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Agro-morphological variability in durum wheat landraces of Morocco

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Abstract

The knowledge about the extent of variability, the distribution and the relationship between descriptors within local germplasm collection are a high value for the improvement and the efficient genetic diversity maintenance and utilization of plant species. The objective of this study was to evaluate the agro-morphological variability in a set of Moroccan durum wheat germplasm collection maintained in the National Gene Bank of Morocco (INRA, Settat). 467 durum wheat (*Triticum turgidum* L. var. *durum*) accessions comprising 444 landraces and 23 improved varieties were planted under field condition and their agro-morphological characters such as days to emergence, days to tillering, days to booting, days to head emergence, days to flowering, days to physiological maturity, plant height, thousands kernel weight, spike shape, and spike density were recorded. Univariate and multivariate analysis of data indicated that thousands kernel weight and plant height presented the highest coefficient of variation with 15.72% and 15.15% respectively. The two-dimensional principal coordinates analysis (2D PCOA) explained 52% of the total variance in the collection and separated the accessions into three main groups, namely, the early maturing and shorter (81 accessions), the moderately late and taller (154 accessions), and the late maturing and taller (232 accessions). The frequency distribution of spike characters showed the prevalence of the pyramidal and the dense spike with 54% and 42% respectively. The results of this study will support efforts of conservation and utilization of landraces in durum wheat breeding programmes.

Keywords: agro-morphological variability; agro-morphological traits; clusters; germplasm conservation; genetic diversity; principal coordinates analysis; *Triticum turgidum* L. var. *durum*.

Abbreviations: DEME: days to emergence; DTIL: days to tillering; DBOO: days to booting; DHEM: days to head emergence; DFLO: days to flowering; DMAT: days to physiological maturity; IPGRI: International Plant Genetic Resources Institute; PLH: plant height; SPSH: spike shape; SPDE: spike density; SPT: spike type; TKW: thousands kernel weight.

Introduction

The earliest known agriculture of the Old World was associated with the Fertile Crescent in the Near East. The dating of this period is considered to be between 9000 and 7000 BC (Murray, 1970), and the earliest domesticated wheat dates back to approximately 7500-6500 BC. According to Feldman (1976), cultivated emmer *Triticum dicoccum* spread to all farming areas of the Near East during the 7th millennium BC. It then spread from the lowlands of Mesopotamia to be taken into cultivation in Egypt, the Mediterranean basin, Europe, Central Asia, India, and Ethiopia over a period from the sixth to the fourth millennium BC. It was the dominant cereal until the first millennium BC, when it was replaced by the free-threshing tetraploid *T. durum* forms. Wheat spread in the Mediterranean countries through the human movements during the historical ages. Among the Mediterranean ecosystems with exceptionally high number of endemic plant, Morocco presents a great diversity of environments and is well known for its rich diversity of many economically important species including durum wheat (*T. turgidum* L. var. *durum*) (Vavilov, 1926; Zarkti et al., 2010). Wheat germplasm from Morocco contributed widely to the wheat improvement in many countries and their characterization is essential for their conservation, evaluation, and utilization in breeding programs. In Morocco, cereals are of paramount importance and their consumption is one of the highest in the world, and cereal demand is almost synonymous with demand for food. Among cereals, durum wheat is grown over an area ranging from 1 to 1.2 million hectares annually, and ranks third after bread wheat and barley (Belaid et al., 2003; MAPM, 2011). Among small cereals, durum wheat has been the subject of renewed interest, because of its adaptation to semi-arid environments and its unique end products. In the West Asia and North Africa (WANA) region, and in the Mediterranean basin, durum wheat has historically received special attention as a major crop (Belaid, 2000); its genetic structure varies from traditional landraces and cultivars to the modern varieties (Bozzini et al., 1998). Genetic diversity of the major crops including the durum germplasm has suffered an overall reduction with time as a consequence of domestication processes and the recurrent use of adapted germplasm (Allard, 1996; Hoisington et al., 1999; Donini et al., 2000). Thus, characterization of crop germplasm collections is very essential preliminarily step for quantifying the extent of genetic diversity within accessions, detecting duplications within germplasm collections, and acts as a starting point for the establishment of “a core collection” where the whole variability spectrum of a species in the collection is represented in a small subset of accessions. Maintaining genetic diversity is also closely associated with the indigenous knowledge of local people that used these biological resources for a long period of time. In some areas of Morocco, farmers still prefer landraces or traditional cultivars under marginal environmental conditions because they yield more. For example, in the mountain and oasis regions of Morocco, durum wheat landraces are still widely grown by farmers, which represent a reservoir of genetic variability that is still not well studied. These observations suggest that these locally adapted germplasm presents interesting adapted traits, thus information on germplasm diversity and genetic relationships among Moroccan durum wheat accessions are critical in wheat breeding programmes and for conservation of genetic resources. Reports indicated that information on germplasm diversity were useful for the efficient germplasm management, genotype selection of genetically distinct parents and to plan crosses for positive traits for different breeding purposes (Fufa et al., 2005; Eivazi et al., 2008). Criteria for the estimation of genetic diversity vary: pedigree analysis (Barrett et al., 1998), morphological traits (Schut et al., 1997; Marić et al., 1998; Casadesus et al., 2007) as well as biochemical markers (Cox et al., 1985; Metakovsky and Branlard, 1998) or molecular markers (Rao and Riley, 1994; Karp et al., 1996; Gupta et al., 1999; Manifesto et al., 2001; Pagnotta et al., 2005). Agro-morphological characterization is a first step towards conservation and utilization of plant genetic resources. When assessing genetic diversity, the use of agro-morphological variation provides greater complementary information to molecular markers characterization (Gomez et al., 2004; Cortese et al., 2010). In this context, our objective was to describe the extent of the intra-specific agro-morphological variability among collection of durum wheat accessions comprising 444 landraces and 23 improved varieties, maintained in the National Gene Bank of Morocco using ten agro-morphological traits.

