Seed and spike traits from remnant populations of *Cenchrus ciliaris* L. in South Tunisia: high distinctiveness, no ecotypes

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In South Tunisia, as part of a native seed production program, a collection of 52 accessions of *Cenchrus ciliaris* was assessed for 12 different traits related to seed and spike. Possible relations between these, eco-geographic origin, and 4 adaptive traits of agronomical value were investigated. We could not establish reliable correlations between traits, nor between traits and eco-geographic origin. However, accessions were very distinct and homogeneous. Our data suggest that native seed production can consider South Tunisia as a single source and target region, but should define sound criteria to select a limited number of accessions for multiplication.

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Keywords: *Cenchrus ciliaris*; south Tunisia; genetic resources; native seed production

Introduction

Cenchrus ciliaris L. and available seed and spike data

Cenchrus ciliaris is a C₄-bunch grass native to West Asia, India and the whole African continent, mostly in dry areas, from sea level to 2000 m altitude (Bogdan, 1977). It has since long been introduced to the arid and semi-arid regions of Australia and America. Besides being apomictic (Taliaferro & Bashaw, 1966; Bashaw & Hanna, 1990), *C. ciliaris* is highly polymorphic and variable for several traits of ecological and agronomic importance. Examples where several true-breeding genotypes have been evaluated for forage seed production are numerous [see, for example, in India, Chakravarty *et al.* (1970), in Australia the varietal description of Bogdan (1977), and the classification of Pengelly *et al.* (1992); in East Africa Boonman (1993)]. In Australia, it was

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introduced deliberately in the 1930s and 1940s (Griffiths Davies & Hutton, 1970; Boonman, 1993), but it was already present in the 19th century (Bogdan, 1977). Tall growing East African genotypes are popular.

The propagule (or diaspore) of *C. ciliaris* consists of one or more caryopses (or seeds) enclosed within paleas, lemmas and glumes, the whole of which is surrounded by a fascicle of bristles, also called an involucre or involucl. The unit composed of caryopses and involucre is variously called a bur, cluster or glomerule, and often confounded with the fascicle or involucre itself. Some authors also confound spikelets with clusters. For the inflorescence, the following terms are used: ear, (seed) head, panicle and spike. In this study, we will use the terms caryopsis, spikelet, involucre, cluster and spike. As far as we know, the only fairly extensive account of these characters in North Africa is botanical in nature. To describe four varieties, Maire (1952) uses variation in spikelets, involucre, and spikelet number per cluster, although he mentions that spike length varies from 3 to 15 cm and spike width from 0.8 to 2 cm. Most published data come from work outside North Africa and are fairly consistent (Table 1).

Cenchrus ciliaris in North Africa

Cenchrus ciliaris is also native to North Africa, but its populations are extremely fragmented. In Tunisia, several authors mention the species is scarce and growth is stunted, because it is selectively overgrazed (Waechter, 1982; Ferchichi *et al.*, 1991; Chaïeb *et al.*, 1992), and because its typical 'flush growth', as a C₄-photosynthesizer in an arid Mediterranean environment, occurs when other grazing resources are scarce. Alternatively, *C. ciliaris* would need high available-phosphorus levels (Christie, 1975) so that continued soil exhaustion could have interacted with overgrazing to cause its decline. *Cenchrus ciliaris* can only complete a phenological cycle when high temperature is combined with adequate soil moisture. In North Africa, this condition is fulfilled with sufficient spring rains (March–May) or early autumn rains (September–October), both of which are erratic events. Widespread soil degradation and truncation in these regions have reduced potential and effective water and nutrient reserves. In this situation of mainly winter rainfall and of enhanced edaphic aridity, *C. ciliaris* and other C₄-grasses are disadvantaged compared to cool-season C₃-grasses (Chaïeb *et al.*, 1990). However, when growth is triggered by abundant rains in spring or early autumn, *C. ciliaris* is so responsive that heading can happen within a few weeks, the exact timing being determined by the temperature regime and photoperiod. Once mature, seed heads shed their diaspores gradually, such that seed can be collected almost throughout the year.

