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Phenotypic Variability and Relations between the Morpho-Physiological Traits of three F5 Populations of Durum Wheat (*Triticum durum* Desf.) Evaluated Under Semi-Arid Conditions

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ABSTRACT

Selection of durum wheat (Triticum durum Desf.) for high yield performance has made little progress In under the semi-arid conditions of the eastern high plateaus of Algeria, due to the sensitivity of plant material selected to harsh abiotic stresses that characterize the region of production. The present study focused on monitoring the variability, heritability and traits association in three cross populations of durum wheat. The results indicated the presence of a sizeable amount genetic variability, for above ground biomass, spike number, spike weight, economical yield and grain yield. These traits expressed moderate to high broad sense heritability and were highly correlated with grain yield, making them a potential selection criteria to improve indirectly grain yield. Physiological traits were generally less variability, had low to moderate heritability and were not significantly correlated with grain yield, suggesting difficulty to select concomitantly for grain yield potential and stress tolerance.

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INTRODUCTION

In Algeria, durum wheat (*Triticum durum* Desf.) is mainly grown under rain fed conditions on the high plateaus. It is subject to terminal stress at the end of the cycle which drastically reduced yield potential [6]. Selection for stress tolerance is, therefore, a prerequisite for combining yield performance and yield stability [10]. Identification of characters that boost both characteristics is an important step in plant breeding [12, 22, 34, 41]. Improvement of grain yield is traditionally achieved through direct selection on the basis of grain yield itself [42, 19], while stability is tentatively approached through resilience to abiotic stress to minimize grain yield variation. Selection for grain yield under drought and heat stress conditions is inefficient due to the low heritability of this trait and the presence of genotype x environment interaction [25, 30, 9, 31]. Ceccarelli *et al*. [13] reported that genetic improvement, under drought and heat stresses, can be achieved through indirect selection for yield in the target environment. Therefore the best option for crop production is to improve both yield and yield stability under stress conditions. Several traits, closely correlated with grain yield, have been identified and proposed as indirect selection criteria to improve simultaneously wheat tolerance to stress and yield potential [33, 14]. Effectiveness of selection for secondary traits to improve yield under drought and heat stresses has been demonstrated in wheat [37]. Expression of high above-ground biomass and efficient partitioning, under stress, are indicative of tolerance to abiotic constraints [16, 38, 40]. Leaf water status, canopy temperature, and membrane stability are utilized to discriminate between tolerant and sensitive genotypes for drought and heat stresses [2, 20, 29, 36]. Relative water content is considered to be a reliable indicator of plant water status [25]. Amani *et al*. [2] and Fischer *et al*. [20] and Oulmi *et al*. [31] reported that the canopy temperature is associated with grain yield performance under water and heat stresses in bread wheat. Drought and heat stresses affect growth, depressing accumulation of above ground and root biomasses. Genotypes which maintain vigorous growth prior to heading can partition assimilates to the root system for the relief of stress and

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to the stem for remobilization during the grain filling phase [11, 38]. Electrolyte leakage from leaf tissues has been suggested as a means of assessing membrane stability, which allows evaluation of the ability of cell membrane to maintain their integrity under stress [7]. Genotypic differences for these traits are often associated with a given operating stress tolerance strategy [8, 32, 17, 35, 39]. The objective of this study is to assess the variability and the relationships among morpho-physiological traits generated in three cross populations of durum wheat (*Triticum durum* Desf.) evaluated under semi-arid conditions.

MATERIALS AND METHODS

Field experiment:

The experiment was conducted at the experimental site of the agricultural Research station of the Field Crop Institute of Sétif (Algeria), during the 2010/2011 cropping season. Three sets of 120 F5 breeding lines derived from Ofanto/Mohammed Ben Bachir, Ofanto/Waha and Ofanto/Mrb_5 durum wheat crosses, along with their respective parents, were seeded on November 2010 in 2 rows, 5.0 m long with 0.2 m row spacing. The parents were repeated every 40 entries. Nutrient deficiencies were prevented with fertilization at sowing, by an application of 100 kg/ha of super phosphate 46% and at jointing, by an application of 100 kg/ha of urea 35%. Weeds were controlled chemically with GranStar [*Methyl Tribenuron]* at 12 g ha-1 rate.

