proposed by Miller et al. (1958) and Johnson et al. (1955) adopted.

A GT biplot analysis, as described by Yan and Rajcan (2002), was constructed to graphically analysis of genotype by trait (GT) in single and across years and for exploring multiple trait data and multi-trait selection.

In GT biplot, the correlation between two traits can be estimated by the cosine angle between the vectors of two traits. Acute angle, right angle and obtuse angles, respectively, shows positive, no correlation and negative correlations. The length of vectors shows the ability of trait to discriminate among test genotypes; a trait with short vector show it is not suitable for genotype discrimination and also is not correlated with other traits (Yan and Rajcan 2002).

All analyses were performed using the R software (R Core Team, 2016) with the packages of GEA-R (Pacheco et al. 2016) and META-R (Alvarado et al. 2016).

Results

Weather conditions

The cropping seasons differed in their weather mainly in amount and monthly rainfall distribution (Fig. 1), thus giving contrasting growing conditions that lead to a range of yield productivity. Annual rainfall was variable during the cropping seasons (2017/18 and 2018/2019) and varied from 521 to 782 mm with considerably increasing than the average long-term (447 mm), especially in second season. In 2017/18, rainfall received mainly in February, April and May

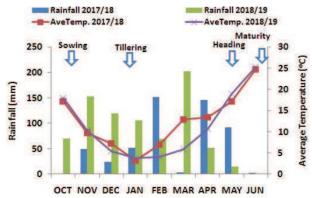


Fig. 1. Average monthly rainfall and average temperature during the experiments conducted at Sararood station (2017/18 and 2018/19)

which coincided with the phenological stages of tillering (Z20-25; Zadoks et al. 1974), stem elongation (Z32-37) and heading (Z55), respectively; while in 2018/19 rainfall received mainly in October, February and March which coincided with sowing-germination (Z10), stem elongation (Z32-37) and heading (Z55), respectively (Fig. 1). Average monthly temperature during the experiment was 12.5 °C (range 3.2° C-24.7°C) in 2017/ 18 and was 14.1 °C (range 2.7° C-28.4°C) in 2018-19; and higher than the long-term average temperature (11.4°C).

Combined analysis of variance

Significant differences (P<0.01) were observed among genotypes (G), years (Y) and genotype \times year (G \times Y) interaction for the investigated traits (Table 2). All the traits (except for RWC, SPAD and grain yield) were significantly influenced by the year effect. The genotypes significantly differed for all the traits, except for GR, RWL, CT, NDVI, Fv/Fm. For these five physiological traits that the genotype effects was not significant, separately analysis of variance per year to revel variance in genotypes were performed (Table 3). Only for Fv/Fm in both years and for GR in second year, genotype effect was significant (P<0.01). The G×Y interaction was significant for all the studied traits except for RWC, CT, NDVI and SPAD. Depending on trait, the genotype accounted for 5.8% (corresponding to GR) to 79.6% (corresponding to PH), followed by the year accounting for 0.3% (correspond to SPAD) to 60.4% (correspond to GR), and G×Y interaction that captured for 6.8% (corresponding to CT) to 41.7% (corresponding to Fv/Fm) of total sum of squares. This indicates that for the majority of traits, the genotype accounted for most of the variation (due to diverse

