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Research Article

Assessment of Agro-Morphological Variability in a Durum Wheat Collection Maintained in the Moroccan Gene Bank

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Abstract: Durum wheat is one of the most important cereal crops in Morocco. The development of high-yielding varieties of durum wheat is one of the major goals of breeding programs in Morocco. Crop genetic diversity should play an essential role in this context. The objective of this study was to assess the genetic variation among 280 durum wheat accessions conserved at the Moroccan Gene Bank by using twelve agro-morphological characters. All accessions are planted in field conditions and arranged in augmented design. The results showed a large variability among the durum wheat accessions. Analysis of variance demonstrated significant to highly significant differentiation between the accessions for the most traits. The number of plants at the emergence stage, the biomass of the plant at the flowering stage, the chlorophyll content, and the number of spikes showed high genetic advance coupled with high heritability (broad sense). Moreover, highly significant coefficients of correlation were revealed between agro-morphological characters offering the possibility of direct or indirect selection. The first two axes of the principal component analysis explained 45% of the total variation and showed that Moroccan landraces and North American cultivars shared similar morphological characters. However, Moroccan cultivars have shown better agronomic characters. This important agro-morphological variation should be utilized as a gene pool in durum wheat improvement to develop novel varieties with high productivity and the best quality.

***Keywords*:** variability, agro-morphological traits, genetic resources, Moroccan gene bank, durum wheat

1. Introduction

Durum wheat (*Triticum turgidum L. subsp. durum* (*Desf*) *Husn*) is one of the most widely cultivated and consumed cereal crops in the Mediterranean Basin [1-3]. It is an economically important crop, ranking third after bread wheat and barley, in terms of cultivation's area and production [4]. It is, also, considered as one of the main sources of proteins and carbohydrates contents in human nutrition [5, 6].

In Morocco, durum wheat is a strategic cereal crop produced on about 1 million hectares [7]. It is cultivated in a wide range of environments, but mostly under rainfed conditions where the precipitations are irregular and crops are often exposed to biotic and abiotic stresses [8]. The development of high-yielding varieties of durum wheat adapted to these agro-climatic environments is one of the major goals of breeding programs in Morocco. Indeed, crop genetic

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diversity should play an essential role in this context. Thus, exploring and identifying useful durum wheat genetic materials is important for specific adaptation to local environmental conditions, given the high level of polymorphism found in durum wheat landraces for commercially valuable traits [9-13]. Several studies on agro-morphological, biochemical, and molecular diversity of Moroccan durum wheat germplasm showed a significant richness of these materials [8, 14-18].

However, to carry out an effective selection for grain yield and its components, the information of the extent of genetic variation present in durum wheat landraces is important [19]. The estimations of the phenotypic and genotypic coefficients of variation, heritability, and genetic advance values would determine the selection method to improve a complex trait such as grain yield [20, 21]. Indeed, Phenotypic (CVP) and Genotypic (CVG) coefficients of variation provide information on the relative magnitude of variation in different populations [22]. While heritability explains the relationship between total genetic variance to phenotypic variance [23]. The most important function of the heritability of quantitative traits is its predictive role in indicating the reliability of the phenotypic value as a guide to genetic value [22, 24-26].

The relationship between durum wheat yield-related traits and their heritability has been extensively studied at the phenotypic level [27-29]. The information collected on the extent and nature of variations is essential in identifying possible genotypes for future crop variety development.

The National Gene bank of INRA-Morocco based in Settat conserves and manages more than 3000 durum wheat accessions (representing old cultivars, local landraces, breeder's materials, elite lines...). The characterization and evaluation of these accessions are very important for utilizing the collection efficiently and effectively. However, information on genetic parameters of yield and yield-related traits for durum wheat accessions maintained at the INRA gene bank is not available. Hence, the present study was undertaken to assess the morphological variability and to estimate the phenotypic coefficient of variation, the genetic coefficient of variation, the heritability, and the genetic advance of agro-morphological traits in a collection of durum wheat consisting of landraces, Moroccan and North American cultivars.