Measured variables:

The length of the vegetative period was determined as the number of calendar days from January first to the day when 50% of the spikes were extruded from fag leaves**.** Plant height was measure in at physiological maturity, from the soil surface to the top of spikes, excluding awns. Above-ground biomass, spike number and spike weight, harvest index, thousand- kernel weight and grain yield were measured, at maturity, from vegetative samples harvested from one row 1.5 m long. Thousand–kernel weight was based on count of 200 seeds. The number of kernels per spike and per square meter were derived from grain yield, thousand- kernel weight and spike number mean values.

Relative water content was determined, at heading, according to the procedure described by Mefti *et al*. [2008]. Three flag leaves, sample from each entry, were placed in pre-weighed plastic tubes containing 10 ml of distilled water (W1), with their cut ends dipped in the water. The tubes were tightly sealed and placed inside a portable ice box, transported to the laboratory and stored, overnight, in a refrigerator to achieve leaves turgor. The tubes were removed from the refrigerator, weighed again (W_2) to get the samples fresh weight (FW =W_2-W_1). The leaves were removed from the tubes, cleaned with a dry cloth and weighted to get the saturated fresh weight (SFW). The leaf samples were oven dried at 70°C for 24 h, to determine the leaves dry weight (DW). Relative water content (RWC) was then determined as the ratio:

$100[(W_2 - W_1) - DW]/(SFW - DW)$.

The percentages of cell injury caused by drought and by heat stress were determined, using flag leaf samples, according to the procedures described by Mekhlouf *et al*. [28]. Canopy temperature was measured at heading stage, with a portable infrared thermometer; model AG-42, Teletemp Corp, Fullerton, CA. Four readings were taken per entry, at three days interval. Flag leaf area and specific leaf dry weight were determined at heading from 5 flag leaves samples. The leaf area was estimated as the product of leaf length x leaf wide x 0.704 [27]. The specific leaf dry weight was determined as the ratio of flag leaf dry weight over flag leaf area [37, 4].

Data analysis:

Data were subjected to statistical analyses to obtain mean and range values for the measured traits. Estimates of phenotypic (σ^2_p), genotypic (σ^2_g) and environmental (σ^2_e) variances and phenotypic (PCV) and genotype coefficients of variation (GCV) were obtained from data of the parents and their offspring according to the method outlined by Cruz *et al*. [15]:

$PCV(\%) = 100\sigma_{pF5}/\bar{Y}_o$

$GPV (\%) = 100\sigma_{gF5}/\bar{Y}_o$

Where $\sigma_{pF5,}$ σ_{gF5} are the phenotypic and genotypic standard deviations of the F_5 generation and $\bar{Y}_o$ is the F_5 mean for the trait under study. Broad sense heritability (h^2_{bs}) was calculated using variance components derived from the parental and the offspring generations' data according to Acquaah [1]:

$h^2bs = 100(\sigma^2_{gF5}/\sigma^2_{pF5})$,

where σ^2_g = genotypic variance, σ^2_p = phenotypic variance. Traits relationship was scrutinized through phenotypic correlation coefficients and regression analyses. Statistical analyzes were performed with LazStats software [24]. The least significant difference at 5% probability level was derived from the analysis of variance of the traits measured on the parental lines which are repeated.

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RESULTS AND DISCUSSIONS

Variability of morphological characters:

The relative comparison of the three populations for the measured agronomic traits is given in figure 1. Taking the mean values of the check cultivar Waha as 100%, the results indicated no differences existed between the three populations and Waha for the number of days to head (DHE). On average, the cross populations expressed low above ground biomass (BIO), low number of kernels per m² (NKM²), low economical yield (YLD_{ec}) and low spike number (SN) than did Waha. However they showed higher average values for plant height (PHT), thousand-kernel weight (TKW), number of kernels per spike (NKS) and harvest index (HI). The advantages of these traits are not highly reflected in grain yield, since only Ofanto/Mrb_5 population presented a higher grain yield average than Waha, Ofanto/Waha had comparable yield to Waha and Ofanto/MBB showed a lower grain yield than Waha (Figure 1). These results indicated that there is large scope to improve traits such as SW, PHT, TKW, NKS and HI in the cross populations' understudy, if these traits exhibit high broad sense heritability and large genotypic variability. However these results suggested also that the improvement of one or more than one or more than one of the above mentioned traits does not necessarily improve indirectly grain yield which is the targeted trait.