									_ /_		-	
Source	df	GR	DH	RWL	RWC	СТ	NDVI	SPAD	Fv/Fm	PH	TKW	YLD
Environment	1	74.12**1	540.0**	0.422	**45.1	358.6**	0.233*	* 10.5	0.0850*	*1044.1**	1748.0**	4562.8
Error	4	0.423	1.439	0.029	56.43	24.78	0.007	14.37	0.001	12.28	15.76	6240.9
Genotype	18	0.396	33.0**	0.053	42.90*	* 8.65	0.004	103.7**	0.009	695.4**	89.07**	9496.5**
GxE	18	0.907**	9.787*	* 0.165	**17.02	5	0.003	17.51	0.0100*	* 105.6**	20.25*	3847.4*
Error	72	0.327	1.115	0.06	19.44	8.51	0.002	19.43	0.00001	2.994	9.588	2692.7
Total	11:	3										
Variance expl	ained	(%)										
Environment		60.4	64.2	4.8	1.6	27.2	43.9	0.3	19.7	6.6	39.1	1
Error		1.4	0.2	1.3	8.2	7.5	5.3	1.6	0.9	0.3	1.4	5.4
Genotype		5.8	24.8	0.9	28.1	11.8	13.6	51.2	37.5	79.6	35.9	36.9
GxE		13.3	7.3 3	33.8	11.1	6.8	10.2	8.6	41.7	12.1	8.2	14.9
Error		19.2	3.3 4	19.2	50.9	46.5	27.1	38.3	0.2	1.4	15.4	41.8
CV (%)		26.88	0.87 2	29.43	5.31%	9.75	9.46	% 8.91%	6 2.97	2.13%	7.48%	28.35%

 Table 2.
 Combined analysis of variance for different agro-physiological traits of 19 durum wheat genotypes across two cropping seasons

GR = Initial growth rate; DH = Days to heading; RWL = Relative water loss; RWC = Relative water content; CT = Canopy temperature; NDVI = Normalized difference vegetative index; SPAD-reading: Chlorophyll content; Fv/Fm = Chlorophyll fluorescence; PH = Plant height; TKW = Thousand kernel weight and YLD = Grain yield.

*, ** significant at 5% and 1% level of probability, respectively.

 Table 3.
 Analysis of variance per year for some physiological traits that the genotype effect in combined ANOVA (Table 2) was not significant

Source	df		2017/18						2018/19				
		GR	RWL	СТ	NDVI	Fv/Fm	GR	RWL	СТ	NDVI	Fv/Fm		
Replication	2	0.463	0.031	29.986	0.012**	0.001**	0.384	0.002*	19.579*	0.002	0.001		
Genotype	18	0.686	0.251	8.486	0.004	0.011**	0.617**	0.072	5.166	0.003	0.008**		
Error	36	0.45	0.167	12.902	0.002	0.001	0.203	0.082	4.116	0.003	0.001		
CV(%)		22.9	45.2	12.8	9.4	1.3	34.1	40.0	6.4	9.2	4.2		

GR = Initial growth rate; RWL = Relative water loss; CT = Canopy temperature; NDVI = Normalized difference vegetative index; Fv/Fm = Chlorophyll fluorescence

*, ** significant at 5% and 1% level of probability, respectively

genetic materials) followed by year and G×Y interaction effects.

Trait performance

Coefficients of variations (CV%) were relatively low for all the traits and was lowest for DH (0.87%) and highest for RWL (29.4 %). Heritability was in general medium and varied between 36.4% (correspond to RWL) and 92.9% (correspond to PH). The heritabilities less than 50% were observed for RWL (36.4%) and GR (43.8%), while the heritabilities between 50-80% were belong to traits of Fv/Fm (64.3%), NDVI (68.6%), CT (68.8%), RWC (78.5) and grain yield (80.0%) and traits with heritabilites higher than 80% were belong to DH (86.7%), SPAD-reading (89.6%), TKW (88.4%) and PH (92.9%).

Mean traits and heritability values for agrophysiological characteristics of genotypes in single and across years are given in Table 4. No similar trends were observed for trait performance and heritability of traits from year to year. Some changes for these attributes from 2017/18 to 2018/19 were positive, while other were negative; and some changes were minimum, while some changes were found to be high. In the case of trait performance, the highest changes

Traits		Trial	mean	Heritability					
	Average	2017/18	2018/19	% Change	Average	2017/18	2018/19	% Change	
GR	2.12	2.93	1.32	54.9	0.438	0.344	0.671	-95.1	
DH	122.00	118.00	126.00	-6.8	0.867	0.963	0.839	12.9	
RWL	0.833	0.893	0.772	13.5	0.364	0.365	0.243	33.4	
RWC	83.00	82.40	83.70	-1.6	0.785	0.217	0.589	-171.4	
СТ	29.90	28.10	31.70	-12.8	0.688	0.134	0.203	-51.5	
NDVI	0.51	0.46	0.55	-19.6	0.686	0.464	0.015	96.8	
SPAD	49.50	49.80	49.20	1.2	0.896	0.97	0.522	46.2	
Fv/Fm	0.69	0.718	0.663	7.7	0.643	0.992	0.911	8.2	
PH	81.40	78.40	84.40	-7.7	0.929	0.994	0.991	0.3	
TKW	41.40	45.30	37.50	17.2	0.884	0.888	0.718	19.1	
YLD	397.50	335.00	459.90	-37.3	0.8	0.333	0.456	-36.9	

