Scientia Horticulturae 185 (2015) 76–81

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Genetic variability in wild cardoon (*Cynara cardunculus* L. var. *sylvestris*) revealed by SSR markers and morphological traits

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ARTICLE INFO

Article history:
Received 11 September 2014
Received in revised form 16 January 2015
Accepted 19 January 2015
Available online 10 February 2015

Keywords:
Cynara cardunculus L. var. *sylvestris*
Wild germplasm
Morphological traits
SSR markers
Differentiation
Gene flow

ABSTRACT

This research aims at characterizing germplasm diversity of wild cardoon (*Cynara cardunculus* var. *sylvestris*) for future breeding. Six populations were identified in in the north of Tunisia and 20 individuals per population were evaluated using UPOV descriptors, and a large variability was revealed between populations for their leaf characteristics. The genetic diversity was also assessed using SSR markers at seventeen loci, which were all polymorphic, and a high genetic variation was found out. A low differentiation (Fst = 0.104) coupled with a high level of gene flow (Nm = 2.25) were observed among populations. Only 10.4% of total genetic variability is shared between populations in comparison to 89.6% within population. Both morphological and molecular characterization showed that population grouping was not clearly related to their geographical distribution and this could be the result of the gene flow between regions. These results highlight a high level of genetic variation in Tunisian wild cardoon germplasm, which represents an interesting material to be conserved and exploited in breeding programs of artichoke.

1. Introduction

Cynara cardunculus L. is a diploid (2*n* = 34) outcrossing perennial species belonging to the Asteraceae family and widespread in the Mediterranean area. It includes three taxa: the globe artichoke (var. *scolymus*), the cultivated cardoon (var. *altilis*) and the wild cardoon (var. *sylvestris*) (Foury, 1989; Rottenberg and Zohary, 1996). Thus, wild and cultivated cardoons represent the most straightforward resource, which can be exploited for globe artichoke improvement. The wild cardoon is considered the presumed wild progenitor of the artichoke and the cultivated cardoon (Foury, 1989). The existence of this complex primary gene pool, containing the wild progenitor and the two crops showing different reproductive strategies, is unique among crop species, making the study of gene pool and germplasm differentiation particularly interesting for the utilization of *Cynara* genetic resources (Raccuia et al., 2004; Pagnotta and Noorani, 2008). Wild cardoon is a non-domesticated robust perennial plant characterized by a rosette of large spiny leaves and branched flowering stems (Wilklund, 1992) and is widely distributed in the Mediterranean area, thriving in warm, dry and low altitude environments. 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. Recent studies have suggested that a high level of differentiation is present in the wild cardoon taxon, and that samples from the Western Mediterranean range more closely resemble the cultivated cardoon than the wild samples from the Eastern Mediterranean region (Wilklund, 1992; Sonnante et al., 2007; Gatto et al., 2013).

Identification of the genetic diversity within crop gene pools is the base of breeding programs. Morphological characterization is the first step in the description and classification of germplasm (Smith and Smith, 1989), whereas markers at the DNA level represent the ideal tool as they can describe the distribution of genetic variation without the effect of the environment (Pagnotta et al., 2009). In the last decades, several molecular markers (RAPD, AFLP, SSR, and ISSR) have been used for the characterization of *C. cardunculus* populations. Among the classes of molecular markers available, the simple sequence repeats (SSR) are highly informative because they are co-dominant and generally highly polymorphic (Koreth et al., 1996).

In Tunisia, despite the richness of wild cardoon germplasm, the identification and characterization of its genetic resources are scarcely investigated. Recently, the study conducted by Ben Ammar et al. (2014) and Khaldi et al. (2012) has revealed a large variability among wild cardoon populations. Therefore, the objective of this

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http://dx.doi.org/10.1016/j.scienta.2015.01.017
0304-4238/

Table 1
Origin of the wild cardoon populations studied.

