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A first linkage map of globe artichoke (*Cynara cardunculus* var. *scolymus* L.) based on AFLP, S-SAP, M-AFLP and microsatellite markers

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Abstract We present the first genetic maps of globe artichoke (*Cynara cardunculus* var. *scolymus* L. $2n=2x=34$), constructed with a two-way pseudo-testcross strategy. A F_1 mapping population of 94 individuals was generated between a late-maturing, non-spiny type and an early-maturing spiny type. The 30 AFLP, 13 M-AFLP and 9 S-SAP primer combinations chosen identified, respectively, 352, 38 and 41 polymorphic markers. Of 32 microsatellite primer pairs tested, 12 identified heterozygous loci in one or other parent, and 7 were fully informative as they segregated in both parents. The female parent map comprised 204 loci, spread over 18 linkage groups and spanned 1330.5 cM with a mean marker density of 6.5 cM. The equivalent figures for the male parent map were 180 loci, 17 linkage groups, 1239.4 and 6.9 cM. About 3% of the AFLP and AFLP-derived markers displayed segregation distortion with a *P* value below 0.01, and were not used for map construction. All the SSR loci were included in the linkage analysis, although one locus did show some segregation distortion. The presence of 78 markers in common to both maps allowed the alignment of 16 linkage groups. The maps generated provide a firm basis for the mapping of agriculturally relevant traits, which will then open the way for the application of a marker-assisted selection breeding strategy in this species.

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Introduction

Globe artichoke (*Cynara cardunculus* var. *scolymus* L.) makes an important contribution to the Mediterranean agricultural economy, producing over 800 kt of crop from more than 80 kha of cultivated land (FAO data 2004: http://www.faostat.fao.org/). Almost 85% of the world artichoke production originates from Europe. The species is also grown in North Africa, the Middle East, South America, the USA and China (FAO data 2004). The edible part of the plant is the head (formally the capitulum), which is the immature composite inflorescence, used as both a fresh and a canned delicacy worldwide. Each plant produces small, medium and large heads, with the largest formed at the apex of the terminal buds along the central stem. The smaller heads develop on the lateral branches. The origin of the artichoke dates back to the era of Theophrastus, the Greek (371–287 BCE) who described their cultivation in Southern Italy and Sicily. In 77 CE, the Roman naturalist Pliny the Elder mentioned their use for medicinal purposes, but it was most probably between 800 and 1,500 CE that the artichoke was domesticated and transformed, presumably in monastery gardens, into the plant which we know today.

Artichoke is a non-fat, zero cholesterol food, rich in folate (vitamin B), vitamin C and minerals, and is a promising source of biopharmaceuticals, such as inulin from its roots (Brown and Rice-Evans 1998), and antioxidant compounds, such as luteolin and di-caffeoylquinic acids from its leaves (Gebhardt 1997). Furthermore, good eating quality oil can be extracted from its seeds (Maccarone et al. 1999) and the whole plant can be used for the production of ligno-cellulosic biomass for energy or paper pulp manufacture (Gominho et al. 2001). Italy is the leading producer of globe artichoke (480 kt per year, FAO data 2004), and also houses the most abundant in situ diversity (Bianco 1990). Distinct varietal groups, well adapted to local environments and local tastes, are generally identified on the basis of harvest time (early- to late-maturing types), size and shape of the head, and

presence/absence of spines on the head bracts. Recently, a living worldwide collection of 89 varietal types was characterized by AFLP profiling (Lanteri et al. 2004), and two major, genetically differentiated groups were identified: group A includes the non-spiny types with elongated or spherical or sub-spherical capitula, and group B the spiny and non-spiny types with medium-small capitula. The fingerprint data provided a demonstration that the traits selected by man have played an important role in shaping the variation and differentiation within cultivated artichoke, and supported the hypothesis that globe artichoke was domesticated from wild cardoon (*Cynara cardunculus* var. *sylvestris*).

Globe artichoke is predominantly cross-pollinating. Cross-fertilization is largely enforced in nature by protandry, so that by applying simple strategies of pollen preservation and application, it is possible to obtain selfed progenies (Mauromicale and Ierna 2000); however, repeated selfing does induce a considerable level of inbreeding depression (Pécaut 1983). At present, commercial production is based mainly on the perennial cultivation of vegetatively propagated clones via crown shoots; although vegetative propagation is costly and is responsible for pathogen diffusion (mainly viruses), it guarantees higher yields of marketable artichokes. Seed-propagated cultivars are becoming popular in some parts of the world, particularly in Israel, Spain and USA (Basnizki and Zohary 1987, 1994; Mauromicale et al. 2004), but they often lack uniformity and their performance is rather unpredictable. At present artichoke breeding is limited to a small number of studies aimed at understanding the inheritance of some major traits (Pécaut 1993; Lopez Anido et al. 1998; Mauromicale et al. 2000). Common breeding aims are to promote earliness, yield and quality, and selection is largely based on intra-clonal variation (Deidda 1967; Abbate and Noto 1981; Pécaut 1993; Mauromicale et al. 2000; Gil and Villa 2003). Few attempts have been made to use hybridization between varietal groups to generate novel genetic combinations (Basnizki and Zohary 1987, 1994; Miller 1975; Scarascia Mugnozza and Pacucci 1976; Tesi 1976). In order to move to a crossing strategy for breeding, some knowledge of artichoke genetics would be advantageous, in particular a framework of linkage relationships, which will facilitate the identification and localization of genes controlling important traits, subsequently opening the way for marker-assisted selection.

The aim of the present work was to develop the first marker-based genetic maps of globe artichoke by applying a combination of marker technologies. The strategy adopted was the double pseudo-testcross, pioneered in *Eucalyptus* by Grattapaglia and Sederoff (1994), and subsequently applied to a number of out-breeding species such as *Poa pratensis* (Porceddu et al. 2002), *Alstroemeria aurea* (Han et al. 2002), *Salix* spp. (Hanley et al. 2002; Barcaccia et al. 2003), *Olea europea* (Wu et al. 2004), *Larix decidua* (Arcade et al. 2000), *Vitis* spp. (Doucleff et al. 2004), *Carya illinoinensis* (Beedanagari et al. 2005) and *Malus* spp. (Kenis and Keulemans 2005). This approach produces two independent maps, one for each parent (Weeden 1994; Atienza et al. 2002; Yin et al. 2002; La Rosa et al. 2003), and is particularly suited to cross-pollinating species, where individuals typically display a high level of heterozygosity.

