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ORIGINAL PAPER

Genetic mapping and annotation of genomic microsatellites isolated from globe artichoke

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Abstract *Cynara cardunculus* includes three taxa, the globe artichoke (subsp. *scolymus* L. Hegi), the cultivated cardoon (var. *altilis*) and their progenitor, the wild cardoon (var. *sylvestris*). Globe artichoke is an important component of the Mediterranean rural economy, but its improvement through breeding has been rather limited and its genome organization remains largely unexplored. Here, we report the isolation of 61 new microsatellite loci which amplified a total of 208 alleles in a panel of 22 *C. cardunculus* genotypes. Of these, 51 were informative for linkage analysis and 39 were used to increase marker density in the available globe artichoke genetic maps. Sequence analysis of the 22 loci associated with genes showed that 9 are located within coding sequence, with the repetitive domain probably being involved in DNA binding or in protein–protein interactions. The expression of the genes associated with 9 of the 22 microsatellite loci was demonstrated by RT-PCR.

Introduction

Cynara cardunculus L. (Asteraceae, $2n = 2x = 34$) contains the three taxa: subsp. *scolymus* L. Hegi (the globe artichoke), var. *altilis* DC. (the cultivated cardoon) and var. *sylvestris* (Lamk) Fiori (the wild cardoon). Globe artichoke is an allogamous plant with an estimated genome size of 1,078 Mbp (Marie and Brown 1993). The immature inflorescences (capitula or heads) provide the edible part of the plant, and are used fresh, canned or frozen for the preparation of a variety of dishes; its leaves have been exploited as hepatoprotectants, and either choleretic or diuretic agents in traditional medicine since Ancient Roman times. In modern times, leaf extracts have been identified as containing cellular protectants against oxidative damage, HIV integrase inhibitors, and bile-expelling and lipid-lowering agents (Gebhardt 1997, 1998; Kraft 1997; Llorach et al. 2002; McDougall et al. 1998; Wang et al. 2003), whilst roots and seeds have been used to extract inulin (Raccuia and Melilli 2004), with high degree of polymerization, and oil (Maccarone et al. 1999; Raccuia and Melilli 2007). The crop is grown across the Middle East, North Africa, South America, China, the USA, and particularly in the Mediterranean region, where it has a significant impact on the rural economy. Italy is the leading global producer (http://faostat.fao.org/). Despite its economic, pharmacological and nutritional value, its improvement through breeding has been rather limited, whilst, unlike other crop species belonging to the same botanical family (such as sunflower, lettuce and chicory), its genome organization remains largely unexplored.

The first molecular maps of globe artichoke have only recently been published (Lanteri et al. 2006). These were largely based on dominant DNA fingerprinting platforms, although a small number of microsatellite (SSR) markers were included (Acquadro et al. 2003, 2005a, b). Although SSRs are widely favoured as a marker platform for genetic mapping and biodiversity studies on account of their allelic variability, it has become clear that some can also act as

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regulatory elements (Iglesias et al. 2004; Martin et al. 2005). The 5′-untranslated region of many monocot and dicot genes contains highly conserved (both with respect to motif and genomic position) SSR sequences (Guo and Moose 2003; Yang et al. 1999), and this has been taken to imply that SSRs can also play a role in gene regulation. The region upstream of the transcription initiation sites in both *Arabidopsis thaliana* and rice genes has been shown to be characterized by a gradient of pyrimidine-rich SSR density (Zhang et al. 2006). SSRs also occur within exons, and their translation products (typically G_x, N_x or P_x) may provide a domain for DNA binding or protein–protein interaction. Such repetitive polypeptide stretches are known to be involved in the activation/de-activation of transcription (Berger et al. 2001; Gerber et al. 1994; Kolaczkowska et al. 2002; Perutz et al. 1994; Toth et al. 2000), and allelic variants in such genic SSRs have been implicated as the genetic determinants of a number of human diseases (Leroy et al. 2000).

Here, we report the development of a set of globe artichoke SSRs, extracted from enriched genomic libraries. We describe their informativeness for diversity analysis and taxonomic discrimination, their genetic map location as well as their annotation and gene ontology (GO) categorization.

Materials and methods

Plant materials and genomic DNA isolation

DNA was extracted from young *C. cardunculus* leaves following Lanteri et al. (2001). The primers developed were applied to DNA of (a) the parents of three established mapping populations, specifically the two diverse globe artichoke genotypes ['Romanesco C3' (C3) and 'Spinoso di Palermo' (Sp-9A)], one cultivated cardoon (A41) and one wild cardoon (Creta 4) genotype; (b) four F1 individuals from each of the segregating populations C3 × Sp-9A, C3 × A41 and C3 × Creta 4; and (c) six globe artichoke genotypes, demonstrated to be representative of Mediterranean Basin germplasm (Lanteri et al. 2004). Linkage analysis was performed on 94 C3 × Sp-9A progeny. Full genotype details are reported in Table 1.

Table 1 The 22 *C. cardunculus* genotypes assayed for genotypic variation

Genotypes	*C. cardunculus* taxa	Cluster[a]
Romanesco C3 (C3)	*scolymus*	A2
Spinoso di Palermo (Sp-9A)	*scolymus*	B1
A41	*altilis*	
Creta 4	*sylvestris*	
Four F_1 genotypes from C3 × Sp-9A	*scolymus*	
Four F_1 genotypes from C3 × A41	*scolymus* × *altilis*	
Four F_1 genotypes from C3 × Creta 4	*scolymus* × *sylvestris*	
Gross Camus	*scolymus*	A1
Hyerois	*scolymus*	A1
Tonda di Paestum	*scolymus*	A2
Violet de Campagne	*scolymus*	B1
Empolese	*scolymus*	B2
Locale di Chioggia Fano	*scolymus*	B2

[a] Globe artichoke clusters are defined in Lanteri et al. (2004)

Enriched libraries

SSR-containing sequences were isolated from ten enriched small-insert genomic libraries following van de Wiel et al. (1999), with minor modifications. *Alu*I, *Rsa*I or *Hae*III (5U) was used to digest 500 ng genomic DNA in the presence of 50 pmol of both 5′-GTTTCAGATCTG GCTCATCGC-3′ (Ada+) and 3′-ACACCAAAGTCTA GACCGAGTAGCG-5′ (Ada−), in a 50 µl reaction containing restriction-ligation buffer (10 mM Tris–HCl pH 7.5, 10 mM MgAc, 50 mM KAc, 5 mM DTT), 1 mM ATP and 5 U T4 DNA ligase. Restriction fragments in the size range 300–1,000 bp were selected by gel electrophoresis extraction and purified from agarose using the NucleoSpin Extract II kit (Macherey-Nagel). These were then amplified using 1 µl of the restricted ligated DNA as template in a 20 µl PCR containing 50 pmol adapter primer (Ada+), 1.5 mM $MgCl_2$, 1 mM dNTP and 1 U Taq polymerase (Invitrogen) in the manufacturer's buffer. The amplification programme was 94°C/120 s, followed by 25 cycles of 94°C/30 s, 50°C/30 s and 72°C/120 s and ending with a 10-min incubation at 72°C. The size-fractioned PCR product was denatured and hybridized to a Nylon+ (Amersham) filter carrying 1.5 µg single-stranded, UV-bound $(GT)_{12}$, $(GA)_{12}$, $(TCT)_{10}$, $(TGT)_9$, $(GAG)_8$, $(GTG)_8$, $(TGA)_9$, $(AGT)_{10}$, $(GCT)_8$, and $(GCC)_7$ for 48 h at 37°C in 5× SSC, 50 mM Na phosphate (pH 7), 7% (w/v) SDS and 50% (v/v) formamide. The filters were consecutively washed in stepwise reducing concentrations of SSC (1.5×, 0.5×, 0.2×, 0× (w/v)) and 1% (w/v) SDS at 62°C. The DNA dissolved in each wash fraction was precipitated by an overnight incubation in 20 µg glycogen, 0.8 M LiCl and 600 µl isopropanol and then resuspended in 0.1× TE. This DNA was re-amplified in a 20 µl PCR, as above. Each PCR product was ligated into pGEM-T (Promega) and introduced into *E. coli* JM109 (Promega). Insert-containing clones were bound to Hybond N+ (Amersham) membranes, which were hybridized with a mixture of the appropriate ^{32}P end-labelled oligonucleotides to select SSR containing clones, which were sequenced by