2. Materials and methods

2.1 *Plant material*

The plant material used in this study comprised 280 accessions of durum wheat, all conserved in the National Gene Bank, Regional Agricultural Research Center of Settat, INRA-Morocco. The durum wheat material consisted of 18 Moroccan cultivars, 185 landraces collected from different agro-ecological regions across the country, and 77 cultivars from North America (Canada and United States).

2.2 *Experimental site and planting design*

The trial was carried out at the Sidi el Aidi experimental station (latitude 33 ° 07 ' 16 " N, longitude 007 ° 37 ' 48 " W, altitude 240 m) during the cropping season 2015/2016. The climate of the experimental site is characterized by an annual rainfall of 386 mm/year with a temperature ranging from -4 to 43 °C. The experimental design was an Augmented Block Design [30], consisting of 14 blocks in which all the accessions were planted in un-replicated plots and Three local checks were replicated 14 times. Each accession was sown in 3 rows of 1 meter long and 30 cm apart. The local check cultivars were "Marzak", "Cariouca" and "Ourgh".

2.3 *Agro-morphological characters*

Observations were recorded on 12 quantitative agro morphological traits. They include a Number of Plants at Emergence stage (NPEM, number), Days to Flowering (DFLO, days), Days to Maturity (DMAT, days), Biomass of Single Plant at Flowering stage (BPFLO, g), Plant Height (PH, Cm), Peduncle Length (Pl, Cm), Chlorophyll content (SPAD, SPAD), Total Biomass (BIO, g), Number of Spikes (NSP, number), Thousand Kernels Weight (TKW, g), Yield per plot (Y, g) and Harvest Index (HI). All the traits were assessed on randomly selected 5 plants of each accession and checks except NSP, TKW, Y, and HI.

2.4 *Statistical data analysis*

The basic descriptive statistics i.e. mean, minimum, maximum, standard deviation, and coefficient of variation were calculated to determine the extent of diversity among the studied accessions and the checks. The Analysis of Variance (ANOVA) was performed for "Augmented Design" as described by Federer [31] using the "Agricolae" package implemented in R software (https://www.R-project.org/). The genetic parameters were estimated from the components of the analysis of variance. Genotypic and Phenotypic Variances (GV (1) and PV (2)), Genotypic and Phenotypic Variation Coefficients (GVC (3) and PVC (4)), broad-sense heritability (h^2) (5), and Genetic Advance (GA) (6) were computed according to the formulas proposed by Burton [32], Jonhson et al. [33], Allard [34], and Falconer et Mackay [22].

$$GV = (MSG - MSE)/r \tag{1}$$

$$PV = GV + (MSE/r) = MSG/r \tag{2}$$

$$GVC\ (\%) = (\sqrt{GV}/X) * 100 \tag{3}$$

$$PVC\ (\%) = (\sqrt{PV}/X) * 100 \tag{4}$$

$$h^2 = (GV/PV) \tag{5}$$

$$GA = h^2 * \sqrt{PV} * K \tag{6}$$

With, MSG: Mean Squares of Genotypes; MSE: Mean Squares of the Error; r: number of replication; √VG: Genotypic standard deviation; √VP: Phenotypic standard deviation; X: mean of the considered traits; K = 2.06 is a selection differential at 5% of the variation

The Pearson correlation coefficient and the Principal Component Analysis (PCA) were carried out on the basis of the agro-morphological traits using respectively R package "Hmisc" and Microsoft Excel.

3. Results

3.1 *Diversity of agro-morphological traits among accessions*

The basic statistical data (mean, minimum, maximum, standard error, and coefficient of variation) for all agro-morphological characteristics in the studied durum wheat accessions and the Moroccan checks are provided in Table 1. High variability was found among the durum wheat accessions. The highest coefficient of variation was shown by Y (29.3%), followed by HI (29.1%), BPFLO (21.6%), and TKW (18.7%). The least value was observed for DFLO (1.2%).