Materials and methods

Plant material and DNA isolation

A controlled intraspecific cross was performed in the experimental fields at the University of Catania in Cassibile (Siracusa, Sicily), using as female a single clone of 'Romanesco C3', and as male a single clone of 'Spinoso di Palermo'. The former is a non-spiny varietal type, while the latter carries long sharp spines on its bracts and leaves. Seeds obtained from the cross were germinated in lightly moistened potting mix at room temperature. Emergence was observed within about 10 days, and healthy seedlings were transferred to the field after 30 days, at which stage there were typically three true leaves. The presence and absence of spines was scored on well-developed leaves of each F_1 plant. Two weeks after transplanting in the field DNA was extracted from each plant following the procedures described by Lanteri et al. (2001), and DNA concentration was estimated by ethidium bromide-fluorometry against DNA standards. After checking each presumptive F_1 plant for hybridity using informative SSR markers (data not shown), 94 progenies were selected for segregation analysis and genetic map construction. However, because a large number of AFLP fingerprints for one individual were not readable, the genetic maps were finally based on a population of 93 individuals.

Marker analysis

For AFLP fingerprinting, we adapted the protocol of Vos et al. (1995), as detailed by Lanteri et al. (2003). Briefly, 5 µl of extracted DNA (400–500 ng) were co-digested with *Eco*RI (or *Pst*I) and *Mse*I, and ligated to standard adapters. The ligation reaction was used as a template for pre-amplification using primers complementary to the adapter sequences plus one selective nucleotide, namely *Eco*RI+A (or *Pst*I+A) and *Mse*I+C. Selective amplification was subsequently carried out using primers carrying two or three selective nucleotides. Amplified fragments were electrophoretically resolved on 5% denaturing polyacrylamide gels and silver stained as described by Bassam et al. (1991). Example of AFLP profiles are shown in Fig. 1. S-SAP fingerprinting (Waugh et al. 1997) used a procedure based on the AFLP protocol above. For the selective amplification, one AFLP primer was replaced with the fluorescence-labelled (IRD-700) *Cyre5* primer designed to anneal to a retroelement LTR (A. Acquadro, E. Portis, A. Moglia, F.Magurno, S. Lanteri, submitted), and the other was an

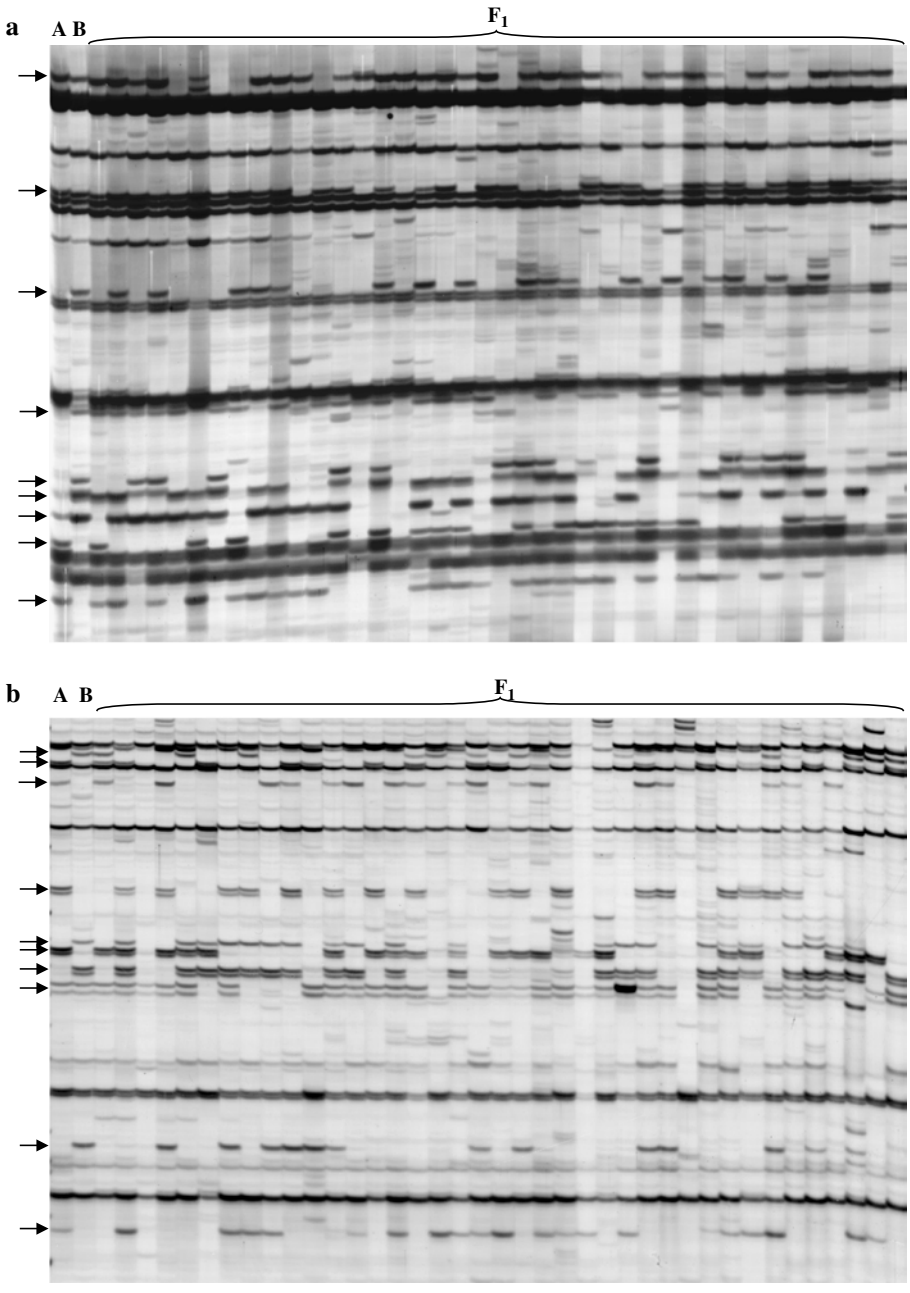

Fig. 1 AFLP patterns of 40 F_1 plants and parents, 'Spinoso di Palermo' (*A*) and 'Romanesco C3' (*B*), amplified with the E33/M48 (**a**) and E33/M50 (**b**) primer combinations. Segregating AFLP markers are indicated by *arrows*

