Research Article

Morphological Variability of Wild Cardoon (*Cynara cardunculus* L. var. *sylvestris*) Populations in North of Tunisia

Imen Ben Ammar,¹ Fethia Harzallah-Skhiri,² and Bouthaina Al Mohandes Dridi¹

¹ High Institute of Agronomy of Chott-Mariem, University of Sousse, 4042 Chott Mariem, Tunisia

² High Institute of Biotechnology of Monastir, University of Monastir, Tunisia

Correspondence should be addressed to Imen Ben Ammar; i.benammar@yahoo.fr

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In north of Tunisia, wild cardoon (*Cynara cardunculus* L. var. *sylvestris* (Lamk) Fiori) is called “khurshef.” It is consumed mainly for its fleshy stems and leafstalks in some traditional dishes. In some regions, heads were used to prepare cheese. North Tunisian germplasm has been currently damaged by severe genetic erosion, pollution, urbanization, and bad farming practices. In order to preserve this species and to assess morphological relationship between accessions, the present study aims to prospect and to characterize individuals in several areas of the north of Tunisia. Six populations were collected and then 20 individuals per population were evaluated using UPOV (International Union for the Protection of New Varieties of Plant) descriptors related to leaves, leafstalks, and heads. Multivariate analyses were used to elucidate relationship among the studied populations. Principal components analysis revealed more diversity within each population. Cluster study reveals large variability among populations. This analysis allows classifying the germplasm of wild cardoon into five groups. Similarities observed between ecotypes despite their distinctiveness of geographic origin suggest a narrow genetic base. These analyses are very useful for the management and the use of wild cardoon in future breeding programs for *Cynara* germplasm.

1. Introduction

Cynara cardunculus L. (Asteraceae), commonly named “cardoon,” is widespread in the Mediterranean area [1]. It comprises two botanical varieties: *C. cardunculus* L. var. *altilis* DC (domestic cardoon) and *C. cardunculus* L. var. *sylvestris* (Lamk) Fiori (wild cardoon), considered to be the wild ancestor of globe artichoke [2, 3]. Two gene pools can be distinguished within wild cardoon: the eastern Mediterranean type, mainly distributed in Italy, Greece, and Tunisia, and the western gene pool, diffused in the Iberian Peninsula. It is a nondomesticated robust perennial plant characterized by a rosette of large spiny leaves and branched flowering stems [4]. The main differences between cultivated and wild cardoon are the larger production potential of the former and the distribution of the assimilates between shoot and root, with the wild type investing more carbohydrates in the roots providing more resistance to adverse climatic conditions [5]. The wild cardoon is considered the presumed wild

progenitor of the artichoke and the cultivated cardoon [2–4, 6]. These three taxa are fully interfertile [3, 7]. Recent studies have suggested that a high level of differentiation is present in the wild cardoon gene pool and that samples from the western Mediterranean range more closely resemble the cultivated cardoon than the wild samples from the eastern Mediterranean one [4, 8]. Assessment of genetic diversity and determining the relationship between ecotypes allow the management of the germplasm and may increase the efficiency of efforts to improve species. Thus, morphological characterization is the first step in the description and classification of germplasm [9]. This study aims to investigate genetic diversity in several Tunisian populations of wild cardoon collected from north Tunisia as inferred by variations in their morphology as a necessary step for the best conservation of wild cardoon gene pool and for future improvement of crop characters. Moreover, it is likely that a source of resistance to *Verticillium* has been found in wild material.

TABLE 1: List of the six populations of wild cardoon included in this study.

Government	Locality	Code
Bizerte	Ain Berda 1	AB1
	Ain Berda 2	AB2
	Beni Amor	BA
Beja	Dhahirat	DH
	Daouar Mahjouba	DM
Siliana	Ain Dissa	SN

FIGURE 1: Localities of the six populations.

2. Materials and Methods

2.1. Plant Material and Study Areas. This study was conducted in 6 localities (Ain Berda 1, Ain Berda 2, Beni Amor, Daouar Mahjouba, Dhahirat, and Ain Dissa) from 3 governorates (Bizerte, Beja, and Siliana) located in the north of Tunisia (Figure 1). Six populations of wild cardoon (AB1, AB2, BA, DH, DM, and SN) (Table 1) were assessed. Twenty samples per population were collected. For each one, samples were coded from 1 to 20.