Results

Agro-morphological variation

The evaluated durum wheat germplasm had a wide range of agro-morphological variation in all traits studied except for days to plant emergence (DEME) (Table 1). Plant height (PLH) averaged 108.31 cm (SE ± 0.76) and ranged from 56.70 to 141.40 cm. Days to booting (DBOO) averaged 133.65 (SE ± 0.42) with a range of 109.00 to 153.90. Days to head emergence (DHEM) ranged from 123 to 165 with a mean of 146.53 (SE ± 0.43). Days to flowering (DFLO) averaged 156.03 (SE ± 0.46) with a range of 129 to 175. Thousands kernel weight (TKW) ranged from 20 to 60.2 g with a mean of 44.45 g (SE ± 0.32). The difference between the minimum and the maximum was high in all traits studied except for DEME. Thousands kernel weight (TKW) and plant height (PLH) presented the highest coefficient of variation with 15.72% and 15.15% respectively, whereas days to emergency (DEME) and days to physiological maturity (DMAT) had lower coefficient of variation with 1.18% and 3.76% respectively. The frequency distributions of the phenotypic data (Supplementary Fig. S2) failed the Kolmogorov-Smirnov test, indicating non-normal distribution. Most of the distributions were negatively skewed. The various spikes found in the collection were classified under 8 phenotypic classes called spike type (Supplementary Fig. S3 and Supplementary Table S2). The frequency of spike shape (SPSH), spike density (SPDE), and spike types (SPT) were presented in Fig. 4. Spike shape, spike density, and spike types showed different frequencies. Accessions with pyramidal spike shape were the most frequent (54%), whereas genotypes with parallel sides were less common with only 7%. Genotypes with fusi-form and cleavate shape were 14% and 15% respectively. Also, accessions with dense spike (43%) prevailed. Accessions with very dense and intermediate spike had almost the same frequency 20% and 22% respectively, whereas accessions with lax spike showed the lowest frequency 7%. Spike type 7 (pyramidal dense spike with square cross section, medium side view, and white color) (26%), and spike type 2 (pyramidal very dense spike with very flat cross section very large side view, and colored spike) (2 %) showed the highest and lowest frequencies respectively. Pearson’s correlation coefficients for agro-morphological traits (Table 2) showed a strong positive correlation between all the traits except for DEME. Moreover, TKW had no association between DMAT and PLH. . A strong and significant relationship was revealed between DHEM and DBOO ($r = 0.99$; $p < 0.001$), DHEM and DFLO ($r = 0.99$; $p < 0.001$) and between DBOO and DFLO ($r = 0.98$; $p < 0.001$). Also the association between PLH and DBOO, DHEM and DFLO was statistically significant ($p < 0.01$)**.**