unlabelled AFLP primer (*Eco*RI, *Mse*I or *Pst*I) with three selective nucleotides. PCR products were separated on a DNA analyser Gene ReadIR 4200 (LI-COR) in 6.5% polyacrylamide gels (Sigma), as described by Jackson and Matthews (2000). The M-AFLP fingerprinting method followed the procedure described by Albertini et al. (2003), using the AFLP pre-amplification product as a template. Briefly, selective amplifications were carried out using a standard two or three selective base AFLP primer (*Eco*RI, *Mse*I or *Pst*I) in combination with an 5′-anchored microsatellite primer [PolyGA: GTC$(GA)_8$ or PolyGT: GAC$(GT)_8$]. PCR products were separated as described for S-SAP fingerprinting. SSR profiling used primer pairs developed in our laboratory (Acquadro et al. 2003, 2005a, b). PCR amplification regimes were as detailed by Acquadro et al. (2003) and amplicons were separated and stained as for AFLP, except that the polyacrylamide content of the gels was increased from 5 to 6%.

Mapping and linkage analysis

Electrophoretic patterns were documented using the Gel Documentation System (Quantity One Programme, BioRad), analysed twice and only reliable markers considered. Markers were separated into three types: (a) maternal testcross markers, segregating only within 'Romanesco C3': i.e., female parent A_1A_2, male parent

A_1A_1 (expected monogenic segregation ratio of 1:1), (b) paternal testcross markers, segregating only within 'Spinoso di Palermo': i.e., male A_1A_2, female A_1A_1, (c) intercross markers, segregating within both parents: i.e., either both parents A_1A_2 (expected segregation ratio = 3:1 for dominant markers, 1:2:1 for co-dominant), or one parent A_1A_2, and the other A_1A_3 (or A_3A_4), (1:1:1:1). The goodness-of-fit between observed and expected segregation data was assessed using the chi-square (χ^2) test. Markers segregating in a Mendelian fashion ($\chi^2 \leq \chi^2_{\alpha=0.1}$) or deviating only slightly from it ($\chi^2_{\alpha=0.1} < \chi^2 \leq \chi^2_{\alpha=0.01}$) were used for map construction, while those showing highly significant segregation distortion ($\chi^2 > \chi^2_{\alpha=0.01}$) were excluded. Markers with missing data for more than 30 of the 93 F_1 individuals were excluded. Two separate data sets were therefore assembled: one was used to construct a linkage map for 'Romanesco C3' (markers a and c) and the other for 'Spinoso di Palermo' (markers b and c). The data were analysed using JoinMap 2.0 software (Stam and Van Ooijen 1995). For both maps, linkage groups were accepted at a LOD threshold of 4.0. To determine marker order within a linkage group, the following JoinMap parameter settings were used: Rec = 0.40, LOD = 1.0, Jump = 5. Map distances were converted to centiMorgans using the Kosambi mapping function (Kosambi 1944). Where a discrepancy arose in the order of markers common to both a maternal and paternal linkage group, the marker order of the '1:1' segregating markers was used as a 'fixed order' to reconstruct the separate parental linkage groups. The order of common markers was then inferred by minimizing the number of singletons between the '3:1' and '1:1' segregating markers in the maternal and paternal data sets. A singleton is assumed to be suspect as a data point because it implies a double recombination event (Han et al. 2002; Isidore et al. 2003).

Linkage maps were drawn using MapChart 2.1 software (Voorrips 2002). AFLP, S-SAP and M-AFLP loci were named according to primer combination (PC) code (Table 1) with multiple markers generated by a given PC ordered by decreasing molecular weight. SSR loci were

Table 1 AFLP, S-SAP and M-AFLP primer combinations used for linkage analysis

	*Eco*RI/*Mse*I template		*Pst*I/*Mse*I template	
	PC	Code	PC	Code
AFLP	E+ACA/M+CAA	e35/m47	P+AC/M+CAA	p12/m47
	E+ACA/M+CAC	e35/m48	P+AC/M+CAT	p12/m50
	E+ACA/M+CAG	e35/m49	P+AC/M+CTT	p12/m62
	E+ACA/M+CAT	e35/m50	P+AG/M+CAA	p13/m47
	E+ACA/M+CTT	e35/m62	P+AG/M+CAT	p13/m50
	E+ACC/M+CAA	e36/m47	P+AG/M+CTA	p13/m59
	E+ACC/M+CAC	e36/m48	P+AG/M+CTC	p13/m60
	E+ACC/M+CTA	e36/m59	P+AG/M+CTG	p13/m61
	E+ACG/M+CAA	e37/m47	P+AG/M+CTT	p13/m62
	E+ACG/M+CAC	e37/m48	P+ATG/M+CAA	p45/m47
	E+ACG/M+CAG	e37/m49	P+ATG/M+CAT	p45/m50
	E+ACG/M+CAT	e37/m50	P+ATG/M+CTA	p45/m59
	E+ACG/M+CTG	e37/m61	P+ATG/M+CTC	p45/m60
	E+ACT/M+CAA	e38/m47	P+ATG/M+CTG	p45/m61
	E+ACT/M+CAT	e38/m50	P+ATG/M+CTT	p45/m62
S-SAP	Cyre5/M+CAA	cyre5/m47	Cyre5/P+AGC	cyre5/p40
	Cyre5/M+CAC	cyre5/m48	Cyre5/P+AGT	cyre5/p42
	Cyre5/M+CAG	cyre5/m49	Cyre5/P+AGG	cyre5/p41
	Cyre5/M+CAT	cyre5/m50		
	Cyre5/E+AAG	cyre5/e33		
	Cyre5/E+ACA	cyre5/e35		
M-AFLP	PolyGA/E+AAG	pGA/e33	PolyGA/M+CAT	pGA/m50(P)
	PolyGA/E+ACA	pGA/e35	PolyGA/P+ATG	pGA/p45
	PolyGA/M+CAT	pGA/m50(E)	PolyGT/P+ATG	pGT/p45
	PolyGA/M+CC	pGA/m16		
	PolyGA/M+CG	pGA/m17		
	PolyGA/M+CTC	pGA/m60		
	PolyGT/E+ACT	pGT/e38		
	PolyGT/M+CAA	pGT/m47		
	PolyGT/M+CC	pGT/m16		
	PolyGT/M+CG	pGT/m17		

named using the original primer nomenclature. Markers that 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 ($\chi^2_{\alpha=0.05} < \chi^2 \leq \chi^2_{\alpha=0.01}$) asterisk (Fig. 2). Independent linkage maps were constructed for each parent using the double pseudo-testcross mapping strategy (Weeden 1994).