2.2. Morphological Traits. At flowering stage, simple random sampling method was followed for collecting the individuals. Forty-three morphological characters were recorded. The morphological traits (Table 2) were evaluated based on the UPOV (International Union for the Protection of New Varieties of Plant) descriptor list for artichoke (since there are no descriptors edited for cardoon). Several specific botanical characters for cardoon were chosen as descriptors. Twenty-five are qualitative traits and eighteen are quantitative traits. The quantitative traits were plant height, the number of leaves per plant, the number of lateral shoots, the number of heads, the length of leafstalk, the width of leafstalk, the depth of leafstalk, the thickness of leafstalk, the length and width of leaf, and the length of spines. The qualitative traits were depth of leaf lobation (determined visually following this scale: from very shallow, shallow, medium, deep, and very deep); leaf and leafstalk color (visually measured); anthocyanin at leafstalk base (visually measured following this scale: no anthocyanin, anthocyanin present in less than 50% of the leafstalk, anthocyanin present in 50% of the leafstalk, and anthocyanin present in more than 50% of the leafstalk); and leafstalk texture (visually measured following this scale: hollow leafstalk, 50% of the leafstalk hollow, and hard and

FIGURE 2: UPGMA dendrogram (based on Gower's coefficient of similarity) revealing morphological diversity among the six populations collected from different regions of north Tunisia.

full leafstalk). For some traits, a discrete classification was adopted. This was particularly important for germplasm characterization. Furthermore, all the characteristics concerning colour are subjective and are often affected by climatic and soil conditions.

2.3. Statistical Analysis. For all data obtained from morphological study, cluster analysis was made using the MVSP (Multivariate Statistical Package for Windows 3.1) and was carried out by UPGMA method. Two trees illustrating the genetic distance among populations (Figure 2) and within ecotypes (Figure 3) were constructed based on Gower's general similarity coefficient [10] for morphological data. Quantitative and qualitative data on morphological traits were subjected to a principal coordinate analysis (PCA) using MVSP statistical software. The first two principal coordinates were used to produce a two-dimensional scatter plot to understand how each axis influenced the variation among groups and whose morphological characteristics have stronger discriminating power.

3. Results

3.1. Cluster Analysis. Morphological characterization revealed high polymorphism among six populations. The dendrogram based on Gower's general similarity coefficient clustered populations into five groups (Figure 2). The first cluster gathered the two populations: Dhahirat (DH) and Ain Berda 2 (AB2) at 67% of similarity. The population Ain Berda 1 (AB1) was the most divergent from other populations (Ain Berda 2 (AB2), Dhahirat (DH), and Daouar Mahjouba (DM)) in cluster 3 ($d = 0.61$). Cluster 4, constituted with populations Siliana Nord (SN) and Beni Amor (BA), presents with cluster 3 (AB1, AB2, DH, and DM) the most level of divergence ($d = 0.50$). Figure 3 presented the dendrogram based Gower's general similarity coefficient which illustrates diversity within accessions. It shows discrimination of ecotypes evaluated on morphological traits into two main groups at 70% of similarity. The first group GI is divided into two subgroups (a) and (b). The subgroup (a) includes 6 subsamples of accessions DM (Daouar Mahjouba) and 1 of DH (Dhahirat). The subgroup

TABLE 2: Morphological traits recorded in the wild cardoon ecotypes.