Days to Flowering (DFLO) of durum wheat accessions ranged from 105 to 118 days, with an average of 111 days. Days to Maturity (DMAT) averaged 140 with a range of 108 to 167 days. However, mean DFLO and DMAT values of checks were respectively, 110 days and 134 days. The Number of Plants at the Emergence stage (NPEM) of durum wheat accessions ranged from 10 to 119 plants with a mean of 66 plants, while the Biomasses of the single Plant at the Flowering stage (BPFLO) varied between 25.37 and 172.21 g with an average of 73.11 g. The mean values of NPEM and BPFLO for the checks were 65 plants and 61.86 g, Plant Height (PH), Peduncle length (Pl) and Biomass (BIO) of the accessions showed wide variations with averages of 130 cm, 36.81 cm and 2.46 kg, respectively, superior to mean values of PH, Pl, and BIO for the checks.

For yield-related traits, the durum wheat accessions displayed relatively low mean values number of spikes (478 spikes), thousand kernels weight (43.16 g), grains yield (0.58 kg), and harvest index (0.24) compared to the checks (Table 1).

The analysis of variance revealed the existence of significant differences among the durum wheat accessions for all the traits except for DMAT, TKW, and HI (Table 2). Significant differences were also observed between durum wheat accessions and the Moroccan checks for all the traits except NPEM. The present findings indicated the existence of wide variability among the studied durum wheat accessions for almost all measured traits.

Table 1. Descriptive analysis of twelve agro-morphological traits for the 280 accessions of durum wheat and 3 checks

Traits	All accession			Checks	CV %
	range		mean ± SE	mean ± SE	
DFLO	105	118	111.27 ± 0.17	109.54 ± 0.20	1.2
DMAT	108	167	139.62 ± 0.35	134.02 ± 0.74	3.6
NPEM	10	119	66.22 ± 1.07	64.69 ± 1.62	15.3
BPFLO	25.37	172.21	73.11 ± 1.50	61.86 ± 2.39	21.6
PH	0.85	1.8	1.3 ± 0.01	1.10 ± 0.02	8.6
Pl	18.3	61.3	36.81 ± 0.42	34.81 ± 0.89	15
SPAD	19.8	63.9	46.87 ± 0.47	54.29 ± 0.71	9.9
BIO	0.8	5.49	2.46 ± 0.03	2.31 ± 0.07	18
NSP	194	984	477.71 ± 7.22	524.47 ± 14.20	13.8
TKW	19	73	43.16 ± 0.59	48.33 ± 1.32	18.7
Y	0.13	1.66	0.58 ± 0.01	0.81 ± 0.02	29.3
HI	0.05	0.58	0.24 ± 0.00	0.36 ± 0.01	29.1

DFLO: Days to Flowering; DMAT: Day to Maturity; NPEM: Number of Plants at Emergence stage; BPFLO: Biomass of one Plant at Flowering; SPAD: Chlorophyll content; PH: Plant Height; Pl: Peduncle length; BIO: Biomass; NSP: Number of Spikes; TKW: Thousands Kernel Weight; Y: Yield; HI: Harvest Index; CV %: Coefficient of Variation; SE: Standard Error

3.2 *Estimation of genetic parameters*

Genotypic variance, phenotypic variance, heritability, Genotypic Coefficient of Variation (GCV), Phenotypic Coefficient of Variation (PCV), Genetic Advance (GA), and genetic advance as a percentage of mean, GA (%) for all the 12 traits were estimated and presented in Table 3.

In the present study, the highest Phenotypic (PV) and Genotypic (GV) variances were observed for NSP and BPFLO while the lowest PVs and GVs were recorded for HI and Y. The Phenotypic Coefficient of Variation (PCV) ranged from 2.57% (DFLO) to 40.04 % (Y), whereas the Genotypic Coefficient of Variation (GCV) varied between 2.28% (DFLO) and 27.69% (BPFLO). Moderate values of PVC were noted for Pl (19.61%), (17.61%), and SPAD (16.69%), while BIO, PH, SPAD, and TKW (13.10%) showed a relatively moderate value of GVC (Table 3).

Broad sense heritability (h^2) estimates ranged from 0.29 to 0.78 for DMAT and DFLO, respectively. Moderately high heritability values were obtained for PH, NPEM, NPS, SPAD, and BPFLO. Concerning genetic advance, the highest value was observed for NSP and the lowest value of genetic advance was revealed for HI (Table 3).