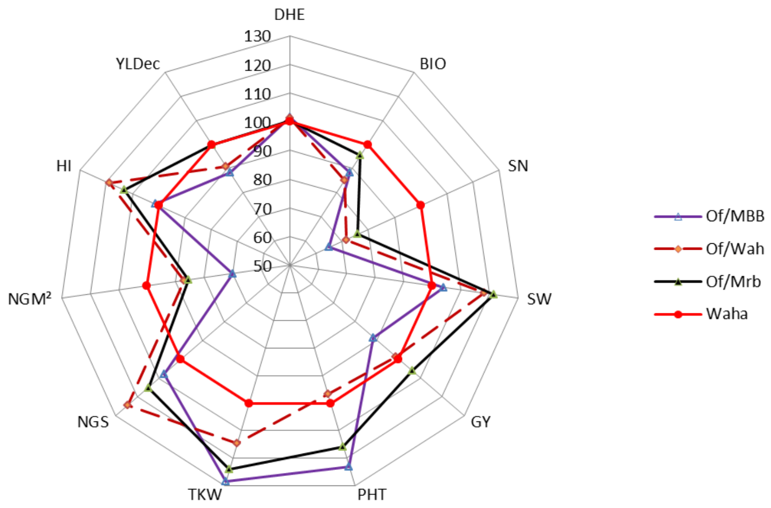

Fig. 1: Average performances, expressed as % of the values of the check cultivar Waha, of the three cross populations for the morphological traits.

Mean values of the number of days to head (DHE) were very close in the three populations. DHE range values varied from 5 days in Ofanto/Waha and Ofanto/Mrb_5 to 7 days in Ofanto/MBB. The largest amplitude expressed in Ofanto/MBB population for this trait suggests the genetic effect of the relatively late cultivar MBB. Indeed, among the crossed parents, MBB is the latest with a DHE average of 132.8 days, comparatively to Ofanto, Waha and Mrb_5 which showed similar degree of earliness with DHE mean values varying from 128.1 to 129.0 days (Table 1). DHE presented a moderate to high coefficient of genetic determination in the three populations, suggesting that this trait could be easily improved; however it presents little variability which results in low genetic gain or response to selection. Comparatively to DHE, above-ground biomass (BIO), spike number (SN), spike weight (SW), grain yield (GY) , number of kernels per spike (NKS) number of kernels per square meter (NKM²) and economical yield (YLD_{eco}) presented a large variability, in the three cross populations. Maximum values of these traits were almost two times higher than the mean values of the base populations (Table 1). Plant height (PHT), thousand-kernel weight (TKW) and harvest index (HI) presented moderate variability since maximum values were 1.2 to 1.5 higher than the mean values of the base populations (Table 1). Compared to the Lsd5% size, the differences between maxi and min values were largely significant for all measured traits. The broad sense heritability values were moderate to high for PHT and HI and very high for BIO, SN, SW, GY, TKW, YLD_{eco}, NKS and NKM². Traits which showed high phenotypic variability and moderate to high broad sense heritability values are more amenable to easy improvement through selection. Therefore BIO, SN, SW, GY, TKW, YLD_{eco}, NKS and NKM² can be easily improved in the three populations understudy. Similar results were reported Amin *et al*. [3] who find that plant height, 1000-kernel weight, grains per spike, days to maturity, harvest index and grain yield showed high heritable, but spike number was moderately heritable.

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Table 1: Mean values of the morphological traits measured in three cross populations of durum wheat.