Table 2 Primer sequences of the 61 CELMS markers and their level of polymorphism

Locus	Forward primer (5′–3′)	Reverse primer (5′–3′)	Repeats	Size	PIC	N_A	LG	S × R	A × R	Sy × R	Acc.	GenBank
CELMS-01	ACAACACAGAAGCGAGGTCA	GAATGAGCCGGATTAGCATT	$(AG)_{17}$	356	0.45	3	11	+	+	+	+	EU744917
CELMS-02	TCCCTCAAGTCAAGCGAGTT	GGAGGGAGGGTTCAAGCTAC	$(TG)_6(GA)_{20}$	307	0.66	5	–	–	+	+	+	EU744918
CELMS-03	GATCAATACGTCGTCGCAGA	CTGAGGCTACCAAGGGTTTG	$(TC)_{18}(AC)_7$	392	0.71	5	3	+	+	+	+	EU744919
CELMS-04	TTTGTCAACCCATACGCAAC	AATCCAATCATATTACCATGTAATA	$(GA)_{19}$	274	0.67	5	10	+	+	+	+	EU744920
CELMS-05	CCCACCTTCTTATCCCATCA	TGGACGTCTGTTTCCTCCTC	$(CT)_{22}(CAG)_4$	309	0.77	7	1	+	+	+	+	EU744921
CELMS-06	CTCCATTCTGTGATGCAGTGA	TGTATCAACCTTGGCCTTCC	$(TC)_{19}(CA)_{10}(AC)_5$	231	0.35	3	–	–	–	+	+	EU744922
CELMS-07	AAGGCAGGGTTAGAGTGACAAC	AGACTCCATGCTTCACACAGAT	$(AG)_{22}$	196	0.30	4	8	+	–	+	+	EU744923
CELMS-08	TTTACAAACTTCCCCTTTCCAC	ACAATACAGATACACGCTCTCCA	$(TC)_{22}(TC)_8(TC)_{10}$	247	0.72	6	1	+	+	+	+	EU744924
CELMS-09	TCATCAATTGATCTGATAAGC	TTCGGTGCTAGGTAGACTT	$(CT)_{19}\cdots(TCT)_7$	195	0.65	4	–	–	+	+	+	EU744925
CELMS-10	TCAGACTTCAGCACCACCTC	GTCGTTCTGGATTCCCACAT	$(AG)_{25}$	315	0.70	4	9	+	+	+	+	EU744926
CELMS-11	GCGAATCAATCCCTTGTCTC	AAGCCATGGATGAAGCAGAG	$(TC)_{21}(TTTG)_3$	258	0.66	6	18	+	+	+	+	EU744927
CELMS-12	TTGATGAATTTTGATCACTA	ACCATTATCCTTTTGCTC	$(GT)_9(GA)_9(TG)_7$	326	0.55	4	16	+	+	+	+	EU744928
CELMS-13	ATGGGACCTTCCTCCAAAATAC	TCCATCATCACCTCACACGTA	$(TA)_7(AC)_{15}$	400	0.73	5	3	+	+	+	+	EU744929
CELMS-14	TCCAGCCATGCAAGAAAAGTAT	CCATCCTGAATCCATAACCAGT	$(AC)_{13}(TC)_7(AC)_{10}(TC)_9$	210	0.61	5	8	+	+	+	+	EU744930
CELMS-15	TGGATGGAAACACTCTTCACAG	TACAGTCCCGATGTGGGTATTT	$(CA)_{15}(TA)_5(ATGT)_{10}(TG)_5$	350	0.62	5	3	+	+	+	+	EU744931
CELMS-16	CTCTCTTTACCCTACTCATAA	CTTTTGGGGTTTCTATACC	$(AC)_{15}(AC)_{14}$	257	0.50	3	1	+	+	+	+	EU744932
CELMS-17	CCCGGATAATAGTCGATGAAGT	CCATGTGAAGATTGGGTGATT	$(GTT)_{32}$	305	0.32	4	–	–	+	+	+	EU744933
CELMS-18	TCCCTCCCATTGTTTCTTCTAA	CTGTTGCTGTTGCTGTAGCTG	$(CAA)_7(CAA)_4$	344	0.23	3	–	–	+	+	–	EU744934
CELMS-19	GATGGTGCTTCTTTCTTTTCCT	TAATATCCCAACCGTCCCC	$(TTG)_5(TTG)_6(TTG)_6(TTG)_6$	297	0.76	5	14	+	+	+	+	EU744935
CELMS-20	TTTTATAATTGCAGACTCAAT	TTCATTTCCAACAAGCCT	$(CAG)_5(CAA)_5(CAA)_8(CAA)_{12}$	218	0.52	3	10	+	+	+	+	EU744936
CELMS-21	TGTCATCAACCCCTACTCAGG	TTCAGATTTACTAACCCAAATGCTT	$(TCT)_4(TTC)_5(TTC)_4(TCT)_{12}$	388	0.56	4	14	+	+	+	+	EU744937
CELMS-22	TTTTCATCATCTCCTTCATGG	GCTTAGAGAAAGGGGAAAGAGG	$(CTT)_{22}(CTC)_6(TTG)_6$	392	0.74	5	–	–	–	+	+	EU744938
CELMS-23	GGCCCTACCTTAAAATGTCTCC	GACGGTGATTGTTGTAGTGGAA	$(CCA)_5(CCA)_5$	241	0.50	2	–	+	+	+	+	EU744939
CELMS-24	ACCAAACTCTGTCGACCACC	GGTTGTGGAGGACCTGGATA	$(CAC)_4(CCA)_{12}$	242	0.61	4	5	+	+	+	+	EU744940
CELMS-25	TTATCAGCCACCTCCACCTC	GACGGGCAATGGTAGTCAAT	$(CCA)_4(CAC)_7$	288	0.69	5	–	+	+	+	+	EU744941
CELMS-26	ACCATGTCACAACAAACCGA	TGATTCTCGTAGGTGGAGGG	$(CCA)_9(CAC)_8$	388	0.55	4	1	+	+	+	+	EU744942
CELMS-27	ACTGTTGTTGCTGGTAAGGGTT	AGAAAGGAGGAGGAAAGCATCT	$(ACC)_6$	367	0.57	4	1	+	–	+	+	EU744943
CELMS-28	GAAAGAAGATGCATAGACCAGGA	CCTCCAGCTGCTGCCTAATA	$(CCA)_4(CCT)_4(CAC)_4$	195	0.24	3	–	–	–	+	+	EU744944
CELMS-29	ATCCCCAAATCCAGCAATTT	TCAATGTGCATGGAAAGAACA	$(CCA)_5(CCT)_4$	296	0.48	2	2	+	+	+	+	EU744945
CELMS-30	TCAGGCACCTCAAACTCCTT	CAGGTGCATGACCACCTAGT	$(ACC)_7$	294	0.56	3	19	+	–	+	+	EU744946
CELMS-31	AAATGGATATTGGAACACCTCC	TATTTGAGGAATGTCTGCTGCT	$(CCA)_4(CCA)_{10}(CCA)_4$	140	0.65	3	4	+	+	+	+	EU744947
CELMS-32	ACCTCCACCACCTTGTCCTC	CATGTAGTGCCTGGATATGG	$(ACC)_5(CAC)_5(CAC)_7$	177	0.46	2	6	+	+	+	+	EU744948
CELMS-33	GATGCACCACTTTCCTCTCAC	ATATGGGCTTTTCTGGTTGTTC	$(CAC)_4(CCA)_{11}$	190	0.65	4	–	+	+	+	+	EU744949
CELMS-34	ACCGCCCGTCGTTGCC	CGCCTAGCAGTTGTGGAAGTGG	$(ACC)_{11}$	169	0.00	1	–	–	–	–	–	EU744950
CELMS-35	CTCCCCCTCCGGTTCAAT	GAACCGATGTGGGGTGGTA	$(CCA)_4(CAC)_6(CCA)_5(CAC)_4$	299	0.42	3	–	–	–	+	+	EU744951
CELMS-36	CACCACTAGTACAATTAACCAT	AGTAGTGGTAGTTGATGTTAGA	$(CAC)_4(ACC)_5(CAC)_5(ACC)_6(CCA)_4$	241	0.63	4	5	+	+	+	+	EU744952
CELMS-37	CGCCGGAATATCAAGATTGT	TACCATCAACTCGGAGAGGG	$(CCA)_8$	300	0.68	5	14	+	+	+	+	EU744953

