

Agronomic and Genetic Diversity of Tunisian Local Durum Wheat Accessions

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Abstract

Background: Durumwheat is the major cereal cultivated in Tunisia for both Human nutrition and feed. As part of the valorization of genetic resources process in the National Gene Bank of Tunisia, we analyzed the genetic diversity of some local durum wheat accessions grown in Tunisia for more than one century. This old germplasm may be used as potential parent genitors in breeding for high yielding phenotypes in semi-arid and arid regions.

Methods: Agronomic characters and microsatellite molecular markers were used to study the genetic diversity among durum wheat accessions. Statistical analyses combined with other computational tools allowed drawing similarity dendrogram, describing genetic distances, and determining the similarity coefficient between accessions.

Results: Seven polymorphic primers revealed the polymorphism, and thus we could distinguish 2 major groups; each of them has common morphological and physiological characteristics. This genetic analysis gives an idea for future cross breeding possibilities within the genetic improvement program, which will contribute to improve resilient lines to climate change.

Key Words: Genetic diversity, SSR, Dendrogram, Genetic improvement

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I. Introduction

Durum wheat (*Triticum turgidum* L. subsp. Durum (Desf.) Husn.) is one of the oldest and most cultivated cereals in the world with more than 17 million hectares, and represents the largest import market for the Mediterranean basin. This is due to the great Mediterranean consumption of durum wheat derivatives (Royo et al., 2009; Nazco et al. 2012). North Africa cultivates around 2.5 million hectares of durum wheat, of which around 1 million hectares are in Tunisia. It is mainly used for the production of pasta and other derived food products. Cereals have been a strategic economic and social activity in Tunisia for thousands of years. They have always occupied large areas and remain the main food resource for Tunisians. The regions of Abyssinia and North Africa are considered secondary centers of diversity for durum wheat (Vavilov 1951). Tunisia, which is part of the secondary center of durum wheat diversity, has a rich diversity in terms of local populations and related wild species (Boeuf 1932; Lala, et al. 2018; Dakhaloui et al 2019). Local varieties of durum wheat are considered valuable parental genetic material for wheat breeding programs around the world.

However, Tunisian agriculture, as rest of the Mediterranean region, faces multiple difficulties such as climate change, which is responsible for fluctuations in durum wheat production. Besides, these changes occurred towards the end of the last century with a warming of the earth of 1°C and a rise in the level of the oceans of about 15 cm (IPCC, 2013). The Mediterranean Basin is one of the regions of the earth most affected and most threatened by global warming. This requires very urgent action in order to find a solution to ensure food security.

The phenotypic homogeneity of cultivars, typical of modern agriculture, caused a dramatic loss of natural variation in the mid-20th century in southern Europe and from the 1970s in North Africa, resulting in genetic erosion of wheat. Most of the landraces are conserved and can be considered as sources of new favorable genes / alleles, which could be introduced into modern cultivars in order to improve them. Tunisian local populations or "landraces" are well adapted to a wide range of environments, they are genetically diverse,

and they are considered to be an important reservoir of useful genes that can be exploited in wheat breeding programs (Daaloul et al. 1991; Khoufi et al. 2012; Babay et al. 2020).

The preservation of the genetic diversity of the national heritage can be used by breeders for the development of new varieties tolerant to these new climatic conditions. The morphological and molecular study of this diversity makes it possible to understand the differences and similarities that exist between ecotypes and to describe the structuring of genetic variability within populations (Khamassi et al. 2012; Khamassi et al. 2013; Khamassi et al. 2014; Dakhaloui et al. 2019). In this context, our work focuses on the analysis of the agronomic and genetic diversity of 12 indigenous accessions of durum wheat cultivated in Tunisia for more than a hundred years by means of molecular markers of the SSR type.

II. Material And Methods

1. Plant material and characterization

The present study was carried out at the National Gene bank of Tunisia (NGBTUN), during the 2020-2021 cropping season. Twelve samples consisting of indigenous Tunisian accessions of durum wheat, that have been cultivated on farms in Tunisia since hundreds of years (Tab. 1) were used in this study. The seeds of these local genotypes are provided by the NGBTUN. The seeds are sown in vegetation pots; each pot contains 3 seeds and three repetition for each pot in a total of 36 pots. A contribution of Nitrogen and Di-Ammoniac Phosphate as well as phytosanitary treatments (insecticide and fungicide), were carried out twice (if necessary) during the vegetative cycle. Weekly irrigations are done to ensure a good development of the plants.