Table 2. Source of variation for the 280 accessions of durum wheat

SV	df	DFLO	DMAT	NPEM	BPFLO	PH	Pl	SPAD	BIO	NSP	TKW	Y	HI
Block	13	1.723	24.08	128.74	187.9	551.0***	45.315	20.57	0.4161*	16000**	61.44	0.04005	0.00535
Entries (Accessions + checks)	282	7.6705***	35.981	291.22**	512.27*	462.59***	48.074	60.850**	0.32591	11977**	89.9	0.04684	0.008171
Checks	2	1.024	2.95	121.88	592.8	403.5	7.797	13.46	0.02712	11630	245.17*	0.01348	0.00643
Acessions	279	8.164***	35.17	322.86***	633.9**	500.1***	51.388*	63.78**	0.39421*	14614***	100.23	0.05967*	0.00813
Checks vs Accessions	1	108.975***	1147.27***	86	4626.1***	13311.9***	146.627*	2010.73***	0.80586	79861***	974.48***	1.95385***	0.50929***
Error		1.767	24.85	101.52	240.4	121	29.62	22.29	0.19323	4463	67.24	0.03277	0.00561

SV: Source of Variation; df: degree free; DFLO: Days to Flowering; DMAT: Day to Maturity; NPEM: Number of Plants at Emergence stage;
BPFLO: Biomass of one Plant at Flowering; SPAD: Chlorophyll content; PH: Plant Height; Pl: Peduncle length; BIO: Biomass;
NSP: Number of Spikes; TKW: Thousands Kernel Weight; Y: Yield; HI: Harvest Index;
Signification codes: '***' 0.001, '**' 0.01, '*' 0.05

Table 3. Estimates of genetic parameters for different agro-morphological traits of durum wheat accessions

Traits	Genotypic variance	Phenotypic variance	PCV %	GCV %	h^2	GA %
DFLO	6.397	8.164	2.57	2.28	0.78	4.61
DMAT	10.32	35.17	4.27	2.31	0.29	3.58
NPEM	221.34	322.86	27.22	22.53	0.69	25.38
BPFLO	393.5	633.9	35.14	27.69	0.62	32.20
PH	0.03791	0.05001	17.61	15.33	0.76	0.35
Pl	21.768	51.388	19.61	12.77	0.42	6.26
SPAD	41.49	63.78	16.69	13.46	0.65	10.70
BIO	0.20098	0.39421	25.73	18.37	0.51	0.66
NSP	10151	14614	24.99	20.82	0.69	172.98
TKW	32.99	100.23	22.84	13.10	0.33	6.79
Y	0.0269	0.05967	40.04	26.89	0.45	0.23
HI	0.00252	0.00813	36.07	20.08	0.31	0.06

DFLO: Days to Flowering; DMAT: Day to Maturity; NPEM: Number of Plants at Emergence stage; BPFLO: Biomass of One Plant at Flowering; SPAD: Chlorophyll content; PH: Plant Height; Pl: Peduncle length; BIO: Biomass; NSP: Number of Spikes; TKW: Thousands Kernel Weight; Y: Yield; HI: Harvest index; GCV %: Genotypic Coefficient of Variation; PCV %: Phenotypic Coefficient of Variation; h^2: Heritability (broad sense); GA %: Genetic Advance

3.3 *Relationships among agro-morphological traits*

Pearson's correlation coefficient (r^2) was estimated among 12 morphological traits in the durum wheat accessions (Table 4). The highest correlation ($r^2 = 0.78$) was recorded between HI and Y, followed by NSP and Y ($r^2 = 0.64$).

Days to Flowering (DFLO) and Days to Maturity (DMAT) were positively correlated with morphological traits (BPFLO and PH) and negatively correlated with yield-related traits (NSP, YI, and HI). Morphological e traits (PH and Pl) showed a significant positive correlation with BIO and a negative association with yield-related traits (Y and HI). The yield was highly and positively correlated with BIO ($r^2 = 0.41$), NSP ($r^2 = 0.64$), TKW ($r^2 = 0.29$), and HI ($r^2 = 0.78$).

