Appendix A Supplementary data.



**Fig. S1**. Graphic representation of the Analysis of molecular variance (AMOVA) based on *Fst* estimator for the partitioning of SSR variation of *C. cardunculs* plants among and within populations identified



**Fig. S2**. Graphic representation of the Analysis of molecular variance (AMOVA) based on *Fst* estimator for the partitioning of SSR variation of wild *C. cardunculs* plants among and within populations identified.



Fig.S3. Dendrogram based on Dice’s Similarity Coefficient and UPGMA clustering for 87 cardoons for the SSRs dataset.

**Table S1** Characteristics of microsatellite primers selected for *Cynara cardunculus* L. Sequences in forward (F) and reverse (R), repeat sequence pattern, individual annealing temperature, original fragment size, and GenBank accession numbers are shown (Acquadro *et al*., 2003, 2009; Scaglione *et al.,* 2009).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Locus | Repeat motif | Primer pair sequence (5¢-3¢) | T (°C) | Genebank | Allele size range (bp)/ (sequenced allele size) |
|
| CELMS 01 | (AG)17 | F:CAACACAGAAGCGAGGTCA | 49 | EU744917 | 356 |
|  |  | R.GAATGAGCCGGATTAGCATT |  |  |  |
| CELMS 10 | (AG)25 | F:TCAGACTTCAGCACCACCTC | 53 | EU744926 | 315 |
|  |  | R:GTCGTTCTGGATTCCCACAT |  |  |  |
| CELMS 11 | (TC)21(TTTG)3 | F:GCGAATCAATCCCTTGTCTC | 55 | EU744927 | 258 |
|  |  | R:AAGCCATGGATGAAGCAGAG |  |  |  |
| CELMS 41 | (ATG)11(GAT)9 | F:CCAAAGCCTTCAGAGCATTC | 53 | EU744957 | 271 |
|  |  | R:GGAATGATGTATGGATCGCC |  |  |  |
| CELMS 48 | (CTG)5(CTG)7 | F:ATAACAGGACGAGGTGTGGAAG | 49 | EU744964 | 321 |
|  |  | R:CTACAGTTGCTTATTGGTCCCC |  |  |  |
| CELMS 58 | (AG)18(AGAA)3 | F:GGATTCCATTGGACTTACAGG | 55 | EU744974 | 259 |
|  |  | R:GGTTTGCCTATCTCTGTCTTTCTT |  |  |  |