Traits	DHE	BIO	SN	SW	GY	PHT	TKW	NKS	NKM²	HI	YLD_{ec}
					Ofanto/MBB						
Max	135	3890	1050	2060	1300.9	129	61.4	71.8	41.9	44.5	2077.6
Min	128	1020	266	510	304.9	58.5	22.3	21.5	7	19	557.8
μ_{F5}	130.9	1966.1	462.4	949.7	602.8	99.3	40.6	33	15.1	31.2	1011.8
Ofanto	129	1759.1	469.8	986.3	652.3	76.8	37.9	37	17.3	37.2	984.4
MBB	132.8	2637.5	587.1	840.9	611.6	118	39.2	26.7	15.7	23.2	1219.4
h²bs	93.7	95.9	90.2	93.5	89.1	96.5	92.2	79.5	88.6	65.1	95.4
					Ofanto/Waha						
Max	133	3780	1095	2130	1256.9	97	45.9	81.6	38.6	67.6	1924.1
Min	128.1	1015	245	520	359.8	56	26.7	18.9	9.4	18	572.3
μ_{F5}	130.4	1894.3	507.6	1079.5	672.2	78	36.2	38.1	18.8	36.6	1038.9
Waha	128.9	2222	710.1	913.8	681.5	80.6	31.6	30.6	21.6	30.8	1143.6
h²bs	89.9	94.9	94.3	96.4	90.7	66.5	88.8	81.1	91.5	76.6	94.7
					Ofanto/Mrb_5						
Max	133	4300	940	1910	1255.7	133	55.6	61	30.5	47.9	2169
Min	128	1010	255	510	406	59	28	19.2	10.7	21.1	623.5
μ_{F5}	129.5	2122.3	540	1110.9	723.4	93.3	39.2	35.2	18.5	34.9	1143.1
Mrb_5	128.1	1941.1	724.1	846	598	95.9	36.5	22.7	16.4	31	1000.9
$Lsd_{5\%}$	0.4	116.7	32.8	51.4	35.5	2.6	0.9	2.3	1.1	2.2	45.1
h²bs	64.7	95.4	93.1	95.7	91.6	96.1	95.4	84.7	89.9	65.6	96.2

DHE= number of days to heading, BIO= Above-ground biomass, g/m²; SN= Spike number per m^2; SW= Spike weight, g/m²; GY = grain yield, g/m², PHT = plant height, cm; TKW = Thousand- kernel weight, NKS = Number of kernels per spike, NKM² = Number of kernels per m², $x10^3$, HI = Harvest index, %; YLDec= economical yield, g/m².

Variability of physiological traits:

Since the best option for crop production, under stressful conditions, is to improve both yield and yield stability through indirect selection, variability and heritability of traits related to stress tolerance need to be studied. Among the traits related to stress tolerance measured in the present investigation, the magnitude of the difference between maximum and minimum values varied from 20.5%, in Ofanto/Mrb_5 population to 26.8% in Ofanto/MBB population for relative water content (RWC); from 11.7 cm², in Ofanto/Mrb_5 population to 18.6 cm² in Ofanto/MBB population for flag leaf area; from 15.2 mg/cm², in Ofanto/MBB population to 19.7 mg/cm² in Ofanto/Mrb_5 population for specific leaf dry weight (Table 2). The differences for the canopy temperature depression, drought and heat stress indices were also high, suggesting sizeable variability expressed in the three cross populations for these characters (Table 2). Among the parents of the cross populations, Mrb_5 presented the highest RWC, the lowest flag leaf area and the highest canopy temperature depression. MBB had the largest flag leaf area, the highest drought and heat stress indices and the lowest canopy temperature depression. Waha expressed the lowest specific leaf dry weight and Ofanto the highest value for this trait (Table 2). MBB, due probably to its lateness appeared to be the most sensitive to heat and drought stress as expression by DSI, HSI and CTD parameters.

Table 2: Mean values of the morphological traits measured in three cross populations of durum wheat.

Traits	RWC	FLA	SLDW	CTD	HSI	DSI
Parameters			Ofanto/MBB			
Max	95.1	31.5	21.8	2.2	69.0	47.2
Min	68.3	12.9	6.6	-4.3	2.9	2.0
μ_{F5}	80.3	20.3	9.8	-2.0	22.0	15.5
Ofanto	68.3	18.1	11.4	1.2	18.4	3.6
MBB	71.8	21.1	9.8	-1.2	28.4	17.1
h^2_{bs}	59.4	70.2	45.6	73.3	95.4	82.2
			Ofanto/Waha			
Max	91.3	27.8	22.2	4.4	63.0	48.7
Min	66.6	15.0	6.3	-1.8	0.0	0.0
μ_{F5}	81.6	20.8	10.0	2.0	23.4	13.9
Waha	82.5	17.2	8.1	0.9	6.5	0.0
h^2_{bs}	18.8	37.6	57.7	74.7	93.4	88.7
			Ofanto/Mrb_5			
Max	90.0	24.5	26.1	4.1	46.0	35.0
Min	69.5	12.8	6.4	-0.2	5.7	1.3
μ_{F5}	83.4	18.7	10.2	2.0	23.1	12.5
Mrb_5	83.0	16.2	9.0	1.5	18.3	15.2
$Lsd_{5\%}$	2.6	1.2	1.3	0.3	2.5	2.3
h^2_{bs}	43.4	64.2	49.9	53.8	90.7	52.2

RWC = Relative water content, %; LA = Flag leaf area, cm²; SLDW = Specific leaf dry weight, mg/cm²: CTD = Canopy temperature depression = T°C air –T°C leaf, °C; DSI = Drought stress index, HSI = Heat stress index.

