Theor Appl Genet (2011) 123:1215–1229
DOI 10.1007/s00122-011-1661-1

ORIGINAL PAPER

Genetic map of artichoke × wild cardoon: toward a consensus map for *Cynara cardunculus*

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Received: 22 March 2011 / Accepted: 9 July 2011 / Published online: 29 July 2011

Abstract An integrated consensus linkage map is proposed for globe artichoke. Maternal and paternal genetic maps were constructed on the basis of an F_1 progeny derived from crossing an artichoke genotype (Mola) with its progenitor, the wild cardoon (Tolfa), using EST-derived SSRs, genomic SSRs, AFLPs, ten genes, and two morphological traits. For most genes, mainly belonging to the chlorogenic acid pathway, new markers were developed. Five of these were SNP markers analyzed through high-resolution melt technology. From the maternal (Mola) and paternal (Tolfa) maps, an integrated map was obtained, containing 337 molecular and one morphological markers ordered in 17 linkage groups (LGs), linked between Mola and Tolfa. The integrated map covers 1,488.8 cM, with an average distance of 4.4 cM between markers. The map was aligned with already existing maps for artichoke, and 12 LGs were linked via 31 bridge markers. LG numbering has been proposed. A total of 124 EST-SSRs and two genes were mapped here for the first time, providing a framework for the construction of a functional map in artichoke. The establishment of a consensus map represents a necessary condition to plan a complete sequencing of the globe artichoke genome.

Communicated by I. Paran.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-011-1661-1) contains supplementary material, which is available to authorized users.

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Introduction

The species complex *Cynara cardunculus* L. includes three *taxa*: the globe artichoke (*C. cardunculus* L. var. *scolymus* (L.) Fiori), the cultivated leafy cardoon (*C. cardunculus* L. var. *altilis* DC.), and the wild cardoon (*C. cardunculus* L. var. *sylvestris* Lam.). This latter botanical entity is widely recognized as the progenitor of the two crops, and the three taxa can be easily crossed producing fertile hybrids (Rottenberg and Zohary 1996; Sonnante et al. 2007). Two genepools can be distinguished within the wild cardoon: the eastern Mediterranean type, mainly distributed in Italy, Greece and Tunisia, and the western genepool, diffused in the Iberian Peninsula (Wiklund 1992). The eastern wild cardoon might have given rise to the artichoke, while the Spanish–Portuguese genotypes might be the progenitors of the cultivated cardoon (Sonnante et al. 2008), and therefore the domestication of the two crops possibly occurred at different times and different places (Foury 1989; Sonnante et al. 2007).

The globe artichoke is widely and traditionally cultivated in the Mediterranean region. More recently, it has also diffused into the Americas, mostly California, Peru, Argentina, and in Asia, mainly China (http://faostat.fao.org). Artichoke immature flower heads are usually consumed as a fresh, frozen, or canned vegetable. Its leaves can be used for the extraction of nutraceutical compounds, mainly flavonoids and chlorogenic acid with its derivatives (dicaffeoylquinic acids), which are also present in the edible part and bioavailable to humans through their diet (Azzini et al. 2007). Artichoke extracts possess anticarcinogenic, anti-HIV, antioxidative, cholesterol-lowering, bile expelling, hepatoprotective, and diuretic activities, as well as antifungal and antibacterial properties (Agarwal and Mukhtar 1996; Gebhardt 1997; Kraft 1997; McDougall et al. 1998; Brown and Rice-Evans 1998; Shimoda et al. 2003; Zhua

et al. 2005). Recently, a number of genes involved in the synthesis of phenylpropanoid compounds have been identified and some of them mapped (De Paolis et al. 2008; Portis et al. 2009; Menin et al. 2010; Sonnante et al. 2010). Another class of enzymes, peroxidases, can participate in a broad range of physiological processes including the biosynthesis of lignin and suberin, defense responses toward stresses, removal of hydrogen peroxide from chloroplasts and cytosol, oxidation of toxic compounds, indole-3-acetic acid regulation, and ethylene biosynthesis. Two full-length cDNAs coding for cationic soluble peroxidases have been recently isolated from artichoke (Cardinali et al. 2011).

The development of molecular marker technology has enhanced not only germplasm characterization and discovery of the genetic basis of complex traits, but also the construction of genetic linkage maps. In plants, genetic markers are useful in breeding programs for marker-assisted selection (Mazur and Tingey 1995), map-based cloning (Tanksley et al. 1995), and anchoring physical maps (Mun et al. 2006; Troggio et al. 2007). Linkage maps are a valuable tool to map genes or markers associated with agronomic and quality traits, as well as to follow traits selected during the domestication process (Kaga et al. 2008; Briggs et al. 2007; Isemura et al. 2010). Further, dense genetic maps are important to prepare contig-based local or genome-wide physical maps, for map-based cloning, and for genome sequencing projects. Since microsatellite (SSR) markers are informative molecular markers for a variety of reasons, high-density microsatellite maps are very effective to reach these objectives.

So far, two linkage maps have been developed in artichoke. The first one was accomplished by analyzing an F_1 population deriving from crossing two artichoke types (Lanteri et al. 2006); a number of microsatellite markers were added to this initial map (Acquadro et al. 2009). The other map was obtained by using an F_1 population derived from a cross between artichoke and cultivated cardoon (Portis et al. 2009). Both maps were based on molecular markers, mainly AFLP, S-SAP, and genomic SSR (gSSR) markers.

A collection of about 36,000 expressed sequence tags (ESTs), originated from randomly selected artichoke cDNAs, is publicly available (http://www.ncbi.nlm.nih.gov/). A set of these expressed sequences also contains SSR arrays, which may be located in the 5′- or 3′-untranslated regions as well as in the coding sequence. About 300 SSR markers derived from these ESTs (EST–SSRs) were used to investigate variation in some artichoke, wild and cultivated cardoon genotypes (Scaglione et al. 2009). EST–SSR markers can be highly informative, since they can be tightly linked with significant allelic variants. They are less expensive in comparison to gSSRs, and are more conserved (Decroocq et al. 2003) and easily transferred to related taxa for comparative analysis (Scott et al. 2000; Gupta et al. 2003). EST–SSR markers have been used to generate linkage maps in a variety of crops such as, rice (Kurata et al. 1994), maize (Chao et al. 1994), wheat (Yu et al. 2004), cassava (Sraphet et al. 2011), etc.

In this study, we report on a new genetic map for artichoke. A total of 192 genotypes were used, from an F_1 hybrid population obtained by crossing a clone of globe artichoke (Mola) with its wild progenitor (Tolfa). The map was based on EST–SSRs, gSSRs, AFLPs, genes involved in the synthesis of caffeoylquinic acids, a gene coding for peroxidase, and two morphological traits. The 124 mapped EST–SSRs and some of the genes had never been mapped before. The alignment with previously published maps allowed proposing a consensus map.

Materials and methods

Plant material and DNA extraction

A single clone of the globe artichoke ‘Mola’ was used as female in an intraspecific cross with a single plant of wild cardoon ‘Tolfa’, used as pollen donor, and about 200 viable F_1 seeds were obtained. After seed germination, plantlets were initially grown in a greenhouse and, at the stage of three leaves, transplanted in an IGV experimental field at Policoro, Italy. Genomic DNA was isolated from young leaves of F_1 plants, using the cetyl trimethyl ammonium bromide (CTAB) method previously described (Sonnante et al. 2002). All plants were checked for hybrid status using the informative Cs-SST1 SSR (Sonnante et al. 2008), and 192 individuals were chosen for further analysis.