Table 1: List of durum wheat accessions and their identifiers in Genesys and NGBTUN.

N°	Accession Name	Genesys ID	NGBTUN ID	N°	Accession Name	Genesys ID	NGBTUN ID
1	Agili Glabre	IG 81978	NGBTUN 5798	7	Biskri Smooth	IG 83251	NGBTUN 5866
2	Sebei Glabre Ac 1	IG 81989	NGBTUN 5808	8	Agili Blanc 1	IG 83301	NGBTUN 5877
3	Biskri Glabre Ac 2	IG 83134	-	9	Biskri	IG 83303	NGBTUN 5879
4	Derbessi Ac 1	IG 83145	NGBTUN 5845	10	D-77	IG 88459	NGBTUN 5915
5	Hamira Ac 4	IG 83152	NGBTUN 5852	11	Hmira Makaou	IG 98614	NGBTUN 6432
6	Jennah Khotifa Ap 10	IG 83157	NGBTUN 5855	12	Souri 428	IG 127580	NGBTUN 6475

NGBTUN: National Gene-Bank of Tunisia, Genesys: online platform for information about Plant Genetic Resources for Food and Agriculture (PGRFA) conserved in genebanks worldwide (<https://www.genesys-pgr.org/>).

1.2. Agronomic characterization

1.2.1. Flag sheet surface

According to Gardner et al. (1985), the area of the flag sheet (SF) (cm²) is measured by the following formula:

$$SF = L \times W \times 0.75$$

- L = length of the flag sheet
- W = width of the base of the leaf

During heading, we calculated the average of the measurement of three flag leaves randomly of each accession in each test run, stressed and control.

1.2.2. Plant length

This parameter is measured in centimeters, from the base of the stem (from the ground) to the top, considering the ear and the beard.

1.2.3. Number of kernels per ear

After husking the ears of each accession, we calculated the average number of kernels per ear for each genotype of each replicate.

1.2.3. Thousand Grain Weight (TGW)

During the harvest, we weighed 100 grains of each replicate for each test. Next, we have to deduce the average weight of a thousand grains.

1.2.4. Agro-morphological statistics

The agro-morphological variability was analysed using the Principal Component Analysis (PCA). The Pearson correlation coefficients ($\alpha = 0.05$, standard PCA without rotation of axes) were calculated by using the software SPSS Statistics 20.

1.3. Molecular characterization

1.3.1. DNA extraction and PCR amplifications

Total genomic DNA was extracted from fresh leaves of each accession after 24 h freeze dry using the 2% cetyltrimethyl-ammonium bromide (CTAB) method (Doyle and Doyle 1987). PCR reactions were carried out following Khamassi et al. (2011) protocol as following: in a reaction mix of 25 μ l total reaction volume containing 1 U of Taq polymerase, 50-100 ng of template DNA, 0.25 μ M of each primer, 0.2 mM of each dNTP, 2 mM of MgCl₂ and 1X PCR reaction buffer. PCR products were separated on 2.5% agarose gels in 0.5 \times TBE buffer. A 100bp DNA Ladder was used to estimate the size of amplified DNA fragments. Putative polymorphisms were detected for each marker. Percentage of the polymorphism obtained by each SSR marker was calculated. In order to investigate the suitability of each SSR marker to assess the genetic diversity among the studied wheat accessions, polymorphism information content (PIC) was determined following Roldan-Ruiz et al. (2000).

1.3.2. Polymorphism Information degree

This setting is referred to as the Information Content of Polymorphism (PIC). It provides information on the ability of a primer to generate polymorphism between the studied accessions (Table 2). It is calculated by the below formula:

$$PIC = 1 - \sum (P_{ij})^2$$

Where P_{ij} is the frequency of the i^{th} band revealed by the j^{th} primer.