Population	Province	Locality	Coordinate points
AB1	Bizerte	Ain Berda	37°30′23"N 9°83′26"E
AB2	Bizerte	Ain Berda	37°30′58"N 9°82′70"E
BA	Bizerte	Beni Amor	37°09′66"N 9°13′88"E
DH	Beja	Dhahirat	36°98′80"N 9°07′78"E
DM	Beja	Daouar Mahjouba	36°96′74"N 9°04′04"E
SN	Siliana	Ain Dissa	35°86′48"N 9°21′47"E

study is to evaluate the genetic diversity and relationships within and among wild cardoon from northern Tunisia. The plant material was studied using leaf morphological traits and SSR markers. This research is a necessary step for a better conservation of the wild cardoon gene pool and for a more efficient use for future improvement of *C. cardunculus*.

2. Materials and methods

2.1. Plant material and morphological analysis

Leaves of 6 wild cardoon populations were collected from their main natural growing regions in different provinces across the north of Tunisia (Table 1). In each region and during the spring season, 20 plants were identified individually in order to sample the greater variability observed in the fields. Plants were quite isolated from one another. For each plant, 3 well-developed leaves were evaluated for 13 morphological traits. Five traits are quantitative: length of leaf (Llf), length of leaf spines (Lslf), length (Llb) and width (Wlb) of the longest lobe, length of lobe spines (Lslb), and eight are qualitative traits: leaf colour (Clf), intensity of green colour (ICV), hue of green colour (HGC), intensity of grey hue (IGH), hairs on the upper side of the leaf (Hlf), number of lobes (Nlb), number of the secondary lobes (Nlbs), shape of the secondary lobes (Slbs).

2.2. DNA extraction and markers analysis

A total of 17 markers were chosen for SSR analysis of genetic diversity, on the basis of their position on an artichoke × wild cardoon genetic map (Sonnante et al., 2011), choosing one marker for each linkage group. Ninety-three genotypes selected from the 6 populations morphologically characterized were used and their seeds were collected at maturity. After seed germination and at stage of three leaves, genomic DNA was extracted from young leaves according to Sonnante et al. (2002) with some modifications.

The PCR reaction mixture (10 μl total volume) consisted of 25 ng genomic DNA, 1.5 mM $MgCl_2$, 200 nM dNTP, 200 nM of each primer, 1× buffer, and 0.5 units Taq polymerase (Invitrogen, Carlsbad, CA, USA). Samples were subjected to the following thermal profile: 3 min denaturation at 94 °C, followed by 38 cycles at 94 °C for 30 s, 50–56 °C for 30 s (specific for each primer, see Table 3), 72 °C for 45 s and ending with 72 °C for 10 min. PCR products were analyzed on an automated sequencer CEQ 8800 (Beckman Coulter, Carlsbad, CA, USA) and peaks were identified by size using the proprietary fragment analysis software of the sequencer.

2.3. Statistical analyses

Mean and standard deviation values of data are calculated from 20 replicates. The data were analyzed statistically by analysis of variance, comparison among means by Duncan's multiple ranges using SPSS version 16.5. The standardized data were analyzed by means of the software package MVSP version 3.1. Principal component analysis (PCA) was carried out. The similarity matrix was measured by means of the Gower general similarity coefficient and a dendrogram was generated using the unweighted pair-group method of the arithmetic averages (UPGMA).

The polymorphic information content (PIC) was according to the formula: $PIC = 1 - \sum P_{ij}^2$ (where P_{ij} is the frequency of the jth allele for the ith marker summed over n alleles). Data matrix of the SSR was analysed using the POPGENE software version 32. Four parameters were computed: average number of alleles per locus (na), number of private alleles, percentage of polymorphic loci (P) and Nei's gene diversity (He). Genetic differentiation among and between populations was assessed by determining F statistics (Fis: fixation index within population; Fit: fixation index total; Fst: differentiation index between populations) and estimating gene flow ($Nm = 1/4\,(1/Fst - 1)$). A UPGMA dendrogram based on Nei's genetic distances was constructed using POPTREE2 with a bootstrap of 1000 replicates.