Cenchrus ciliaris is clearly a very promising candidate for native seed production in arid North Africa. Nevertheless, there has been no attempt so far to use it to restore primary and secondary production of degraded drylands, and to lessen chronic forage shortages of the oversized population of small ruminant livestock. This is especially true for South Tunisia, where the former common steppes are being replaced by olive orchards and cereal fallows. During the set-up of a seed production unit of native forage species in South Tunisia, we established a collection of 52 accessions from distinct South Tunisian eco-geographic regions, and from abroad. In this paper, we investigate the variation and the relationships of several seed and spike-related traits of *C. ciliaris*. We specifically address the following questions:

- How are seed and spike traits related to one another and to some traits of agronomic importance?
- Are these traits related to eco-geographic variation?

Table 1. Data on seed and spike traits collected from published references. Variables **in bold** were also observed in this study

	Caryopsis colour, shape and size	Spikelets per cluster	Caryopses per cluster	Clusters per spike	Caryopses per spike	Spike length (cm)	Weight 1000 clusters (g)	Weight 1000 Caryopses (g)
Maire (1952)	ellipsoid, compressed, or truncated 2 mm	1-3 varies between 4 North-African 'varieties'				3-15		
Whyte <i>et al.</i> (1959)							5-11.1 2-3	0.5-1.0
Bogdan (1966)	Light brown, somewhat rectangular, dorsally compressed 1.5-2 mm	1-3					single-seeded clusters	
Evers <i>et al.</i> (1969)		1.21-1.83	0.25-0.94	94-209	36-110	8.0-9.4		
		averages per genotype, influenced by photoperiod						
Chakravarty <i>et al.</i> (1970)				35-250		4.8-10.7	1.2-5.87	
Winkworth (1971)			1-2					
Yadav <i>et al.</i> (1974)				14-51 per 2 cm spike length		7.0-12.3		
Nicholson <i>et al.</i> (1985)								0.520-0.980
Hacker & Ratcliff (1989)			1-4 for individual clusters of the same introduction					
Hacker & Ratcliff (1989)			0.58-2.15 averages per introduction from Southern Africa					
Pengelly <i>et al.</i> (1992)			1.70-1.01 averages per grouping of accessions					
Neffati (1994)			1-2					1.0006

Material and methods

Experimental site

The collection was established at the experimental site of the IRA (Institute of Arid Regions) located in El Fje, 22 km east of Médenine and 5 km inland (33°30'N; 10°40'E). The plot is 78 × 24 m and is on a gentle slope. Its soil characteristics are considered as typical for the cereal fallows of South Tunisia. The soil has a calcium-rich sandy horizon (90% particle size > 50 µm) that overlays a gypsum crust at a depth of about 0.4 m depth. This gypsum horizon was partially destroyed by deep plowing during field preparation. Organic C- and P-concentrations of such soils are extremely low: organic C < 0.5% and Olsen extractable P < 10 ppm (Floret & Pontanier, 1982; Ewing, 1995; Visser, unpublished data). No fertilizer was applied at the start or during the observations. Rainfall data were recorded at the institute's weather station at 0.5 km.

Collection and establishment

A collection of 52 accessions of *C. ciliaris*, labelled 1–52 according to decreasing latitude (variable ACCESS) was established from seed: 47 accessions originating from the arid and desertic regions of Tunisia, two from Morocco, one from Senegal, and two from Western Australia. Seed of all accessions was collected between 1993 and 1996.

In South Tunisia, a *C. ciliaris* site is usually not more than an isolated cluster of one to a few closely spaced individuals. Unprotected, these clusters are heavily grazed and extremely scarce. Most of the time, tufts survive in situations protected from grazing, for example on escarpments or slopes of wadi beds, or close to cereal fields and orchards. In these protected situations, mature seed is almost always present. With regard to the extra work involved, the question was asked whether it was necessary to harvest these tufts individually or not. Previous studies of variation between progenies of different tufts from such sites (Visser, unpublished data) suggest that the co-existence of several different genotypes on this spatial scale is rather unlikely. Because the number of individuals and seed bearing tillers per accession was usually very limited, seed was bulked over as many individuals per accession as possible. This number was usually between 1 and 10. The National Park of Bou Hedma is the only place in South Tunisia where *C. ciliaris* is fairly common. Here, we have deliberately looked for isolated clusters and harvested these as a whole. When not enough seed was present on the site, small transplants were grown in a nursery to produce more seed for the collection. Of every accession, latitude and longitude were recorded with a GPS device, as well as site-specific characteristics. The 52 accessions fall into seven eco-geographic groups (Figure 1):

- (1) Mezzouna, a northern arid group of which five from the National Park of Bou Hedma, (ACCESS 1–11, wherein Bou Hedma ACCESS 3–7);
- (2) Kebili-Tozeur, an eastern desertic group (ACCESS 12 and 17);
- (3) Jerba, an island group (ACCESS 13 and 14);
- (4) Jeffara, a southern arid group (ACCESS 15, 16, and 18–39);
- (5) Sidi Touï, a subdesertic National Park group (ACCESS 40–45);
- (6) Dhehiba, a southern desertic group (ACCESS 46 and 47);
- (7) Foreign, the non-Tunisian accessions (ACCESS 48–52).