Marker analysis

AFLP analyses were performed as established by Vos et al. (1995) with some modifications. Genomic DNA was digested for 3 h at 7°C with 5 U of *Eco*RI and 1 U of *Mse*I, ligated to standard adapters, and amplified by a pair of primers carrying one selective nucleotide (*Eco*RI + A and *Mse*I + C). The preselective PCR step was achieved using 2 min of initial denaturation at 94°C, 35 cycles of 30 s DNA denaturation at 94°C, 40 s annealing at 56°C, and 2 min of extension at 72°C, followed by a final incubation at 72°C for 5 min. Afterward, 34 *Eco*RI/*Mse*I primer combinations with each primer carrying three selective nucleotides were employed to amplify pre-selective amplification products. Selective PCR step conditions were: an initial denaturation at 94°C for 2 min followed by ten cycles-run of 94°C for 20 s, 94°C for 30 s, 66°C down by 1° every cycle to reach 56°C at the last cycle of the

period, 2 min elongation at 72°C, followed by 20 cycles of 94°C for 30 s, 56°C for 30 s, 2 min elongation at 72°C, and ending with a 30 min of incubation at 60°C. At first, 34 primer combinations were screened for their informative segregation on the parental genotypes and on six progeny plants. Only primer combinations producing high-quality and polymorphic amplification products were used to generate genotypic data for the construction of the genetic map. *Eco*RI primer was labeled with IRD700 or Cy5 at the 5′ end. PCR products were analyzed by capillary electrophoresis using a CEQ 8800 sequencer (Beckman Coulter, Fullerton, USA). Presence or absence of informative AFLP fragments was visually scored. Each polymorphic marker segregating as heterozygous in one parent and null in the other (expected segregation ratio 1:1) was given a suffix according to the primer combination and the molecular weight. Only clear-cut fragments were recorded and markers with more than 20% missing data were not included in further analyses.

A set of 301 microsatellite primer pairs obtained from previously published studies or maps (Lanteri et al. 2006; Sonnante et al. 2008; Acquadro et al. 2009; Scaglione et al. 2009) was initially tested on the parental genotypes and six randomly picked plants of the segregating population. Most markers were CyEM EST-SSRs (218, see Scaglione et al. 2009) and CELMS gSSRs (45, see Acquadro et al. 2009). For some CyEM markers, for which no amplification was obtained, reverse primers were redesigned (supplementary Table S1). Three primers were used for the amplification of each SSR locus: a forward primer including a 5′ extension of an M13 18 pb sequence (TGTAAAACGACGGCCAGT), a reverse primer, and an M13 primer (5′ TGTAAAACGACGGCCAGT 3′) fluorescently labeled at the 5′ end for detection by capillary electrophoresis (Schuelke 2000). PCR reactions were performed in a 9700 thermal cycler (Applied Biosystems, Foster City, CA) in 10 µl reaction mixtures containing 2.5 ng template DNA, 0.07 µM forward primer, 0.2 µM reverse primer, 0.2 µM M13-labeled primer (Sigma-Aldrich, Milan, Italy), 0.2 mM of each dNTP, 1 µl 10× buffer, 0.4 U *Taq* DNA polymerase (Invitrogen, Carlsbad, CA) and 1.5 mM $MgCl_2$. Amplification was achieved using 3 min of initial denaturation at 94°C, 38 cycles of 30 s at 94°C, 30 s annealing at optimal primer temperature and 45 s synthesis at 72°C, followed by a final 10 min incubation at 72°C. PCR products were analyzed on an automated sequencer CEQ 8800 (Beckman Coulter) and peaks were identified by size using the proprietary fragment analysis software of the sequencer. Any ambiguous genotype was re-run, re-amplified, or identified as unknown. Polymorphic markers, heterozygous in at least one parental genotype, were applied to the full mapping population, for a total of 149 polymorphic SSR primers (supplementary Table S1). The maximum number of accepted missing data was 38 (20%) for each marker.

Gene mapping

New markers were developed for most genes analyzed in this study (Table 1). SNP markers were used to map the genes HQT (hydroxycinnamoyl-CoA:quinate hydroxycinnamoyltransferase, homologous to accession no. DQ915589), C3′H (*p*-coumaroyl ester 3′-hydroxylase, homologous to accession no. FJ225121), PAL1 (phenylalanine ammonia-lyase, accession no. AM418560), and 4CL (4-coumarate:CoA ligase, accession no. JF338138), C4H (cinnamate-4-hydroxylase, accession no. AM690437). Briefly, for HQT, C3'H, 4CL, and C4H, sequences were amplified in the two parental genotypes and PCR products directly sequenced, to detect SNPs. For PAL1, the intron specific to this member of the artichoke PAL family (De Paolis et al. 2008) was amplified and sequenced. For all the above genes, SNPs were genotyped in the F_1 progeny using high-resolution melting (HRM) approach in a RotorGene 6000 (Corbett Research, Mortlake, AU). A final volume of 10 µl contained 1.5 mM $MgCl_2$, 0.5 U Taq DNA recombinant polymerase (Invitrogen), 1× EvaGreen (Biotium, Hayward, USA), 0.2 mM dNTP, 0.2 µM each specific forward and reverse primer (Table 1), and 12.5 ng DNA. Amplification cycles included a denaturation step at 94°C for 2 min, followed by 40 cycles at 94°C for 30 s, 57°C for 20 s, 72°C for 20 s, and a final elongation step at 72°C for 5 min. HRM was performed in a temperature range varying according to the gene, increasing the temperature by 0.2°C every 2 s. Fluorescent data were acquired at the end of each extension step during PCR cycles and each of the HRM steps with automatic gain optimization. To verify the correct size of amplification products obtained and the correct SNP, for each gene some PCR samples were separated on 1.5% agarose gel and sequenced. Fluorescent data acquired during PCR reaction and DNA melting were analyzed using the RotorGene 6000 series software (Corbett Research), and samples with a high C_T (>30), an end point fluorescence lower than 50% of average fluorescence of all samples, or a low amplification efficiency (<1.4) were repeated or discarded from the analysis. High-resolution melting curve analysis was carried out using the HRM module, and the melting data were normalized by adjusting the start and end fluorescent signals of all the samples analyzed to the same levels.

In HQT2 gene (accession no. FM244907), a mononucleotide (A) repeat detected in the intron sequence and polymorphic between Mola and Tolfa was applied to screen the mapping population (Table 1). The HCT (hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase, homologous to accession no. DQ104740)

Table 1 Mapped genes, primer sequence, marker type, and its corresponding positon in sequence, polymorphism in parental lines, segregation ratio, position on consensus map linkage groups, and accession number of gene sequence

Gene	Primer sequence	Marker type	Position bp[a]	Polymorphism		Segregation ratio	Consensus map	Accession no.
				Mola	Tolfa			
C3′H	F: GATGGATTTCTAATGCCCTTAGTC	SNP	486	C/T	T/T	1.1	LG_XI	JF338141[b]
	R: GCTCCCAGATAACCTTTAACCA							
C4H	F: CAGTGGTGGACGATGTGAAG	SNP	508	A/G	A/G	1:2:1	LG_II	AM690437
	R: AGGATCATCCTCGCTCTC							
4CL	F: TCTCGCTGCCTAAAACACAA	SNP	144	C/T	T/T	1:1	Mola-18	JF338138
	R: CTCCACGTCAGCATAGGTGT							
PAL1	F: TTTTTGTGGGTAAAGTGAAGTCAT	SNP	193 int	T/T	T/C	1:1	LG_XI	AM418560
	R: TATTCCGGCGTTCAAAAATC							
HQT	F: ACGGCTGGGTAACGATGG	SNP	271	G/G	A/G	1:1	LG_XVI	JF338140[b]
	R: CAATGCTACAATCCGCTTCA							
HQT2	F: GGGTTGGCAAGATTGTTGTA	SSR	1110 int	418–419	418–418	1.1	LG_VIII	FM244907
	R: AAATGAGTCACCTGGCGAAC							
HCT	F: ACCGAATCAACACCAACCAT	Indel	5′UTR	120–129	120–120	1:1	LG_III	JF338139[b]
	R: GCTTGATTGACTTTGAAGAAACG							
PAL3[c]	F: ACTTGCCTTCTCTTGTGCCTACCT	SSR	5′UTR	204–229	190–204	1:1:1:1	LG_I	AM418586
	R: TCCGCAACCATTCTCTTCACCTCA							
1-SST[c]	F: AAGCACAACTGGATCCATTC	SSR	3′UTR	266–296	261–261	1:1	LG_I	AM4978438
	R: AAATATAATCTCACAAGTGGA							
PRX	F: ATGAGCCAATTGAGCGTATTG	SSR	3′UTR	268–269	268–268	1:1	Mola-18	GU145300
	R: GGAACACAACAAACCATATATTCA							