**Table S2**. Summary of allelic data and statistics for the five SSR loci across five cardoon populations. Na = number of different alleles; Ne = number of effective alleles; I = Shannon's information index; Ho = Observed Heterozygosity; He = Expected Heterozygosity; F = Fixation Index.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | ***CAL-CAS-W*** | ***CAL-TRE-W*** | ***CAL-SIB-W*** | ***SAR-UTA-W*** | ***SAR-UTA-C*** | ***WHOLE*** |
| **CELMS48** |  |  |  |  |  |  |
| **Na** | 3 | 3 | 2 | 1 | 2,000 | 2,200 |
| **Ne** | 2,091 | 2,253 | 1,862 | 1 | 1,923 | 1,826 |
| **I** | 0,786 | 0,898 | 0,655 | 0 | 0,673 | 0,602 |
| **Ho** | 0,5 | 0,231 | 0,273 | 0 | 0,000 | 0,201 |
| **He** | 0,522 | 0,556 | 0,463 | 0,000 | 0,480 | 0,404 |
| **F** | 0,042 | 0,585 | 0,411 | #N/D | 1,000 | 0,509 |
|   |   |   |   |   |   |   |
| **CELMS41** |  |  |  |  |  |  |
| **N** | 22 (44) | 25 (50) | 21 (42) | 5 (10) | 5 (10) | 15,600 |
| **Na** | 5 | 4 | 7 | 4 | 2,000 | 4,400 |
| **Ne** | 2,086 | 2,546 | 4,768 | 2,778 | 2,000 | 2,835 |
| **I** | 1,011 | 1,058 | 1,688 | 1,168 | 0,693 | 1,124 |
| **Ho** | 0,545 | 0,720 | 0,619 | 0,8 | 1,000 | 0,737 |
| **He** | 0,521 | 0,607 | 0,790 | 0,640 | 0,500 | 0,612 |
| **F** | -0,048 | -0,186 | 0,217 | -0,250 | -1,000 | -0,253 |
|   |   |   |   |   |   |   |
| **CELMS11** |  |  |  |  |  |  |
| **N** | 25 (50) | 25 (50) | 22 (44) | 5 (10) | 5 (10) | 16,400 |
| **Na** | 6 | 3 | 4 | 5 | 2,000 | 4,000 |
| **Ne** | 3,141 | 2,654 | 2,527 | 3,125 | 2,000 | 2,689 |
| **I** | 1,316 | 1,036 | 1,048 | 1,359 | 0,693 | 1,090 |
| **Ho** | 1 | 1 | 1 | 0,8 | 1,000 | 0,960 |
| **He** | 0,682 | 0,623 | 0,604 | 0,680 | 0,500 | 0,618 |
| **F** | -0,467 | -0,605 | -0,655 | -0,176 | -1,000 | -0,581 |
|   |   |   |   |   |   |   |
| **CELMS58** |  |  |  |  |  |  |
| **N** | 23 (46) | 26 (52) | 23 (46) | 5 (10) | 5 (10) | 16,400 |
| **Na** | 5 | 5 | 8 | 4 | 1,000 | 4,600 |
| **Ne** | 2,972 | 3,440 | 4,831 | 3,333 | 1,000 | 3,115 |
| **I** | 1,279 | 1,336 | 1,750 | 1,280 | 0,000 | 1,129 |
| **Ho** | 0,696 | 0,923 | 0,739 | 0,8 | 0,000 | 0,632 |
| **He** | 0,664 | 0,709 | 0,793 | 0,700 | 0,000 | 0,573 |
| **F** | -0,048 | -0,301 | 0,068 | -0,143 | #N/D | -0,106 |
|   |   |   |   |   |   |   |
| **CELMS01** |  |  |  |  |  |  |
| **N** | 23 (46) | 26 (52) | 24 (48) | 5 (10) | 5 (10) | 16,600 |
| **Na** | 5 | 5 | 8 | 4 | 2,000 | 4,800 |
| **Ne** | 2,997 | 2,998 | 5,878 | 2,941 | 2,000 | 3,363 |
| **I** | 1,271 | 1,313 | 1,894 | 1,221 | 0,693 | 1,278 |
| **Ho** | 0,739 | 0,808 | 0,833 | 0,6 | 1,000 | 0,796 |
| **He** | 0,666 | 0,666 | 0,830 | 0,660 | 0,500 | 0,665 |
| **F** | -0,109 | -0,212 | -0,004 | 0,091 | -1,000 | -0,247 |
|  |  |  |  |  |  |  |
| **CELMS10** |   |   |   |   |   |   |
| **N** | (38) 19 | 25 (50) | 23 (46) | 3 (6) | 5 (10) | 15,000 |
| **Na** | 6 | 6 | 6 | 1 | 2,000 | 4,200 |
| **Ne** | 4,719 | 4,771 | 4,266 | 1 | 2,000 | 3,351 |
| **I** | 1,636 | 1,647 | 1,592 | 0 | 0,693 | 1,114 |
| **Ho** | 0,842 | 0,960 | 0,826 | 0 | 1,000 | 0,726 |
| **He** | 0,788 | 0,790 | 0,766 | 0 | 0,500 | 0,569 |
| **F** | -0,069 | -0,215 | -0,079 | #N/D | -1,000 | -0,341 |
|  |  |  |  |  |  |  |
| **Overall loci** | ***CAL-CAS-W*** | ***CAL-TRE-W*** | ***CAL-SIB-W*** | ***SAR-UTA-W*** | ***SAR-UTA-C*** | ***WHOLE*** |
| **N** | 22,33 (45) | 25,50 (51) | 22,50 (51) | 4,67 (9) | 5,0 (10) | 16 |
| **Na** | 5,000 | 4,333 | 5,833 | 3,167 | 1,833 | 4,033 |
| **Ne** | 3,001 | 3,110 | 4,022 | 2,363 | 1,821 | 2,863 |
| **I** | 1,216 | 1,215 | 1,438 | 0,838 | 0,574 | 1,056 |
| **Ho** | 0,720 | 0,774 | 0,715 | 0,500 | 0,667 | 0,675 |
| **He** | 0,640 | 0,659 | 0,708 | 0,447 | 0,413 | 0,573 |
| **F** | -0,117 | -0,156 | -0,007 | -0,120 | -0,600 | -0,191 |
| **number of polymorphic loci** | 6,000 | 6,000 | 6,000 | 4 | 5,000 |  |
| **percentage of polymorphic loci** | 100% | 100% | 100% | 66,67% | 83,330 | 90% |