Cluster analysis

The result of the two-dimensional principal coordinates analysis (2D PCOA) based on the 8 agro-morphological traits revealed a great polymorphism among the 467 genotypes (Fig. 2). The first and the second axis together explained 52% of the total variance in the standardized data set of the 467 accessions. The first and second axis demonstrated 39 % and 13 % of the variation, respectively. Based on this agro-morphological data, the 2D PCOA separated the local germplasm into three main groups, each group with distinctive features. The group 1 contained the early maturing and shorter accessions (81 accessions). This group included the early maturing landraces and the recent varieties of durum wheat, listed in the national official catalogue between 1980 and 2000. These varieties comprised, ‘Karim’, ‘Belbachir’, ‘Sebou’, ‘Oum Rabia’, ‘Sarif’, ‘Tensift’, ‘Massa’, ‘Isly’, ‘Anouar’, ‘Jawhar’, ‘Yasmine’, ‘Amjad’, ‘Tarek’, ‘Ouregh’, and ‘Marjana’. It also included the variety ‘Haj Mouline’ released in 1974, and the varieties used as checks: ‘Tomouh’, ‘Marzak’, and the earliest variety ‘Acsad 65’. The group 2 contained landraces that were moderately late and taller (154 accessions). It included the older variety ‘Kyperounda’. The group 3 contained the late maturing and taller genotypes of the landraces (232 accessions). It also included the old varieties of durum wheat released between 1949 and 1980. They were ‘Zeramek’, ‘Selbera’, and ‘Jori’. Based on the continuous-Manhattan’s dissimilarity index, the cluster analysis was performed using weighted neighbor-joining method (Fig. 3). The analysis had demonstrated the ability of agro-morphological traits to detect the variability among the accessions. Furthermore, the analysis had classified the accessions into 3 groups, namely, A, B, and C. The group A contained the majority of early maturing and shorter accessions that were present in group 1. In this class we founded the early and modern cultivars including the earliest

Table 1. Agro-morphological variation in durum wheat germplasm accessions.

Descriptors	Days to emergency (DEME)	Days to tillering (DTIL)	Days to booting (DBOO)	Days to head emergence (DHEM)	Days to flowering (DFLO)	Days to physiological maturity (DMAT)	Plant height (PLH)	Thousands kernel weight (TKW)
Mean	18.12	45.70	133.65	146.53	156.03	184.51	108.31	44.45
SE	0.01	0.10	0.42	0.43	0.46	0.33	0.76	0.32
Median	18.12	47.00	135.00	148.00	158.00	185.00	111.80	43.77
CV (%)	1.18	4.48	6.70	6.22	6.34	3.76	15.15	15.72
Min	17.62	39.00	109.00	123.00	129.00	154.90	56.70	20.00
Max	18.63	48.00	153.90	165.00	175.00	198.00	141.40	60.20
Skewness	-0.06	-1.63	-0.60	-0.64	-0.78	-0.30	-0.65	-0.24
SE. Skewness	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11
Kurtosis	7.09	1.59	-0.33	-0.34	-0.13	-0.54	-0.27	0.12
SE. Kurtosis	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22

Notes: SE: standard error of the mean; Min: minimum; Max: maximum; CV: Coefficient of variation, S.E. Skewness: standard error of the skewness, and S.E. Kurtosis: standard error of the kurtosis.

Table 2. Pearson's correlation coefficients among eight agro-morphological traits.