[a] Position in cds, or UTR sequence. For PAL1 and HQT2, position of the SSR from the beginning of the intron (int)

[b] Accession numbers refer to sequences obtained in Mola genotype and homologous, respectively, to HCT (accession no. DQ104740), HQT (accession no. DQ915589), and C3′H (accession no. FJ225121)

[c] PAL3 and 1-SST gene markers correspond to Cs-Pal03 and Cs-1SST markers, respectively (Sonnante et al. 2008)

gene was amplified in the two parental genotypes using primers designed in the 5′ and 3′ UTR regions. The presence of a 9-bp gap in the 5′ UTR allowed obtaining a useful marker (Table 1). PAL3 and 1-SST (sucrose:sucrose 1-fructosyltransferase, homologous to accession no. AM4978438) genes were mapped as a result of polymorphic SSRs present in the 5′ UTR and 3′ UTR, respectively (De Paolis et al. 2008; Sonnante et al. 2008, see Table 1). Peroxidase gene (PRX, accession no. GU145300) was sequenced in Mola and Tolfa and a mononucleotide SSR in the 3′ UTR was used for deriving a marker (Table 1).

Morphological traits

The parental lines are characterized by the presence or absence of spines on the head bracts, in particular Mola is a non-spiny varietal type, while Tolfa is a spiny wild type. For each plant of the F_1 progeny, the presence/absence of spines on the head bracts was scored. Another character considered in this study was flower color: Mola has purple flowers, while Tolfa is a white flower genotype. Flower color was scored for each plant of the F_1 progeny.

Genetic mapping

Marker segregation was analyzed with regard to goodness of fit to the expected Mendelian ratio using the Chi-square test. Slightly distorted markers ($0.005 \leq P \leq 0.05$) were kept in the linkage analysis unless they were of low quality and affected the marker order within a linkage group (LG). Genotypic data were subjected to linkage and recombination analysis with JoinMap®4.0 software (van Ooijen 2006) applying the Kosambi function to estimate genetic map distances (Kosambi 1944). LOD (logarithm of the odds) score thresholds equal to 6 with some exceptions (3 LGs in Tolfa with LOD equal to 5) were used to determine LGs; furthermore, the following JoinMap®4.0 parameters were used: Rec = 0.40, LOD = 1, Jump = 5. Markers were grouped as maternal or paternal testcross markers with an expected segregation ratio of 1:1; intercross markers had an expected segregation ratio of 3:1 for dominant markers and

Table 2 Number of markers screened for the artichoke mapping population Mola × Tolfa

Marker type	Screened markers	Polymorphic markers				Unlinked markers	Mapped markers	Markers in the integrated map
		Total	Maternal	Paternal	Intercross			
AFLP	34 PCs[a]	235	159	76	/	11	224	192
SSR	301	149	82	22	45	4	145	137
Morphological markers	2	2	2	0	0	1	1	1
Genes[b]	11	10	6	2	2	0	10	8

[a] PCs primer combinations

[b] Genes as in Table 2

1:2:1 or 1:1:1:1 for codominant ones. Separate linkage maps were constructed for each parent using the double pseudo-testcross mapping strategy (Grattapaglia and Sederoff 1994). Fully informative markers with four or three segregating alleles (ab × cd, ef × eg), double heterozygous markers (hk × hk), and markers segregating only from the maternal genotype (lm × ll) were used to construct the female map; fully informative markers, double heterozygous markers, and markers segregating only from the paternal genotype (nn × np) were used to build the male map.

An integrated consensus map was obtained by using all marker sets together. The resulting LGs were numbered according to Portis et al. (2009) when bridge markers linked the two maps (12 LGs), and serially in descending order of their genetic length for the remaining LGs. The maternal and paternal LGs were numbered following the same criterion as for the integrated map, except for Mola-18, since this LG remained unaligned (see results session).

Results

AFLP and SSR markers

A total of 34 AFLP primer combinations were tested to determine the levels of polymorphism between parents (Table 2). Out of these primer pairs, 15 (Mcac_Eaca, Mcac_Eagg, Mcag_Eaac, Mcag_Eaat, Mcag_Eagt, Mcat_Eaag, Mcat_Eagc, Mcta_Eagg, Mctc_Eagc, Mctg_Eaac, Mctg_Eaag, Mctg_Eaat, Mctg_Eaca, Mctg_Eacg, Mctt_Eacg) were effective in revealing visually clear polymorphic loci between Mola and Tolfa. The selected primer combinations yielded 238 polymorphic and unambiguously scorable bands, with sizes ranging from 63 to 627 bp. The number of bands varied among the different pairs of selective primers. Mcag_Eaac and Mctg_Eaca combinations gave the lowest number of polymorphic bands (10), while Mctc_Eagc combination produced the highest number of polymorphic bands (23). Out of the 235 AFLP markers used for mapping, 159 (67.7%) were present only in Mola while 76 (32.3%) only in Tolfa (Table 2, supplementary Table S1).

For SSR analyses, an approach of PCR amplification using M13-tailed and labeled primers was used (Schuelke 2000). Out of 301 SSR primer pairs initially analyzed on the parental lines, 90 either did not show any amplification or produced non-specific products, whereas 61 were homozygous and therefore 151 primer pairs were discarded. A total of 149 microsatellite markers were informative and exhibited clear and unambiguous single locus amplification on the parents, segregating in the six progeny samples initially tested, and could be therefore used for further linkage analysis (Table 2, supplementary Table S1). Of these, 124 were CyEM EST–SSRs (56.9% of the initial CyEM tested), which, to our knowledge, were mapped here for the first time. In general, Tolfa showed a lower level of heterozygosity than Mola. Out of the 149 SSRs used for mapping, 82 (55%) segregated only in Mola and 22 (14.8%) only in Tolfa, while 45 (30.2%) segregated in both parents: 42 segregated consistently with a 1:1:1:1, 2 with a 1:2:1, and 1 with a 3:1 ratio (Table 2, supplementary Table S1).

Gene mapping

Eleven genes mainly involved in the synthesis of caffeoylquinic acid in artichoke were searched for polymorphisms (SNPs, SSRs or indels) in the two parental lines. SNP genotyping was performed using HRM technology.

Three artichoke HQT genes have been isolated so far (Comino et al. 2009; Sonnante et al. 2010). HQT gene homologous to DQ915589 was sequenced in Mola (accession no. JF338140) and Tolfa, and subsequently mapped using an SNP at position 271 from the ATG (Table 1). HRM analysis provided two types of curves, one for the homozygous (GG) and the other for the heterozygous (AG) genotypes (Fig. 1), with segregation 1:1. The HQT2 gene (accession no. FM244907) marker was heterozygous only in the female parent Mola and segregated 1:1 (Table 1), whereas HQT1 gene (accession no.