Table 2 continued

Locus	Forward primer (5′–3′)	Reverse primer (5′–3′)	Repeats	Size	PIC	N_A	LG	S × R	A × R	Sy × R	Acc.	GenBank
CELMS-38	ACTGGGGTTTACAAGCTGTGAT	CTCCTGATGTGTTGTTCTTGATG	$(ATC)_9(TCC)_4(TCA)_9$	409	0.40	3	–	–	–	+	+	EU744954
CELMS-39	ATTCCAATCACCTCTGTGGC	ACTGTATGGTGAAGTCGTTA	$(GAT)_5(GAT)_{14}$	182	0.42	3	10	+	+	+	+	EU744955
CELMS-40	TGGATTAAGGCACACACTGAAC	TGATGATAACAAAGGAGGGGAT	$(ATC)_4(ATC)_{16}(TCT)_9$	388	0.75	6	1	+	+	+	+	EU744956
CELMS-41	CCAAAGCCTTCAGAGCATTC	GGAATGATGTATGGATCGCC	$(ATG)_{11}(GAT)_9$	271	0.59	4	2	+	+	+	+	EU744957
CELMS-42	AAGCTGAAGTCGAGGAACCA	TGGGATGAAGATTCCCAGAG	$(TGA)_4(TGA)_8(GAT)_5$	358	0.66	3	3	+	+	+	+	EU744958
CELMS-43	CCTTCACCCCTGTCTACAAGAT	GGGGAGGCACGATGAG	$(ATG)_{10}$	288	0.00	1	–	–	–	–	–	EU744959
CELMS-44	GTTCCACGTTTGAAGCGAGT	TTTGCTATTGTCCATAAAAGATTGA	$(CTA)_{16}(ACT)_4(ACT)_4(TAC)_4(ACC)_4$	249	0.50	2	5	+	+	+	+	EU744960
CELMS-45	TTCTGTGGAGAGTTTCATCCAA	TAGCTTGCTCACGCTCAGTG	$(TCT)_{103}$	426	0.48	5	–	+	+	+	+	EU744961
CELMS-46	CATTAGCGTATCTAGTGGAGAAAGACT	GCCATCTTCTTCTTCTACTCAGG	$(AGA)_{52}(AGA)_4$	250	0.00	1	–	–	–	–	–	EU744962
CELMS-47	TGGAAAGGGGAGAGAAACAA	CTGGTGATCAAGGCCAGAGT	$(AGA)_{28}(AAG)_6$	222	0.00	1	–	–	–	–	–	EU744963
CELMS-48	ATAACAGGACGAGGTGTGGAAG	CTACAGTTGCTTATTGGTCCCC	$(CTG)_5(CTG)_7$	321	0.57	3	2	+	+	+	+	EU744964
CELMS-49	AGCAACAGCCACAACAACTTC	TGGACCTTGAACATAACCTTGA	$(CAG)_6(CAG)_4$	215	0.29	3	–	–	+	–	+	EU744965
CELMS-50	AACAGCAGCAGCAACAAATAAG	GGACGAAAGAAAAGGAACACAG	$(CAG)_5(AGC)_5$	190	0.00	1	–	–	–	–	–	EU744966
CELMS-51	CTTGTTGATGCTGTTGTCGAGT	TAGGGCTGTGTTTTGACCTTTT	$(CTG)_6(TGC)_4$	226	0.00	1	–	–	–	–	–	EU744967
CELMS-52	TGCAGCAAATTCTTTTGTGG	TGTGGGAACCTCTATAATCTCTTTG	$(CT)_{18}$	301	0.53	4	1	+	+	+	+	EU744968
CELMS-53	TTTGTTCACGGAATTCAACG	GCCCTGTCCTCGATAAGATG	$(GA)_{18}$	235	0.00	1	–	–	–	–	–	EU744969
CELMS-54	CGAAAAGAGTTCAAGAGGGAAA	GCACCTGAAGCATCTGAGG	$(GAA)_n$	180	0.00	1	–	–	–	–	–	EU744970
CELMS-55	CTCTAGTCGCAGAGGATGGA	TGCCACATTTAAAGCAACCA	$(GAGAAG)_2$	318	0.00	1	–	–	–	–	–	EU744971
CELMS-56	CCTAGGGATGATGCCCATAC	ATGGAGTCGATTCACCTTGC	$(TGA)_6(GAT)_4$	250	0.00	1	–	–	–	–	–	EU744972
CELMS-57	GTTGGGGTGTCAAAACGAAT	CCAAGGGGATGACTAAGAGC	$(TCT)_{10}$	243	0.41	3	–	–	+	+	+	EU744973
CELMS-58	GGATTCCATTGGACTTACAGG	GGTTTGCCTATCTCTGTCTTTCTT	$(AG)_{18}(AGAA)_3$	259	0.66	4	1	+	+	+	+	EU744974
CELMS-59	TCCGTTATTTCTTGCGGTTA	TACCTCTCCGGTTGGAATTG	$(CT)_{16}(TC)_8$	399	0.29	3	2	+	+	+	+	EU744975
CELMS-60	TGGTGGGAAAAGGAGTGTTT	CATACCCACCCTGCAAGTTA	$(GA)_5(GA)_{10}(GA)_{12}(GA)_{14}(GA)_6(AG)_5$	381	0.59	3	19	+	+	+	+	EU744976
CELMS-61	TGCAAACCAGAAACTGCTTG	TGCAGACTTTACCTCCACCA	$(CT)_{18}(GT)_8$	170	0.32	3	–	–	+	+	–	EU744977

'+' and '−' denote, respectively, a polymorphic or a monomorphic locus

PIC polymorphic information content, N_A number of alleles, *LG* linkage group, *S* × *R* progeny Sp-9A × C3, *A* × *R* progeny A41 × C3, *Sy* × *R* progeny Creta 4 × C3, *Acc.* globe artichoke genotypes

Greenomics™ (Wageningen, The Netherlands). From these sequences, primer pairs were designed by Primer 3.0 (Rozen and Skaletsky 2000), http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi), adopting default parameter settings. A tailing primer strategy was used, as described by Oetting et al. (1995). The newly developed SSR markers were identified by a number, prefixed by CELMS (*Cynara* Enriched Library MicroSatellite) (Table 2).

