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QTLs detection and mapping for yield-related traits in globe artichoke

Eugenia A. Martin[a,*], Vanina P. Cravero[a], Fernando S. López Anido[b], Enrique L. Cointry[a]

[a] Cátedra de Mejoramiento Vegetal y Producción de Semillas, IICAR-CONICET, Campo Experimental J.F. Villarino CCNo. 14, S2125ZAA Zavalla, SF, Argentina
[b] Cátedra de Genética, IICAR-CONICET, Campo Exp. J.F. Villarino CCNo. 14, S2125ZAA Zavalla, SF, Argentina

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ABSTRACT

The genetic basis of yield-related traits in *Cynara cardunculus* L. was studied in a F_1 population derived from an inter-botanical varietal cross between wild cardoon and "Estrella del Sur FCA" globe artichoke. The aim of this work was identify and localize QTLs (Quantitative Trait Loci) associated with yield-related traits as a first step to marker-assisted selection. Sixty-eight polymorphic markers (SSR) were used to analyze the segregating population by ANOVA approach. A second analysis based in previous linkage maps of the species was conducted in two seasons by SIM (Simple Interval Mapping) procedure. A total of 66 QTLs associated with seven morphological traits were detected and mapped in the first evaluated season, of these 42 were validated among both seasons. All QTLs identified are major QTLs (explain >10% of phenotypic variation) and 27 of these are responsible for >20% of phenotypic variation. This work represents an initial platform for the dissection of a complex trait, such as yield in the species. Although the QTLs detected need to be more precisely mapped, they will be targets in breeding programs of globe artichoke to improve yield-related traits.

1. Introduction

Cynara cardunculus L. (allogamous *Asteraceae*, $2n = 34$) includes two domesticated taxa, globe artichoke and cultivated cardoon, as well as the wild cardoon, commonly considered the wild ancestor of both domesticated forms.

Globe artichoke is typically grown on the Mediterranean countries, but it is also cultivated in South America, North America, and China (FAO, 2013), as an important horticultural crop. The edible parts are the heads (immature inflorescences), consumed as fresh, canned or frozen vegetable. It represents a source of natural antioxidants by its high content of phenolic and flavonoid compounds (Moglia et al., 2008; Pandino et al., 2011). To date, in artichoke breeding, studies on the inheritance of a small number of main traits such as precocity, color head, spiny, compacity, and yield has been conducted (López Anido et al., 1998; Mauromicale et al., 2000; Cravero et al., 2005; Martin et al., 2008; López Anido et al., 2010; Portis et al., 2012; Martin et al., 2013; Portis et al., 2014).

Most agricultural important traits such as yield, quality and precocity are controlled by multiple genes and are known as quantitative traits. The regions within genomes that contain genes associated with a particular quantitative trait are known as quantitative trait loci (QTLs). The QTL analysis is based on the detection of association between phenotype and the genotype of markers. Single-marker analysis (or single-point analysis) is the simplest method for detecting QTLs associated with single markers. The statistical methods used for this analysis include *t*-tests, analysis of variance (ANOVA) and linear regression. Linear regression is the most commonly used because the coefficient of determination (R^2) from the marker explains the phenotypic variation arising from the QTL linked to the marker. Another statistical method for detecting QTLs is simple interval mapping (SIM), where a linkage map is required and intervals between adjacent pairs of linked markers are analyzed simultaneously (Collard et al., 2005).

Due to the genetic complexity of quantitative traits, the detection of QTL is rather difficult, since these one are highly polygenic and influenced by the environment. The artichoke yield is a complex traits highly influence by the temperature, irrigation or management of the crop (Shinohara et al., 2011).

The aim of this work was identify and localize QTLs for yield-related traits in *C. cardunculus* L. to be used as tool for selection in breeding programs.

* Corresponding author.
E-mail address: eamartin@unr.edu.ar (E.A. Martin).