 Table 4.
 Mean values and heritability for agro-physiological traits in single and across years

GR = Initial growth rate; DH = Days to heading; RWL= Relative water loss; RWC = Relative water content; CT = Canopy temperature; NDVI = Normalized difference vegetative index; SPAD-reading: Chlorophyll content; Fv/Fm = Chlorophyll fluorescence; PH = Plant height; TKW = Tthousand kernel weight and YLD = Grain yield

 Table 5.
 Characteristics of the durum wheat genetic materials differing in origin for different agro-physiological traits across two years

Genetic materials	Statistics	Traits										
		GR	DH	RWL	RWC	СТ	NDVI	SPAD	Fv/Fm	PH	TKW	YLD
Modern cultivars	AVG	2.15	120	0.814	80.7	29.3	0.50	52.5	0.705	85.3	41.5	502
	MIN	2.09	119	0.701	76.9	29.1	0.47	50.1	0.69	80	37.8	485
	MAX	2.22	120	0.926	84.5	29.5	0.54	54.9	0.72	90.5	45.3	520
ICARDA	AVG	2.06	121	0.833	82.8	30.4	0.52	50.3	0.708	80.7	45.4	402
	MIN	1.81	120	0.73	79.6	29.9	0.48	48.9	0.673	75.5	42.0	324
	MAX	2.5	121	0.935	85.1	31.0	0.54	52.1	0.75	85.5	47.9	455
IDGB	AVG	2.09	124	0.889	83.7	29.8	0.51	48.8	0.690	86.9	42.3	404
	MIN	1.71	121	0.754	81.4	28.4	0.49	42.4	0.605	75.3	37.0	380
	MAX	2.65	128	1.02	87.9	33.2	0.55	55.0	0.758	122.3	44.7	443
CIMMYT	AVG	2.19	121	0.790	83.3	29.9	0.50	48.6	0.677	76.0	38.3	359
	MIN	2.00	120	0.718	81.1	28.2	0.48	41.3	0.638	73.7	34.2	290
	MAX	2.57	122	0.939	88.1	31.2	0.54	56.9	0.728	77.8	41.3	422

AVG = Average, MIN = Minimum, MAX = Maximum; ICARDA = The International Centre for Agricultural Research in the Dry Areas; CIMMYT = The International Wheat Maize and Wheat Improvement Center and IDGB = international durum gene bank

across years was observed for GR (54.9%), followed by grain yield (37.3%) and NDVI (19.6%); and the least value was found for SPAD-reading (1.2%) followed by RWC (1.6%) and heading date (6.8%). In the case of heritability, the least changes were found for plant height (0.3%), followed by Fv/Fm (8.2%) and heading date (12.9%), while the highest changes were observed for RWC (171.4%) followed by NDVI (96.8%) and GR (95.1%).

Comparison of durum wheat genotypes differing in origin

Table 5 summarizes the characteristics of durum wheat genotypes differing in geographical regions across years. Among the regions, the grain yield of genotypes originated from IDGB (356 g/plot) and new cultivars (352 g/plot) was higher than those originated from CIMMYT (308 g/plot) in 2017/18, while in 2018/ 19 the new cultivars (652 g/plot) and ICARDA lines (463 g/plot) expressed highest mean yield.