SSR genotyping

The SSRs were tested for their informativeness on the 22 genotypes reported in Table 1. PCRs were performed and the resulting products analysed as reported by Acquadro et al. (2005b). Briefly, amplification products were mixed with 5–50 µl of formamide dye, denatured and quenched, and then electrophoresed on a DNA analyser Gene ReadIR 4200 (LI-COR). The PCR products were scored as band presence (1) and absence (0), thus generating a binary data matrix. From this, the polymorphic information content (PIC) was calculated for each locus as described by Anderson et al. (1993) using Microsoft Office Excel software.

Linkage analysis

The segregation of alleles for those SSR markers informative between C3 and Sp-9A was followed in the C3 × Sp-9A population developed by Lanteri et al. (2006). Separate linkage maps were constructed for each parent using the double pseudo-testcross mapping strategy (Weeden 1994), incorporating previously scored genotypic data. Markers were separated into three types: maternal testcross markers, segregating only in C3 (expected segregation ratio 1:1); paternal testcross markers, segregating only in Sp-9A (1:1); and intercross markers, segregating within both parents (either 1:2:1 or 1:1:1:1). The goodness-of-fit between observed and expected segregation data was tested by χ^2, and only markers fitting or deviating slightly from expectation ($\chi^2_{\alpha=0.1} < \chi^2 \leq \chi^2_{\alpha=0.01}$) were used for map construction, using JoinMap v2.0 (Stam and Van Ooijen 1995). For both maps, linkage groups (LGs) were accepted on the basis of a LOD threshold of >4.0. To determine marker order within a LG, the parameter settings were Rec = 0.40, LOD = 1.0, Jump = 5. Map distances were converted to centiMorgans (cM) using the Kosambi mapping function (Kosambi 1944). Linkage maps were drawn using MapChart v2.1 (Voorrips 2002). A method-of-moments type estimator (Hulbert et al. 1988), as proposed in 'method 3' by Chakravarti et al. (1991), was used to estimate the genome length (*G*) of each parent.

Sequence annotation

Sequences were analysed with the BlastX or BlastN algorithm (Altschul et al. 1997). The non-default BlastX parameters applied were as follows: database = reference proteins; organism = Viridiplantae; max target sequences = 50; matrix = BLOSUM62; filter = low complexity regions. No threshold was set. The BlastN parameters applied were as follows: database = reference mRNA sequences; organism = Viridiplantae; optimize for = highly similar sequences ("MegaBlast"); filter = low complexity, species-specific repeats for *Arabidopsis*. The target database contained all the available Viridiplantae sequences (3,592,723 entries for BlastX, 32,825,875 for MegaBlast, March 2008). MegaBlast analyses covering six *Asteraceae* genera (*Lactuca*, *Helianthus*, *Chicorium*, *Taraxacum*, *Centaurea*, *Carthamus*) were executed using the same parameters, with a threshold of $1.0e^{-8}$. In some cases, local alignment hits with an *e* value below the threshold were considered, where their annotation was interpretable. A second annotation was performed with the Blast search tool of AmiGO (http://amigo.geneontology.org/cgi-bin/gost/gost.cgi) using default parameter settings. GO annotation terms were reported for each CELMS locus (Table 3), considering the biological process (P), the cellular component (C) and molecular function (F). The gene structure prediction system Gene Builder (http://l25.itba.mi.cnr.it/%7Ewebgene/genebuilder.html) was used to confirm the presence of significant (>45 residues) open reading frames (ORFs), using parameters derived from *A. thaliana*. The CELMS loci which did not align with any GenBank entry were analysed using CENSOR (Jurka et al. 1996), applied in genome projects to identify and mask repetitive elements. Loci which contained an SSR motif within an ORF were designated as coding SSRs.

Experimental confirmation of expressed SSRs

The transcription of each CELMS locus was assayed by RT-PCR. Total RNA was extracted from 8-week-old leaves of Sp-9A using the NucleoSpin RNA plant extraction kit (Macherey-Nagel), and 2 µg of this RNA was denatured at 70°C for 5 min and then reverse transcribed at 42°C for 1 h in a 20 µl reaction containing 100 U M-MuLV reverse transcriptase (Fermentas), 0.5 mM dNTP and 0.8 µg dT_{15} in the buffer supplied by the manufacturer; 5 µl of a 1:10 dilution of this reaction was provided as template for a 20 µl PCR containing 10 pmol of each CELMS primer (Table 2), 1.5 mM $MgCl_2$, 0.2 mM dNTP, 1 U GoTaq (Promega) in the buffer supplied by the manufacturer. The cycling conditions consisted of a denaturation of 94°C/60 s, followed by 27 cycles of 94°C/30 s, 55°C/30 s and 72°C/60 s, terminated with a 10-min