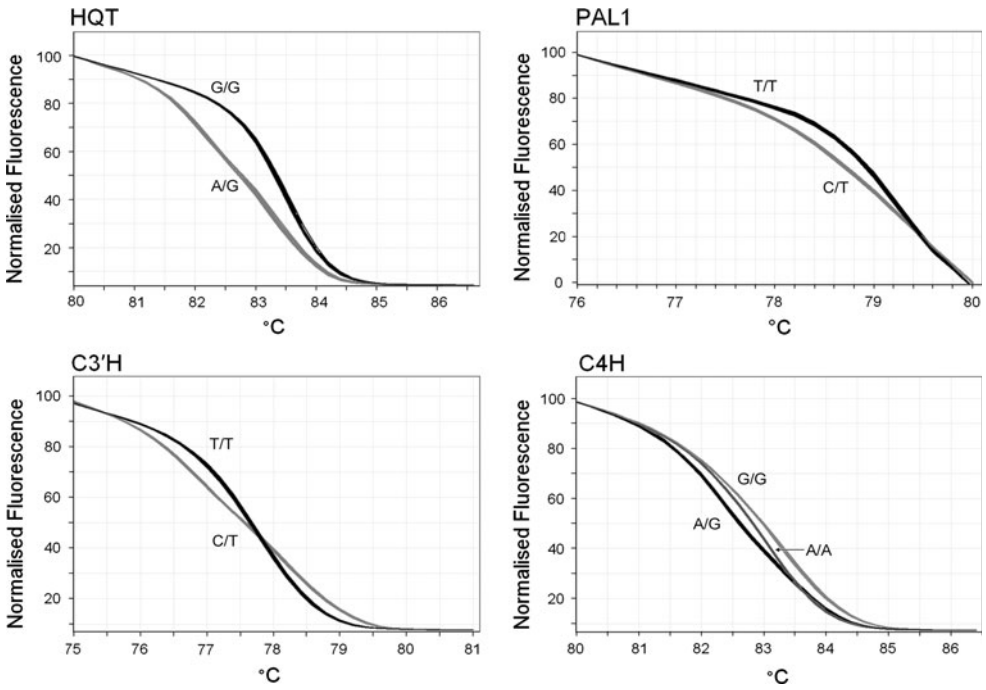


Fig. 1 High-resolution melting analysis of HQT, PAL1, C3′H, and C4H genes. Homozygous or heterozygous curves are indicated

EU677935) was not mapped since both parents were homozygous for all the polymorphisms detected.

The HCT gene was sequenced in Mola (accession no. JF338139) and Tolfa, and was mapped using a 9 nucleotide gap in the 5′ UTR region upstream of the ATG; the male parent was homozygous and the female parent heterozygous. The amplification of the region including this gap yielded a fragment in Tolfa (120 bp) and two fragments in Mola (120–129 bp), with a segregation of 1:1 in the F_1 mapping population (Table 1).

The PAL gene family has been partially isolated and characterized in artichoke (De Paolis et al. 2008). PAL1 (accession no. AM418560) was mapped using an SNP at 193 bp from the beginning of the intron. The male parent was heterozygous (C/T) and the female parent was homozygous (T/T), producing two different curves in HRM analysis (Fig. 1), with a segregation of 1:1 (Table 1). For mapping the PAL3 gene (accession no. AM497826), an SSR marker present in the 5′ UTR region, just upstream the ATG, was used (Sonnante et al. 2008).

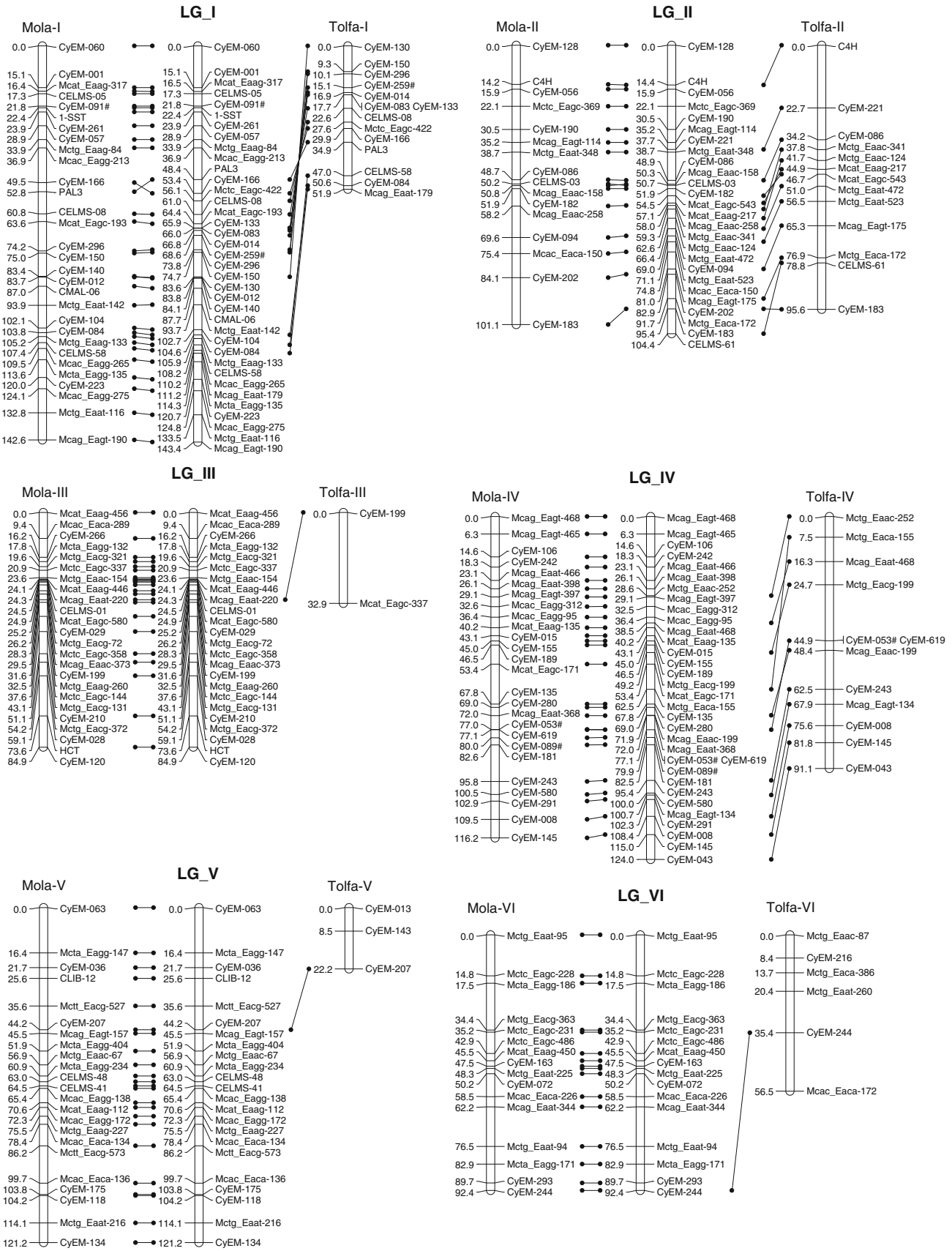
C3′H gene was sequenced in Mola (accession no. JF338141) and an SNP was detected in the second exon at position 486 from the ATG, considering the cds sequence (Table 1). This SNP was mapped only in the female parent Mola (C/T), the male parent Tolfa being homozygous (T/T). Also in this case, HRM analysis evidenced two types of curves (Fig. 1), with a segregation of 1:1.

The putative 4CL gene was first completely isolated from Mola artichoke. For this purpose, three artichoke EST sequences (GE578240, GE581210, GE583703), included in contig CL3632 by Scaglione et al. (2009) and similar to 4CL from other plant species, were used to design primer pairs and amplify a fragment of the gene sequence, which was completed at the 3′ end by RACE PCR. The whole sequence was obtained from Mola (accession no. JF338138), and screened for the presence of SNPs or indels. The chosen SNP was at 144 bp from the ATG (cds sequence), for which Mola was heterozygous (C/T) and Tolfa was homozygous (T/T; Table 1).

Putative artichoke C4H gene (accession no. AM690437) was sequenced in both parents and an SNP (position 508 bp from ATG, cds sequence) for which both Mola and Tolfa were heterozygous (A/G) was chosen to develop a marker for genetic mapping (Table 1, Fig. 1).

PRX gene marker was a mononucleotide SSR, for which Mola was heterozygous and Tolfa homozygous, with a segregation 1:1 (Table 1). For the 3′ UTR SSR already described for 1-SST gene (De Paolis et al. 2008), the female parent was heterozygous and the male parent homozygous. As expected, the observed segregation ratio was 1:1 (Table 1).