3.4 *Principal component analysis*

Principal Component Analysis (PCA) was performed based on the 12 studied agro morphological traits (Figure 1 and Table 5). The five Principal Components (PCs) contributed to 73.5% of the variation among the durum wheat accessions. The first two principal components (PC 1 and PC 2) accounted for more than 44% of the total variation. The traits, which contributed more positively to PC 1, were NSP, Y, and HI. While, BIO, Pl, PH, and NSP have contributed more positively to PC 2.

The biplot of the 280 accessions based on the 12 agro morphological traits allowed the distribution of the collection into three main groups (Figure 1). Moroccan cultivars were isolated from landraces and North American cultivars which formed a large group. The Moroccan durum wheat landraces and North America durum wheat accessions were characterized by the highest mean values of the morphological and physiological traits, whereas the Moroccan durum wheat cultivars were characterized by the highest mean values of the agronomic traits.

Table 4. Pearson's correlation coefficients (r^2) among 12 agro-morphological characters

Traits	DFLO	DMAT	NPEM	BPFLO	PH	Pl	SPAD	BIO	NSP	TKW	Y
DMAT	0.29**										
NPEM	-0.02ns	-0.04ns									
BPFLO	0.24**	0.3**	-0.05ns								
PH	0.25**	0.39**	0.14*	0.34**							
Pl	0.07ns	0.13*	0.08*	0.21**	0.5**						
SPAD	-0.08ns	-0.11*	-0.18**	-0.02ns	-0.32**	-0.09ns					
BIO	0.07ns	0.16ns	0.3**	0.16**	0.35**	0.35**	0.04*				
NSP	-0.16**	-0.26**	0.38**	-0.33**	-0.12*	0.02ns	0.09ns	0.4**			
TKW	0.01ns	-0.02ns	-0.14*	0.13*	0ns	0.09ns	0.21**	0.19**	-0.02ns		
Y	-0.14**	-0.33**	0.14*	-0.24**	-0.24**	0.07ns	0.3**	0.41**	0.64**	0.29**	
HI	-0.21**	-0.46**	-0.03ns	-0.39**	-0.48**	-0.14**	0.28**	-0.2**	0.42**	0.19**	0.78**

ns, *, **: non-significant and significant correlations at the 5% and 1% threshold, respectively
DFLO: Days to Flowering; DMAT: Day to Maturity; NPEM: Number of Plants at Emergence stage; BPFLO: Biomass of one Plant at Flowering; SPAD: Chlorophyll content; PH: Plant Height; Pl: Peduncle length; BIO: Biomass; NSP: Number of Spikes; TKW: Thousands Kernel Weight; Y: Yield; HI: Harvest Index