Table 3 Annotation of the CELMS sequences and their putative function

Locus	ORF	RT-PCR	SSR position	Stretch	dbEST, Asteraceae	Similarity (protein)	Identity (DNA)	*e* value[a]	Algorithm	Putative function	Function (F)	Process (P)	Component (C)
Similarities with genes													
CELMS-03	188–367	–	SSR after ORF	–	–	NP_199800	NM_124367.3	$2e^{-08}$	BlastX	CLC-C (chloride channel C)	Voltage-gated chloride channel	Chloride transport	Membrane
CELMS-04	41–165	–	SSR after ORF	–	–	NP_181138	NM_129153.2	$1e^{-08}$	BlastX	BLH1 (BLH1)	Transcription factor	Regulation of transcription	Nucleus
CELMS-05	146–466	+	Coding SSR	$(SL)_n$	EL399062, *C. tinctorius*, $9e^{-62}$	NP_680740	NM_148374.2	$2e^{-18}$	BlastX	Peptidoglycan-binding LysM domain-containing protein	–	Cell wall catabolic process	–
CELMS-06	364–783	–	SSR before ORF	–	–	NP_191366	NM_115669.2	$7e^{-09}$	BlastX	GIS (glabrous inflorescence stems)	Nucleic acid binding	Regulation of transcription	Intracellular
CELMS-10	–	–	–	–	–	NP_180289	NM_128279.3	$9e^{-06}$	BlastX	Kelch repeat-containing ser/thr phosphoesterase	Phosphoprotein phosphatase	–	Intracellular
CELMS-15	395–587	–	SSR after ORF	–	–	NP_192095	NM_116416.3	$4e^{-14}$	BlastX	Transducin/WD-40 repeat family protein	–	–	–
CELMS-16	169–215[b]	–	SSR before ORF	–	AB274889, *L. sativa*, $9e^{-40}$	–	EV203634	$3e^{-39}$	MegaBlast	Transcribed locus, *B. napus*	–	Electron transport/ photosynthesis	Chloropl./ thylak./ PSI
CELMS-17	60–144; 357–456	–	Coding SSR	–	–	NP_200631	NM_125208.3	$9e^{-17}$	BlastX	MSI1 (multicopy suppressor of IRA1)	MSI type nucleosome	Chromatin assembly	–
CELMS-18	123–436	+	Coding SSR	$(Q)_n$	–	NP_173356	NM_101780.2	$3e^{-13}$	BlastX	ARF19 (auxin response factor 11)	Transcription factor	–	–
CELMS-19	90–460	–	Coding SSR	$(N)_n$	–	NP_197569	NM_122076.1	$2e^{-04}$	BlastX	LRR transmembrane protein kinase	Protein kinase activity/ receptor	Phosphorylation	–
CELMS-20	59–487	+	Coding SSR	$(Q)_n$	–	NP_567896	NM_119407.2	$1e^{-05}$	BlastX	LUG (LEUNIG); transcriptional co-repressor	DNA binding	Development/cell differentiation	Nucleus
CELMS-33	1–644	–	Coding SSR	(P)n	EL456618, *H. tuberosus*, $5e^{-93}$	NP_177361	NM_105874.3	$1e^{-29}$	BlastX	SEC14 cytosolic factor/ phosphoglyceride transfer family	Transporter activity	Transport	Intracellular/ membrane
CELMS-37	132–497	+	Coding SSR	$(P)_n(LXXL)_n$	–	NP_001053250	NM_001059785.1	$1e^{-20}$	BlastX	LRR plant protein	Protein binding	–	–
CELMS-38	184–660	+	Coding SSR	$(H)_n$	DW060955, *L. sativa*, $3e^{-40}$	–	DW060955	$1e^{-38}$	MegaBlast	*L. saligna* cDNA/ WRKY DNA binding protein like	ATP-dependent DNA helicase	DNA repair	–
CELMS-44	1–138; 241–531	–	SSR between two ORF	–	–	NP_850630	NM_180299.1	$3e^{-18}$	BlastX	MSH7 (Muts Homolog 6-2)	Damaged/mism. DNA binding	Mismatch repair	–
CELMS-47	1–802	+	Coding SSR	$(K)_n$	–	NP_001053494	NM_001060029	$3e^{-08}$	BlastX	MIP, major intrinsic protein family protein, aquaporin like	Receptor activity/H^+ ion transporter	ATP synthesis	Chloropl./ thylak./ PSI

Table 3 continued

Locus	ORF	RT-PCR	SSR position	Stretch	dbEST, Asteraceae	Similarity (protein)	Identity (DNA)	*e* value[a]	Algorithm	Putative function	Function (F)	Process (P)	Component (C)
CELMS-48	199-8	+	Coding SSR	$(Q)_n$	EH757759, *C. solstitialis*, $3e^{-71}$	–	EH757759.1	$3e^{-71}$	MegaBlast	*C. solstitialis* cDNA clone	DNA topoisomerase	DNA change during replication	Chromosome
CELMS-49	241–555	+	Coding SSR	$(L)_n$	CX944987, *H. annuus*, $1e^{-29}$	–	EV203711	$4e^{-28}$	MegaBlast	Transcribed locus, *B. napus*	Transcription factor	–	Phloem
CELMS-52	212-24	+	SSR before ORF	–	EH739359, *C. maculosa*, $4e^{-75}$	NP_973993	NM_202264.2	$1e^{-31}$	BlastX	Putative NMT2	Methyltransferase	Metabolic process	–
CELMS-57	82–182; 232–364	–	SSR after ORF	–	–	NP_177784	NM_106308.3	$7e^{-03}$	BlastX	TF (squamosa promoter-binding-like protein 16, SPL16)	Oxidoreductase	Electron transport	Intracellular
CELMS-60	426–565; 598–702	–	Coding SSR	$(ER)_n$	–	NP_171911	NM_100296.1	$4e^{-17}$	BlastX	C2 domain-containing protein	Glycosyl transferase activity	–	–
CELMS-61	134–181; 309–381	–	SSR between two ORF	–	EH767288, *C. solstitialis*, $4e^{-15}$	–	EH767288	$4e^{-15}$	MegaBlast	*C. solstitialis* cDNA clone	Receptor	Cell–matrix adhesion/ signalling	Integrin complex
Similarities with mobile elements													
CELMS-21	–	–	–	–	–	–	ATENSPM12	80%	Censor	EnSpm like element (DNA transposon)	–	–	–
CELMS-23	–	–	–	–	–	–	RIRE7_I	71%	Censor	Ty3-Gypsy like element (LTR Retrotransposon)	–	–	–
CELMS-24	–	–	–	–	–	–	CEREBA_I	69%	Censor	Ty3-Gypsy like element (LTR Retrotransposon)	–	–	–
CELMS-25	–	–	–	–	–	–	SZ-6IN	66%	Censor	Ty1-Copia like element (LTR Retrotransposon)	–	–	–
CELMS-26	–	–	–	–	–	NP_001046979	NM_001053514.1	$4e^{-10}$	BlastX	Retrotransposon gag protein	RNA-directed DNA polymerase	Integration	Nucleus
CELMS-30	–	–	–	–	–	–	ATGP8	78%	Censor	Gypsy like element (LTR Retrotransposon)	–	–	–
CELMS-34	–	–	–	–	–	–	TEMPINDAS	70%	Censor	hAT-like (DNA transposon)	–	–	–
CELMS-35	–	–	–	–	–	–	EnSpm5_OS	67%	Censor	EnSpm like element (DNA transposon)	–	–	–
CELMS-36	–	–	–	–	–	–	NonLTR-5_CR	61%	Censor	Non LTR Retrotransposon like	–	–	–
CELMS-39	–	–	–	–	–	–	SHACOP23_MT	78%	Censor	LTR Retrotransposon like	–	–	–

Table 3 continued

Locus	ORF	RT-PCR	SSR position	Stretch	dbEST, Asteraceae	Similarity (protein)	Identity (DNA)	*e* value[a]	Algorithm	Putative function	Function (F)	Process (P)	Component (C)
CELMS-40	–	–	–	–	–	–	Copia40-PTR_I	90%	Censor	LTR Retrotransposon like	–	–	–
CELMS-41	–	–	–	–	–	NP_001061216	NM_001067751	$1e^{-10}$	BlastX	*Oryza sativa* Copia protein	Nucleic acid/zinc ion binding	DNA integration	–
CELMS-54	–	–	–	–	–	NP_194886	NM_119307.3	$2e^{-02}$	BlastX	SRZ-22 (serine/arginine-rich 22)	Nucleic acid/zinc ion binding	DNA integration/RNA splicing	–
CELMS-55	–	–	–	–	–	NP_001043197	NM_001049732.1	$5e^{-03}$	BlastX	PDR-like ABC transporter (PDR3 ABC transporter)	Nucleic acid/zinc ion binding	DNA integration/viral reprod.	–
CELMS-56	–	–	–	–	–	–	SHAMUDRAV_MT	70%	Censor	MuDr like DNA transposon	–	–	–

[a] When the CENSOR algorithm was used, a similarity value is reported

[b] The ORF found is less than 45 amino acids but is present at the end of the CELMS locus

incubation at 72°C. Control reactions were derived from template produced in the absence of reverse transcriptase. In some cases, primers had to be re-designed (Table 4). RT-PCR products were separated by agarose gel electrophoresis and visualized by EtBr staining.