Fig. 2 Genetic maps of the globe artichoke Mola (female parent) on the *left*, wild cardoon Tolfa (male parent) on the *right*, and the integrated consensus linkage map in the *middle*. LGs with fewer than four markers are shown as unnumbered minor groups at the *bottom*, unless useful for linking the two maps. Intercross markers are indicated by *connecting lines*. Molecular marker names are on the *right side* of each linkage group; genetic distances are on the *left* (*cM* Kosambi mapping function) ▶


LG_I
Mola-I
0.0 CyEM-060
15.1 CyEM-001
16.4 Mcat_Eaag-317
17.3 CELMS-05
21.8 CyEM-091#
22.4 1-SST
23.9 CyEM-261
28.9 CyEM-057
33.9 Mctg_Eaag-84
36.9 Mcac_Eagg-213
49.5 CyEM-166
52.8 PAL3
60.8 CELMS-08
63.6 Mcat_Eagc-193
74.2 CyEM-296
75.0 CyEM-150
83.4 CyEM-140
83.7 CyEM-012
87.0 CMAL-06
93.9 Mctg_Eaat-142
102.1 CyEM-104
103.8 CyEM-084
105.2 Mctg_Eaag-133
107.4 CELMS-58
109.5 Mcac_Eagg-265
113.6 Mcta_Eagg-135
120.0 CyEM-223
124.1 Mcac_Eagg-275
132.8 Mctg_Eaat-116
142.6 Mcag_Eagt-190
Tolfa-I
0.0 CyEM-130
9.3 CyEM-150
10.1 CyEM-296
15.1 CyEM-259#
16.9 CyEM-014
17.7 CyEM-083 CyEM-133
22.6 CELMS-08
27.6 Mctc_Eagc-422
29.9 CyEM-166
34.9 PAL3
47.0 CELMS-58
50.6 CyEM-084
51.9 Mcag_Eaat-179
LG_II
Mola-II
0.0 CyEM-128
14.2 C4H
15.9 CyEM-056
22.1 Mctc_Eagc-369
30.5 CyEM-190
35.2 Mcag_Eagt-114
38.7 Mctg_Eaat-348
48.7 CyEM-086
50.2 CELMS-03
50.8 Mcag_Eaac-158
51.9 CyEM-182
58.2 Mcag_Eaac-258
69.6 CyEM-094
75.4 Mcac_Eaca-150
84.1 CyEM-202
101.1 CyEM-183
Tolfa-II
0.0 C4H
22.7 CyEM-221
34.2 CyEM-086
37.8 Mctg_Eaac-341
41.7 Mctg_Eaac-124
44.9 Mcat_Eaag-217
46.7 Mcat_Eagc-543
51.0 Mctg_Eaat-472
56.5 Mctg_Eaat-523
65.3 Mcag_Eagt-175
76.9 Mctg_Eaca-172
78.8 CELMS-61
95.6 CyEM-183
LG_III
Mola-III
0.0 Mcat_Eaag-456
9.4 Mcac_Eaca-289
16.2 CyEM-266
17.8 Mcta_Eagg-132
19.6 Mctg_Eacg-321
20.9 Mctc_Eagc-337
23.6 Mctg_Eaac-154
24.1 Mcat_Eaag-446
24.3 Mcag_Eaat-220
24.5 CELMS-01
24.9 Mcat_Eagc-580
25.2 CyEM-029
26.2 Mctg_Eacg-72
28.3 Mctc_Eagc-358
29.5 Mcag_Eaac-373
31.6 CyEM-199
32.5 Mctg_Eaag-260
37.6 Mctc_Eagc-144
43.1 Mctg_Eacg-131
51.1 CyEM-210
54.2 Mctg_Eacg-372
59.1 CyEM-028
73.6 HCT
84.9 CyEM-120
Tolfa-III
0.0 CyEM-199
32.9 Mcat_Eagc-337
LG_IV
Mola-IV
0.0 Mcag_Eagt-468
6.3 Mcag_Eagt-465
14.6 CyEM-106
18.3 CyEM-242
23.1 Mcag_Eaat-466
26.1 Mcag_Eaat-398
29.1 Mcag_Eagt-397
32.6 Mcac_Eagg-312
36.4 Mcac_Eagg-95
40.2 Mcat_Eaag-135
43.1 CyEM-015
45.0 CyEM-155
46.5 CyEM-189
53.4 Mcat_Eagc-171
67.8 CyEM-135
69.0 CyEM-280
72.0 Mcag_Eaat-368
77.0 CyEM-053#
77.1 CyEM-619
80.0 CyEM-089#
82.6 CyEM-181
95.8 CyEM-243
100.5 CyEM-580
102.9 CyEM-291
109.5 CyEM-008
116.2 CyEM-145
Tolfa-IV
0.0 Mctg_Eaac-252
7.5 Mctg_Eaca-155
16.3 Mcag_Eaat-468
24.7 Mctg_Eacg-199
44.9 CyEM-053# CyEM-619
48.4 Mcag_Eaac-199
62.5 CyEM-243
67.9 Mcag_Eagt-134
75.6 CyEM-008
81.8 CyEM-145
91.1 CyEM-043
LG_V
Mola-V
0.0 CyEM-063
16.4 Mcta_Eagg-147
21.7 CyEM-036
25.6 CLIB-12
35.6 Mctt_Eacg-527
44.2 CyEM-207
45.5 Mcag_Eagt-157
51.9 Mcta_Eagg-404
56.9 Mctg_Eaac-67
60.9 Mcta_Eagg-234
63.0 CELMS-48
64.5 CELMS-41
65.4 Mcac_Eagg-138
70.6 Mcat_Eaag-112
72.3 Mcac_Eagg-172
75.5 Mctg_Eaag-227
78.4 Mcac_Eaca-134
86.2 Mctt_Eacg-573
99.7 Mcac_Eaca-136
103.8 CyEM-175
104.2 CyEM-118
114.1 Mctg_Eaat-216
121.2 CyEM-134
Tolfa-V
0.0 CyEM-013
8.5 CyEM-143
22.2 CyEM-207
LG_VI
Mola-VI
0.0 Mctg_Eaat-95
14.8 Mctc_Eagc-228
17.5 Mcta_Eagg-186
34.4 Mctg_Eacg-363
35.2 Mctc_Eagc-231
42.9 Mctc_Eagc-486
45.5 Mcat_Eaag-450
47.5 CyEM-163
48.3 Mctg_Eaat-225
50.2 CyEM-072
58.5 Mcac_Eaca-226
62.2 Mcag_Eaat-344
76.5 Mctg_Eaat-94
82.9 Mcta_Eagg-171
89.7 CyEM-293
92.4 CyEM-244
Tolfa-VI
0.0 Mctg_Eaac-87
8.4 CyEM-216
13.7 Mctg_Eaca-386
20.4 Mctg_Eaat-260
35.4 CyEM-244
56.5 Mcac_Eaca-172