III. Results and Discussion

1. Molecular characterization

Ten pairs of microsatellite primers were used to study the genetic diversity of 12 genotypes of wheat grown in Tunisia since more than one hundred years. Seven primers generated polymorphic profiles (Table 2). Two primers generated monomorphic profiles, and one did not amplify any locus; and were therefore discarded from the study.

108 polymorphic bands were obtained, with an average of 9.81 bands per accessions and 13.5 bands per primers. For each accession and for each primer, the polymorphic bands, at a determined level of migration are denoted by 1 (presence of band) and 0 (absence of band). Thus, for each primer, we measured the degree of polymorphism through the calculation of the PIC. It reflects the ability to discriminate polymorphism for each marker as a function of the number of alleles at a locus (Table 2). The value of PIC varies between 0.24 for primer wmc116 and 0.81 for primer wmc789, and an average value of $PIC = 0.49$. The polymorphic microsatellite markers used in this study were very informative. According to Sundaram et al. (2007), markers with values of $PIC = 0.5$ or greater are very informative for genetic studies and they are extremely useful in distinguishing the rate of polymorphism.

Table 2: Primers information; sequences, PIC, and temperature of annealing ($T^{\circ}a$)

Primers	5'-3'	PIC	$T^{\circ}a$
WMC 553	F: cggagcatgcagctagtaa R: cgcctgcagaattcaacac	0,48	61
WMC 619	F: ttcccttcccctcttccg R: tacaatgccacgagcacct	0,57	61
WMC 329	F: acaaaggctcattcgtaga R: aacacgcatcagttcagt	0,57	51
WMC 798	F: gtgtgtagttagctccaaaag R: gttagcatggcacatagaagcag	0,81	61
WMC 168	F: aacacaaaagatccaacgacac R: cagtatagaaggattttgagag	0,40	51
WMC 110	F: gcagatgagttgattggattg R: gtacttggaaactgtgtttggg	0,40	61
WMC 116	F: acggcaccacgagaacctagtgcg R: gcgtcgtctgtctgcacatgg	0,24	61

1.1. Dendrogram and genetic distance

The dendrogram obtained (Figure 1) based on the similarity matrix (Table 3) presents information on the genetic relationships between the different accessions as a function of the allelic distribution of the loci. The coefficient

of similarity varies in the order of 34 to 90%. A dendrogram at the level of 60% similarity, allowed us to obtain three main groups (A and B).

Groupe A

The accessions of this group have a minimum of similarity percentage of 69%, this first group is composed with main accessions (Agili Glabre, Sebei Glabre, Biskri glabre, Derbessi, Sourì 428, Jennah Khotifa, Agili Blanc and Hamira Makaou). These accessions share some morphological characters such as: they are all glum hairless (absence of pilosity on the glume) and similarity in the spike shape.