Results

SSR development and evaluation of marker polymorphism

A total of 279 positive clones were selected, producing 179 unique sequences. Of these, 99 were amenable to primer design, the remaining 80 were discarded because they either contained very little flanking sequence or the sequences were refractory to primer design. In all, 61 primer pairs (Table 2) reproducibly amplified a product, which consisted of two alleles per template; the remainder amplified poorly, or generated complex profiles. The recovery efficiency was thus 22% (61 out of 279).

Of the 61 CELMS loci, 51 were informative in one of the mapping populations; specifically 39 in C3 and Sp-9A, 43 in C3 and A41, and 50 in C3 and Creta 4. The germplasm panel showed variation at 49 loci (Fig. 1a), allowing for the identification of 208 alleles (2–7 alleles per locus, mean 3.8). The PIC values varied from 0.23 to 0.77 (mean 0.52 ± 0.02); CELMS-05 had the highest PIC, and CELMS-18 the lowest. Each genotype was uniquely distinguished by its combined SSR profile.

Linkage analysis

Of the 39 informative loci between C3 and Sp-9A, 12 segregated only within the female parent C3, 6 only within the male parent Sp-9A, and 21 (15 as 1:1:1:1 and 6 as 1:2:1) within both (Fig. 1b). Four loci suffered from mild segregation distortion, but only CELMS-33 showed a severely distorted segregation and was, therefore, excluded from the mapping exercise. Markers which segregated with only a minor deviation from the expected ratio are identified with one $(\chi^2_{\alpha=0.1} < \chi^2 \leq \chi^2_{\alpha=0.05})$ or two $(\chi^2_{\alpha=0.5} < \chi^2 \leq \chi^2_{\alpha=0.01})$ asterisks in Fig. 2. In all, 29 SSR loci were placed on the C3 map, distributed across 11 of the 18 major (containing a minimum of four markers) LGs described by Lanteri et al. (2006). Seven mapped to LG1. CELM-60 was linked to a previously orphan AFLP marker, thus generating a new LG (LG19, Fig. 2). On the Sp-9A map, 25 loci were placed on 11 of the 17 major LGs, including 6 on LG1; 2 loci allowed the definition of new LGs (LG18 and LG19, Fig. 2). One intercross (CELMS-23) and two female-testcross (CELMS-25 and CELMS-45) loci remained unlinked.

Table 4 Sequence of RT-PCR primers targeted to ORF sequences

Locus	Primer sequence (5′–3′)[a]	
	Forward	Reverse
CELMS-03	ATGGATGGTAACGTTGATATAGAATG	CTCGTAATCAAGAGATTCGATTGG
CELMS-04	AATGGACAGATGACGGTGGT	AGTCACCAGCAGCAGGCATA
CELMS-06	TCCTAATCAGGTGGCTGGAC	CTTTGCCTCTTGGCAAACTC
CELMS-15		TGGGGATCTTCGTGGTAATC
CELMS-18	AACCAAACAAACCAACTTGTGA	
CELMS-20	CAACAGCTCATGTTGCAG	
CELMS-33	TCACAACAAAAATCGCCTCA	GACGACGTCGGTTCTTTCAT
CELMS-37		TCAAACGCAAAGTGAAATCG
CELMS-48	AGAATGCGAAGGCGTCAAC	TGACTTCAACCATGGTATCTTTG
CELMS-52	CCGCTCAAGAGCAAAAGAGA	AATTTGCTGCACAGCTGGAT
CELMS-57	GCAGATGCGACCTCTGGT	ATTCGTTTTGACACCCCAAC
CELMS-60	GGTGGGTATGGAAAGAAGACA	GAAGAACGCGTGTGTTTCAC

[a] When the primer is omitted, the original primer as reported in Table 2 has been used

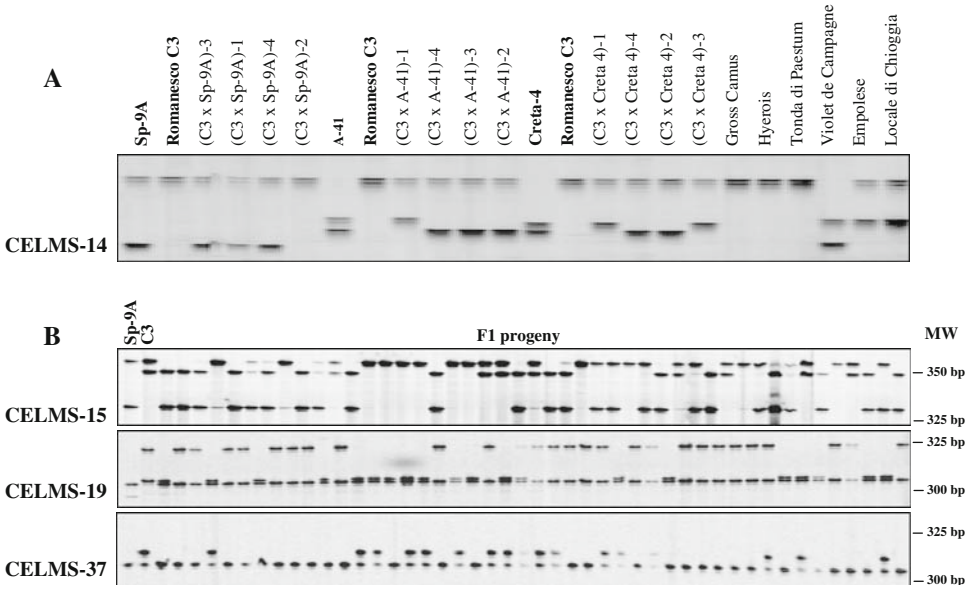


Fig. 1 **a** CELMS-14 profile of the germplasm panel; **b** segregation patterns for three CELMS loci amongst progeny of the cross C3 × Sp-9A

As a result of the integration of the SSR loci, the 19 maternal LGs now comprise 239 markers, spanning 1,373.0 cM with a mean inter-marker distance of 5.7 cM, and cover 53.2% of the estimated *G*. Similarly, the 19 paternal LGs comprise 212 markers, spanning 1,294.9 cM (54.3% of *G*) with a mean inter-marker distance of 6.1 cM. The maternal and paternal maps share all 19 mapped SSR intercross markers, allowing for the definition of homologous LGs. In summary, 35 SSR loci were added to the genetic map, covering 12 of the 16 homologous LGs in addition to three non-aligned groups (Fig. 2).

Sequence analysis and annotation

The annotation pipeline resulted in 39 non-genic CELMS loci and 22 genic CELMS loci that contained at least one ORF (Table 3). Of the 39 non-genic loci, 15 were related to transposon-like elements, and 24 showed no similarity to any existing sequence. Of the 22 which shared sequence homology with database entries, 5 matched a transcription factor, 5 a transport protein, 4 a gene encoding a specific enzyme, 4 a protein involved in the signal transduction cascade, 3 a protein involved in the DNA repair processes and 1 in chromatin assembly (Fig. 3a; Table 3). Nine of the

genes contained a protein–protein interaction domain associated with protein/DNA binding. The majority possess polyglutamine/asparagine, or polyproline tracts, known to be involved in protein–protein interactions (Berger et al. 2001). MegaBlast analysis within the Asteraceae produced nine high *e* value hits in which the CELMS sequence aligned with an EST (http://cgpdb.ucdavis.edu/database/Database_Description.html; Table 3). In 12 loci, the repeat motif was present within an ORF. Of these, CELMS-18, CELMS-20 and CELMS-48 had conserved polyglutamine stretches, matching, respectively, auxin response factor 16 (ARF-16), a transcriptional co-repressor (LUG, Fig. 3b) and phytochrome 1 (PFT1). CELMS-33 and CELMS-37 carried polyproline stretches, matching, respectively, the cytosolic factor family protein 14 (SEC14) and a leucine-rich repeat-like protein. CELMS-38 had a polyhistidine stretch, homologous to a WRKY DNA binding protein (Table 3).