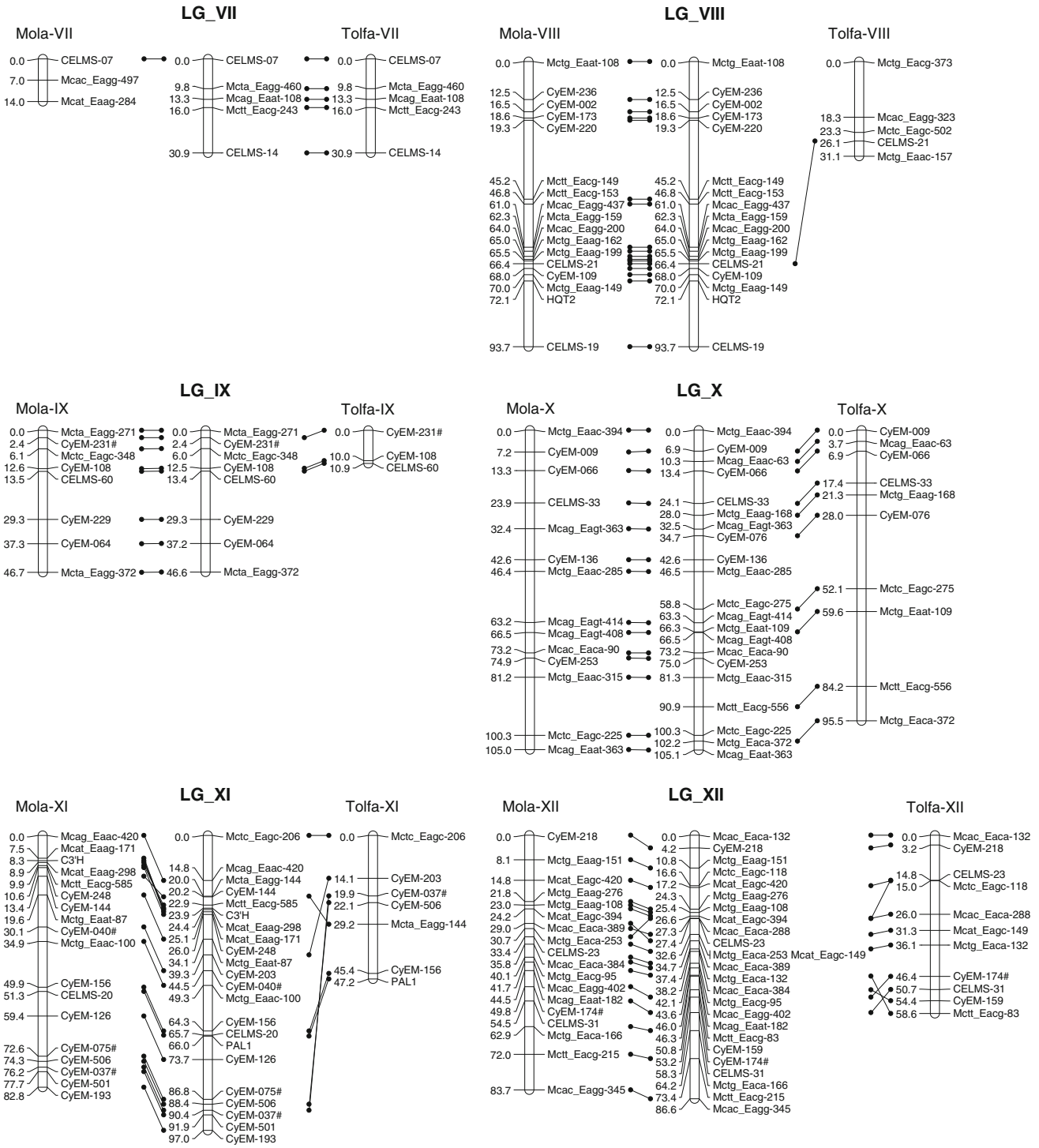


Fig. 2 continued

Morphological traits

Flower head spinosity and flower color were found to segregate in the Mola × Tolfa mapping population. Presence/absence of spines segregated in a 1:1 ratio, confirming that this trait is controlled by a single gene with two alternative alleles: dominant non-spiny (*Sp*) and recessive wild-type spiny (*sp*) (Basnizki and Zohari 1994). Tolfa was homozygous recessive *spsp*, while Mola was heterozygous *Spsp*.

Crosses between Mola (purple flowers) and Tolfa (white flowers) produced hybrid F_1 plants showing a flower color

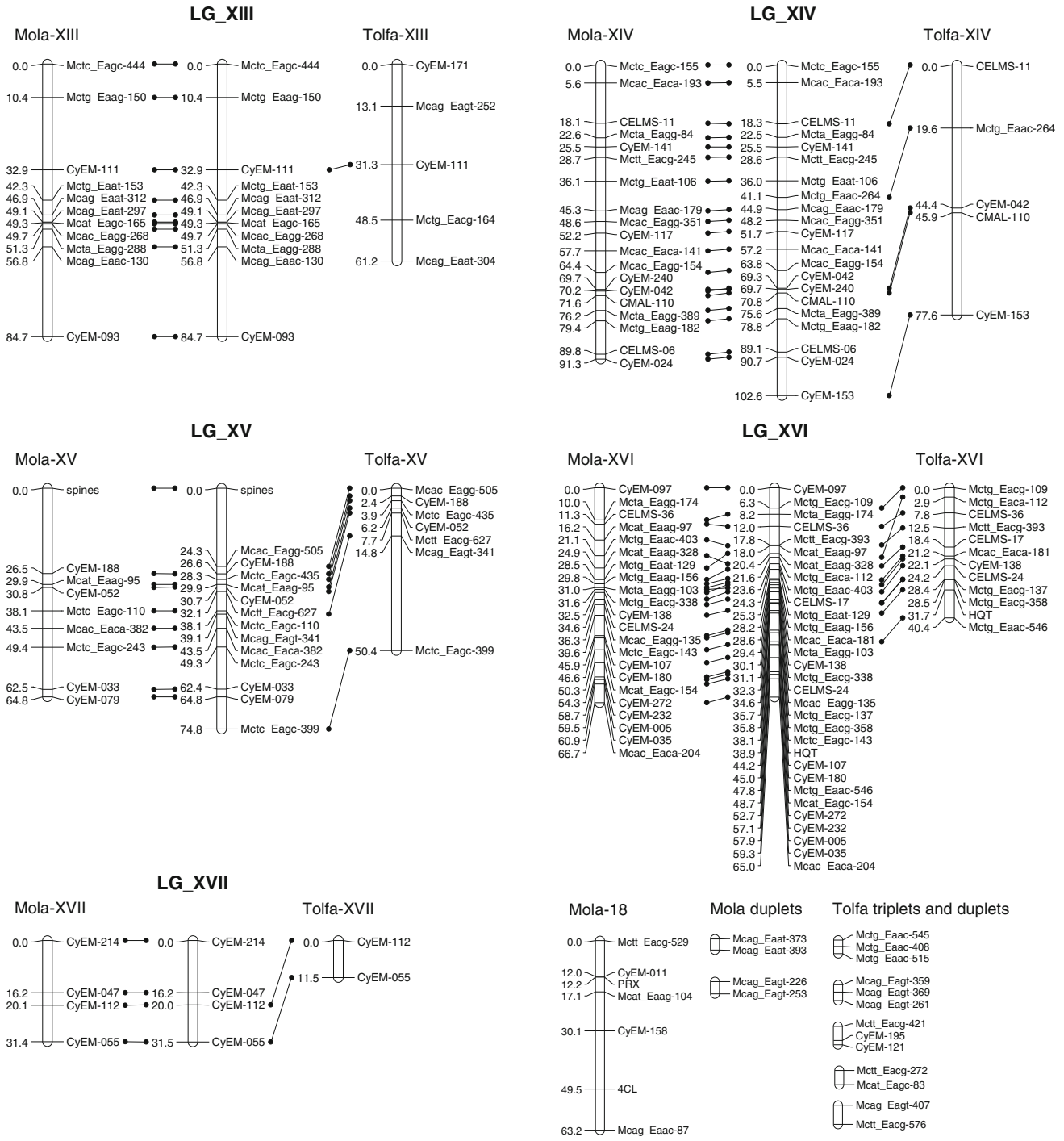


Fig. 2 continued

segregation ratio of 1:1 light-purple:white. Flower color inheritance was analyzed by Foury and Aubert (1977), who described the absence of color as a monofactorial recessive trait. Therefore, our genotypes would be: *Bb* (purple) and *bb* (white), even though we observed a lighter color in the F_1 purple flowers, compared to the maternal genotype.