Based on GR, the highest initial growth rate was belong to CIMMYT lines in 2017/18, while new cultivars had the highest GR values in 2018/19, and across years the CIMMYT lines had the highest GR values. The new cultivars were earliest in heading among different geographical regions, while the IDGB lines were the latest in heading. In the case of RWL, the ICARDA lines in 2017/18 and the new cultivars in 2018/19 had the least water loss, while across two years the CIMMYT lines with the least RWL and IDGB lines with the highest RWL values were found as desirable and undesirable germplasms. The highest RWC values were belonged to ICARDA and IDGB lines in 2017/18 and 2018/19, respectively. In general, the IDGB and CIMMYT lines had the highest RWC and the new cultivars vice versa. The new cultivars compared with other germplasm had cooler canopy and highest SPAD-reading. The ICARDA germplasm lines were better than other genetic resources for NDVI and Fv/Fm, indicating that the better stay-green characteristics in these genetic materials than others. In the case of plant height, across the years, the IDGB lines were tall in stature while the CIMMYT lines were short in stature. The new cultivars slightly shorter than the IDGB lines, and ICARDA lines were in medium.

Across the years, the ICARDA lines had the highest TKW (45.4 g), while the CIMMYT lines had the lowest value (38.3 g).

Associations between agro-physiological traits and grain yield

The phenotypic correlation among the yield and agrophysiological traits in the both cropping seasons is given in Table 6. In general, the correlations were moderate to low. The strongest associations were observed between TKW and NDVI in 2018/19 with rvalue equal to 0.70 (P<0.01), while in 2017/18 was non-significant (r=0.40). All correlations between measured traits and yield were low; the maximum value was r =0.52 (P<0.05), between TKW and yield in 2018/ 19. Correlations greater than r = 0.5 were observed also between NDVI and PH in both seasons, and between CT and RWL in 2018/19; and between DH and PH; GR and NDVI; RWL and PH; and between CT and Fv/Fm in 2017/18, and between RWC and DH in 2018/19. All other correlations were smaller than 0.5.

Genotype classification based on agrophysiological traits

The dendrogram in Fig. 2 shows the similarities of the examined durum wheat genotypes for the studied traits. The pattern map of the agro-physiological traits for each genotype allowed the identification of five

Traits	GR	DH	RWL	RWC	СТ	NDVI	SPAD	Fv/Fm	PH	TKW	YLD
GR		0.49*	0.03	0.32	0.08	-0.16	-0.23	0.21	0.42	-0.18	-0.25
DH	-0.43		-0.29	0.54*	-0.46	-0.09	-0.19	0.29	0.48*	-0.25	-0.19
RWL	-0.28	0.48*		-0.02	0.70**	0.04	0.07	-0.01	-0.24	0.14	0.09
RWC	-0.04	0.08	-0.21		-0.09	-0.04	-0.16	0.21	0.16	-0.19	-0.15
СТ	0.26	-0.25	-0.09	-0.12		-0.27	0.07	-0.16	-0.38	-0.09	-0.11
NDVI	-0.60**	0.29	0.29	0	-0.18		0.25	0.09	0.57**	0.70**	0.4
SPAD	0.03	-0.04	-0.08	0.24	-0.29	-0.36		0.02	0.1	0.07	-0.02
Fv/Fm	-0.07	0.21	-0.11	0.48*	-0.56**	-0.12	0.31		0.4	0.32	0.35
PH	-0.21	0.64**	0.50*	0.25	-0.15	0.31	0.12	0.31		0.39	0.12
TKW	-0.44	0.01	0.21	-0.06	0.34	0.4	-0.05	-0.13	0.29		0.52*
YLD	0.07	0.43	0.13	-0.04	0.11	0.06	-0.08	-0.23	0.26	0.08	

Table 6.Phenotypic correlations between grain yield and physiological traits for 19 durum wheat genotypes in 2017/18
(below diagonal) and 2018/19 (above diagonal) cropping seasons

GR: initial growth rate; DH: days to heading; RWL: relative water loss; RWC: relative water content; CT: canopy temperature; NDVI: normalized difference vegetative index; SPAD-reading: chlorophyll content; Fv/Fm: chlorophyll fluorescence; PH: plant height; TKW: thousand kernel weight; YLD: grain yield