The variable that corresponds to these labels (1–7) is coded as GROUP.

The field was divided in three parallel blocks in which the 52 accessions were laid out following a randomized complete block design. Each block consists of two adjacent series of 26 accessions (six series in total). An accession was sown in three consecutive

Figure 1. Eco-geographic grouping of the South Tunisian accessions and study site as determined by latitude and longitude of the original sites. Dhehiba (□); Sidi-Touï (△); Jeffara (*); Jerba (▲); Keb-Toz (■); Mezzouna (+); study site (□).

rows spaced one m apart. Each row contains five pockets spaced 0.6 m apart. In March 1997, after a rainfall of 30 mm, these pockets were sown with five seed clusters each. During the period March–November 1997, the collection was regularly irrigated to ensure maximal survival, but has been entirely rain-fed since December 1997. Emergence rates were generally high but variable. Therefore, empty pockets have been replanted using transplants from the same collection and accession in September 1997, after a rainfall of 26 mm. In March 1998, the whole collection was cut at 5 cm. March and April 1998 had 16 and 15 mm of rain respectively, and prompted immediate regrowth. In this paper, we use variables recorded on seed traits collected on the harvest before the first cut, and variables related to spike size and production and tuft dimensions after that cut.

Data collection

Variables belong to four categories: (1) five variables recorded once for each accession; (2) two variables related to seed characteristics recorded on three replicates per accession; (3) nine variables recorded in the experimental field on four central (non-border) tufts out of the 15 in each replicate, and (4) two ratios derived from the original variables.

- (1) The variables recorded once for each accession are latitude (LAT), longitude (LONG), and three seed traits recorded as qualitative variables on seed harvested just before the first cut:
 - Shape (SHA) with three classes: long L, round R and ellipsoid O;
 - Colour (COL) with three classes: light L, dark D and brown B;
 - Size (SIZ) with three classes: large L, small S and intermediate M.
- (2) Three lots of 500 clusters (WEICLU, g per 1000 clusters) and 500 naked caryopses (WEICAR, g per 1000 caryopses) per accession were weighed with a 10^{-4} g precision balance.

- (3) Of the 15 tufts per replicate, the six border tufts were discarded and from the remainder nine, four even-aged tufts were chosen for the following observations. Heading date was recorded as number of days evolved since the first cut for the first spike to emerge (HEADAY, days). At 39 days after the first cut, total number of spikes per tuft (SPITUF, count), average diameter (DIATUF, cm) and average height (HEITUF, cm) of the tufts was recorded. At the end of the flowering period, one spike per tuft, on the most developed tiller, was chosen for the measurements total spike length (LENSPI, cm), maximal spike width (WIDSPI, cm), total number of clusters per spike (CLUSPI, count) and number of fertile clusters per spike (CLUFER, count). On the same tiller the total number of spikes (SPITIL, count) was recorded.
- (4) With the variables recorded on the seed, the contribution of the caryopses to overall cluster weight was calculated as WEICAR:WEICLU (CARCLU, %). With variables recorded on four spikes of the four tufts per replicate, the fertility rate of the spikes was calculated as CLUFER:CLUSPI (FERSPI, %).

Data analysis

One-way analyses of variance (ANOVA) were carried out on variables of categories 2, 3 and 4 to assess differences between accessions on three different levels. First, groups 1–6 (Tunisian accessions) as a whole were tested against Group 7 (non-Tunisian accessions). Secondly and thirdly, ACCESS and GROUP were used as grouping factors. Simple one-way ANOVA was used for variables of Category 2 and for CARCLU (three replicates per accession, no blocking and no repeated sampling). For variables of Category 3 and for FERSPI (three replicates, grouped in three experimental blocks with four observations per replicate), the one-way ANOVA model includes additional sources of variation due to blocking and to subsampling.