Parameters number	Code	Parameters and descriptive value
Character of plant		
1	H	Height of plant (cm)
2	Nlf/p	Number of leafs/plant
3	Nlsh	Number of lateral shoots on main stem
4	Nh	Number of heads
5	Hp	Heads position (1: terminal/2: lateral)
6	H	Habit (1: erect/2: semierect/3: horizontal)
Characters of leafstalk		
7	Lls	Length of leafstalk (cm)
8	Wls	Width of leafstalk (cm)
9	Dls	Depth of leafstalk (cm)
10	Thls	Thickness of leafstalk (cm)
11	Tex	Texture of leafstalk (1: hollow/2: medium/3: strong)
12	Ca	Colour of anthocyanin at leafstalk base (1: absent/2: sparse/3: medium/4: strong)
13	Cls	Colour of leafstalk (1: whitish; 2: greenish white; 3: whitish green; 4: light green; 5: green; 6: green ash; 7: grayish green)
14	Hls	Hairs on leafstalk (1: absent/9: present)
15	Lsls	Length of spines at leafstalk (cm)
Characters of leaf		
16	Llf	Length of leaf (cm)
17	Clf	Leaf colour (1: yellowish green; 2: light green; 3: greenish; 4: dark green; 5: green ash; 6: grayish green)
18	Icv	Intensity of green colour (1: light/2: medium/3: dark)
19	HGC	Hue of green colour (1: absent/2: yellowish/3: grayish)
20	Igh	Intensity of grey hue (1: weak/2: medium/3: strong)
21	Hlf	Hairs on the upper side of the leaf (1: absent/9: present)
22	Lslf	Length of spines at leaf (cm)
Characters of lobations		
23	Shlb	Shape of lobes (1: acute/2: nearly right angle/3: obtuse)
24	Nlb	Number of lobes (3: few/5: medium/7: many)
25	Llb	Length of the longest lobe (cm)
26	Wlb	Width of the longest lobe (cm)
27	Nlbs	Number of the secondary lobes (1: none or very few/3: few/5: medium/7: many/9: very many)
28	Slbs	Shape of the secondary lobes (1: acuminate/2: acute/3: rounded)
29	Lslb	Length of spines at lobes (cm)
Characters of head		
30	HD	Diameter of head (cm)
31	HS	Size of head (1: small/2: medium/3: large)
32	Sc	Shape of central head (1: circular/2: broad elliptic/3: ovate/4: triangular/5: transverse broad elliptic)
33	Hc	Colour of central head flower (1: violet; 2: purplish blue; 3: white)
34	Ta	Time of appearance (1: early/2: medium/3: late)
35	Drec	Diameter of receptacle (cm)
36	Trec	Thickness of receptacle (cm)
37	Srec	Shape of receptacle (1: flat/slightly depressed/strongly depressed)
38	D	Density of inner bracts (1: sparse/2: medium/3: dense)
Characters of outer bract		
39	Sob	Shape of outer bract (1: broader than long/2: as broader as long/3: longer than broad)
40	Saob	Shape of apex of outer bract (1: acute/2: flat/3: emarginated)
41	Cob	Colour of outer bract (1: green/2: green striped with violet/3: violet striped with green/4: mainly violet/5: entirely violet)
42	Tob	Thickness at base of outer bract (3: thin/5: medium/7: thick)
43	Lsob	Length of spines of outer bract (cm)

TABLE 3: Means, maximal and minimal values of the recorded quantitative traits of subgroups obtained after classification.