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2. Materials and methods

2.1. Plant material

An inter-varietal crossing between an individual of a local accession of wild cardoon (used as female progenitor) and a single genotype of the Argentinean globe artichoke cultivar "Estrella del Sur FCA" (used as male parental) was used to develop a F_1 segregating population described by Martin et al. (2013). The hybrid status of each individual of the segregating population was checked using the SSR (simple sequence repeat) CELMS-30 and -37 (Acquadro et al., 2009). Both progenitors and 115 F_1 individual plants were grown and evaluated at the horticultural area of the Experimental Field Station of the Universidad Nacional de Rosario (33°1′S; 60°53′W; 24 m a.s.l.). This site experiences a mild weather and an average annual rainfall of 1000 mm, and the soil is a medium-heavy clay soil, with up to 3% organic matter and pH value ~6.0. Rosario (Santa Fe province) and La Plata (Buenos Aires province) are the typical zones in Argentina where most of the country's globe artichoke is produced (Cravero et al., 2010; personal communication). The seeds of the F_1 generation were germinated in Speedling traits (72 cells, vol. 43 cm^3 per cell) on a greenhouse and were transplanted on field at the stage of four true leaves (~40 days after germination, middle April). The spacing between plants within each row was 80 cm and the inter-row spacing was 1.4 m. When necessary, supplementary drip irrigation (Isiplasttape™ 15 ml/h) was supplied from transplant to harvest time.

2.2. Phenotypic evaluation

Three clones of each parental genotype and all progeny individuals were harvested and evaluated over two growing seasons: 2013 and 2014 (from September to November in both years). A total of seven agronomic traits related with marketable yield were evaluated: number of head (capitula) per plant (NHP); fresh weight (g), diameter (cm) and length (cm) of the main head (WMH, DMH, and LMH, respectively) and fresh weight (g), diameter (cm) and length (cm) of the second heads (W2H, D2H, and L2H, respectively) developed from the ramification of the main stem. In both evaluation seasons the parental genotypes were used as testers.

2.3. Molecular evaluation and QTL identification

DNA of both parents and each progeny individuals was extracted from fresh leaf using a DNeasy Plant Mini Kit (Qiagen). A total of 241 SSRs, previously developed for the species by Acquadro et al. (2009) and Scaglione et al. (2009), were used to evaluate the genotypes (supplementary material). An initial screen was performed using DNA of both parents and a set of 6 F_1 individual. Only SSRs that showed polymorphism among parental genotypes and segregate in the F_1 set were used for genotyping all the F_1 individuals. The PCR conditions and the separation of the amplicons were applied followed the recommendations of the assay developer (Acquadro et al., 2009; Scaglione et al., 2009).

2.4. Statistical analysis of the phenotypic traits

The normal distribution of the morphological traits in the F_1 generation for both seasons was verified by a Shapiro–Wilks test (Shapiro and Wilk, 1965) and distribution histograms. The *t*-Student test (Snedecor, 1964) was used to compare the mean values between parents and the Pearsonís coefficient was used to determinate the correlation between traits in both years evaluated. Segregation of a trait was considered as transgressive when at least one progeny individual presented a value higher or lower by at least two standard deviations than the higher or lower recording parental. These statistical analyses and graphics were carried out with InfoGen software (Balzarini and Di Rienzo, 2004).

2.5. Marker-QTL association

The SSR markers were tested for an expected Mendelian segregation ratio 1:1 (segregate in only a parental) or 1:2:1 (segregate in both parents) in the F_1 population using JoinMap4.0 software (Van Ooijen, 2006). Goodness-of-fit between the observed and expected segregation ratios was determined by a χ^2 test. Only SSR markers showing a monogenic segregation ($\chi^2 \leq \chi^2_{\alpha=0.1}$) were used for QTLs association.

The association between SSR markers and traits was evaluated at 2013 season and it was determined by a one-way ANOVA, in which genotype at marker loci was used as the classifying variable. A *p*-value <0.05 was defined as threshold to determine the association between a QTL and a SSR locus. The percentage of total phenotypic variation explained by each QTL was determined by R^2 values. The SSR-QTL associations were examined with InfoGen software.