RWC and SLDW presented low broad sense heritability, FLA and CTD showed moderate h²bs while DSI and HIS exhibited high h²bs (Table 2). The low h²bs of RWC and SLDW suggested that these traits are affected by the environment conditions which inflate the environmental component of variance. Therefore selection to improve RWC or SLDW may be less effective. Selection to improve DSI and HSI will be effective due to the high variability associated with high broad sense heritability. These results suggested that there is little scope to improve RWC and SLDW in the populations understudy, however there are possibilities to improve drought and heat tolerance measure as through cell membrane injury caused either by drought or by heat.

Relations between variables:

Development of varieties which are resilient to moisture stress conditions and able to express high grain yield potential is an important objective in many breeding programs [5]. This requires the identification and selection for traits which are associated with both yield potential and tolerance to the stresses prevailing in the targeted environment for which varieties are to be developed [18, 38, 23]. Traits association is usually approached through regression and correlation analyses, even though path analysis specifies the direct and indirect effects of the causing traits on the caused character [21]. Since grain yield is the caused character, and for selection for high grain yield and stress tolerance to be effective, a thorough understanding of yield contributing characters and their interrelationships among themselves and with yield is necessary. In the present study mutual relationship between grain yield and its contributing characters showed within each of the three populations, yield is largely determined by YLD_{ec}, NKM², SN, SW, and BIO which showed correlation coefficients greater than 0.700 (Table 3). YLD_{ec}, NKM², SW and BIO are difficult to measure with an acceptable precision, in the field, they usually exhibit high coefficient of variation greater making them less desirable than grain yield as selection criteria. However the number of spikes is a trait easily assessed visually, and could be a useful indirect selection criterion to improve grain yield potential.

Table 3: Simple coefficients of correlations between yield and morpho-physiological characters measured on three durum wheat cross populations.

	Ofanto/MBB	Ofanto/Waha	Ofanto/Mrb_5
DHE	-0.133^{ns}	-0.115^{ns}	0.072^{ns}
BIO	0.863*	0.805*	0.852*
SN	0.828*	0.719*	0.715*
SW	0.909*	0.869*	0.852*
PHT	0.413*	0.500*	0.413*
TKW	0.244*	-0.025^{ns}	0.244*
NKS	0.187*	0.286*	0.187*
HI	0.036^{ns}	0.113^{ns}	0.036^{ns}
NKM²	0.913*	0.927*	0.913*
YLD_{ec}	0.952*	0.934*	0.949*
CTD	-0.015^{ns}	0.182*	-0.015^{ns}
DSI	0.097^{ns}	0.072^{ns}	0.097^{ns}
HSI	0.101^{ns}	0.105^{ns}	0.101^{ns}
FLA	0.319*	0.207*	0.319*
SLDW	-0.113^{ns}	-0.068^{ns}	-0.113^{ns}
RWC	-0.039^{ns}	0.011^{ns}	-0.039^{ns}

DHE= number of days to heading, BIO= Above-ground biomass, g/m²; SN= Spike number per m^2; SW= Spike weight, g/m²; GY = grain yield, g/m², PHT = plant height, cm; TKW = Thousand- kernel weight, NKS = Number of kernels per spike, NKM² = Number of kernels per m², $x10^3$, HI = Harvest index, %; YLDec= economical yield, g/m², RWC = Relative water content, %; LA = Flag leaf area, cm²; SLDW = Specific leaf dry weight, mg/cm²: CTD = Canopy temperature depression = T°C air –T°C leaf, °C; DSI = Drought stress index, HSI = Heat stress index. Ns, * = non-significant and significant correlation coefficient at 5% probability level ($r_{5\%}$= 0.180).