Traits*		a**	b**	c**	d1**	d2**	d3**	d4**	d5**	d6**
H	Mean	84.67 ± 5.2	85.39 ± 32.6	88.86 ± 25.4	67.51 ± 11.2	52.17 ± 10.4	71.06 ± 17	77.57 ± 20	58.35 ± 15.1	43.92 ± 22.3
	Max.	93	162	118	52.5	74.2	98.5	114	87.9	92.2
	Min.	77	36	71	20	34	35	36	35.2	23
Nlf/p	Mean	20 ± 6.4	25.83 ± 5.3	21.66 ± 7.4	21.4 ± 5.2	16.88 ± 4.2	16.03 ± 4.6	18.73 ± 5.6	16.1 ± 3.6	20.62 ± 9.2
	Max.	32	35	30	31	26	25	33	24	36
	Min.	14	18	16	11	9	9	10	12	9
Nlsh	Mean	5.71 ± 2.4	5 ± 1.3	4 ± 0	2.72 ± 0.6	2.27 ± 1.4	2.65 ± 1.3	2.63 ± 0.7	2.9 ± 1.9	2 ± 0.5
	Max.	11	7	4	4	6	7	4	8	3
	Min.	4	3	4	2	1	1	2	2	1
Nh	Mean	11.14 ± 5	14.94 ± 4.9	6.33 ± 4.7	9.83 ± 2.7	3.55 ± 1.6	4.84 ± 2.8	5.68 ± 3.3	5.4 ± 3.4	3.5 ± 1.8
	Max.	21	25	10	12	7	13	15	15	7
	Min.	7	7	1	2	2	2	2	3	2
Llf	Mean	41.01 ± 9	40.49 ± 4	35.72 ± 13	45.77 ± 5.4	33.79 ± 10.8	34.99 ± 7.6	33.03 ± 4.4	40.38 ± 4.5	27.42 ± 5.1
	Max.	53.10	49.97	45.67	39	49.7	52.3	41.43	47.8	38.67
	Min.	28.79	32.93	21.33	19.7	19.6	21.2	25.47	34.3	20.7
Lslf	Mean	0.81 ± 0.3	0.77 ± 0.2	0.58 ± 0.6	0.83 ± 0.1	0.49 ± 0.2	0.66 ± 0.2	0.65 ± 0.1	0.76 ± 0.4	0.44 ± 0.1
	Max.	1.4	1.24	1.26	0.74	0.96	1.28	0.9	1.4	0.7
	Min.	0.5	0.4	0	0.3	0	0.4	0.4	0	0.3
Lls	Mean	65.7 ± 14.0	63.68 ± 14	58.03 ± 15.1	59.71 ± 5.3	42.28 ± 8.5	58.33 ± 14.3	51.42 ± 8.6	47.38 ± 8.8	31.62 ± 12
	Max.	89	99.4	69	43	54.7	77.2	75.2	60	60.2
	Min.	46.7	33.6	40.8	25.9	28.8	16.2	36.1	31.2	22
Wls	Mean	1.83 ± 0.4	2.04 ± 0.5	1.46 ± 0.5	2.53 ± 0.4	1.43 ± 0.3	1.26 ± 0.3	1.37 ± 0.4	1.34 ± 0.4	1.25 ± 0.2
	Max.	2.2	3.35	1.75	2.2	2.1	1.9	2.24	2.04	1.42
	Min.	0.9	1.6	0.9	1	0.9	0.66	0.71	0.63	0.96
Dls	Mean	1.08 ± 0.5	1.75 ± 0.3	1.41 ± 0.7	1.75 ± 0.2	0.98 ± 0.2	1.08 ± 0.2	1.29 ± 0.4	0.89 ± 0.2	0.92 ± 0.2
	Max.	1.6	2.51	2.2	1.35	1.5	1.75	1.9	1.18	1.2
	Min.	0.52	1.17	0.94	0.7	0.58	0.78	0.5	0.6	0.6
Thls	Mean	0.62 ± 0.19	0.80 ± 0.17	0.63 ± 0.19	1.11 ± 0.14	0.56 ± 0.13	0.49 ± 0.12	0.57 ± 0.18	0.57 ± 0.2	0.56 ± 0.18
	Max.	0.83	1.24	0.85	0.95	0.77	0.8	0.91	0.91	0.8
	Min.	0.4	0.52	0.46	0.44	0.3	0.3	0.3	0.26	0.3
Lsls	Mean	1.48 ± 0.2	1.65 ± 0.2	0.77 ± 0.2	2.17 ± 0.1	1.33 ± 0.1	1.07 ± 0.1	1.33 ± 0.2	1.38 ± 0.2	1.24 ± 0.2
	Max.	2.2	2.25	1.5	1.4	2.09	1.48	2.1	3.2	1.9
	Min.	1.15	0.87	0	0.74	0.5	0.76	0.8	0.24	0.6
Llb	Mean	13 ± 2.7	12.16 ± 2.3	13.2 ± 4	13.73 ± 1.5	12.29 ± 3.3	11.20 ± 2.1	10.64 ± 1.8	15.58 ± 2.4	10.08 ± 1.9
	Max.	15	16.3	16.7	10.2	18	15	14	19.7	13
	Min.	7.8	8.2	8.9	5.6	6.7	7.8	7.1	11.4	6.6
Wlb	Mean	1.95 ± 0.8	2.31 ± 0.9	3.16 ± 1.2	2.3 ± 0.5	2.13 ± 0.6	2.27 ± 0.5	2.25 ± 0.4	2.93 ± 0.5	2.1 ± 0.4
	Max.	3.1	4.3	4.3	2.1	3.2	3.7	3.2	3.6	2.6
	Min.	0.7	0.8	2	0.7	0.7	1.4	1.7	2	1.4
Lslb	Mean	1.10 ± 0.2	1.20 ± 0.3	0.81 ± 0.7	0.93 ± 0.2	0.69 ± 0.4	0.96 ± 0.3	1.02 ± 0.2	0.76 ± 0.4	0.95 ± 0.3
	Max.	1.4	1.9	1.4	0.8	1.73	1.7	1.5	1.24	1.44
	Min.	0.8	0.6	0	0.24	0	0.45	0.65	0	0.4
Nlbs	Mean	4.14 ± 1.6	4.22 ± 1.7	1.66 ± 1.2	6.83 ± 1.3	4.22 ± 1.6	4 ± 1.7	4.57 ± 1.3	2.6 ± 1.6	2.5 ± 1.4
	Max.	7	7	3	7	7	7	7	5	5
	Min.	3	1	1	3	1	1	3	1	1
HD	Mean	6.45 ± 0.4	5.47 ± 0.5	4.14 ± 0.4	7.73 ± 0.9	3.05 ± 0.7	3.98 ± 0.8	4.84 ± 0.6	3.04 ± 0.4	2.95 ± 0.5
	Max.	6.8	7	4.5	5	4.14	5.5	6.04	3.66	3.67
	Min.	5.74	5	3.71	1.73	1.94	2.62	3.85	2.6	2