Trait	Days to plant emergence (DEME)	Days to tillering (DTIL)	Days to booting (DBOO)	Days to head emergence (DHEM)	Days to flowering (DFLO)	Days to physiological maturity (DMAT)	Plant height (PLH)	Thousands kernel weight (TKW)
Days to plant emergence (DEME)	1.00							
Days to tillering (DTIL)	-0.03	1.00						
Days to booting (DBOO)	-0.02	0.82***	1.00					
Days to head emergence (DHEM)	-0.02	0.82***	0.99***	1.00				
Days to flowering (DFLO)	-0.01	0.84***	0.98***	0.99***	1.00			
Days to physiological maturity (DMAT)	-0.04	0.69**	0.87***	0.87***	0.86***	1.00		
Plant height (PLH)	0.02	0.43**	0.36**	0.37**	0.41**	0.22**	1.00	
Thousands kernel weight (TKW)	0.03	-0.10*	-0.20**	0.21**	-0.20**	-0.08	0.08	1.00

Notes: *Significant at P < 0.05; **significant at P< 0.01; ***significant at P < 0.0001;

variety 'Acsad 65' used as checks. The group B contained accessions of group 2 that were moderately late maturing and tall including the variety 'Kyperounda'. The group C contained the majority of taller and late maturing accessions that were represented in the group 3. In this group, we can further distinguish two subgroups, C1 and C2, which differ mainly with respect to plant height character (the accessions of C2 were taller than C1). C2 included tallest (MGB# 560 and MGB# 5966, very late maturing (MGB# 5042 and MGB# 6005) and highest thousand kernel weight (MGB# 3034 and MGB# 3186) accessions.

Discussion

Characterization of local landraces of durum wheat is an important process for the evaluation and preservation of indigenous germplasm. Such germplasm have undergone local adaptations through selection for a particular geographic region over many generations. Our study has shown considerable variations in all the agro-morphological characteristics except for DEME. TKW and PLH showed the highest coefficient of variation compared to other traits. The ranges for many of the trait values were higher than those described for Tunisian durum germplasm by Sourour et al. (2010), indicating a high degree of polymorphism present in the durum wheat collection from Morocco. This shows that there are diverse combinations of traits at the individual genotype level, indicating ample possibilities for obtaining desirable trait combinations in specific accessions to meet the demands of breeders, farmers, consumers and the industry.

The substantial morphological diversity observed in durum wheat germplasm of Morocco may be associated with cultural and ecological adaptation to diverse environments where the crop had been cultivated for centuries, followed by intensive selection by remote native communities in these diverse environments. For centuries, farmers have been selecting seed, multiplying it, and sharing it with their neighbors. It is also likely that the introduction of landraces by farmers in other communities (exchange of seeds) has been a prerequisite for hybridization; this hybridization was a prerequisite for gene flow, and gene flow a prerequisite for introgression or permanent incorporation of genetic information from one genetic material into another (Andersson and De Vicente, 2009). Continued cultivations of the new genetic materials under harsh inhospitable environments like arid shallow mountains, successive

Fig 1. Map showing the distribution of the collecting sites of durum wheat landraces in Morocco. A point indicates a site where a landrace of durum wheat was sampled.

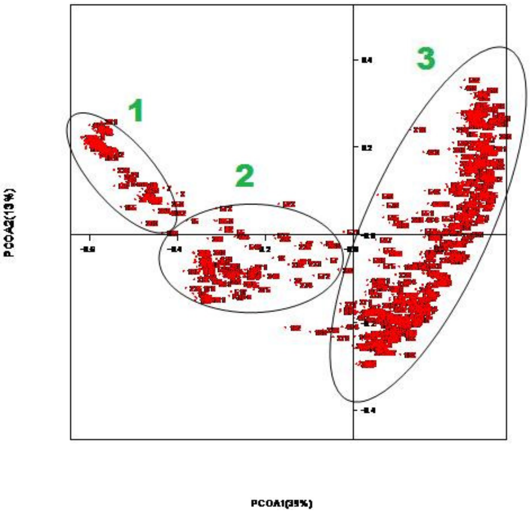

Fig 2 The two-dimensional principal coordinates analysis of the Moroccan durum wheat collection examined based on 8 agro-morphological traits. The entries are presented by numbers; PC1 and PC2 are first and second principal components, accounting for 52 % of total variance. Details regarding group 1, 2 and 3 are discussed in the text.