To assess correlations between traits and homogeneity of accessions, a principal components analysis (PCA) was carried out on the category 3 variables, not averaged per accession. Category 2 variables could not be included because the three replicates did not correspond to the three experimental blocks. Category 4 variables could not be included because they are derived from other variables, hence not independent. To assess relations of some variables not included in the PCA, Pearson correlation coefficients have been calculated between Category 2 and 3 variables averaged over their respective replicates per accession.

Finally, accessions were clustered using the furthest neighbour criterion on a matrix of Euclidean distances. Variables used were all those of the PCA (category 3), but now averaged over 12 replicates, and category 2 variables WEIBUR and WEICAR, averaged over three replicates. The qualitative variables SHAP, COL and SIZ were concatenated into one label (SHAP + COL + SIZ) to identify accessions in the dendrogram resulting from the clustering procedure.

All analysis was carried out with the computer package SPSS version 10.0, except for the one-way ANOVAs for category 3 variables and FERSPI, which were carried out with S-plus 2000.

Results

One-way ANOVA's (Table 2)

Where comparison is possible, values and ranges for individual variables lay grossly within the ranges reported in Table 1. Spike fertility, with rates from 63 to 95%, is never higher than 100%, suggesting the rarity of 2 or more fertile spikelets or caryopses per

Table 2. Mean values of seed and spike traits of South Tunisian and non-Tunisian accessions and results of variance ratio tests for differences between accessions means: overall, grouped per eco-geographic origin and as Tunisian versus non-Tunisian accessions

Variable		HEITUF	DIATUF	HEADAY	SPITUF	SPTIL	LENSPI	WIDPSI	CLUSPI	CLUFER	FERSPI	WEICLU WEICAR CARCLU			
Unit		cm	cm	count	count	count	cm	cm	count	count	%	g	g	%	
Accession values															
South Tunisian	Mean	28	46	30	24	2	7.2	1.8	86	67	77.9	1.560	0.906	61.2	
	Min.	14	25	21	2	1	4.6	1.0	49	32	63.4	0.891	0.617	28.3	
	Max.	42	65	36	87	8	9.2	2.5	135	107	95.3	2.966	1.165	109.6	
Non-Tunisian	Mean	27	53	24	34	3	8.2	2.0	109	85	78.2	1.924	0.721	39.3	
	Min.	20	39	21	5	1	6.3	1.1	61	44	73.0	1.447	0.606	29.6	
	Max.	41	75	29	79	4	10.3	2.6	193	152	84.3	2.143	0.989	68.3	
ANOVA															
Sampling error (%)		7.4	5.5	3.2	12.0	25.5	7.8	6.9	7.5	9.9	5.3				
Based on MS with 468 df.															
South Tunisian (47) versus foreign (5) accessions															
Var. Ratio	1/152 df. →	0.24	27.32	82.43	20.16	5.39	34.48	10.54	35.46	37.16	0.12	1/154 df. →	12.62	26.60	24.32
Prob.		0.628	0.000	0.000	0.000	0.021	0.000	0.001	0.000	0.000	0.730		0.001	0.000	0.000
Exp. Error (%)		23.8	21.0	16.2	64.1	67.2	14.6	16.4	31.7	31.7	9.5		4.3	2.6	5.0
Between all accessions (52)															
Var. Ratio	51/102 df. →	86.320	124.910	265.727	241.390	68.561	46.155	66.823	204.474	113.771	34.549	51/104 df. →	95.510	110.528	101.630
Prob.		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.000
Exp. Error (%)		5.4	4.3	2.0	9.5	13.5	*	1.7	2.3	3.7	2.2		23.9	11.6	26.9
Between eco-geographic groups (7)															
Var. Ratio	6/147 df. →	22.749	25.302	24.511	9.323	16.117	30.356	6.76	83.697	25.361	2.49	6/149 df. →	2.409	24.437	6.71
Prob.		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.03	0.000	0.000
Exp. Error (%)		21.7	19.5	15.4	63.4	63.0	12.9	16.2	29.1	28.8	9.5		23.6	14.8	27.7

Variables in **bold** also mentioned in Table 1. HEITUF = tuft height, DIATUF = tuft diameter, HEADAY = heading date, SPITUF = number of spikes per tuft, SPTIL = number of spikes per tiller, LENSPI = spike length, WIDPSI = spike width, CLUSPI = number of clusters per spike, CLUFER = number of fertile clusters per spike, FERSPIKE = fertility rate of spike, WEICLU = weight of 1000 clusters, WEICAR = weight of 1000 caryopses, CARCLU = caryopsis weight relative to cluster weight.