*, ** significant at 5% and 1% level of probability, respectively

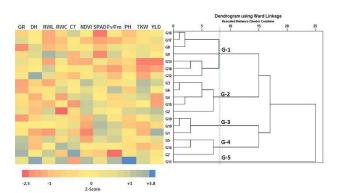


Fig. 2. Cluster heat map of 19 durum wheat genotypes performance across two years. Hierarchical clustering analysis expresses the visual representation of genotypes in different groups based on similarity as well as the correlation with different traits. GR: initial growth rate; DH: days to heading; RWL: relative water loss; RWC: relative water content; CT: canopy temperature; NDVI: normalized difference vegetative index; SPAD-reading: chlorophyll content; Fv/Fm: chlorophyll fluorescence; PH: plant height; TKW: thousand kernel weight; YLD: grain yield

genotype groups with desirable variants of each trait for the use as potential genetic materials in durum wheat breeding program. Based on the results, the tested genotypes showed different levels of combined high grain yield and/or physiological characteristics and good agronomic performance. The group G-1 consisted of seven genotypes (G16, G17, G13, and G18 originated from CIMMYT and G8, G9 and G12 originated from IDGB) as group with low yield and late heading. Group G-2 comprised five genotypes of G3, G6, G4 (originated from ICARDA), G15 (originated from CIMMYT) and G2 (originated from Iran) which totally can be characterized as a group with early heading, high NDVI, highest TKW and moderate grain yield. The group G-3 included three genotypes of G10 (from IDGB), G19 (CIMMYT) and G1 (Iran) with early heading, the highest SPAD-reading and grain yield. The fourth group (G-4) consisted of three genotypes of G5 (ICARDA), G14 (CIMMYT) and G7 (IDGB) that were characterized as genotypes with highest GR, RWL, CT and low yielding. The last group (G-5) consisted of only genotype G11 (from IDGB) with moderate grain yield, higher growth rate, highest RWC, NDVI, Fv/Fm, SPAD reading, and highest plant stature and lowest CT.

Trait profiles of genotypes

The GT biplot (Fig. 3) was used to identify genotypes

with the highest values for one or more traits in single and across years. The variation accounted for by two principal components (PC1 + PC2) was 48.5% in 2017/ 18, 46.7% in 2018/19 and 41.9% across years. In 2017/18 (Fig. 3) the genotype G7 had the highest values for grain yield, TKW and CT. Genotype G11 was late in heading with higher NDVI, PH and RWL values. The G5 was the earliest genotype in heading with highest GR value, while genotype G1 was the best in RWC, SPAD-reading and Fv/Fm among the genotypes. In 2018/19, genotype G3 had the highest values for grain yield, NDVI, TKW, SPAD and RWL. As previous year, G11 was late in heading but with higher PH, NDVI, RWC and GR, while the G18 had the highest CT. Across the years, genotype G11 was late in heading, higher in plant height, RWC and Fv/ Fm, with high grain yield. The genotype G7 was higher in canopy temperature, RWL and growth rate, while the genotype G1 had the highest SPAD-reading.

The association between any two traits in Fig. 3 can be approximated by the cosine of the angle between their vectors. The most prominent associations between traits under rainfed conditions in 2017/18 were: a positive correlation between YLD, TKW and NDVI; between DH, PH and RWL; between SPAD, RWC and Fv/Fm; and between GR and CT, as indicated by the acute angles between their vectors. A negative association between GR with the traits of DH, PH and RWL was observed as indicated by the large obtuse angles between their vectors. This negative association was high because of their vectors were considerably also long (Fig. 3).

The traits with strong positive associations are tending to discriminate genotypes in similar fashions and those with negative associations tend to discriminate genotypes in opposite direction. For instant, the genotypes (i.e., G8 and G2) discriminated by the YLD, TKW and NDVI are different from those (i.e., G5) discriminated by GR. Similarly the genotypes (i.e., G1) discriminated based on SPAD, RWC and Fv/Fm are different from those selected by the YLD and TKW. The length of the trait vector also is a good marker to show the ability of traits in discriminating genotypes; the traits with longer vector will all more discrimination among genotypes. Accordingly, the NDVI, PH, Fv/Fm, CT and TKW had the highest discriminating ability, while the grain yield followed by GR and RWC had the least discriminating ability.