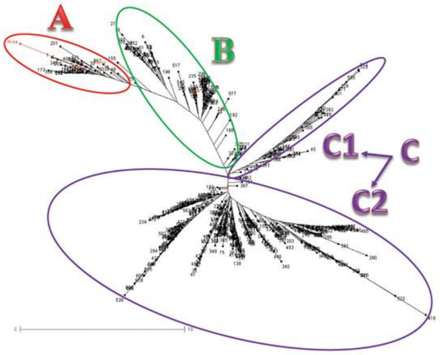

Fig 3 Tree of 467 accessions of durum wheat based on Weighted Neighbor-Joining analysis. The entries are presented by numbers. Details regarding groups A, B and C (C 1 and C 2) are discussed in the text.

selections, and the autogamy system of wheat have contributed to preserve the identity and the wide phenotypic diversity of this crop in Morocco. Studying this phenomenon in Morocco would be a model for *in situ* conservation of the crop genetic resources as affected by geographical isolation of a community and adaptation to different local habitats. Many of agro-morphological traits are of interest for improving breeding efficiency. Such traits are used as a means of distinguishing between varieties or as markers in segregating populations, or of interest for their direct or indirect selection. Indirect selection is a breeding method where some traits can improve genetic gain for a correlated trait of economic importance (Falconer and Mackay, 1996). For example, some of the traits such as plant height and flowering time had clearly distinguished the accessions. These traits have high heritability and strong genetic correlation with grain yield in wheat (Richards, 1991), and therefore could be used for indirect selection. In their study, Murphy et al. (2008) reported that plant height, 1000 kernel weight and leaf area index were positively correlated with yield, and they were responsible for approximately 38% of the variation in yield among 63 spring wheat landraces and modern cultivars. They also found a negative association between weed weight and plant height, and suggested that indirect selection on plant height may be effective in improving weed suppression ability of wheat. As indicated in Table 1 and Supplementary Fig. S2, the distributions of the values for agro-morphological traits were negatively skewed and biased towards higher phenotypic values. Assuming that selection (by the climate and by human) for these agro-morphological traits occurred over the course of the domestication of this species, as reported by Moragues et al. (2005), negatively skewed distributions for these characteristics would be expected because major genes conditioning these traits were rapidly pyramided. The two-dimensional principal coordinates analysis (2D PCOA) used for analysis of the agro-morphological traits differentiated the accessions of Morocco into 3 major groups namely, the early maturing and shorter, the moderately late and taller, and the late maturing and generally taller. Majority of landraces in the collections were late maturing compared to controls such as 'Acsad 65' and 'Tomouh' (which are specifically bred for semi-arid regions). These late maturing landraces were collected from mountains with prolonged high rainfall and cool climatic conditions, where the crop might have adapted for a long favorable growing seasons. This group contains also 3 old varieties adapted to the northern mountainous region. Recent varieties are included in the early maturing and shorter genotypes group. This observation is expected because the modern cultivars are bred for early maturity and shorter heights in order to adapt to the semi-arid conditions, where early maturity pre-empt the drought season (De Vita et al., 2007). The distance tree constructed with (WNJ) analysis matched those obtained with the 2D PCOA, and highlights the variability present in each group. This variability is most likely due to anthropogenic impact through dynamic storage practices, diverse needs and end-uses, seed exchange (gene flow) by farmers and geographic environmental conditions. It has been documented that genetic diversity of landraces had been related to their adaptability (Cooper et al., 2000; IPGRI, 1991) and farmers have played a greater role in maintaining the genetic diversity (Zeven, 2002). Morphological diversity found for ear descriptors showed a polymorphism of spike characters with 8 groups and the prevalence of the pyramidal and the dense spike in the collection studied. These results indicated that germplasm with dense spike seems to have been subjected to more conscious selection than other types,