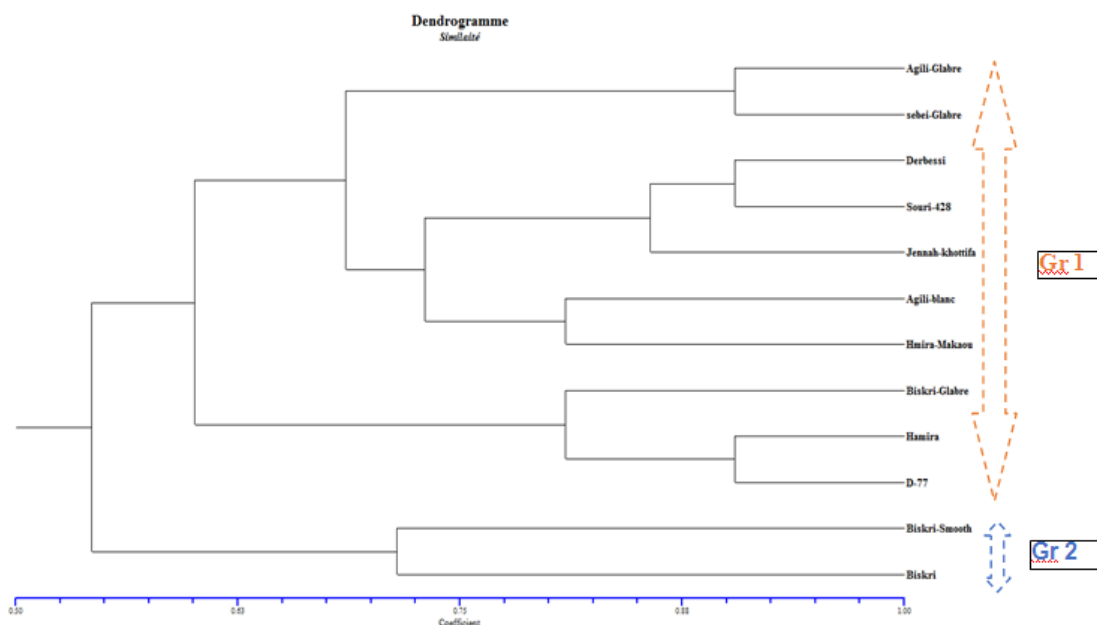
Groupe B

This group contains only "Biskri Smooth" and "Biskri". The two accessions are selected from a mixture in 1908/1909 (Tunis Test and Acclimatization Garden, created in 1893) and originating from Biskra (Algeria) (Deghais et al. 2007). The two accessions share the majority of the morphological characters.

Table3: Similarity matrix

	Agili-Glabre	Sebei-Glabre	Biskri-Glabre	Derbessi	Hamira	Jennah-Khottifa	Biskri-Smooth	Agili-Blanc	Biskri	D-77	Hmira-Makaou	Souri-428
Agili Glabre	1,00											
Sebei Glabre	0,90	1,00										
Biskri Glabre	0,62	0,71	1,00									
Derbessi	0,81	0,71	0,52	1,00								
Hamira	0,62	0,52	0,81	0,62	1,00							
Jenneh Khotifa	0,71	0,62	0,52	0,81	0,62	1,00						
Biskri Smooth	0,52	0,43	0,52	0,52	0,62	0,52	1,00					
Agili Blanc	0,62	0,52	0,43	0,62	0,52	0,81	0,71	1,00				
Biskri	0,43	0,34	0,43	0,43	0,62	0,52	0,71	0,52	1,00			
D-77	0,62	0,52	0,81	0,62	0,90	0,62	0,71	0,62	0,52	1,00		
Hmira Makaou	0,71	0,62	0,52	0,71	0,71	0,71	0,71	0,81	0,62	0,62	1,00	
Souri 428	0,81	0,71	0,62	0,90	0,71	0,90	0,62	0,71	0,52	0,71	0,81	1,00

Figure 1: Dendrogram of genetic similarities based on UPGMA method.



2. Agronomic characterization

2.1. Plant length

The length of plants is variable, between 91 cm for “Agili Blanc” and 125 cm for “Hmira Ac4” (Figure 2). All of these accessions are relatively long compared to the improved commercial varieties (between 70 and 90 cm) widely cultivated nowadays. Tunisian farmers have long made a selection of their local varieties. Long straw and especially amber and vitreous grains characterize this one. These characteristics meet a double need: (i) Use of straw for livestock feed during times of scarcity (between harvest and spring). (ii) A high semolina yield; semolina being the basic product for making couscous and durum wheat bread