Estimation of genome length

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. In this method, G is given by the expression $N(N\text{-}1)X/K$, where N is total number of mapped markers in the major groups, X is the observed maximum distance between two adjacent framework markers in centi-Morgans at a certain minimum LOD score, and K the number of markers pairs with a LOD value at the same minimum LOD score.

Results

AFLP, S-SAP and M-AFLP markers

Sixty-four AFLP PCs (four *Eco*RI primers × eight *Mse*I primers and four *Pst*I primers × eight *Mse*I primers) were used to generate fingerprints of both parents and six F_1 progenies. On the basis of the number of polymorphic markers detected, the 30 most informative PCs (15 *Eco*RI/*Mse*I and 15 *Pst*I/*Mse*I, Table 1) were taken forward for mapping. In all, 352 polymorphic AFLP markers were identified, of which 66% were heterozygous in one parent and absent in the other (testcross markers), with the remainder being heterozygous in both parents (intercross markers). The number of polymorphic AFLP markers per PC ranged from 8 to 25, with a mean of 11.7 markers per PC (Table 2). For S-SAP, the same group of 30 PCs were applied to the parents and sample progenies, and nine of these were used for mapping (Table 1). The number of polymorphic S-SAP markers per PC ranged from 3 to 10 (mean 4.6 per PC), and 41 S-SAP markers were identified, of which 25 were testcross and 16 were intercross markers (Table 2). Following a screen of 36 M-AFLP PCs, 13 were used for mapping (Table 1). The number of polymorphic M-AFLP markers ranged from 1 to 6 per PC, and generated a total of 38 (32 testcross, 6 intercross) informative markers (mean 2.9 per PC). Of the 295 testcross markers, 160 (54%) were heterozygous in 'Romanesco C3' and the remaining 135 (46%) in 'Spinoso di Palermo'. An analysis of genotype frequencies showed that about 14% of these AFLP and AFLP-derived markers suffered from segregation distortion ($\chi^2 > \chi^2_{\alpha=0.1}$). Alleles in the male parent showed more segregation distortion than did those in the female parent (16 vs. 10%). Twelve highly distorted markers ($\chi^2 > \chi^2_{\alpha=0.01}$) were discarded prior to the construction of linkage maps.

Microsatellite markers

Twelve of the 32 SSR primer pairs assayed were informative: these were CDAT-01, CLIB-04, CLIB-12 (Acquadro et al. 2003), CMAL-06, CMAL-8, CMAL-21, CMAL-24, CMAL-108, CMAL-117 (Acquadro et al. 2005a), CMAFLP-04, CMAFLP-07 and CMAFLP 08 (Acquadro et al. 2005b). CLIB-04, CMAL-06 and CMAL-117 segregated only in the female parent, while CMAL-08 and CMAL108 segregated only in the male parent. The remaining loci segregated in both parents either in the ratio 1:1:1:1 (CDAT-01 and CLIB-12) or 1:2:1, and were thus located on both the male and female linkage maps. All the SSR loci were included in the linkage analyses, as minor segregation distortion ($\chi^2_{\alpha=0.05} < \chi^2 \leq \chi^2_{\alpha=0.01}$) was observed only for one locus (CMAL-08).

Map construction

After the exclusion of 12 markers showing highly significant levels of distortion, 432 markers were available for map construction (including presence/absence of spines). In all, the maps were built from 300 loci for 'Romanesco C3' (maternal) and from 275 for 'Spinoso di Palermo' (paternal). Of these, 143 loci were in common between both maps.

For the maternal map, 204 markers were assignable to 18 major linkage groups (LGs), each containing a minimum of four markers (Fig. 1), and a further 19 markers were distributed as five triplets and two doublets. Fifteen testcross (9%) and 62 intercross (42%) markers remained unlinked. The length of the individual LGs varied from 27.0 to 132.4 cM (mean 73.9 cM), comprising 4–26 loci per LG (mean 11.3). The mean inter-marker distance was 6.5 cM, and the longest gap of 26.6 cM was found on LG4. Although the majority (76%) of map intervals were less than 10 cM, some large gaps remain in the map. The markers generated by *Eco*RI/*Mse*I and *Pst*I/*Mse*I were evenly distributed across all 18 LGs, without any obvious clustering of markers generated by any one PC. Only four LGs (LG6, 13, 14 and 15) were composed solely of AFLP loci. Microsatellite loci (SSR and M-AFLP) and S-SAP fragments were distributed over, respectively, nine and eight LGs. Seventeen loci with minor segregation distortion were mapped to 12 LGs (14 at $\alpha = 0.05$ and three at $\alpha = 0.01$), and groups of distorted loci in close linkage to one another were detected on LG1, LG10 (three loci per LG) and LG5 (two loci).