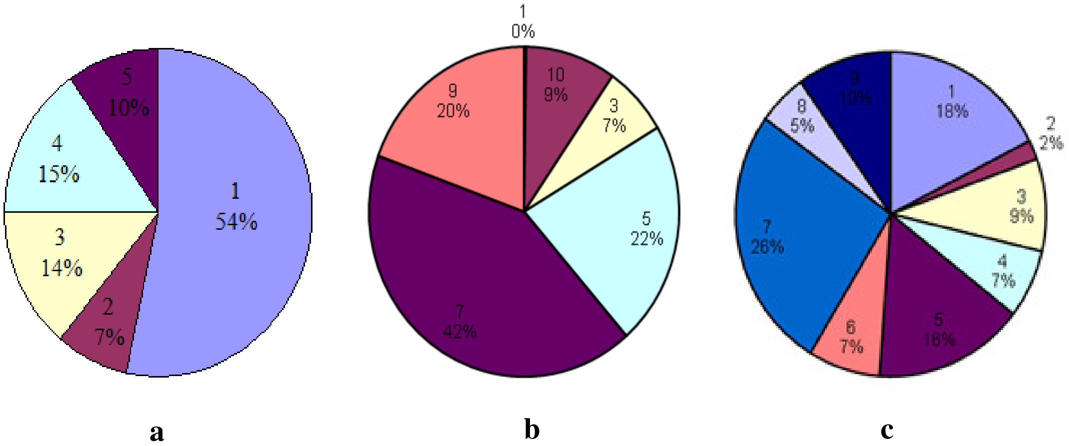

Fig 4. The frequency of various spike shapes (a), spike densities (b) and spike types (c) observed in landraces of Morocco. Spike shapes (a): 1- Tapering (pyramidal), 2- Oblong (parallel sides), 3- Fusi-form, 4- Clavate and 5- Unknown; spike densities (b): 1 - Very lax, 3 – Lax, 5 – Intermediate, 7 – Dense, 9 -Very dense and 10 – Unknown; spike types (c): description of spike types 1-8 are presented in supplementary Table S2 and supplementary Fig. S3.

and support the hypothesis that farmer's concept of a variety corresponds to a set of seed producing plant with similar spike characteristics. This social dynamics of landraces may also be viewed as a means of crop *in situ* conservation of locally adapted material especially in marginal environments. These finding are in good agreement with those of Pecetti et al. (2001) where they found that accessions with dense and very dense spikes prevailed in the Bulgarian germplasm (eastern Europe), and they seems to have been subjected to intense selection, taken into consideration the concept that spike density is a possible indicator for the degree of crop improvement (Evans, 1981). Results of our evaluation are congruent with those of Elings (1993) and Annicchiarico et al. (1995) where they suggested that climatic features of collecting sites or sites of origin can markedly influence morphological and adaptive traits of durum wheat landraces and agronomic features of landraces. Moroccan durum wheat diversity, as expected, might also associated with variation in environments.

Materials and methods

Plant materials

A set of 467 accessions of durum wheat (*Triticum turgidum* L.var. *durum*, 2n = 4x = 28, AABB genome) comprising 444 landraces and 23 improved varieties (Supplementary Table S1) collected in Morocco was used. Four improved varieties namely 'Kyperounda', 'Tomouh', 'Marzak' and 'Acsad 65' were included as checks in the study. All the seed samples used in our experiments were conserved in the National Gene Bank, Centre Régional de la Recherche Agronomique de Settat, INRA, Settat, Morocco. The Moroccan durum landraces were collected from a wide geographic area across the country, differing in altitude, rainfall, maximum, and minimum temperatures. Distribution of the sampled sites is shown in the Fig. 1.