In a second step, the putative localization of the QTLs yield-related traits was determined using a simple interval mapping (SIM) based on the wild cardoon and globe artichoke linkage maps previously developed by Martin et al. (2013). The critical LOD (Logarithm of Odds) score = 3.0 was used as basis to identify QTL candidates. The QTLs detected were described by the markers closest to the region corresponding to the QTL and each QTL name was formed by the abbreviated form of the trait followed by the relevant linkage group (LG). SIM analysis was performed with JoinQTL v.6 (Van Ooijen, 2009), and linkage maps and QTLs position were drawn using MapChart 2.2 software (Voorrips, 2002).

Since the SSR markers used in the ANOVA analysis were previously mapped by Martin et al. (2013), a comparison between the QTLs detected and localized by both statistical methods was performed. QTLs detected and mapped in the first season (2013), were also scored and evaluated in the second season (2014) to determinate their consistency.

3. Results

3.1. Phenotypic analysis

All the morphological traits showed normal distribution among the F_1 progeny ($W > 0.94$) in the first season evaluated. In 2014 a normal distribution ($W > 0.90$) was observed for most of the traits, except for length of the main head ($W = 0.80$). The parental genotypes were significantly different ($p < 0.01$) for all traits analyzed in both seasons (Table 1). The inter-trait correlations revealed that diameter and length of the main and secondary heads were the most important head components influencing fresh weigh, with a high positive correlation that ranged from 0.82 to 0.93 for the first season and from 0.82 to 0.97 for the second one. The number of head per plant showed positive correlation values with all the other traits evaluated, it was from 0.38 to 0.60 (1st season), and from 0.62 to 0.66 (2nd season). The same trait showed transgressive segregation (extreme phenotypes in the F_1 population) toward the wild cardoon parent in both seasons, where a high number of transgressive individuals were observed in the progeny (~33% in the 1st season and ~20% in the 2nd one).

3.2. Molecular analysis and QTLs association

A total of 70 SSR markers were polymorphic among parents and segregated in the F_1 population. Sixty-eight SSRs (97%) presented a monogenic Mendelian segregation ($\chi^2 \leq \chi^2_{\alpha=0.1}$). From the association analysis, 22 QTLs were significant at 5% (Table 2). Seven

Table 1
Mean values, standard error (SE), and standard deviation (SD) for each evaluated head trait of Wild Cardoon (WC) and Estrella del Sur FCA globe artichoke (ES): number of head per plant (NHP), fresh weight, diameter and length of the main head (WMH, DMH, and LMH, respectively) and fresh weight, diameter and length of the second heads (W2H, D2H, and L2H, respectively). Significant mean differences between parentals are indicated (*$P > 0.05$). Mean values, SE, minimum and maximum values, and Shapiro–Wilks test for each trait in the F_1 ($W > 0.90$ indicate normal distribution of the trait).

Traits	1st Season											
	Genotypes											
	WC			ES				F_1				
	Mean	SE	SD	Mean	SE	SD		Mean	SE	Min	Max	W
NHP	17.00	1.08	2.16	4.00	0.41	0.82	*	20.16	0.76	9.00	39.00	0.94
WMH	22.93	1.54	3.07	147.03	8.06	16.13	*	73.49	2.05	34.10	123.20	0.97
DMH	4.30	0.09	0.18	7.20	0.09	0.18	*	5.56	0.07	4.10	7.00	0.97
LMH	4.45	0.10	0.21	8.48	0.83	1.65	*	6.76	0.06	5.30	7.80	0.96
W2H	14.25	1.62	3.24	62.28	4.21	8.43	*	47.42	1.53	24.50	78.20	0.95
D2H	3.83	0.18	0.35	5.65	0.21	0.42	*	4.8	0.07	3.50	6.10	0.97
L2H	4.13	0.19	0.39	6.00	0.79	1.57	*	6.01	0.06	4.80	7.25	0.98
2nd Season												
NHP	15.50	1.20	1.98	4.00	0.35	0.75	*	19.46	0.89	6.00	39.00	0.97
WMH	23.50	1.13	2.91	152.05	9.05	17.20	*	77.24	1.94	35.00	131.20	0.97
DMH	4.65	0.10	0.15	8.30	0.89	0.16	*	7.3	0.2	3.30	9.90	0.95
LMH	5.21	0.10	0.17	9.13	0.97	1.54	*	7.63	0.19	3.60	9.50	0.80
W2H	16.35	1.45	3.5	71.10	4.55	9.13	*	53.27	1.54	26.50	89.00	0.95
D2H	4.12	0.19	0.39	6.14	0.30	0.52	*	5.38	0.19	2.00	8.15	0.96
L2H	4.53	0.19	0.41	7.52	0.81	1.65	*	6.44	0.19	2.80	8.65	0.90