3. Results

3.1. Morphological characterization

Significant differences were found for all morphological traits, except for the colour of leaves and the hue of green colour, indicating that there is a high degree of phenotypic diversity among the populations analyzed (Table 2). DM population showed the most hairy leaves. The low intensity of grey hue characterizing DH population is due to the absence of hairs on the upper side of the leaf. Plants from this population provided also the longest leaves. DM showed the highest number of lobes while the highest number of secondary lobes was observed in AB2. Regarding the length of

Table 2
Leaf characteristics of *Cynara cardunculus* L.var.*sylvestris*.

	AB1	AB2	BA	DH	DM	SN	Sig. degree
Clf	3.4 ± 1.98	4.1 ± 1.48	4.2 ± 1.36	3.7 ± 2.06	3.6 ± 1.57	4.2 ± 1.33	ns
ICV	4.6 ± 1.39^{b}	4.5 ± 1.70^{b}	5.7 ± 1.49^{a}	6.1 ± 1.02^{a}	5.8 ± 1.50^{a}	6.6 ± 0.82^{a}	***
HGC	2.5 ± 0.68	2.4 ± 0.88	2.2 ± 0.91	2.2 ± 0.76	2 ± 0.91	2.2 ± 1.00	ns
IGH	4.3 ± 1.49^{ab}	4.7 ± 1.49^{b}	4.2 ± 1.50^{ab}	3.6 ± 1.14^{a}	4.1 ± 1.77^{ab}	3.9 ± 1.02^{ab}	ns
Hlf	1.6 ± 1.14^{bc}	2.5 ± 1.82^{ab}	2.8 ± 1.4^{a}	1 ± 0^{c}	2.8 ± 2.50^{a}	1.9 ± 1.21^{abc}	***
Nlb	5.7 ± 1.34^{b}	6.6 ± 0.82^{a}	5.5 ± 1.57^{b}	5.9 ± 1.37^{ab}	6.6 ± 0.82^{a}	6.3 ± 0.97^{b}	**
Nlbs	4.1 ± 1.51^{ab}	4.7 ± 1.49^{a}	3.1 ± 1.37^{b}	4.2 ± 1.64^{ab}	4 ± 1.77^{ab}	3.1 ± 1.65^{b}	**
Slbs	1.3 ± 0.65^{cd}	1 ± 0^{d}	1.8 ± 0.76^{ab}	1.3 ± 0.47^{cd}	1.6 ± 0.68^{bc}	2.2 ± 0.95^{a}	***
Llf (cm)	38.2 ± 4.67^{a}	32.4 ± 6.59^{b}	25.5 ± 4.60^{c}	40.4 ± 7.37^{a}	32.0 ± 6.66^{b}	39.8 ± 5.03^{a}	***
Lslf (cm)	0.7 ± 0.20^{a}	0.6 ± 0.20^{ab}	0.4 ± 0.13^{c}	0.7 ± 0.26^{ab}	0.6 ± 0.18^{bc}	0.7 ± 0.41^{ab}	**
Llb (cm)	11.8 ± 2.30^{b}	10.0 ± 1.60^{c}	8.7 ± 2.32^{c}	11.8 ± 2.14^{b}	11.8 ± 2.83^{b}	14.9 ± 2.10^{a}	***
Wlb (cm)	2.2 ± 0.68^{b}	2.2 ± 0.61^{b}	1.6 ± 0.72^{c}	2.1 ± 0.56^{b}	2.5 ± 0.72^{ab}	2.7 ± 0.64^{a}	***
Lslb (cm)	1.1 ± 0.24^{a}	1.0 ± 0.26^{ab}	0.6 ± 0.32^{d}	1.0 ± 0.18^{ab}	0.9 ± 0.36^{bc}	0.7 ± 0.47^{cd}	***

Means with different letters in the same line indicate significant differences at $P < 0.05$ ns: no-significant at 5%. *Abbreviations used*: Llf: length of leaf, Clf: leaf colour, ICV: intensity of green colour, HGC: hue of green colour, IGH: intensity of grey hue, Hlf: hairs on the upper side of the leaf, Lslf: length of spines on leaf, Nlb: number of lobes, Llb: length of the longest lobe, Wlb: width of the longest lobe, Nlbs: number of the secondary lobes,Slbs: shape of the secondary lobes, Lslb: length of spines on lobes.