In the ten remaining CELMS, the SSR motifs were located either up- or downstream of the ORFs, or within an intron. Only 4 loci, out of the 22 genic CELMS, showed evidence of transcriptional activity in leaf tissue (CELMS-5, -38, -47, -49), but when new primer pairs were designed targeted to the coding sequence (Table 4), a further 5 such loci (CELMS-18, -20, -37, -48, -52) were identified.

Discussion

SSR development and evaluation of marker polymorphism

Until March 2008, only 173 *Cynara* spp. DNA sequences were present in the GenBank database; these included 32 SSR-containing sequences previously developed (Acquadro et al. 2003, 2005a, b) of which 12 were mapped in the globe artichoke genetic map (Lanteri et al. 2006). The main objective of the present work was to develop additional informative SSR markers from enriched genomic libraries to improve the genetic maps of *C. cardunculus*. At the same time, their usefulness for genotype identification and phylogenetic studies was assessed.

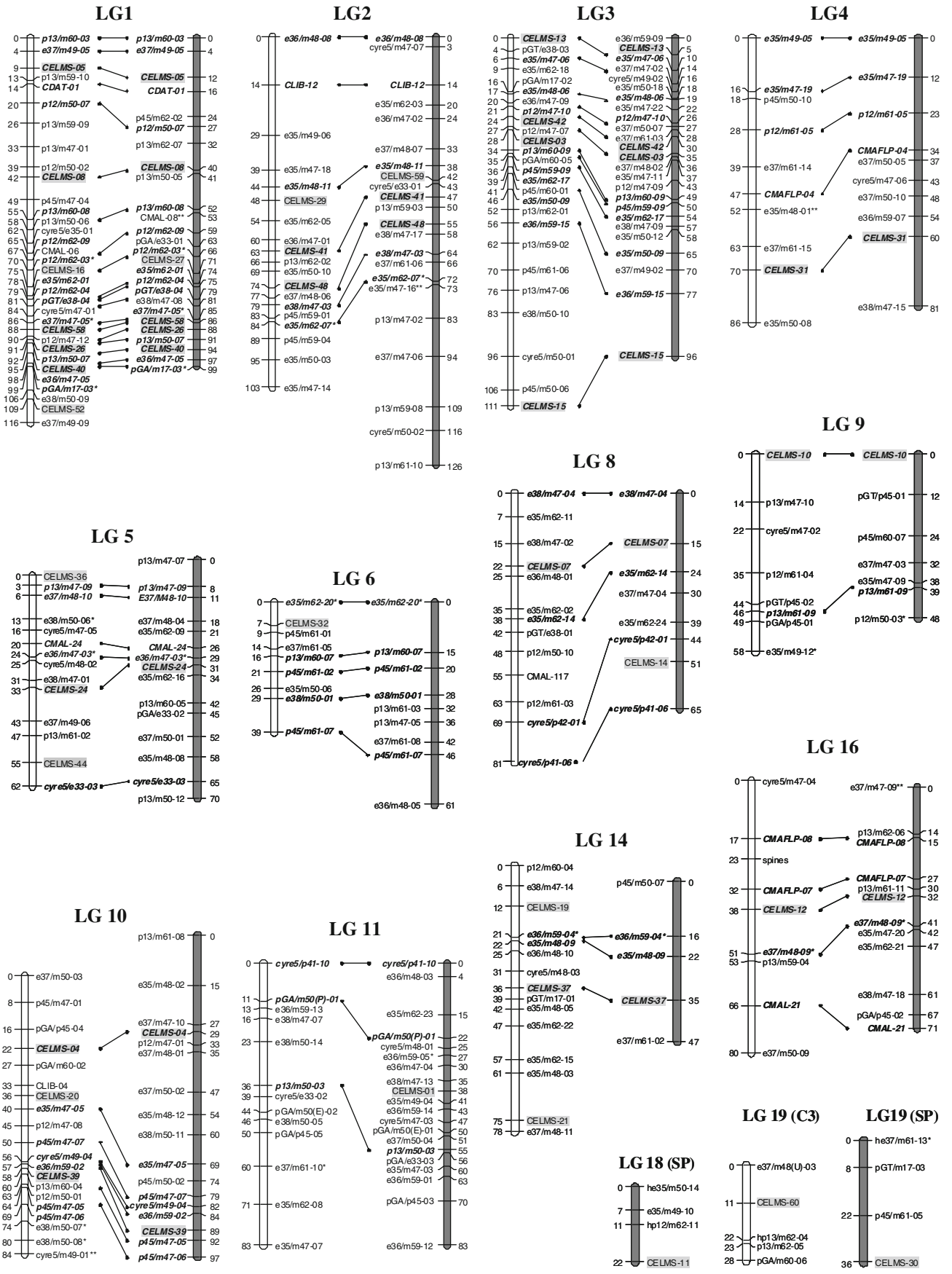
In conventional methods for SSR isolation from genomic libraries, the efficiency of recovery is rather low, varying from 0.045 to 12% (Zane et al. 2002). The necessary procedures tend to be time and labour intensive, and thus costly. As a result, a number of library enrichment methods have been proposed (Acquadro et al. 2005b; Squirrell et al. 2003). Oligo hybridization capture techniques, based on either probe immobilization on filters or on streptavidin-coated magnetic beads, improve the recovery rate of SSR-containing sequences to 20–90% across a variety of taxa (Zane et al. 2002). The enrichment protocol used here was based on the targeting of ten repetitive di- or trinucleotide motifs known to occur frequently in the coding regions of plant genomes (Morgante et al. 2002). A surprisingly high level of redundancy was encountered, resulting in the loss of 100 out of the original 279 positive clones. Duplication of clones was assumed to have occurred during the enrichment phase, and may be associated with the two-step PCR procedure, each comprising 25 cycles. Our subsequent experience has indicated that 15–20 cycles per PCR does reduce the extent of clone redundancy.

The informativeness of the CELMS SSRs was comparable with what has been demonstrated for an earlier set of both globe artichoke (Acquadro et al. 2005b), sunflower (Tang et al. 2003; Paniego et al. 2002) and lettuce (van de Wiel et al. 1999) SSRs. Furthermore, the application of three CELMS (-9, -14, -40) markers for addressing the pattern of genetic diversity of a collection of Sicilian globe artichoke landraces from small-holdings made it possible to gather information on the evolution and domestication of the species (Mauro et al. 2009).