For the paternal map, 180 loci were arranged into 17 LGs, with three triplets and three doublets (Fig. 1). Seventeen testcross (13%) and 62 intercross (42%) markers remained unlinked. The length of the LGs varied from 26.5 to 126.7 cM (mean 72.9 cM). The number of loci per LG varied from 4 to 21 (mean 10.6), giving a

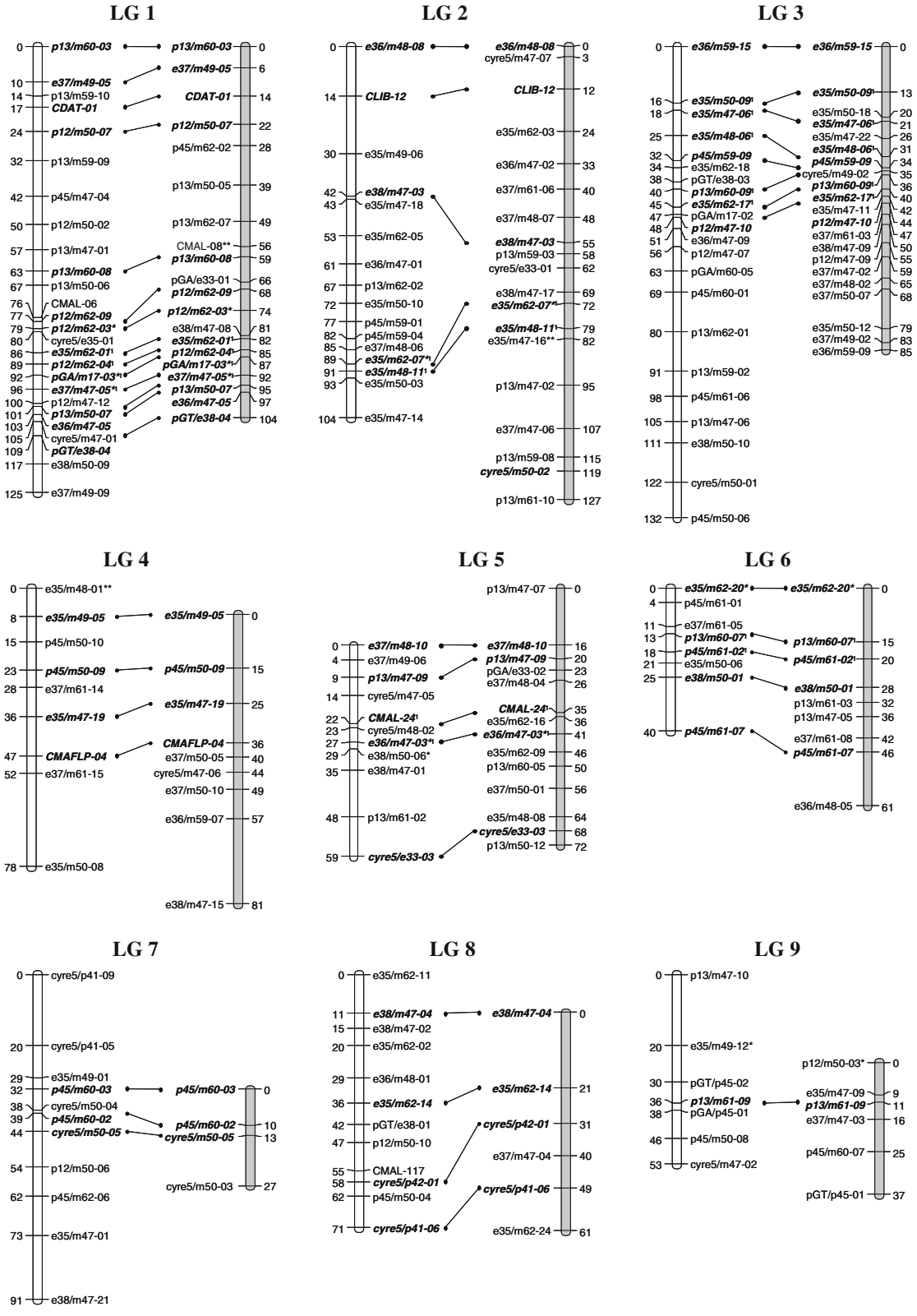

Fig. 2 Genetic maps of the globe artichoke varietal types 'Romanesco C3' (female parent, *white* LGs on the *left*) and 'Spinoso di Palermo' (male parent, *grey* LGs on the *right*). Aligned LGs are presented side-by-side. For the female map, marker names are shown on the *right* of each linkage group with map distances (in centiMorgans) on the *left*; for the male map, the mirror arrangement applies. Intercross markers are shown in *italics*, and in *bold* for those used to align LGs, which are connected with a *line*. LGs with fewer than four markers are shown as 'minor groups'. Markers showing significant levels of segregation distortion are indicated by *asterisks* ($*0.1 > P \geq 0.05$, $**0.05 > P \geq 0.01$). The 'fixed order' of bridge markers identified with superscript 1 was imposed by minimizing the number of singletons between the '3:1' and '1:1' segregating markers