Construction of parental and integrated maps

To develop the linkage map for the female parent, Mola, a total of 296 markers were used (Fig. 2). Of these, 289 markers were ordered on 18 LGs, including one triplet (group of three markers) used to align to paternal maps

Table 3 Main characteristics of the linkage groups in maternal, paternal, and integrated maps

Mola				Mola x Tolfa				Tolfa				Published maps	
LG	No. of markers	Size (cM)	AVI[a] (cM)	LG	No. of markers	Size (cM)	AVI[a] (cM)	LG	No. of markers	Size (cM)	AVI[a] (cM)	Acquadro et al. (2009)	Portis et al. (2009)
Mola-I	30	142.6	4.7	LG_I	37	143.4	3.9	Tolfa-I	14	51.9	3.7	LG1	LG I
Mola-II	16	101.1	6.3	LG_II	26	104.4	4.0	Tolfa-II	13	95.6	7.3	LG3	LG II
Mola-III	24	84.9	3.5	LG_III	24	84.9	3.5	Tolfa-III	2	32.9	16.4	LG11	LG III
Mola-IV	26	116.2	4.5	LG_IV	33	124.0	3.8	Tolfa-IV	12	91.1	7.6	–	–
Mola-V	23	121.2	5.3	LG_V	23	121.2	5.3	Tolfa-V	3	22.2	7.4	LG5	LG V
Mola-VI	16	92.4	5.8	LG_VI	16	92.4	5.8	Tolfa-VI	6	56.5	9.4	–	–
Mola-VII	3	14.0	4.7	LG_VII	5	30.9	6.2	Tolfa-VII	5	30.9	6.2	LG8	LG VII
Mola-VIII	17	93.7	5.5	LG_VIII	17	93.7	5.5	Tolfa-VIII	5	31.1	6.2	LG14	LG VIII
Mola-IX	8	46.7	5.8	LG_IX	8	46.6	5.8	Tolfa-IX	3	10.9	3.6	LG19	LG IX
Mola-X	14	105.0	7.5	LG_X	21	105.1	5.0	Tolfa-X	10	95.5	9.5	–	LG X
Mola-XI	18	82.8	4.6	LG_XI	22	97.0	4.4	Tolfa-XI	7	47.2	6.7	LG10	LG XI
Mola-XII	18	83.7	4.6	LG_XII	25	86.6	3.5	Tolfa-XII	11	58.6	5.3	LG4	LG XII
Mola-XIII	11	84.7	7.7	LG_XIII	11	84.7	7.7	Tolfa-XIII	5	61.2	12.2	–	–
Mola-XIV	19	91.3	4.8	LG_XIV	21	102.6	4.9	Tolfa-XIV	5	77.6	15.5	LG18	LG XIV
Mola-XV	9	64.8	7.2	LG_XV	14	74.8	5.3	Tolfa-XV	7	50.4	7.2	–	–
Mola-XVI	22	66.7	3.0	LG_XVI	31	65.0	2.1	Tolfa-XVI	12	40.4	3.4	LG5	LG XVI
Mola-XVII	4	31.4	7.8	LG_XVII	4	31.5	7.9	Tolfa-XVII	2	11.5	5.7	–	–
Mola-18	7	63.2	9.0	–	–	–	–	–	–	–	–	–	–
Total	285	486.4	5.2		338	1,488.4	4.4		122	865.5	7.1		

Triplets and duplets are considered only when useful for the connection between the parental maps. For the integrated map (Mola × Tolfa), LGs are numbered according to Portis et al. (2009) when common markers are available, or in descending order for the other LGs. Distances are in cM

[a] AVI: average marker interval

(Table 3), plus two unnumbered duplets (groups of two markers), while 7 markers remained unlinked. The total length of Mola map was 1,486.4 cM with an average distance of 5.2 cM between markers. The largest LG consisted of 30 mapped markers with a length of 142.6 cM (Table 3). AFLP markers were quite evenly scattered over 17 LGs, with some clustering in LG_V, LG_VIII, and LG_XIII. SSR markers were distributed over all the 18 LGs, with 103 newly mapped CyEM markers on 17 LGs. Eight genes were located on the maternal map, on six different LGs. The newly mapped PRX gene mapped on Mola-18 together with the 4CL gene. HQT2 gene was also mapped for the first time on LG Mola-VIII (see below).

For the linkage map of the male parent, Tolfa, 149 molecular markers were used (Fig. 2). Of these, 135 were mapped: 122 into 17 numbered LGs including two triplets and two duplets (used to align to maternal map), while three triplets and two duplets were not numbered. Fourteen markers remained unlinked (Table 3). The map length was 865.5 cM with an average distance of 7.1 cM between markers. The largest LG consisted of 13 mapped markers with a length of 95.6 cM (Table 3). AFLP markers were distributed over 15 LGs, while SSR markers were present in all LGs. Four genes mapped on the male map, on four different LGs, among which was the newly mapped PAL1 on LG Tolfa-XI.

Initially, the deviating markers (χ^2 test, $P < 0.005$) were excluded from linkage analysis to avoid false linkage. However, after constructing a robust map, we tried to add one by one the distorted markers. In each parental line, 29 (Mola) or 24 (Tolfa) loci were distorted, representing 9.8 or 16.1% of the total markers used for mapping, respectively. Of these, 26 in Mola (9% of mapped markers) and 15 in Tolfa (11% of total mapped markers) were added to the female or male map, respectively.

As for morphological traits, spinosity could be located only on the maternal map, on Mola-XV (Fig. 2), since the female parent was heterozygous for this trait. Conversely, flower color could not be included in any LG and therefore remained unmapped.

The integrated map was developed using 356 molecular markers, and one morphological marker, and consisted of a total of 337 linked molecular and one morphological markers ordered into 17 LGs (Fig. 2). Maternal Mola-18 remained as a single LG, since no bridge marker was shared between this group and any of the paternal LGs. Mola-18 also contained 4CL and the newly isolated PRX genes. In the integrated map, 18 markers from the female or male map were unmapped; of these, 2 segregated in the female parent, and 16 in the male parent. The LGs on the integrated map were numbered from I to XVII (Table 3, see below). The integrated map covered 1,488.8 cM distance with an average distance of 4.4 cM between markers. The largest LG consisted of 37 mapped markers with a length of 143.4 cM. In total, 44 intercross markers of the integrated map were shared between the male and female maps, mainly EST–SSRs (CyEM), together with PAL3 and C4H genes. CyEM markers were distributed over 16 LGs in the integrated map.

Alignment with other maps and LG numbering

To obtain a consensus map for artichoke, our integrated map was aligned to other available maps (Lanteri et al. 2006; Acquadro et al. 2009; Portis et al. 2009). For this purpose, the position of common bridge SSR markers and genes was compared. In total, 31 SSRs and genes were in common between the present map and previous maps, distributed over 12 LGs of the integrated consensus map and on an unlinked LG (supplementary Table S2). Among the bridge markers distributed over 12 LGs, 24 were SSRs (21 CELMS, 2 CMAL, 1 CLIB) and 7 genes: PAL3, 1-SST, C4H, HCT, C3′H, HQT, and HQT2 (supplementary Table S2, see below). In general, for the LGs containing more than two bridge markers, the order of markers was maintained in the consensus map compared to the map by Portis et al. (2009).

The gene 4CL (LG Mola-18) was added to the previously published map on a new small unlinked LG by Menin et al. (2010). Menin et al. (2010) also mapped two acyltranferases (acyltrans_1 and acyltrans_2) from artichoke. We aligned their gene sequences to HQT1 and HQT2 described in Sonnante et al. (2010) and found that acyltrans_1 displayed 99% identity to HQT2, and 79% to HQT1, whereas acyltansf_2 exhibited 99 or 79% identity to HQT1 or HQT2, respectively. Therefore, we can assume that acyltranf_1 corresponds to HQT2, and acyltransf_2 is an allelic form of HQT1. This is confirmed by the fact that HQT2 maps on the same LG_VIII like acyltransf_1, and we can hence suppose that HQT1 maps on LG_II, like acyltransf_2.

In our map, LGs were numbered according to Portis et al. (2009) when common markers could be identified. For LGs showing no common bridge markers, a number was assigned on the basis of LG length and content, each of these LGs being identified for the presence of specific CyEM markers (Fig. 2).

Discussion

For obtaining the linkage map described here, a fairly large F_1 population derived from crossing a globe artichoke with a wild cardoon was screened. The two parental lines, Mola and Tolfa, differed for several characters, such as the presence of spines, flower head shape and size, flowering

time, plant height and shape, flower color, content of polyphenols, etc. Since artichoke is a highly heterozygous crop, a strategy based on a two-way pseudo test cross was chosen for mapping (Grattapaglia and Sederoff 1994). The linkage map was mainly based on, EST–SSR, gSSR, AFLP markers, and several genes, most of which are involved in the synthesis of caffeoylquinic acids.