*Experimental MS < Sampling MS.

cluster, but is much higher than the rates that can be calculated from Evers *et al.* (1969). Significance tests for the differences of means indicate high distinctiveness of accessions, which lessens for most but not all traits when these accessions are grouped. Concomitantly, coefficients of variation (CV) of the experimental error are low at the accession level, but greatly increase when accessions are grouped. On the other hand, CVs of the sampling error for variables of category 3 and for FERSPI are higher but of the same order of magnitude as CVs of the experimental error at the accession level. Consequently, the within-accession unexplained variation is not noticeably larger than the among-accession unexplained variation. We attribute the higher value of the CVs of SPITIL to the nature of the variable (counts with just a few possible outcomes). When the ANOVAs are recalculated without Group 7, the differences keep the same significance levels for all variables (data not shown). So the Tunisian accessions are also very distinct among themselves.

Compared to the non-Tunisian accessions, Tunisian *C. ciliaris* tends to have shorter spikes, but with equal numbers of clusters and caryopses and equal fertility rates. Clusters weigh less but caryopses are heavier, yet these differences are not significant. However, their combination in CARCLU leads to a significantly greater contribution of the caryopses to the cluster weight of Tunisian accessions. In addition, Tunisian *C. ciliaris* heads on average one week later, and produces fewer spikes per tuft. Lower absolute spike numbers could logically be due to later heading date but also to their smaller tuft diameters compared to the non-Tunisian accessions. However, this supposes equal tuft densities (number of tillers per unit of area) and this variable has not been analysed here. At least in this early regrowth stage, no differences in tuft height have appeared between Tunisian and non-Tunisian accessions.

PCA (Figs 2 and 3)

The total variation of the data set explained by the three first axes is 68%, being partitioned as 38% (PC1), 17% (PC2) and 13% (PC3) respectively. The other components have eigenvalues < 1 and have not been interpreted. PC1 mainly accounts for positive covariation in size of productive and reproductive parts. PC2 mainly accounts for the variation in heading date, that is negatively correlated with spike number per tuft. PC3 shows a pattern somewhat opposite to those of PC1 and PC2. The number of clusters and number of fertile clusters per spike are the only variables that correlate positively with each other on all three axes (Fig. 2). So, although the ANOVAs show that these traits, together with fertility rate, vary extensively between accessions, a quite constant proportion of the clusters contains a fertile spikelet. Figure 3 is a score plot of all accessions, each of them represented by 12 scores (3 replicates $\times$ 4 measurements per replicate) in the (PC1, PC2)-plane. Each accession is a distinct unit because its 12 scores are closely spaced and separate well from the 12 scores of neighbouring accessions. No clusters of similar accessions appear. Instead, accessions of different groups are intermingled. As a consequence, the accessions rather than the eco-geographic groups behave as homogeneous units, and should be evaluated as such.

Correlations

Correlations between variables not included in the PCA are weak or absent, and so are their relationships with category 3 variables (matrix not shown). Specifically, cluster and caryopsis weight are uncorrelated ($r = -0.189$, $p = 0.180$), showing that cluster weight is not a reliable measure of caryopsis weight. Of the weak relationships with category 3 variables, the most striking is the positive correlation between caryopsis weight and heading date ($r = 0.285$, $p = 0.040$) and the negative correlation between caryopsis

Figure 2. Loadings of the original variables on the three first principal components of the PCA. HEITUF = tuft height, DIATUF = tuft diameter, HEADAY = heading date, SPITTUF = number of spikes per tuft, SPITIL = number of spikes per tiller, LENSPI = spike length, WIDSPI = spike width, CLUSPI = number of clusters per spike, CLUFER = number of fertile clusters per spike, FERSPIKE = fertility rate of spike, WEICLU = weight of 1000 clusters, WEICAR = weight of 1000 caryopses, CARCLU = caryopsis weight relative to cluster weight. PC1 (■); PC2 (▒); PC3 (□).

weight and number of spikes per tuft ($r = -0.306$, $p = 0.027$). Early heading would therefore produce not only more spikes but also lighter seeds than late heading. However, these correlations are not very strong. They correspond to variation accounted for by PC2 only, and should not be generalized.