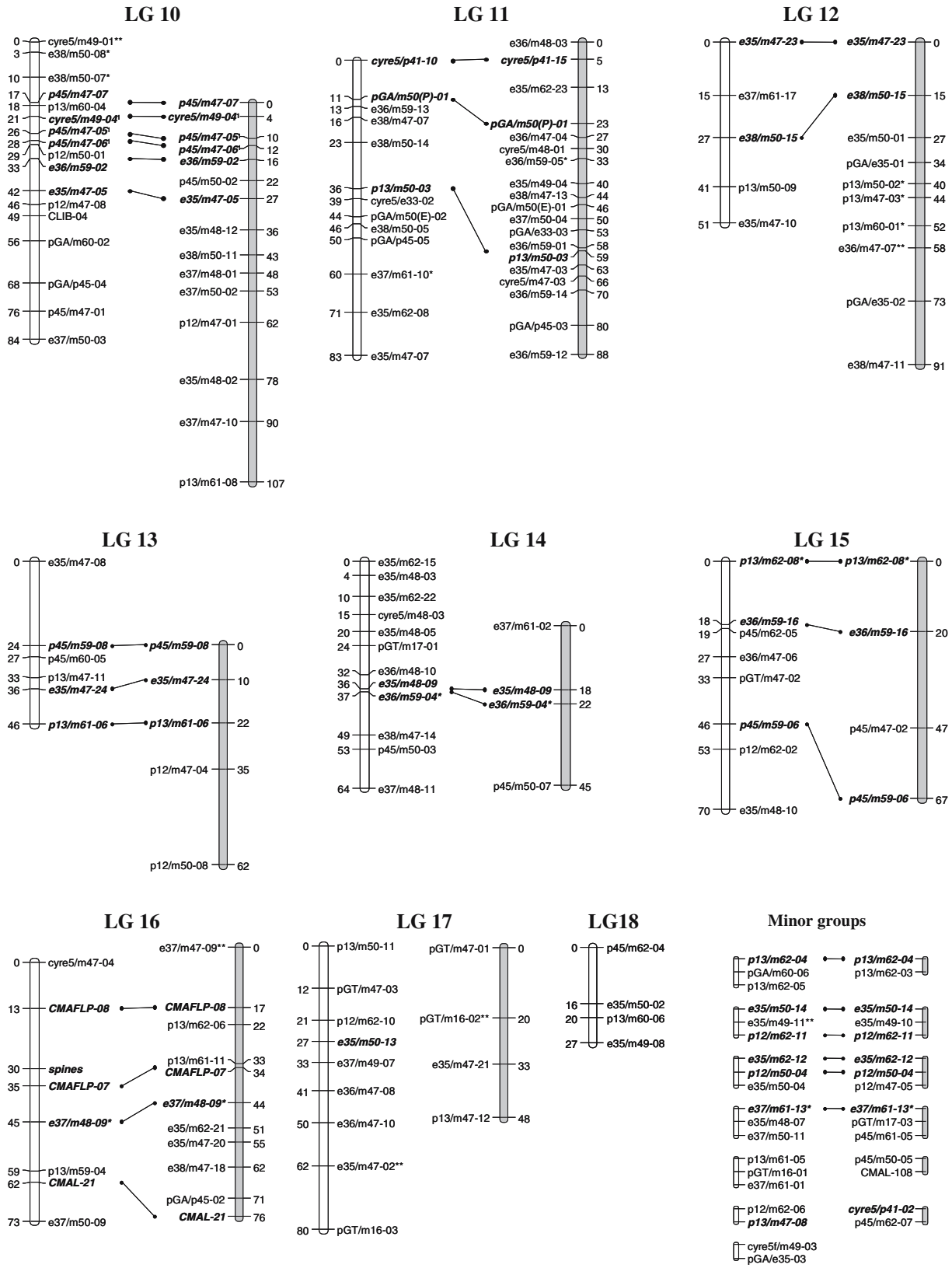
LG 10
0 cyre5/m49-01**
3 e38/m50-08*
10 e38/m50-07*
17 p45/m47-07
18 p13/m60-04
21 cyre5/m49-04
26 p45/m47-05
28 p45/m47-06
29 p12/m50-01
33 e36/m59-02
42 e35/m47-05
46 p12/m47-08
49 CLIB-04
56 pGA/m60-02
68 pGA/p45-04
76 p45/m47-01
84 e37/m50-03
p45/m47-07 0
cyre5/m49-04 4
p45/m47-05 10
p45/m47-06 12
e36/m59-02 16
p45/m50-02 22
e35/m47-05 27
e35/m48-12 36
e38/m50-11 43
e37/m48-01 48
e37/m50-02 53
p12/m47-01 62
e35/m48-02 78
e37/m47-10 90
p13/m61-08 107
LG 11
0 cyre5/p41-10
11 pGA/m50(P)-01
13 e36/m59-13
16 e38/m47-07
23 e38/m50-14
36 p13/m50-03
39 cyre5/e33-02
44 pGA/m50(E)-02
46 e38/m50-05
50 pGA/p45-05
60 e37/m61-10*
71 e35/m62-08
83 e35/m47-07
e36/m48-03 0
cyre5/p41-15 5
e35/m62-23 13
pGA/m50(P)-01 23
e36/m47-04 27
cyre5/m48-01 30
e36/m59-05* 33
e35/m49-04 40
e38/m47-13 44
pGA/m50(E)-01 46
e37/m50-04 50
pGA/e33-03 53
e36/m59-01 58
p13/m50-03 59
e35/m47-03 63
cyre5/m47-03 66
e36/m59-14 70
pGA/p45-03 80
e36/m59-12 88
LG 12
0 e35/m47-23
15 e37/m61-17
27 e38/m50-15
41 p13/m50-09
51 e35/m47-10
e35/m47-23 0
e38/m50-15 15
e35/m50-01 27
pGA/e35-01 34
p13/m50-02* 40
p13/m47-03* 44
p13/m60-01* 52
e36/m47-07** 58
pGA/e35-02 73
e38/m47-11 91
LG 13
0 e35/m47-08
24 p45/m59-08
27 p45/m60-05
33 p13/m47-11
36 e35/m47-24
46 p13/m61-06
p45/m59-08 0
e35/m47-24 10
p13/m61-06 22
p12/m47-04 35
p12/m50-08 62
LG 14
0 e35/m62-15
4 e35/m48-03
10 e35/m62-22
15 cyre5/m48-03
20 e35/m48-05
24 pGT/m17-01
32 e36/m48-10
36 e35/m48-09
37 e36/m59-04*
49 e38/m47-14
53 p45/m50-03
64 e37/m48-11
e37/m61-02 0
e35/m48-09 18
e36/m59-04* 22
p45/m50-07 45
LG 15
0 p13/m62-08*
18 e36/m59-16
19 p45/m62-05
27 e36/m47-06
33 pGT/m47-02
46 p45/m59-06
53 p12/m62-02
70 e35/m48-10
p13/m62-08* 0
e36/m59-16 20
p45/m47-02 47
p45/m59-06 67
LG 16
0 cyre5/m47-04
13 CMAFLP-08
30 spines
35 CMAFLP-07
45 e37/m48-09*
59 p13/m59-04
62 CMAL-21
73 e37/m50-09
e37/m47-09** 0
CMAFLP-08 17
p13/m62-06 22
p13/m61-11 33
CMAFLP-07 34
e37/m48-09* 44
e35/m62-21 51
e35/m47-20 55
e38/m47-18 62
pGA/p45-02 71
CMAL-21 76
LG 17
0 p13/m50-11
12 pGT/m47-03
21 p12/m62-10
27 e35/m50-13
33 e37/m49-07
41 e36/m47-08
50 e36/m47-10
62 e35/m47-02**
80 pGT/m16-03
pGT/m47-01 0
pGT/m16-02** 20
e35/m47-21 33
p13/m47-12 48
LG18
0 p45/m62-04
16 e35/m50-02
20 p13/m60-06
27 e35/m49-08
Minor groups
p13/m62-04
pGA/m60-06
p13/m62-05
e35/m50-14
e35/m49-11**
p12/m62-11
e35/m62-12
p12/m50-04
e35/m50-04
e37/m61-13*
e35/m48-07
e37/m50-11
p13/m61-05
pGT/m16-01
e37/m61-01
p12/m62-06
p13/m47-08
cyre5f/m49-03
pGA/e35-03
p13/m62-04
p13/m62-03
e35/m50-14
e35/m49-10
p12/m62-11
e35/m62-12
p12/m50-04
p12/m47-05
e37/m61-13*
pGT/m17-03
p45/m61-05
p45/m50-05
CMAL-108
cyre5/p41-02
p45/m62-07

Fig. 2 (Contd.)