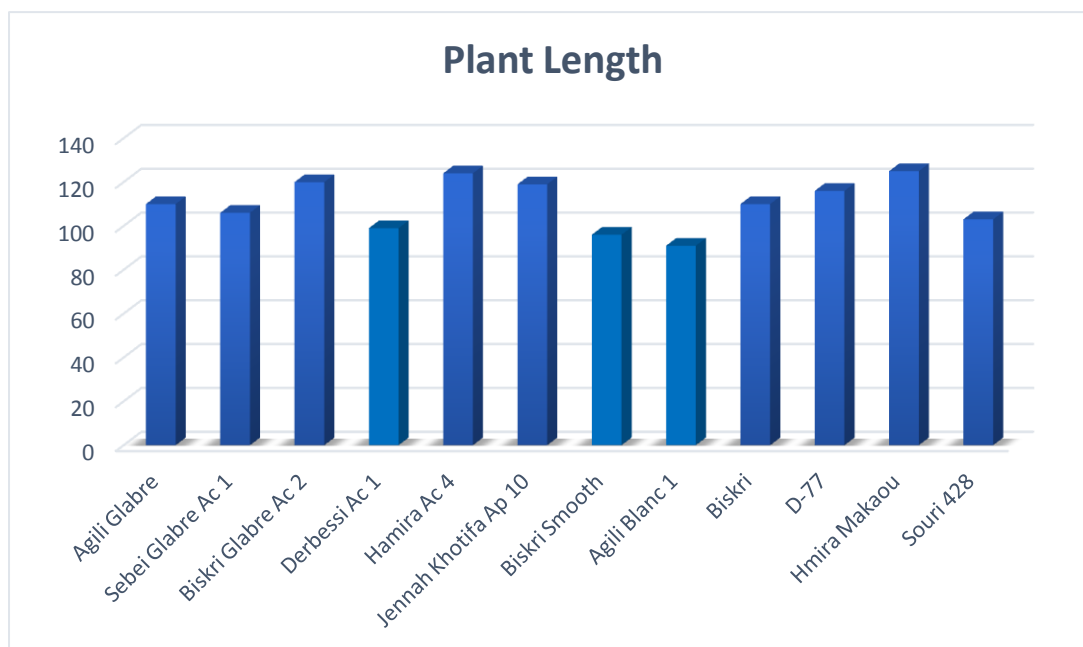


Figure 2: Variation in length of wheat accessions

1.2. *Flag leaf area*

Concerning the leaf surface of the flag leaves, we notice that the average for the accessions varies between 15 cm² for “Hamira Ac4” and 38.5 cm² for “Biskri smooth” (Figure 3).