TABLE 3: Continued.

Traits*		a**	b**	c**	d1**	d2**	d3**	d4**	d5**	d6**
Drec	Mean	6.03 ± 0.6	4.36 ± 1.1	3.11 ± 0.4	6.58 ± 0.3	2.30 ± 0.7	3.41 ± 0.9	4 ± 0.6	2.13 ± 0.5	2.08 ± 0.5
	Max.	6.9	5.81	3.5	3.9	4.16	5.76	5.37	2.84	2.8
	Min.	5.36	0.37	2.75	2.8	1.03	2.25	2.82	1.41	1.53
Trec	Mean	2.16 ± 0.3	1.58 ± 0.2	1.27 ± 0.2	2.37 ± 0.1	0.96 ± 0.2	1.16 ± 0.3	1.44 ± 0.3	1.01 ± 0.2	1.05 ± 0.2
	Max.	2.7	1.94	1.4	1.55	1.24	1.9	2.25	1.37	1.4
	Min.	1.75	1.2	1.04	1.1	0.6	0.7	1	0.8	0.85
Lsob	Mean	0.85 ± 0.2	1.96 ± 0.3	0.69 ± 0.3	1.89 ± 0.3	0.75 ± 0.3	0.91 ± 0.3	0.82 ± 0.2	0.67 ± 0.3	1.07 ± 0.4
	Max.	1.27	1.81	0.94	1.62	1.15	1.61	1.17	1	1.9
	Min.	0.55	0.75	0.35	0.53	0.13	0.49	0.6	0	0.61

* See Table 2; ** see Figure 3.

(b) gathered 13 subsamples of AB1 (Ain Berda 1), 3 of AB2 (Ain Berda 2), and 2 of DH (Dhahirat). The major group GII is subdivided into 7 subgroups (c), (d1), (d2), (d3), (d4), (d5), and (d6). Subgroup (c) comprises only 3 subsamples. The major subgroup is (d3) that assembles 26 subsamples of DH (Dhahirat), DM (Daouar Mahjouba), AB1 (Ain Berda 1), and AB2 (Ain Berda 2). Group (c) presents the highest groups (88.86 cm); however, the highest ecotypes belong to group (b) with 162 cm. Table 3 shows the main quantitative data of several groups obtained by classification with the dendrogram presented in Figure 3. Group (b) presented the group of the highest number of leaves/plant (39) with also the maximal value (35 leaves/plant) and a good potential to produce lateral shoots (5 lateral shoots). Group (a) included the cluster of ecotypes with high number of lateral shoots (5.71) with also the maximal value (11 lateral shoots for DM13). On basis of the length of spines on different organs, it can be noticed that groups representing the group of ecotypes with the lowest length of spine, respectively, are (d2) on leaf (0.44 cm), (c) on leafstalk (0.77 cm), (d2) on lobation (0.69 cm), and (d1) on outer bracts (0.67 cm). Figure 2 illustrates also the genetic distance among the studied ecotypes of wild cardoon gathered into several groups. It is noticed that the nearest distance ($d = 0.90$) was exhibited among the ecotypes AB14 and AB210 representing the highest similarity, while the farthest genetic distance (0.69) was exhibited between AB11 and (AB18, AB15, AB113, and AB12) subgroup on group (b) from the same accession and the same area. The cluster showed also a high diversity within DH14, DH5, and SN5 (0.70), knowing that DH and SN are geographically very distant but clustered in the same subgroup.