Experimental site and planting the materials

The study was carried out in INRA (Institut National de la Recherche Agronomique) experimental research station at Sidi Al Aidi (latitude 33° 07' 16'' N, longitude 007° 37' 48'' W, altitude 240 m), near Settat, during the cropping season 2005 – 2006. The soil type in this site is a Vertic Calci Argixeroll (USDA taxonomy; pH 8.0) rich in clay and has a depth of 90 to 120 cm. Water retention by weight is estimated to be 30% at -0.033 MPa and 15% at -1.5 MPa for 100-cm depth. In this semi-arid area, the rainy season is usually from November through March but drought can occur at any time during the growing season of wheat, this low average rainfall is characterized by large fluctuations from one year to another (Karrou, 1998). During the growing season, temperature conditions were characterized by large contrasts. January was the coldest-month with an average low temperature of 4.84 °C. Temperature increased during the month of May, and sometimes reached 41°C. Rainfall was above average and uniform until the end of March, and then was deficient (Supplementary Fig. S1). The four checks used in this experiment were augmented with 16 randomly allocated new accessions (entries) so that each block had 20 plots: 16 tested accessions plus 4 checks. The experiment was laid out as an augmented block design. Each accession was sown in 2 rows of 1 meter long and 20 cm apart. Weeds were controlled manually. Diseases and pests were controlled by conventional pesticides.

Agro-morphological data collection

For each accession, the following variables were recorded: the numbers of days to plant emergence (DEME), days to tillering (DTIL); days to booting (DBOO); days to head emergency (DHEM); days to flowering (DFLO); days to physiological maturity (DMAT); plant height (PLH); thousands kernel weight (TKW); spike shape (SPSH); and spike density (SPDE). The phenological data were observed according to the scale of Zadoks et al. (1974). Spike characters were recorded as described in the (Supplementary Table S2A), following the official methodology for wheat variety description adopted by Office National de Sécurité Sanitaire des Produits Alimentaires (ONSSA), Morocco, referencing to the International Union for the Protection of New Varieties of Plants (UPOV).

Univariate and multivariate analysis

The descriptive statistics of each trait was conducted and followed by an analysis of variance (ANOVA). The frequency distribution of the scored values for the spike characters was carried out. These statistical analyses were performed using appropriate procedures of the SAS Software version 9.1 (SAS 9.1 Institute Inc., Cary, NC, USA). Pearson's correlation analyses among phenological stages, PLH, and TKW were also carried out. The agro-morphological data were also used for multivariate analysis (considering the phenological stages, PLH, and TKW) with the major objectives to display the variability among durum wheat genotypes on the basis of multiple traits, identify traits that are positively or negatively associated, identify traits that can be used for an indirect selection for another trait, and determine the main characters that differentiate between accessions. The principal coordinates analysis (PCOA) has been used for analysis of the agro-morphological traits. The two-dimensional PCOA was performed based on the dissimilarity matrix using the GenStat (GenStat 11th edition, http://www.genstat.com) software. To generate a graphical display, "testers" are used equivalently to traits and "entries" to genotypes. The genotype-by-trait two-way table of data was first trait-standardized; the standardization (centering) is necessary to remove the trait effects, because different traits used had different units. Therefore, we assumed an equal ability of all the traits to discriminate among genotypes. Data are standardized by a mixed model, then used to calculate Manhattan distances and decomposed into principal component (PC). The first two principal components (PC1) and (PC2) were used to generate a two-dimensional graphical display. Manhattan's dissimilarity index was calculated and used to construct weighted neighbor-joining (WNJ) analysis (Bruno et al., 2000).

Conclusion

In this study, we analyzed the variability of durum wheat collection maintained in the Gene Bank of Morocco on the basis of the agro-morphological characters. Indirect estimation of genetic diversity based on the agro-morphological characterization of individual accessions was higher within the Moroccan durum wheat accessions, and it provides a good genetic background for future quantitative trait loci studies. This variability among accessions is expressed in differences of earliness, plant height, thousands kernel weight and spike characters and they could be used to select advantageous adaptive traits for crosses in the breeding programs with the participation of farmers, to improve adaptation to the Moroccan environments and adaptation to climate change. Such strategy has been developed in Italy (Boggini et al., 1990); and in Tunisia (Daaloul et al., 1990). Further investigations through biochemical and molecular markers are in progress to study the genetic variations in Moroccan durum wheat collection.

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