Table 2 Markers generated for the genetic mapping in 'Romanesco C3' and 'Spinoso di Palermo'

	No. of PC screened	No. of PC used	Maternal[a] (Romanesco C3)	Paternal[a] (Spinoso di Palermo)	Intercross[b]	Total	Average number per PC
AFLP	64	30	121	112	119	352	11.7
S-SAP	30	9	17	8	16	41	4.6
M-AFLP	36	13	19	13	6	38	2.9
SSR	32	12	3	2	7	12	1.0
Total	162	64	160	135	148	443	7.1
Distorted markers $\chi^2_{\alpha=0.1} < \chi^2 \leq \chi^2_{\alpha=0.05}$ (%)			6 (4%)	8 (6%)	14 (9%)	28 (6%)	0.5
Distorted markers $\chi^2_{\alpha=0.05} < \chi^2 \leq \chi^2_{\alpha=0.01}$ (%)			7 (4%)	11 (8%)	2 (1%)	20 (5%)	0.3
Distorted markers $\chi^2 > \chi^2_{\alpha=0.01}$ (%)[c]			3 (2%)	3 (2%)	6 (4%)	12 (3%)	0.2
Total distorted markers (%)			16 (10%)	22 (16%)	22 (15%)	60 (14%)	1.0
Total mapped markers (%)			142 (89%)	115 (85%)	81 (55%)	338 (76%)	5.5
Unlinked markers			15	17	61	93	1.5

[a]Markers segregating 1:1
[b]Markers segregating 3:1. 1:2:1 or 1:1:1:1
[c]Markers not included in the linkage analysis

marker density of one per 6.9 cM, with the largest gap (27.1 cM) being on LG13. Most of the genetic intervals (77%) were below 10 cM, but large gaps were present in some regions. AFLP loci were evenly distributed over the 17 LGs, with no obvious clustering of markers generated by any one PC. Only four LGs (LG6, 12, 13 and 18) were composed solely of AFLP loci. Microsatellite loci (SSR and M-AFLP) and S-SAP fragments were distributed over, respectively, 13 and 10 LGs. Eighteen loci (9.9%) with minor segregation distortion were mapped to 11 LGs (13 at $\alpha = 0.05$ and 5 at $\alpha = 0.01$), and groups of distorted loci in close linkage to one another were detected on LG1, LG12 (four loci per LG) and LG2 (two loci).

Map length, genome coverage and alignment

The 18 maternal LGs spanned 1330.5 cM, and covered 53.4% of the estimated G of 2491.0 cM. Similarly, the 17 paternal LGs spanned 1239.4 cM (54.6% of G, 2268.0 cM). The maternal and paternal maps shared most of the intercross markers (78 out of the 82 mapped). On the basis of these bridge markers, 16 aligned LGs were identified and could therefore be given a consensus LG number. The number of bridging markers for each consensus LG varied from 1 to 14 (mean 4.6). No common loci were mapped on the remaining LGs (maternal LG17 and LG18, and paternal LG17). Intercross markers also defined four common minor LGs (Fig. 2).

Presence/absence of spines

Presence:absence of spines segregated in a 1:1 ratio ($\chi^2 = 0.04$), confirming that this trait is controlled by a single gene with two alternative alleles: dominant non-spiny (*Sp*) and wild-type spiny (*sp*) (Pochard et al. 1969; Basnizki and Zohary 1994). 'Spinoso di Palermo' is homozygous recessive *spsp*, while 'Romanesco C3' is heterozygous *Spsp*. Because only the female parent is heterozygous, *Sp* could only be located on the maternal map, where it was located on LG16, flanked by two SSR loci: CMAFLP-08 and CMAFLP-07.

Discussion

Mapping population

We have created a F_1 mapping population and applied the two-way pseudo-testcross strategy to construct genetic maps of two well-differentiated globe artichoke genotypes (Lanteri et al. 2004). 'Romanesco C3' is a late maturing type, producing large, green spherical, non-spiny heads between March and June, while 'Spinoso di Palermo' is an early maturing type, which can be forced to produce medium, violet, elongated heads between autumn and spring, and carries long sharp spines on its bracts and leaves. The parents also differ from one another for other morpho-physiological and agronomic characters, some of which may be key breeding traits. In globe artichoke, cross-pollination is promoted by protandry, but self-pollination is not precluded. The stigmatic surface is receptive to pollen 2–3 days after pollen shedding, and therefore fertilization of peripheral florets can be effected by the pollen of more internal ones, since flowering progresses from the periphery to the centre of the head. Some self-pollination is also possible through pollen transfer between heads, as each plant produces four to six asynchronously flowering capitula. For this reason, it was necessary to pre-screen the mapping population for hybridity with SSR markers to avoid the inclusion of individuals which were not the result of the crossing of the two parents in the mapping population.