Although often showing a lower level of polymorphism than genomic microsatellites, EST–SSRs are very effective molecular markers for the production of functional maps, since they are directly linked to expressed genes and therefore allow the straight mapping of agronomically important loci (Nicot et al. 2004). However, the knowledge on artichoke genome is still scarce and the only publicly available genomic resource at the moment is represented by about 36,000 ESTs, which have been assembled in unigenes and searched for SSRs (CyEM loci, Scaglione et al. 2009). Out of a total of 218 CyEM markers tested, we were able to map nearly 57%, and the remaining either did not amplify, produced uninterpretable amplification products or were homozygous in both parents. The lack of amplification of some EST–SSR primers could be due to the presence of large introns in the region between the two primers, primers designed on intron–exon junctions, or to a lower efficiency of M13 primers compared to directly labeled PCR primers (Spiller et al. 2011). In 13 cases, we redesigned reverse primers on the same EST regions that did not produce an amplification product, obtaining an amplicon of the expected size; a larger fragment was found in one case (CyEM-091), possibly due to the presence of an intron. The 124 functional CyEM loci mapped on almost all the 17 LGs of the integrated map, and on an LG of the female parent unlinked to the male map. To our knowledge, these EST–SSR markers had never been mapped before, and therefore this represents a step toward the construction of a functional map for artichoke. The availability of functional genetic linkage maps combined with gene ontology information can provide beneficial contribution to comparative genomics and to marker-assisted breeding by direct gene selection for target traits (Gadaleta et al. 2009).

The integrated map contained 17 LGs, which is the haploid chromosome number of *C. cardunculus*. The male map was smaller than the female and integrated maps. The relatively smaller size might be due to lower rates of recombination, and greater genome coverage could be obtained by increasing the number of molecular markers (Riaz et al. 2006). Indeed, the wild male parent retained a much higher level of homozygosity compared to the cultivated artichoke used as female parent. This was already observed by analyzing the distribution of SSR diversity in artichoke, wild and cultivated cardoon (Sonnante et al. 2008). For artichoke, it has been suggested that, like for other vegetatively propagated crops, farmers might have selected propagation material on the basis of heterotic traits associated with high-level of heterozygosity (Sonnante et al. 2007, 2008). However, it cannot be excluded that during the domestication and differentiation of artichoke, some seed-propagated material showing heterotic traits might have been added to vegetatively propagated material, as in other crops like cassava (Pujol et al. 2005). Even nowadays, in some Mediterranean areas under traditional agricultural systems, farmers cultivate seed-propagated artichokes not belonging to modern seed propagated cultivars and maintain vegetatively only those plants possessing the desired traits (Sonnante et al. 2007). Similar to our observations for wild cardoon, the level of heterozygosity was found to be lower than in artichoke also in the seed-propagated cultivated cardoon (Portis et al. 2009).

To establish a consensus map for artichoke, all the SSR and gene markers mapped in the published maps for *C. cardunculus* (Acquadro et al. 2009; Lanteri et al. 2006; Portis et al. 2009) were screened in our parental genotypes. A total of 31 bridge markers were positioned on our integrated consensus map and were in common with at least one of the published maps. These markers allowed to link 12 of our LGs to the map based on a cross between artichoke and cultivated cardoon (Portis et al. 2009). All the other LGs could be identified for the presence of specific EST–SSRs. The shared bridge markers maintained the same groupings and generally the same order in the LGs, as in the previous published maps. When considering different mapping populations, rearrangements in marker order can be partly explained by different recombination events or by small population sizes (Spiller et al. 2011). In fact, the reliability of genetic maps depends on the marker saturation of the map and on the size of the experimental populations, marker order inversions being a general problem in populations of about 100 individuals (Ferreira et al. 2006). For this reason, we decided to analyze a fairly large segregating population of 192 individuals, removing markers with more than 20% missing data.

Our female Mola map (1,486.4 cM) has the same length as the C3 female map (1,486.8 cM) in Portis et al. (2009), while our integrated consensus map cannot be directly compared to other artichoke maps, since, to our knowledge, this is the first integrated map for *C. cardunculus* published so far.

Several genes involved in the synthesis of chlorogenic acid were mapped. In most cases, SNPs were detected and subsequently analyzed using high-resolution melt approach, a post-PCR technique initially employed in biomedical research to detect mutations and distinguish SNPs (Liew et al. 2004; Herrmann et al. 2007) that can be used for mutation scanning and genotyping (Gundry et al. 2003). In plants, HRM has been successfully applied to discover and map SNPs in barley (Lehmensiek et al. 2008),

almond (Wu et al. 2009), and tomato (Shirasawa et al. 2010). For all the genes analyzed in this study, comparison of resultant melt profiles allowed homozygous and heterozygous sequence variants to be distinguished on the basis of differing shape and position of the melt curve. Therefore, HRM proved to be an efficient, rapid, and cost-effective method for mapping SNPs also in artichoke.

In the case of gene families, we were able to map more than one gene member. In particular, two HQT and two PAL genes were mapped. The acyltransferase-coding genes HQT1 and HQT2 are both involved in the latest steps of chlorogenic acid synthesis in artichoke. They both possess an intron of different length and sequence, display differential expression levels in artichoke organs and tissues, and in various physiological stages of the plant; moreover, recombinant proteins retain a differential substrate specificity: HQT1 is more active in the formation of caffeoylquinate (chlorogenic acid), while HQT2 shows a higher affinity toward coumaroylquinate (Sonnante et al. 2010). The ectopic expression of HQT1 in *Nicotiana* led to an increase in the content of chlorogenic acid and its derivative cynarin (dicaffeoylquinate). We were able to map HQT2 (LG_VIII), but not HQT1, since for this latter gene both parents were homozygous even in the promoter region. By sequence similarity, we have assumed that HQT1 and HQT2 correspond to acyltransf_2 and acyltrans_1 in Menin et al. (2010), respectively. In fact, HQT2 maps on the same LG as acyltrans_1 and we infer that HQT1 maps on LG_II, like acyltranf_2. Another partially characterized HQT gene (Comino et al. 2009) was mapped on the same group (LG_XVI) as in a previous map (Portis et al. 2009).

PAL genes catalyze the first step of the phenylpropanoid pathway. In a previous work, four PAL coding sequences were isolated and partially characterized, showing differential expression in artichoke tissues (De Paolis et al. 2008). Two members of this family were mapped on different LGs; while PAL3 marker had already been mapped (Sonnante et al. 2008; Portis et al. 2009), PAL1 was mapped here for the first time and was located on LG_XI.

The presence of spines on flower head bracts was mapped on LG_XV. Portis et al. (2009) recorded spines on leaves and, although both parents were supposed to be heterozygous for the trait (non-spiny), spinosity was only mapped on the male parent on LG_XIII. Since spines were recorded on different organs and no other common bridge marker was located on the LG mapping spines, we decided not to align the two LGs.

Conclusions

We have established a consensus linkage map for artichoke, based on the analysis of an F_1 population obtained by crossing artichoke x wild cardoon. The map includes EST-derived and genomic SSRs, AFLPs, genes, and one morphological trait. In particular, 124 EST–SSRs, and the genes PAL1 and PRX have been mapped here for the first time. A number of common bridge markers and genes have allowed the alignment of the present map to previously published maps for artichoke. Numbering of LGs has been proposed. Although further markers need to be added to obtain a more saturated map, an integrated consensus map opens new perspectives for the identification of artichoke chromosomes using physical mapping strategies and represents a necessary condition to plan a complete sequencing of the artichoke genome.

Acknowledgments We are grateful to Giuseppe Sonnante and Donatella Danzi for technical assistance. This work was partially supported by MiPAAF (Ministero delle Politiche Agricole Alimentari e Forestali), project "CarVarVi—Valorizzazione di germoplasma di carciofo attraverso la costituzione varietale ed il risanamento da virus" and by MiPAAF, project "Implementazione nazionale trattato FAO risorse genetiche vegetali".

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