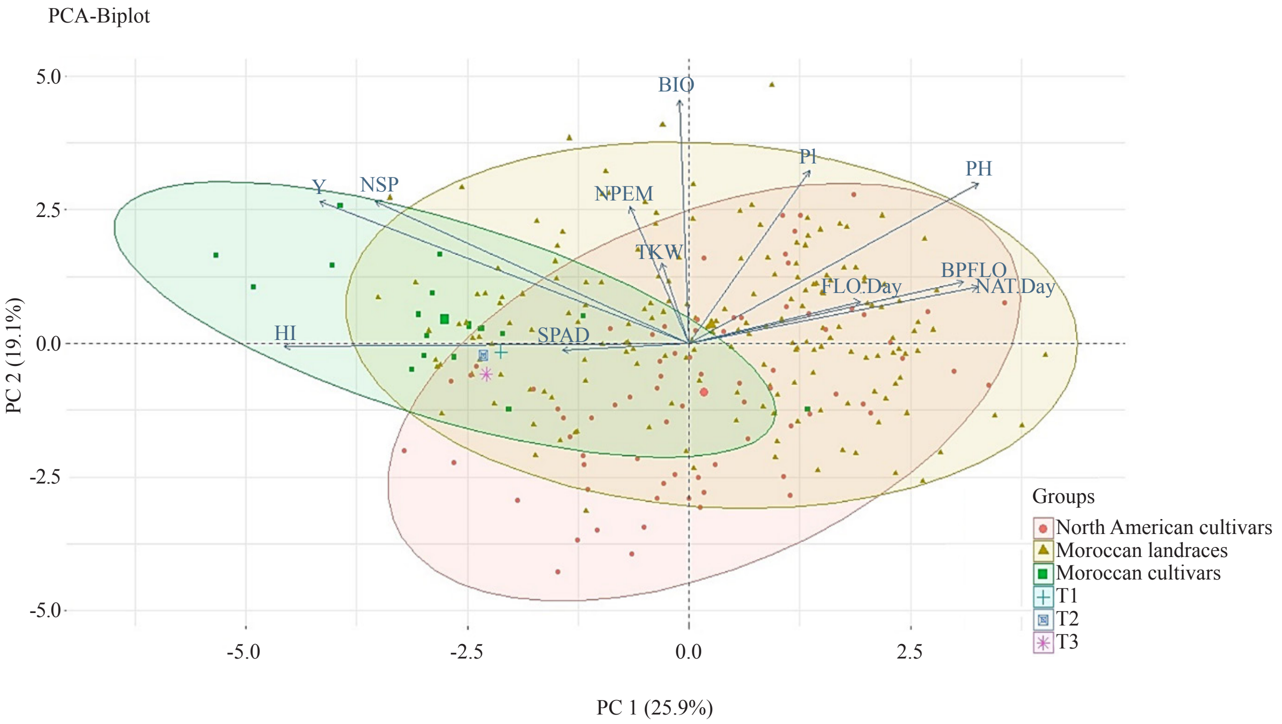

Figure 1. Scatter diagram of the first to PCA components (PC 1and PC 2) for the 280 accessions based on 12 agro-morphological traits. DFLO: Days to Flowering; DMAT: Day to Maturity; NPEM: Number of Plants at Emergence stage; BPFLO: Biomass of one Plant at Flowering; SPAD: Chlorophyll content; PH: Plant Height; Pl: Peduncle length; BIO: Biomass; NSP: Number of Spikes; TKW: Thousands Kernel Weight; Y: Yield; HI: Harvest Index

Table 5. Correlation matrix between the first five PCA axes and the 12 agro-morphological traits

Traits	PC 1	PC 2	PC 3	PC 4	PC 5
DFLO	-0.35	0.14	0.21	0.54	0.64
DMAT	-0.60	0.19	0.15	0.32	-0.05
NPEM	0.12	0.47	-0.57	0.27	-0.12
BPFLO	-0.57	0.21	0.33	0.03	-0.15
PH	-0.60	0.55	-0.12	-0.19	0.13
Pl	-0.25	0.60	0.01	-0.52	0.13
SPAD	0.26	-0.02	0.61	0.27	-0.43
BIO	0.01	0.84	0.04	0.13	-0.25
NSP	0.65	0.49	-0.26	0.18	-0.03
TKW	0.05	0.27	0.70	-0.21	0.05
Y	0.77	0.49	0.22	0.01	0.13
HI	0.84	-0.01	0.19	-0.08	0.32
eigenvalue	3.10	2.28	1.56	0.97	0.88
percentage of variance	25.89	19.08	13.02	8.15	7.358
cumulative percentage of variance	25.89	44.97	58.00	66.15	73.50

DFLO: Days to Flowering; DMAT: Day to Maturity; NPEM: Number of Plants at Emergence stage;
BPFLO: Biomass of one Plant at Flowering; SPAD: Chlorophyll content; PH: Plant Height; Pl: Peduncle length;
BIO: Biomass; NSP: Number of Spikes; TKW: Thousands Kernel Weight; Y: Yield; HI: Harvest Index

4. Discussion

The aim of a plant breeding program is to improve the crop's agronomic and economic traits [35, 36]. Knowledge of the extent of genetic variation and diversity available in germplasm could help researchers and breeders to design better effective breeding strategies in durum wheat varieties.