Marker generation

We exploited two categories of markers: (1) anonymous sequences which require no a priori genome sequence information (AFLP and M-AFLP), and (2) markers where some prior sequence knowledge was necessary—

i.e., retrotrasposon-based S-SAP, and SSRs which had been specifically developed from artichoke by Acquadro et al. (2003, 2005a, b, A. Acquadro, E. Portis, A. Moglia, F. Magurno, S. Lanteri, submitted). The AFLP technique was chosen on the basis of good reproducibility, and because, in our experience, most AFLP markers segregate monogenically (Lanteri et al. 2004; Portis et al. 2005a, b). Thus they generally generate large amounts of good quality genotypic data, with a relatively low input of labour, producing finally 352 robust, and genomically widely distributed markers. The M-AFLP technique is closely related to AFLP, and the distribution of these markers was expected to mirror that of AFLPs. However, it has been demonstrated that the frequency of microsatellite motifs is significantly higher in transcribed regions than in non-transcribed DNA (Morgante et al. 2002), so M-AFLPs may be more efficient than AFLP for gene tagging (Albertini et al. 2003). S-SAP profiling requires prior knowledge of the retrotransposon LTR sequence. Although retrotransposons are generally abundant, conserved and dispersed in plant genomes, until now their presence and distribution in *C. cardunculus* has not been characterized. Finally, some SSR markers were included, as these are ideal for linkage mapping on account of their robustness, polymorphism, co-dominant inheritance and conservation at the intraspecific level. (Wu et al. 2004). These features ensured their effectiveness in identifying shared LGs between the maps obtained from different mapping experiments.

Marker segregation

We targeted a F_1 population to avoid inbreeding depression, which has been associated with segregation distortion and therefore bias in the estimation of linkage distances (Tavoletti et al. 1996). About 86% of the markers segregated in a strict monogenic Mendelian fashion, with segregation distortion being detected at only 14% of loci, consistent with the level found in similar studies (Conner et al. 1997; Barreneche et al. 1998; Casasoli et al. 2001; Han et al. 2002; Scalfi et al. 2004; Wu et al. 2004; Pekkinen et al. 2005). The origin of segregation distortion can be associated with statistical bias or errors in genotyping and scoring, but is mainly a consequence of biological factors, such as chromosome loss, non-random union of gametes, zygotic survival, changes in genetic load (Bradshaw and Stettler 1994) or null alleles (Pekkinen et al. 2005). Cervera et al. (2001) have reported that where distorted markers are ignored, a significant part of a linkage group can be excluded, and therefore suggested the inclusion of markers that deviate from Mendelian segregation at the 5% level (but not at the 1% level) in order to reduce the probability of type I (false linkage) errors. Similarly Doucleff et al. (2004) concluded that the exclusion of markers showing distortion at $<1\%$ leads to the loss of a large segment of two linkage groups. In contrast, the inclusion of highly distorted markers in linkage analysis has been demonstrated to be beneficial in some mapping studies (Kuang et al. 1999; Fishman et al. 2001). In the present work, we have chosen to include only markers deviating at 1% and above. Clusters of such markers were found on linkage groups LG1, 5 and 10 in the female map, and LG1, 2 and 12 in the male map. These regions were unidirectionally biassed in the sense that all distorted markers showed an excess of the presence of the parental allele. This is suggestive of a biological mechanism underlying segregation distortion, as opposed to random bias caused by scoring errors or chance (Fishman et al. 2001).

Map construction and marker distribution

Two comprehensive maps have been developed—one consisting of 204 markers arranged on 18 LGs, with a total length of 1330.5 cM and a mean inter-marker distance of 6.5 cM, while the other included 180 markers ordered into 17 LGs, covering 1239.4 cM, with a marker density of 6.9 cM. In addition, a small number of unlinked triplets and doublets were generated in both maps. Since the number of LGs defined in the male map is equal to the haploid chromosomal number of artichoke, the formation of 18 LGs in the female map, as well as the presence of various minor groups and unlinked single loci in both maps indicates that further analysis will be necessary to genetically define all the chromosomes. The two maps were aligned with 78 common intercross markers, and these identified 16 LGs. Intercross markers are less informative than testcross markers where they are of the dominant type *A*/*a*, since only in the homozygous recessive *aa* individual is it unambiguous as to which allele came from which parent (Crespel et al. 2002). However, this limitation does not apply for co-dominant markers where the two parents share no alleles (i.e., one is A_1A_2 and the other A_3A_4).

A clustering of markers was observed in some LGs, and in most cases was concentrated in the middle of an LG. Such clustering is not uncommon (Keim et al. 1990; Reiter et al. 1992; Tanksley et al. 1992; Vallejos et al. 1992), particularly, although not exclusively, in centromeric regions, where reduced recombination usually applies (Tanksley et al. 1992). Microsatellite-based loci (SSR and M-AFLP) were randomly distributed across most of the LGs. The 27 loci mapped in the female map were present on 13 of the 18 LGs, with up to four per LG, generally unclustered. A similar distribution applies to the male map. In all, 39 microsatellite loci were placed on the aligned map, covering 13 of the 16 LGs, as well as two non-aligned groups (one for each map). S-SAP loci were also well distributed: 19 loci were placed on the female map, covering 10 out of the18 LGs, with 1–4 loci per LG. Clusters of S-SAP loci were present on LG5 and LG7 (three and four fragments, respectively) (Fig. 1). On the male map, 14 S-SAP loci were mapped to eight of the 17 LGs, with some clustering on LG2 and LG11 (three fragments per LG). A total of 27 common S-SAP loci were present on the aligned map, which covered 12 out of the 16 aligned LGs. A cluster of five S-SAP loci was detected on LG7.

Conclusion

To our knowledge, this is the first description of a marker-based linkage map in globe artichoke. Linkage relationships among molecular markers represent the initial step for the future identification of chromosomal regions carrying genes of interest and their future targeting in breeding programmes aiming to incorporate marker-assisted selection. Since globe artichoke can be easily vegetatively propagated, the mapping population described here is immortal, and thus can be grown in contrasting environments to facilitate the identification of QTL which have a significant effect on key agronomic characters. *C. cardunculus* includes both the cultivated forms globe artichoke and cultivated cardoon, as well as their progenitor wild cardoon. We are currently planning the construction of marker-based genetic maps based on F_1 populations involving combinations between 'Romanesco C3' and either cultivated or wild cardoon accessions, as these will allow us to undertake comparative QTL mapping studies. Wide cross populations of this type will facilitate the exploration of the genetic control of quantitative characters in exotic genetic backgrounds. Although we have relied heavily on common AFLP or AFLP-derived markers for map alignment, we aim in the future to move to common SSR markers, which will require a significant effort in the development of new microsatellite assays.

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