In 2018/19 the strong positive correlations were found between grain yield, TKW and NDVI; between

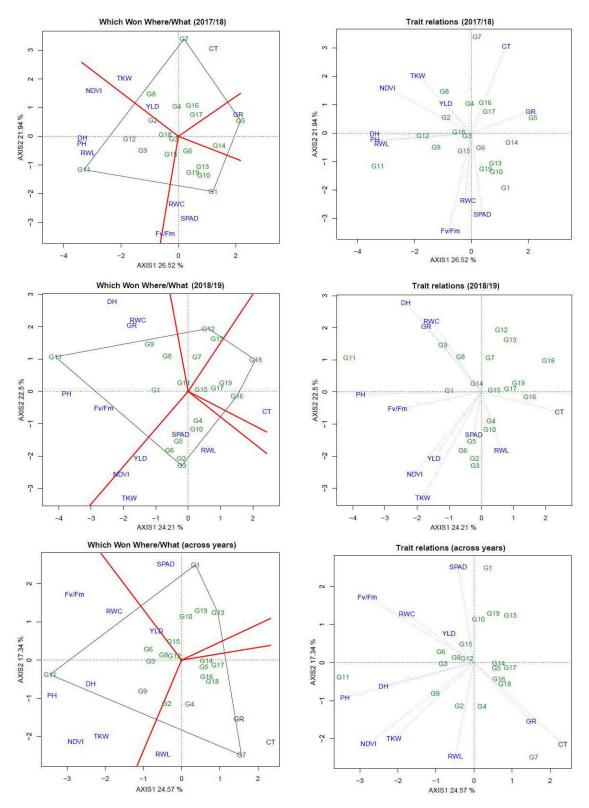


Fig. 3. The GT-biplot showing which-for-what pattern and trait relations among studied traits of 19 durum wheat genotypes in single and across years (2017/18 and 2018/19). GR: initial growth rate; DH: days to heading; RWL: relative water loss; RWC: relative water content; CT: canopy temperature; NDVI: normalized difference vegetative index; SPAD-reading: chlorophyll content; Fv/Fm: chlorophyll fluorescence; PH: plant height; TKW: thousand kernel weight; YLD: grain yield

DH, GR and RWC; and between PH and Fv/Fm. The traits TKW, NDVI, PH and DH were among the traits with the highest discriminating ability, while in contrast the traits SPAD and RWL had the least discriminating ability. Across the years, positive correlations were found between: TKW and NDVI, where they tend to discriminate the breeding line G9; and between DH and PH, which tend to discriminate the breeding line G11. The positive relationships between Fv/Fm, RWC, SPAD and YLD allowed similar ranking the genotypes, where the G15 and G6 were the best genotypes based on these traits. A strong positive correlation was observed between GR and CT, which tend to discriminate the breeding line G7. Genotypes with long vectors are those that have extreme levels for one or more traits. Such genotypes could be either selected for further trials or for parents. Accordingly, among the genotypes, the G7, G11 and G1 had different extreme levels for one or more traits.

Discussion

The tested durum wheat genotypes showed a high phenotypic variability for the traits studied under drought condition. Comparing the genetic materials indicated that the genotypes originated from ICARDA were better adapted to rainfed conditions. They were superior to the new cultivars for some physiological and agronomic traits such as NDVI, Fv/Fm and 1000kernel weight. In general, the new cultivars were superior in grain yield and SPAD-reading. Among the traits, heading date, plant height, SPAD index and 1000-kernel weight with higher heritability than grain yield would be useful for indirect selection. This is in agreement with the other studies that suggesting the use of selected traits for improving of grain yield under the Mediterranean rainfed conditions (Gizaw et al. 2016; McIntyre et al. 2010). However, correlation analysis between the traits is needed to understand correlated inheritance (Lopes et al. 2012). The magnitude of correlation of NDVI with other physiological traits were higher under very late sown (VLS) conditions relative to late sown (LS) conditions. NDVI is strongly correlared with thousand kernel weight under LS and VLS conditions making it as strong favourable trait for selection (Sunil et al. 2020) The negative phenotypic correlation between grain yield and heading date indicates the possibility of identifying genotypes with a combination of high grain production and early heading. Based on the earlier studies (De Vita et al. 2007; González-Ribot et al. 2017), selection for early heading will be increased grain yield under drought condition, because it prevent terminal drought stress. 1000-kernel weight, as main component of grain yield, showed significant positive associations with grain yield, showing the importance of this trait to improve grain productivity under rainfed condition.