3.2. Principal Component Analysis. Principal component analysis (PCA) was performed taking into account all parameters. The eigenvalue obtained by PCA indicates that the first two PCA axes described 28.89% of the total of variance (Table 4). The first axis accounts for factor 19.75% of the variability. It is correlated with the following traits: H, Nlf/p (number of leaves per plant), Nlsh (number of lateral shoots), Nh (number of heads), Lslf, Lls, lls, Dls (depth of leafstalk), Thls (thickness of leafstalk), Lsls (length of spines

of leafstalk), Lslb (length of spines of lobation), HD (diameter of head), HS (size of head), DO (degree of opening), Drec (diameter of receptacle), Trec (thickness of receptacle), Tob (thickness of outer bract), and Lsob (length of spines on outer bracts) (Figure 4).

The second axis represents 9.14% of the variance; it represents Hp (head position), Llf (length of leaf), Cls (colour of leafstalk), Llb (length of lobation), Wlb (width of lobation), Ta (time of appearance), and Srec (shape of receptacle) (Figure 4).

4. Discussion and Conclusion

The main objective of this study was to analyze morphological variation among and within several accessions of wild cardoon from the north of Tunisia. On one hand, morphological analysis based on different characters allowed clustering the six populations into five groups. On the other hand, 120 ecotypes were assessed and split into two groups demonstrated by the topology of the dendrogram based on Gower's general similarity coefficient. This study allows grouping ecotypes of several accessions into one cluster in spite of differences between the origins and the environmental conditions. There is no association between clusters with geographic location of populations; this reflects a genetic basis of the plant form in wild cardoon. In agreement with [4, 7], this discrimination may be based on the hypothesis that wild cardoon gene pool is differentiated according to its geographical distribution. In addition, on the basis of the geographical isolation, Lanerti et al. [11] identified two distinct gene pools, the Sardinian and Sicilian populations, which are clearly differentiated. Moreover, the plant width is an indicator of plant's growth habit. Indeed, most of Tunisian ecotypes have a semierect and horizontal habit. This descriptor is important in terms of crop management because it can help in terms of defining the area of each plant, harvesting, and feasibility of farming practices in the breeding program. The influence of environmental conditions is strongly correlated with quantitative traits but not with the qualitative ones, particularly the shoot number and leafstalk texture [12]. This is not in agreement with Lanerti et al. [11] study that proved that the pigmentation

TABLE 4: Definition of the first two components of PCA made on the basis of morphological traits of wild cardoon accessions.

Principal components	PCA1	PCA2
% variance	19.75	9.14
Cumulative %	19.75	28.89
Traits*	Eigenvalue	
H	0.213	-0.116
Nf	0.071	0.236
Nc	0.177	-0.073
Npl	0.195	0.044
Pc	-0.010	-0.014
Pt	-0.156	0.173
Long f	0.068	-0.260
Léf	0.125	-0.165
Cf	-0.171	0.064
Icv	-0.185	-0.184
Tcv	0.124	0.174
Icg	0.080	0.223
Plf	-0.095	0.189
Longp	0.175	-0.217
Larp	0.191	0.075
Pp	0.207	0.066
Ep	0.057	0.153
Lép	0.097	0.013
Text	-0.101	0.259
Ca	-0.041	0.197
Cp	-0.171	0.128
Plp	0.034	0.293
Fl	0.000	0.000
Nl	0.011	-0.135
Longl	-0.043	-0.254
Largl	-0.011	-0.236
Lél	0.218	-0.121
Nls	0.212	-0.035
Fls	-0.217	-0.053
D	0.229	0.016
Tc	0.226	-0.021
Cc	0.196	0.081
Ea	-0.221	-0.029
Dr	0.208	-0.035
Er	0.213	0.045
Fr	-0.121	-0.249
Ds	0.234	-0.063
Fb	0.136	-0.010
Fa	0.092	0.086
Cb	0.001	0.199
Ep	0.188	0.021
Léb	0.098	0.232

