M-AFLP BASED DEVELOPMENT OF MICROSATELLITES FOR CHARACTERISATION OF CYNARA CARDUNCULUS L. GERMPLASM

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Summary
Nine microsatellite markers for *Cynara cardunculus* L. were developed using a two-step 'primer extension' procedure, based on the microsatellite-AFLP (M-AFLP) technique. Polymorphism was explored in 24 plants of wild cardoon (*C. cardunculus* L. var. *sylvestris*) and transferability was tested on accessions of both globe artichoke (var. *scolymus*), and cultivated cardoon (var. *altilis*).

Keywords
AFLP, artichoke, cardoon, microsatellite-AFLP, SSRs

Introduction

Cynara cardunculus L. is a diploid ($2n = 2x = 34$) species, native to the Mediterranean basin, which includes globe artichoke (var. *scolymus* L.), cultivated cardoon (var. *altilis* DC) and wild cardoon [var. *sylvestris* (Lamk) Fiori]. The latter is considered the ancestor of both cultivated forms on the basis of molecular [1], cytogenetic and isozyme [2] studies. We have previously generated a set of 23 microsatellite primer pairs in globe artichoke through either an enriched library approach, database sequence searches [3] and a novel approach, named MAL (microsatellite amplified library), [4] which is a combination of the AFLP methods with a primer-extension based enriched library.
Here we report the development of a new set of nine polymorphic SSR markers in *Cynara cardunculus*, using a two step "primer extension" procedure, based on the microsatellite-AFLP (M-AFLP) technique [5].

Materials and Methods

In the first step, total DNA was restricted-ligated (with *Eco*RI and *Mse*I restriction enzymes) and pre-amplified; M-AFLP selective amplifications were carried out using an *Eco*RI adapter directed primer with three selective bases in combination with an 5'-anchored microsatellite primer [GTCG$(AG)_7$, GACG$(TG)_7$, and CAGC$(TC)_7$,]. Amplified fragments were resolved on poly-acrylamide gels and silver stained. Selected M-AFLP fragments were excised from gel, re-amplified and directly sequenced. From the derived sequence of each positive amplicon, a primer (the forward primer) directed towards the microsatellite motif and a nested primer (forward nested) were designed. For two loci (CMAFLP-07, CMAFLP-11) additional internal SSRs were detected and internal specific microsatellite-flanking primers were designed.
In the second step, the opposite microsatellite flanking sequence was amplified using the restriction-ligation reaction as template for the amplification with the previously developed forward primer, in combination with the *Mse*I primer with no selective nucleotide. For nested

PCR 1µl of 100-fold diluted first PCR reaction was used as template using the nested primer and the *Mse*I primer. Only single band products where directly sequenced. A second primer (reverse) was designed for each positive sequence.

Results and Discussion

Nine SSRs were tested for their informativeness on twenty-four *C. cardunculus* L. var. *sylvestris* accessions collected in Palazzolo (Sicily, Italy) together with two accessions of globe artichoke, and two of cultivated cardoon. Seven microsatellites showed compound repeats three of which were 'perfect' and four 'imperfect'; only two SSR loci showed non-compound repeats (Table 1). All loci were polymorphic in the 24 wild cardoon individuals assayed and the number of alleles per locus varied from 2 to 13. Observed (H_O) and expected (H_E) heterozygosity ranged from 0.19 to 0.77 and from 0.18-0.89, respectively. Wright's fixation index (F_{IS}) values ranged from 0.06 to 0.23. Only one of the nine loci (CMAFLP-18) showed significant deviation from HW equilibrium. This can be attributed to genotyping artefacts, such as the presence of null alleles. No significant pairwise linkage disequilibrium was found among loci.

Allele frequencies ranged from 0.02 to 0.87. Nineteen (30%) were rare alleles ($p < 0.05$) of which eight were exclusive of one genotype. All primer sets also amplified more than one allele per locus without additional optimization in globe artichoke and cultivated cardoon accessions, suggesting that these markers are potentially useful for characterizing genetic variation in the *Cynara cardunculus* species. Ongoing research is involved in applying these markers for the construction of a genetic-molecular map of the species.

Table 1 Characterization of microsatellites developed from *Cynara cardunculus* (n = 24 wild cardoon accessions). N_A is number of alleles detected; H_E and H_O are expected and observed heterozygosity, respectively; F_{IS} is Wright's Fixation index.

Locus	Repeat motif	Allele size range (bp)	N_A	H_E	H_O	F_{IS}
CMAFLP-01	$(AT)_6\,(AC)_3\,(GA)_2$	210-230	10	0.84	0.77	0.09
CMAFLP-04	$(TC)_{11}\,(TA)_9\,(GT)_{13}$	310-360	10	0.87	0.77	0.11
CMAFLP-05	$(TC)_4\ldots(TA)_3(CA)_{12}$	150-152	2	0.48	0.50	-0.05
CMAFLP-07	$(GA)_{12}\ldots(GA)_9$	210-235	8	0.85	0.75	0.12
CMAFLP-08	$(AG)_6\ldots(ATC)_4\ldots(CAT)_6$	380-420	6	0.71	0.67	0.06
CMAFLP-11	$(GCA)_6$	165-175	2	0.18	0.19	-0.06
CMAFLP-13	$(GA)_4\ldots(TA)_6$	260-320	6	0.74	0.71	0.04
CMAFLP-15	$(GA)_{11}$	80-95	6	0.80	0.71	0.11
CMAFLP-18	$(CA)_8\,(TA)_6\,(CA)_5\,(TA)_4\,(GT)_5$	200-250	13	0.89	0.69	0.23*

* Significant departure ($P < 0.05$) from Hardy-Weinberg equilibrium.

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