Cluster analysis

The dendrogram generated by the cluster analysis (Fig. 4) does not reproduce the eco-geographic groupings of Fig. 1 and within one cluster, seed morphology remains variable. Even the smallest of the clusters on the lowest level contain accessions from different groups, in which furthermore seed morphology still varies extensively. Of all groups except one (Kébili-Tozeur), accessions are spread over different clusters. For the three groups composed of pairs of accessions, those of Jerba and Dhehiba, although geographically close, belong to different clusters. By contrast, the pair of Kebili-Tozeur, although geographically distant, belongs to the same cluster, but their seed morphologies still differ.

The two largest and most distant eco-geographic groups, Mezzouna and Jeffara, are quite intricate, even for their outlying accessions. Of the first level, all clusters but one contain at least one accession from both Mezzouna and Jeffara. In general, the Mezzouna group represents the highest number of outliers. The five Bou Hedma

Figure 3. Projection of the accession scores of the PCA (12 replicates per accession) in the (PC1, PC2)-plane. Dhehiba ($\square$); Sidi-Touï (Δ); Jeffara ($\ast$); Jerba ($\blacktriangle$); Keb-Toz ($\blacksquare$); Mezzouna ($+$); study site ($\square$).

accessions included in our collection (ACCESS = 3–7) occur in five different clusters of the dendrogram. On the other hand, the six accessions of the National Park of Sidi Touï are split in just two groups of three accessions by the cluster analysis.

On the highest level, there is only one non-Tunisian accession (Senegal) that separates from the South Tunisian accessions; the others are intermingled with the Tunisian accessions. This accession is also the most extreme outlier (most positive PC1-score) in the PCA-plot of Fig. 3.

Discussion

Seed and spike traits for selection purposes

The first question we sought to answer was if, within South Tunisian populations of *C. ciliaris*, suites of co-varying traits exist so that the determination of one of them gives a reliable clue to the values of the others. The answer is no. Although some broad trends could be extracted by PCA, most traits we measured are ‘promiscuous’ in the sense that they are shown to vary with several other traits at once and in opposite directions. PCA does not produce variation along mainly one PC, and cluster analysis does not lead to clear-cut groups to which suites of trait values can be attributed. This does not seem atypical for *C. ciliaris*. In general, workers elsewhere have either failed to find correlations that account for a considerable portion of the variation, or else present contradictory suites of traits. Chakravarty *et al.* (1970), in selecting suitable genotypes of *C. ciliaris* for seed and forage production among 65 accessions, stated that no genotype existed that combined all desired traits. Some years later, however, Yadav *et al.* (1974), in an attempt to separate genetic and environmental components of phenotypical variation

Figure 4. Dendrogram resulting from the cluster analysis (furthest neighbour criterion applied on Euclidean distances calculated from nine variables averaged per accession). Eco-geographic group names are preceded by a concatenated label indicating shape, colour and size of the seeds (in that order), and followed by individual accession number.

between 28 accessions of *C. ciliaris*, reported that plant height, leaf breadth, spike length and fodder yield were significantly and positively correlated with one another, and that breeders should concentrate on leaf breadth and cluster number because of their high genotypic correlations with fodder yield. This could also hold in our case if PC1 of the PCA would have accounted for a higher proportion of the total variation of the data set. In that case, accessions with $PC1 > 0$ would be preferred. Lovelace *et al.* (1972), having worked on 7–15 genetic lines of *C. ciliaris*, could not establish significant correlations between dry matter digestibility (DMD) and some agronomic field traits, only a highly negative correlation between seed head density and rhizome development. That correlation would agree with the classical trade-off between reproductive and vegetative allocation of resources, and resembles the negative correlation between heading date and number of spikes per tuft in our data set, expressed by their opposite loadings on PC2. For high grazing value, $PC2 < 0$ would be preferred. On the other hand, Wilson *et al.* (1989) have tried to link DMD with easily measurable morphological and anatomical leaf and stem characteristics on 21 genotypes of *C. ciliaris* and related *Cenchrus* species. They state that leaf DMD is negatively correlated with leaf size and specific leaf weight (SLW), and that stem DMD is negatively correlated with number of vascular bundles in hand-cut stem sections. Furthermore, genotypes with 'lax' (non-erect) leaves and small vegetative tillers (visually scored for) tend to have higher DMD. Small vegetative tillers would correspond with $PC3 > 0$ in our PCA. Because leaf size and rigidity, SLW, tiller size and vascular bundle density are easily observable in the field, they could be useful selection criteria for rapid screening for DMD within a large breeding population. However, this should happen at low selection pressure because correlation does not explain a high proportion of the total variation and because of the possible trade-offs with other useful criteria (Wilson *et al.*, 1989). In total therefore, it seems that correlations between traits are not reliable enough to simplify selection criteria for *C. ciliaris*.