*See Table 2.

intensity (explained by the anthocyanin color at the base of leafstalk in our study) is known to be very sensitive to temperature. Our results suggest that man's traits selection is significant to understand role in the variation within wild cardoon accessions. For example, thorn length is a main character. So it seems interesting to select ecotypes with short thorn in order to use them in breeding programs. Indeed, we found some ecotypes in groups (c), (d2), and (d1) without spines on leaf and on lobation. Other ecotypes, one in group (c) and one in group (d5), are without spines, respectively, on leafstalk and on outer bract. The results that have shown the good potential of some ecotypes to produce biomass (number of leaves and lateral shoots) may be explained by the particular adaptability of the *Cynara* genus into the Mediterranean environment. This agrees with the study of Foti et al. [13]. The genetic diversity may be related to natural hybridization and fluctuations in environmental conditions. Plants might respond to their environments through developmental plasticity in many aspects of their phenotypes [14]. Some groups, such as (a) and (b), showed the highest number of heads (flowers). This could be attributed to the natural habitats, which may exert some stress from the surrounding environments. Consequently, these ecotypes tend to produce more flowers as an attempt to maintain their existence and to ensure the reproduction under unpredictable conditions. Sultan [15] proved that plants increased their reproductive output when they grow in rich resource conditions. Archontoulis et al. [16] proved that, under water stress, the light and nitrogen distributions are more complicated because water stress affects not only appearance and elongation of leaves and uptake and partitioning of nitrogen, but also morphological aspects of leaf positioning, leaf angle, and azimuth angle.

Moreover, in 2000, Sultan [17] reported that plants might respond to environmental conditions not only by altering their offspring through changes in the quantity and quality of seed provisioning. Notable differences in the obtained values of leaves number (from 9 to 36 leaves) and leaf dimensions (from 19.9 cm to 53.10 cm) were found. It may be concluded that variability in leaf traits seems to be an environmentally affected character. The above finding is in agreement with the study of Balaguer et al. [18] who reported that the variability in leaf size affects the ability of plants to capture light and therefore compete with neighbors. *Cynara* is a rather complex crop in terms of morphology and growth. Moreover, studies concerning temperature and photoperiod effects on the development rate of *Cynara* are lacking in the literature, however, irrespective of the rate of development that varies among regions and varieties. Under Mediterranean climate, the biomass yield of this crop is very variable and depends on the variation in air temperature [19].

The morphological characterization is the first step in the description and the classification of germplasm [9]. According to Greene et al. [20], plant breeders can use genetic similarity information to complement phenotypic information in the development of breeding populations. Morphological markers have been traditionally used to highlight differences among cultivars. However, this type of characterization does not always reflect the real genetic variation, because the phenotype is determined by genetic

FIGURE 3: UPGMA dendrogram (based on Gower's coefficient of similarity) of the 120 ecotypes presenting morphological variability within the six populations.

information of the individual and also the results of its interaction with the environment; thus, in many cases a trait may be the expression of phenotypic plasticity [21].

The cluster analysis based on morphological traits showed an important diversity in the germplasm of north Tunisian wild cardoon. The traits used in this study can provide reliable information on the variability in wild cardoon populations when we reduce the number of descriptors

without decreasing the discrimination between populations. Similarities observed among several ecotypes and some populations, despite their distinctiveness of geographic origin, suggest a narrow genetic base. Cluster analysis, obtained with characterization, will be utilized to choose ecotypes from within clusters to be then considered for inclusion in the core collection. Thus, it is very interesting to manage these genetic resources by establishment, for example, of ex

FIGURE 4: PCA scatter plot of first two principal components detecting the relationship among and within 120 ecotypes of six populations of Tunisian wild cardoon based on morphological traits.

situ collection. Therefore, it is necessary to accomplish this work with estimating the genetic diversity of the accessions understudied by using the molecular and biochemical characterization.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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