In the present study, twelve agro-morphological traits were used for the characterization of 280 durum wheat accessions maintained at the Moroccan Gene Bank. The presence of significant ($p < 0.05$) to highly significant ($p < 0.001$) differences and a large spectrum of ranges between the maximum and minimum mean values for most of the traits reflect the existence of broad morphological variability within the accessions. This variability offers large flexibility for the development of well-adapted varieties to different agro-ecological regions. The morphological variability of accessions could be attributed to farmers' management of local varieties. In fact, farmers in Morocco maintain and produce the seeds themselves in fields where several cultivars coexist. This promotes gene flow between them, thus contributing to the spread of genetic diversity. Such observations were reported by Zarkti et al. [15], Nsarellah et al. [8], Chentoufi et al. [16], and Sahri et al. [18] in Moroccan durum wheat germplasm.

Otherwise, the Phenotypic Coefficients of Variation (PCV) were higher than the Genotypic Coefficients of Variation (GCV) for all the agro-morphological traits indicating a relatively low influence of the environment on these characters [22]. The estimations of PCV and GCV reported in this study are higher for the yield-related traits, namely BIO, NSP, TKW, Y, and HI. This suggested that the impact of environmental factors on the expression of these traits is relatively low and these traits are responsive to selection. Similar results were obtained for durum wheat by Maniee et al. [37], Abinasa et al. [29], and Wolde et al. [20].

Nevertheless, the coefficients of variation are indicators of the level of variability that exists between genotypes for a trait but do not indicate the heritable and non-inheritable part of this variability [38]. According to Jonhson et al. [33], estimation of heritability and genetic advance is essential for the selection based on phenotypic expression.

Therefore, high heritability combined with moderate to high genetic advance could be effective for selection based on morphological traits [32]. In the current study, high heritability together with high genetic advance was recorded for NPEM, BPFLO, SPAD, and NSP. Similar findings were also reported by Shankarrao et al. [39], Kumar et al. [40], Saleem et al. [41] and Kumar et al. [42]. However, grain yield exhibited a moderate estimate of heritability with a relatively intermediate value for genetic advance. Wolde et al. [20], Abinasa et al. [29] and Tsegaye et al. [43] also noticed intermediate values of broad-sense heritability and genetic advance for grain yield in durum wheat. Thus, the selection of high-yielding genotypes on the basis of grain yield would not be as effective as selection for its components [44].

In this study, grain yield was positively correlated with BIO, NSP, TKW, and HI but negatively associated with the phenological and developmental traits. These results imply that enhancing grain yield could be achieved through the indirect selection of these traits. Similar observations were reported by [43] and Aghaee et al. [45] for durum wheat; Kumar et al. [42] and [46] for bread wheat; and Singh et al. [47] and [48] for barley.

Furthermore, a principal component analysis was performed to study the structure of the 280 accessions based on twelve agro-morphological characters. The studied accessions were clustered into three sub-groups. The first and the second groups include Moroccan durum wheat landraces and North American durum wheat cultivars, respectively. The accessions of these two groups were characterized by the highest mean values of morphological characters, such as BPFLO, Pl, and PH. Whereas the third group was formed by the Moroccan durum wheat cultivars and the three checks ‘Marzak’, ‘Cariouca’ and ‘Ourgh’, characterized by the highest mean values of agronomic traits, such as NSP, Y, and HI. Similar results were reported by Zarkti et al. [15] for Moroccan durum wheat accessions; Sourour et al. [49] for Tunisian durum wheat accessions and Hailu et al. [50] and Mengistu et al. [51] for Ethiopian durum wheat accessions.

5. Conclusion

The results of this study showed a large variability among the 280 durum wheat accessions for many desirable traits, indicating the high potential of the durum wheat genetic resources maintained at the Moroccan gene bank for effective crop improvement.

The estimation of genetic parameters revealed very little influence of environmental factors on the expression of important traits that have resulted in low differences between the phenotypic and genotypic coefficients of variation.

In addition, high broad-sense heritability coupled with high expected genetic gain has been observed for several traits. Moreover, traits association analysis indicates that grain yield is highly and positively correlated with its components. Improvement by direct or indirect selection is, therefore, possible for these characters.

However, molecular and biochemical analyses would complement the results of this study.

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Conflict of interest

The authors declare no conflicts of interest.

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