Grain yield is positively correlated, to a lesser degree, with plant height, number of kernels per spike and flag leaf area. The relationship of TKW with grain yield is variable and cross- dependent, since this trait showed positive correlation with grain yield only within Ofanto/MBB and Ofanto/Mrb_5 cross populations. Similar results were reported by Majumder *et al*. [26] who reported from study of correlations that spikes per plant, grains per spike, 1000-kernel weight and harvest index were positively and significantly associated with grain yield, suggesting the utilization of these traits in breeding program to improve grain yield. Exception of canopy temperature depression which showed positive correlation with grain within Ofanto/Waha population, the other physiological traits measured (DHE, FLA, HI, DSI, HSI, RWC, SLDW) didn't express significant relationships with grain yield, suggesting the independence of both characteristics: i.e. yield potential and stress tolerance, but it may be possible to select high yield breeding lines with acceptable tolerance to stress within each of the three cross populations understudy (Table 3).

Path coefficient analysis was performed to disclose the causes and effects of the contributing characters with yield. Estimates of positive direct effects on yield were exhibited by above ground biomass, number of kernels per square meter, harvest index and thousand-kernel weight. The number of spikes and the number of kernels per spike exhibited sizable negative direct effects. The physiological traits had no direct effect on grain yield (Table 4). Stepwise regression retained BIO, TKW, NKS, HI and NKM² as significant determinants of grain yield (Table 4).

Table 4: Direct effects and partial correlation coefficients of measured traits on grain yield, averaged over the three cross populations

Traits	Beta	b	t (b =o vs b≠0)
BIO	0.676	0.206	**
SN	-0.167	-0.218	ns
PHT	0.011	0.099	ns
TKW	0.147	4.270	**
NKS	-0.104	-2.820	*
HI	0.324	11.240	**
NKM²	0.589	19.950	**
CTD	-0.005	-0.355	ns
DSI	0.005	0.062	ns
HSI	0.010	0.236	ns
FLA	0.016	0.633	ns
SLDW	0.015	0.147	ns
RWC	-0.014	-0.431	ns

DHE= number of days to heading, BIO= Above-ground biomass, g/m²; SN= Spike number per m^2; SW= Spike weight, g/m²; GY = grain yield, g/m², PHT = plant height, cm; TKW = Thousand- kernel weight, NKS = Number of kernels per spike, NKM² = Number of kernels per m², $x10^3$, HI = Harvest index, %; YLDec= economical yield, g/m², RWC = Relative water content, %; LA = Flag leaf area, cm²; SLDW = Specific leaf dry weight, mg/cm²: CTD = Canopy temperature depression = T°C air –T°C leaf, °C; DSI = Drought stress index, HSI = Heat stress index. Ns, * = non-significant and significant correlation coefficient at 5% probability level ($r_{5\%}$= 0.180).

The results of the study of the relationship between yield and morpho-physiological characters through phenotypic correlations and path coefficients suggests that above ground biomass, harvest index number of kernels per m², and 1000-grain weight were the most important characters as they exhibited high direct effects on grain yield. Majumder *et al*. [26] reported positive direct effects of number of kernels per spike, thousand kernel weight, number of spikes and harvest index on bread wheat gain yield. Therefore, selection for these characters would give better response to yield improvement, since they showed moderate to high variability and broad sense heritability. Further studies should be made with more characters related to stress tolerance to find out ways to concomitantly select for both characteristics.

Conclusion:

Grain yield is the principal character of a cereal crop targeted in crop improvement, however under low input stressful environment it is necessary to associate stress tolerance and high yield potential, to minimize grain yield variation. Thus selection for desirable genotypes should not be based on yield only, but traits related to stress tolerance should also be considered. Therefore information is needed on genetic variability, heritability of yield, yield components and traits influencing stress tolerance and on mutual association between these characters. The present study indicates the presence of high variability in morphological and physiological characters of three cross populations of durum wheat. The correlation study indicates that above ground biomass, harvest index, number of kernels per m², and 1000-kernel weight are the most important characters determining grain yield as they exhibited positive correlation coefficient and high and positive direct effects on grain yield. Thus selection should be based on these traits for the development of high yielding durum wheat breeding lines, since these characters showed moderate to high broad sense heritability and sizeable variability. Physiological traits had low to moderate variability and heritability and were not significantly correlated with grain, suggesting difficulty to concomitantly select for both traits: yield potential and stress tolerance, in the cross populations under study. To combine between yield performance and stress tolerance, the use of a selection index can be useful.

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