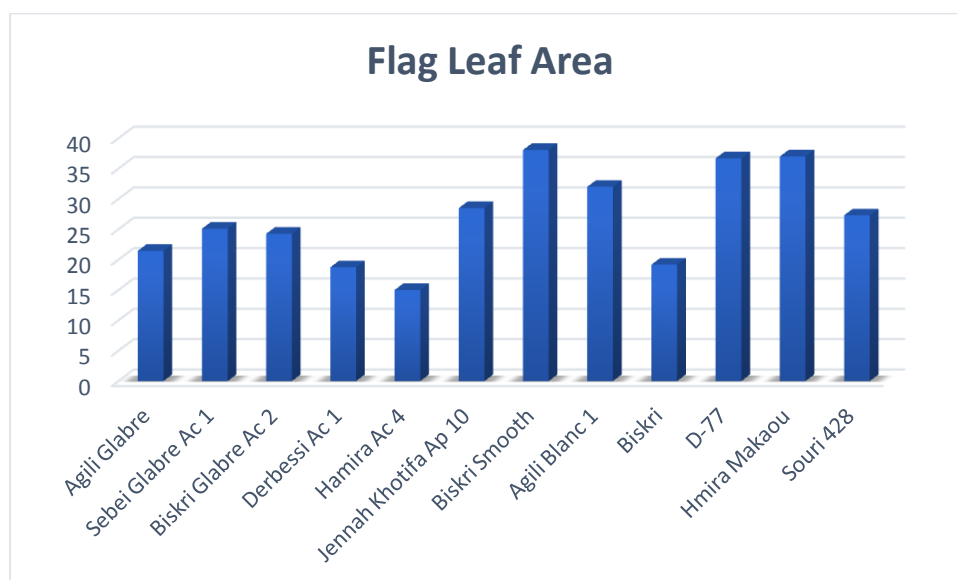


Figure 3: Variation of flag leaf area of wheat accessions

1.3. Spike grain number

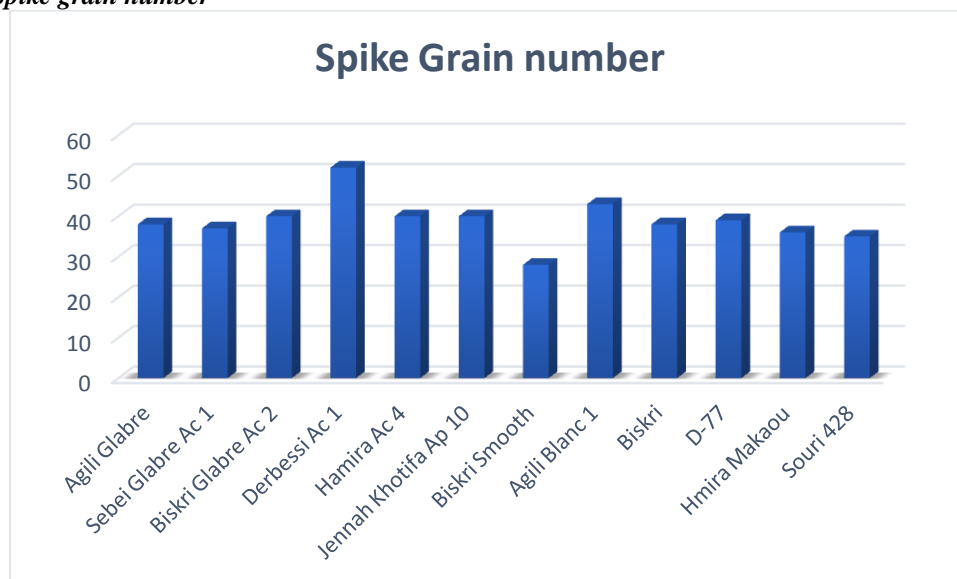


Figure 4: Variation of spike grain number of wheat accessions

According to Figure 4, accession Derbessi Ac1 is the most productive in terms of grain yield per ear, followed by Agili blanc Ac1. In contrast, accession Biskri smooth has the lowest number of kernels per ear.

1.4. Thousand kernel weight (TKW)

Concerning the TKW, we notice that the average for the accessions varies between 34,8 g for “Biskri” and 57.1 g for “Agili glabre” (Figure 5). Thousand-kernel weight is an important parameter for the evaluation of variety breeding. TKW is not only directly related to the grain yield and milling quality of grain, but also has an impact on the seedling vigor and growth indirectly affecting the yield (Wenhua Wu et al. 2018).

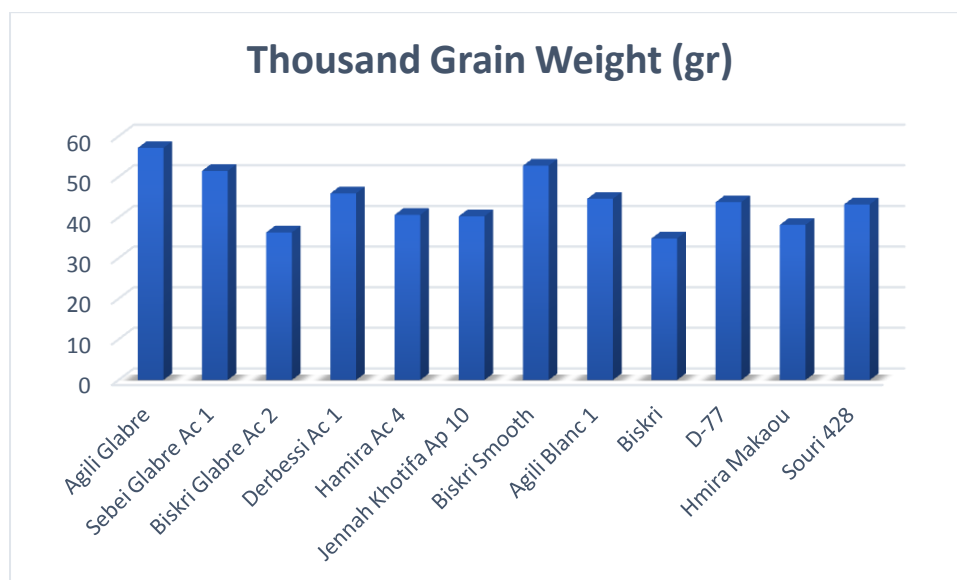


Figure 5: Variation of thousand-grain weight (TKW) of wheat accessions

1.5. Principal Component Analysis (PCA)

The first three PCA axes accounted for 75% of the total variability expressed by agro-morphological traits (Figure 6). The first component accounts for 42 % of the total variation. A variability of accessions showed by the axes 1 and 2 which explained 75% of the total variability, revealed group and two isolated accessions (Derbessi and Biskri smooth).