Eco-geographical variation

The second question was whether eco-geographical variation of these traits suggests the existence of ecotypes, this is, genotypes that have evolved in response to specific environmental conditions, and have become locally adapted. The development of ecotypes rarely involves just one adaptive trait, but generally suites of related traits. For example, for annual species, desert ecotypes would tend to have more compact growth cycles than arid ecotypes, and invest more in reproductive effort (Aronson *et al.*, 1992, 1993; Ewing, 1995). Sometimes, but not always, desert ecotypes have been shown to exhibit greater phenotypic plasticity in phenological development (Aronson *et al.*, 1992, 1993). In the case of *C. ciliaris*, there would be extensive ecotypic variation with regard to seed dormancy and germination capacity (Butler, 1985; Hacker & Ratcliff, 1989; Venter & Rethman, 1992), for example in function of annual rainfall and the presence of a prolonged dry season of the area of origin (Hacker & Ratcliff, 1989). However, the link between variation in adaptive traits and the environmental variables to which *C. ciliaris* should adapt is rarely well established.

At least five traits we studied are adaptive: HEADAY (heading date), SPITUF (number of spikes per tuft), SPITIL (number of spikes per tiller), HEITUF (tuft height) and DIATUF (tuft diameter). Both PC1 and PC2 of the PCA have high loadings for four of them (not HEADAY in PC1 and not SPITIL in PC2). ANOVAs on individual variables show the existence of significantly different eco-geographical group means for each trait separately. However, the increased experimental error of the ANOVAs when accessions are grouped (Table 2) and intricacy of these groups in the PCA score plot (Fig. 3) according to PC1 and PC2, as well as in the dendrogram (Fig. 4) suggests that group means are quite meaningless because of singular outlying accessions. For example, the variation of heading date and number of spikes per tuft

(main loadings of PC2) across eco-geographic groups does not indicate earlier heading of desertic accessions as compared to arid accessions. Our results therefore do not support ecotypic differentiation of *C. ciliaris* within South Tunisia.

South Tunisia is nevertheless a typical transition zone between arid and desertic rainfall patterns, where different vegetation types are recognised in response to variation in edapho-climatic aridity (Floret & Pontanier, 1982). Arguments why no ecotypes emerge from our analysis are wide-ranging.