Fig. 2 Genetic maps of C3 (female parent of mapping population, *white* LGs on *the left*) and Sp-9A (male parent, *grey* LGs on *the right*). The 35 mapped SSR loci are shaded *light grey*. Intercross markers are shown in *italics* and in *bold*; aligned LGs are presented side-by-side. LG-7, -12, -13, -15, and -17 are not reported since they are not covered by CELMS markers. Markers showing significant levels of segregation distortion are indicated by *asterisks* ($0.1 > *P \geq 0.05$, $0.05 > **P \geq 0.01$) ▶

Linkage analysis and marker distribution

About 10% of the SSR loci suffered from segregation distortion, consistent with the level found for the markers used by Lanteri et al. (2006) to construct the first globe artichoke genetic maps. Segregation distortion has been associated with statistical bias or errors in genotyping and scoring, but stems mainly from a number of biological phenomena affect meiosis, fertilization and embryogenesis (Bradshaw and Stettler 1994). The presence of null alleles, which is not uncommon in the context of SSR loci (due to failure of one or both primers to anneal), can also contribute to apparent skewing, as homozygotes become indistinguishable from non-null allele containing heterozygotes (Pekkinen et al. 2005). In the present work, we have chosen to include markers deviating at 1% level and above; although the inclusion of distorted loci into the map increases the chance of type I errors of false linkage, these loci can be useful in increasing our knowledge on specific regions of the genome. The newly developed SSR set has increased the number of mapped SSRs from 10 to 39 in the C3 map, and from 8 to 34 in the Sp-9A map. The number


LG1
LG2
LG3
LG4
LG 5
LG 6
LG 8
LG 9
LG 10
LG 11
LG 14
LG 16
LG 18 (SP)
LG 19 (C3)
LG19 (SP)

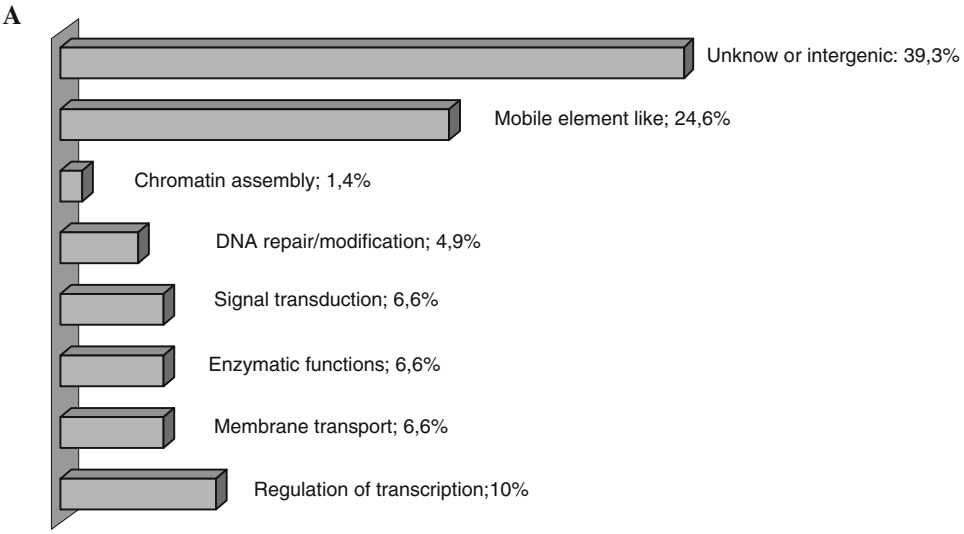


Fig. 3 **a** Gene ontology of CELMS loci; **b** the relationship between a CELMS sequence and its *A. thaliana* homologue based on the alignment of both nucleotides and amino acids. *Boxes* show conserved motifs

of intercross SSRs, which serves as bridge markers between the two maps, was increased from 7 to 26, resulting in the identification of 12 homologous and 3 non-aligned LGs covered by 1–7 SSR markers.

The new female map spanned 1,373.0 cM with a mean inter-marker distance of 5.7 cM, representing only a 3% increase in the total length of the map, but a ~12% decrease in the mean inter-marker distance. Similarly, the male map was increased by ~5% in length, with a ~12% decrease in the mean inter-marker distance.

Since the CELMS markers appear to be well distributed along the LGs, it is likely that SSR loci are dispersed throughout the globe artichoke genome. Some clustering of SSRs has been observed around the putative centromeric region of LG1, -2, -3 and -10, a pattern which is not unusual (Arens et al. 1995; Bhattramakki et al. 2000; Gill

et al. 2006; Jones et al. 2002; McCouch et al. 2002; Ramsay et al. 2000). The addition of new markers has allowed the filling of some of the gaps in the base maps, especially on LGs 4, 8 and 14; and the addition of a second bridge marker to LG9. However, the distal region of LG13 remains sparsely populated, and the gaps in LG6, -14 and -15 remain unfilled. Increasing marker density and the addition of genes underlying phenotypic traits to a map require the creation of mapping populations from parents which segregate for the latter, but retain common sets of markers (Hayes et al. 1996; Weeden et al. 2000). Examples of such consensus maps have been reported for several crops (Ellis et al. 1992; Kleinhofs et al. 1993; Tanksley et al. 1992). Markers in common across populations can serve as anchors to locate important genes to a particular LG, thereby allowing the location of genes underlying phenotype even in populations where these do not segregate. We are currently constructing genetic maps based on crosses between Romanesco C3 and cultivated or wild cardoon, which are genotypically/phenotypically highly divergent to facilitate comparative QTL mapping. A high proportion (49 out of the 61) of the CELMS markers was suitable for mapping in multiple populations, and thus represents a set of robust and informative anchor points in *C. cardunculus* populations.

By blasting all the CELMS loci against the Asteraceae dbESTs, we found nine hits, putative orthologues loci from lettuce, sunflower and chicory. Four of them (CELMS-5, -16, -52 in LG1 and CELMS-48 in LG2) were placed on the globe artichoke linkage maps and might be used as anchor markers for map alignment within the Asteraceae family.

Sequence annotation

We have annotated the CELMS loci in an attempt to convert anonymous markers to those associated with specific biological functions. The sequence of the SSR loci provides a handle on putative function, provided that it shares homology with already characterized orthologous sequences. This approach led to the assigning of putative function to about one-third of the CELMS sequences. Most of these (20 out of 22) were amongst the trinucleotidic motif sequences; the two dinucleotidic types (CELMS-05 and CELMS-60) were both "coding SSRs" carrying GA_n/CT_n as stretches of glutamate–arginine or serine–leucine. The dominance of trinucleotidic motifs in genic SSRs has been reported in both *A. thaliana* and soybean (Morgante et al. 2002).

The sequences of CELMS-18, -20, -33, -37, -38, -47, -48 are likely to be orthologues of genes with known function, as they both show a high level of sequence similarity and retain the SSR sequence in the equivalent position. In CELMS-18 and -20, the orthologous sequences are conserved in the flanking regions, but not in the SSR itself (CAA in globe artichoke and CAG in *A. thaliana*), a pattern which has been previously noted in comparisons between rice and *A. thaliana* (Zhang et al. 2006).

As previously performed in the Solanaceae (Wu et al. 2006, 2009), Fabaceae (Phan et al. 2007; Hougaard et al. 2008; Ellwood et al. 2008) and Asteraceae (Chapman et al. 2007) families, a COS marker approach may represent an effective mean for generating molecular markers. Our comparative analysis amongst the Asteraceae species showed similarity values up to 100% between sequences from globe artichoke and those from the yellow starthistle (*Centaurea solstitialis*) or safflower (*Carthamus tinctorius*); accordingly, an exploration of the Asteraceae dbEST seems very promising for new microsatellite markers mining as well as for synteny studies.

Conclusions

We have developed, annotated and mapped a set of 61 new genomic globe artichoke SSR markers, with the aim to extend the limited number of co-dominant markers currently available; these markers represent valuable tools for genetic analysis of the species. The new SSRs were uniformly distributed in the already developed globe artichoke maps, thus improving their coverage and contributing in future alignment of the new maps under development.

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