**Table S3**. Analysis of molecular variance (AMOVA) for 87 individulas of *C. cardunculs* using SSR: \* Significance test after 99 permutations

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Source of variance | df | SS | MS | EstimatedVariance | % | F-Statistics |  | Value |  | P-*value\** |  |
| Among Regions | 1 | 14,338 | 14,338 | 0,221 | 9% | **Frt** |  | 0,085 |  | 0,010 |  |
| Among Pops | 3 | 39,316 | 13,105 | 0,290 | 11% | **Fsr** |  | 0,121 |  | 0,010 |  |
| Among Indiv | 82 | 182,345 | 2,224 | 0,115 | 4% | ***Fst*** |  | 0,195 |  | 0,010 |  |
| Within Indiv | 87 | 173,500 | 1,994 | 1,994 | 76% | ***Fis*** |  | 0,054 |  | 0,020 |  |
| Total | 173 | 409,500 |  | 2,620 | 100% | ***Fit*** |  | 0,239 |  | 0,010 |  |

Table S4. Analysis of molecular variance (AMOVA) for 87 individulas of *C. cardunculs* using SSR: \* Significance test after 99 permutations

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Source of variance | df | SS | MS | Estimated Variance | % | F-Statistics | Value | P-value\* |  |
| Among Regions | 1 | 8,907 | 8,907 | 0,211 | 8% | **Frt** | 0,082 | 0,010 |
| Among Pops | 2 | 26,166 | 13,083 | 0,210 | 8% | **Fsr** | 0,089 | 0,010 |
| Among Indiv | 78 | 179,945 | 2,307 | 0,157 | 6% | ***Fst*** | 0,164 | 0,010 |
| Within Indiv | 82 | 163,500 | 1,994 | 1,994 | 78% | ***Fis*** | 0,073 | 0,010 |
| Total | 163 | 378,518 |  | 2,572 | 100% | ***Fit*** | 0,225 | 0,010 |

**Table S5.** Pairwise Matrix of the genetic distance between five populations of *C. Cardunculus* using *Fst* and Nei ‘s Index (both identity and distance)

|  |  |  |  |
| --- | --- | --- | --- |
| Populations | *Fst* | Nei D | Nei I |
| *CAL-CAS-W* | ***CAL-TRE-W*** | 0,129 | 0,432 | 0,649 |
| *CAL-CAS-W* | ***CAL-SIB-W*** | 0,091 | 0,393 | 0,675 |
| *CAL-TRE-W* | ***CAL-SIB-W*** | 0,044 | 0,143 | 0,867 |
| *CAL-CAS-W* | ***SAR-UTA-W*** | 0,212 | 0,896 | 0,408 |
| *CAL-TRE-W* | ***SAR-UTA-W*** | 0,165 | 0,547 | 0,579 |
| *CAL-SIB-W* | ***SAR-UTA-W*** | 0,123 | 0,511 | 0,600 |
| *CAL-CAS-W* | ***SAR-UTA-C*** | 0,336 | 1,804 | 0,165 |
| *CAL-TRE-W* | ***SAR-UTA-C*** | 0,347 | 1,704 | 0,182 |
| *CAL-SIB-W* | ***SAR-UTA-C*** | 0,271 | 1,419 | 0,242 |
| *SAR-UTA-W* | ***SAR-UTA-C*** | 0,433 | 1,827 | 0,161 |