- (1) Aronson *et al.* (1992, 1993) established ecotypes of three Mediterranean annuals within a geographical range smaller than that of South Tunisia but with more pronounced annual rainfall differences. Although there are rainfall differences within South Tunisia, these might still be too similar to cause ecotypic differentiation. By contrast, Hacker & Ratcliff (1989) compared introductions from widely separated regions of sub-Saharan Africa with different combinations of temperature and rainfall regimes to establish ecotypes of *C. ciliaris*. In addition, present South Tunisian populations are reduced to remnants patchily concentrated in scarce 'refuges' of the landscape (Noy-Meir, 1996). It is these 'refuges' that we have sampled, after almost accidental discoveries (except for the National Park of Bou Hedma). These situations are not always representative for the zonal climate or 'average' edaphic aridity. Often, these particular microsites receive extra run-off or infiltration water, and/or nutrients, or else their relief protects tufts from overgrazing. However, in case of recently man-made 'refuges', *C. ciliaris* has not always enjoyed the extra water, nutrients or protection it enjoys now, and might have been exposed to heavy grazing until very recently.
- (2) Traits adaptive to climatic variation, such as heading date and spike productivity, and possibly also tuft height and diameter, can also be adaptive in response to other environmental variables, such as grazing pressure. Being generally drought-resistant but very selectively grazed, adaptation to grazing pressure has obviously shaped North African, in this case South Tunisian populations of *C. ciliaris*. The influence of 'minor' climatic differences within the Mediterranean belt may well have been smoothed by the overriding pressure to become grazing resistant and/or tolerant. If this is true, North African populations should be more adapted to intense grazing by small ruminants than non-Tunisian accessions. The individual differences established between Tunisian and non-Tunisian accessions (two of which are Moroccan) suggest this might make sense, as Tunisian accessions are shown to be smaller in diameter (not in height), head later, and bear fewer and smaller spikes that are more fertile and contain heavier caryopses. However, cluster analysis does not separate these from non-Tunisian accessions. Other variables, such as dry matter production and persistence under intense cutting, rhizome development, tillering pattern, tuft density and leafiness are better suited to study this hypothesis than the variables we used in this paper. Despite distinctiveness as a whole, South Tunisian accessions remain highly variable amongst each other, because factors related to genetic structuring of the population are possibly still more determining to explain genetic variation than local adaptation to climatic or grazing pressure differences.
- (3) Our analysis strongly suggests that most accessions are composed of a single distinct genotype. Even accessions from neighbouring 'refuges' are distinct. So former populations must have possessed an overall fine-grained genetic structure, composed of adjacent genetic islands maybe just the size of the actual 'refuges', with gene flow restricted to seed dispersal in a rather random manner. *C. ciliaris* is an apomictic and long-lived perennial. Within populations of such species, the process of local adaptation is typically slow, and genetic structure poorly explained by environmental variables (Loveless & Hamrick, 1984). In addition,

on-going local adaptation processes can be disrupted by recurrent migration events (Heywood, 1991). Because of the fluffiness and stickiness of *C. ciliaris* diaspores, and because North African human populations used to be extremely mobile, migrations and even long-range transports (in camel wool for example) were probably recurrent events until this century. All these factors hamper ecotypic differentiation.

Comparisons of a limited number of accessions and neglect of the possibility of a fine-grained population structure at perpetual non-equilibrium with the current environment, as deduced here, can lead to odd interpretations, leading to the recognition of ecotypes if observed variation happens to correspond with expectations about the influence of environmental variation. For example, in a common garden experiment, Ferchichi *et al.* (1991) studied phenology and growth of two accessions of *C. ciliaris*: a more vigorous one from the National Park of Bou Hedma and a less vigorous one from Chahbania (a recently protected range in the middle of the Jeffara coastal plain). They interpreted differences between both accessions in terms of strong 'backward' selection forces (toward less productive genotypes and stunted growth) outside the National Park. However, our analysis does not support this view. The accession of Chahbania is indeed identical to ACCESS = 27 of our collection. It is an outlier with the third-highest score on PC2, and clusters on the lowest level of the dendrogram with ACCESS = 6, another outlier with the lowest score on PC3, collected in the National Park of Bou Hedma. So we would state the opposite, were it not that all accessions of Bou Hedma are dissimilar. We therefore cannot find evidence for 'backward' selection of *C. ciliaris* outside protected sites, but we do find evidence for a wider range of genetic variation in larger sites, in agreement with the suggested fine-grained genetic structure of former *C. ciliaris* populations.

Consequences for native seed production

Native seed production differs from conventional seed production in that it aims to produce seed that, once reintroduced in the target region, can safely reproduce and evolve without human interference (Knapp & Dyer, 1998). The general principle is to provide 'local yet ample' genetic variation and it is left to the seed-producers to define what this 'local yet ample' comes down to. In a context such as South Tunisia, the use of seed mixtures of native genotypes so as to recreate fine-grained genetic structure within reconstituted populations would be more appropriate than the use of cultivars composed of a single genotype. But the need for genetic variation does not exclude selection of more productive, yet grazing resistant, genotypes. Our data suggest that traits vary quite independently from one another and that the presence of ecotypes within South Tunisia is improbable. So all South Tunisian material can be considered as 'local', and potential seed producers have a large choice to create superior yet variable seed mixtures. Nevertheless, they still need to define a sound set of selection parameters. In this respect, there is a need for data about variation in germination capacity and in seedling vigour in relation to caryopsis and cluster weight. Furthermore, the cutting regime imposed on the collection since March 1997 will yield more essential information with regard to grazing value. A last remark concerns the value of new material, coming from sites close to sites of accessions already included in the collection. Our data suggest that there are no reasons to disregard them, because the chances are high that they include unique and new genotypes.

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