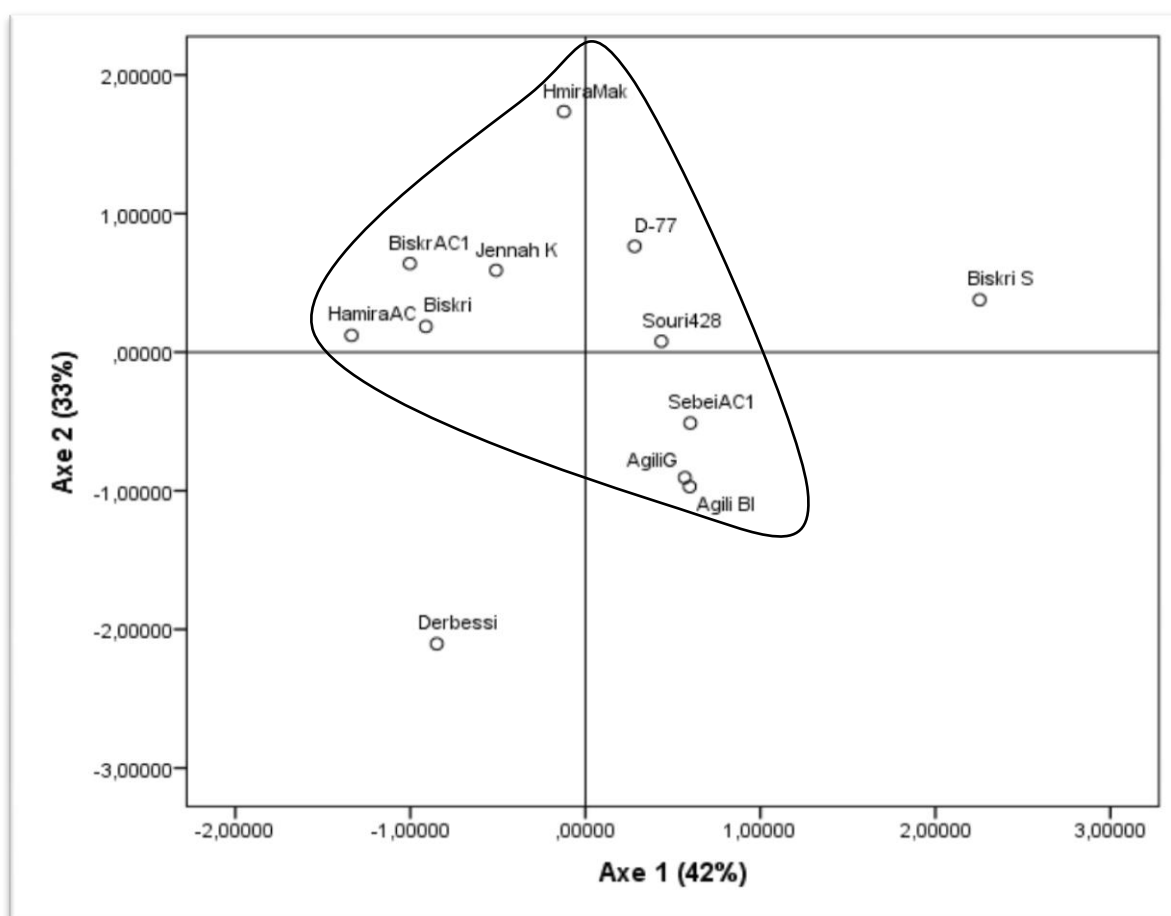


Figure 6: Principal component analysis of agro morphological data of wheat accessions

IV. Conclusions

Both molecular and agro-Morphological techniques are complementary to better characterize and study the diversity of wheat and all cereal species. The genetic diversity obtained by SSR markers is relatively consistent and compatible with some agro-morphological characters of accession. SSR molecular-marker can be employed in assaying the level of polymorphism and diversity. The characterization of some durum wheat genotype using agro-morphologic and SSR markers may provide useful information for long-term improvement in yield, qualities, resistance (biotic and abiotic stresses) and for germplasm identification and conservation. As preliminary germplasm assessment, Biskri smooth accession looks interesting to go deeper characterization and valorization.

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