In some cases, however, no correlation between increase yield and earlier heading in wheat is reported (Chairi et al. 2018; Flohr et al. 2018). The limited genetic gains incorporating early maturity may be due to reduced time available for assimilate partitioning required for high grain yield (Royo et al. 2007) may partly explained by the negative association between grain weight and heading date (Zhou et al. 2007).

Biplot analysis revealed that grain yield was positively associated with TKW and NDVI; and negatively correlated with GR and CT. However, breeders are looking for new yield related traits to screen genotypes, detect yield differences and find traits with strong associations with grain yield (Fufa et al. 2005; Gutierrez et al. 2012). Several studies declared that grain yield improvement has been significantly associated with increased TKW and NDVI (Marti et al. 2007; Morgounov et al. 2010; Zheng et al. 2011; Aisawi et al. 2015; Tattaris et al. 2016).

In this study, significant differences (P<0.01) were observed among genotypes for heading date, SPAD index, RWC, plant height, TKW and grain yield. High genotypic variation in chlorophyll content (SPAD) and the rate of leaf senescence (higher SPAD index) has been detected during grain filling in wheat (Rampino et al. 2006; Lopes and Reynolds 2012). Several other studies have reported positive associations between the stay-green trait with grain yield and grain weight in spring wheat under rainfed conditions (Blake et al., 2007; Cossani and Reynolds 2012; Lopes et al. 2012). Therefore, a delay in leaf senescence would increase the amount of fixed carbon available for grain filling. This is in agreement with the results from the ICARDA materials that with the highest values of SPAD, NDVI and Fv/Fm had the highest values of 1000-kernel weight. Positive correlations between grain yield with green flag leaf duration and total flag leaf photosynthesis have also been reported in spring wheat (Spano et al. 2003; Wang et al. 2008). Thus, delay in leaf senescence (higher SPAD index) resulting in increase the amount of fixed carbon available for grain filling. Chlorophyll content is a useful attribute for improving grain yield in wheat. Various reports indicate that breeding advances in the combination of this trait with new wheat cultivars showing slightly high chlorophyll content compared to older cultivars (Beche et al. 2014). Positive and moderate correlation between chlorophyll content and grain yield has been reported by other studies (Lopes et al. 2012; Gao et al. 2017). The stay-green associated with maintaining chlorophyll content can be effective in increasing wheat yield (Cossani and Reynolds 2012). Similarly, Lopes and Reynolds (2012) reported positive correlation between stay-green and grain yield under drought and heat stress conditions in spring wheat. Therefore, the use of the stay-green to select promising wheat genotypes is likely to enhance the genetic progress for wheat adaptation in rainfed conditions (Christopher et al., 2018). Thus, the positive correlation between physiological traits (i.e., SPAD, NDVI and Fv/Fm), that explaining stay-green in wheat, with grain yield can be targeted for cultivar development (Lopes et al. 2012; Beche et al. 2014). Genotypic variation for grain yield, and yield related traits under rainfed conditions in this study is of great interest to breeders because selected genotypes with desirable traits can be used as parents in future breeding programs. The study also revealed some merit/defects of the tested genotypes and identified some useful traits for indirect selection that can be explored for durum wheat breeding program.

Authors' contribution

Conceptualization of research (RM); Designing of the experiments (RM, PS); Contribution of experimental materials (RM); Execution of field/lab experiments and data collection (RM, FS, AI); Analysis of data and interpretation (RM, MD, FS); Preparation of manuscript (RM, FS).

Declaration

The authors declare no conflict of interest.

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