Table 2
SSR marker associated with yield-related traits in the F_1 (1st season) by ANOVA analysis. SSR name (marker); phenotypic trait: number of head per plant (NHP), fresh weight, diameter and length of the main head (WMH, DMH, LMH) and fresh weight, diameter and length of the second heads (W2H, D2H, L2H); phenotypic variation explained by a QTL (R^2), *p*-value and localization in linkage maps (LG). QTLs significant at 1% level are shown in **bold**.

Marker	Traits	R^2	*p*-Value	LG
CELMS-02	WMH	0.06	0.045	Est_VIIIA
	W2H	0.06	0.0499	
	D2H	0.08	0.0164	
CELMS-10	**W2H**	**0.18**	**0.0027**	Est_III
	D2H	0.11	0.0173	
	L2H	0.08	0.0437	
CyEM_02	D2H	0.06	0.0355	SSR-Ref LGVIII
CyEM_244	L2H	0.06	0.0335	Est_IV
CyEM_293	**W2H**	**0.13**	**0.0020**	Est_IV
	D2H	**0.10**	**0.0062**	
CELMS-57	**WMH**	**0.18**	**0.0013**	Wild_XI
	DMH	**0.17**	**0.0020**	
CELMS-36	D2H	0.11	0.0119	Wild_Xva
CyEM_188	**DMH**	**0.10**	**0.0075**	Wild_VIII
CyEM_234	WMH	0.08	0.0104	SSR-Ref LGI
	DMH	0.08	0.0151	
	W2H	**0.08**	**0.0080**	
	D2H	0.08	0.0141	
	L2H	0.06	0.0338	
CyEM_53	W2H	0.08	0.0134	Wild_XII
	D2H	0.06	0.0341	
CyEM_86	D2H	0.06	0.0333	Wild_II

of these associations (31.8%) were significant at 1% level (highlighted in bold on Table 2). All QTLs were linked to eleven SSR markers and six of these molecular markers showed association with more than one trait. The trait with the highest number of phenotypic/molecular associations was diameter of the second heads, with a total of eight QTLs detected. Moreover, three SSRs were associated with fresh weight of the main head, three with diameter of the main head, five with fresh weight of the second heads and three with length of the second heads. QTLs for number of head and length of the main head were not found in this first association analysis. The percentage of total phenotypic variation explained by each QTL ranged from 6 to 18% and the highest R^2 values were found for QTLs that control weight of the main and weight of the second heads. Eight of the detected QTLs (36.4%) accounted more than 10% of phenotypic variation each one.

3.3. QTLs localization

At the first evaluated season (2013), the SIM analysis ($LOD_{min} = 3.0$) identified a total of 66 QTL regions across wild cardoon and globe artichoke linkage maps (Fig. 1.a and b). Under the same statistical analysis and $LOD_{min} = 4.0$, a total of 31 QTL regions were identifies (47% of the QTLs identified at $LOD_{min} = 3.0$). The number of QTLs per trait, at LODmin = 3.0, ranged from six to 14 (mean 9.4) (Table 3). A total of 13 QTLs related with different evaluated traits were identified at LG Wild_VII. At Est_XI were localized 10 QTLs related with number of head per plant, length and diameter of the main and second heads, and weight of the second heads. At the homologous linkage group Wild_XI were identified 7 QTLs associated with number of head per plant, weight of the main head, length of the main and second heads, and diameter if the main head. Nine QTLs related with length and diameter of the main and second heads were localized at Wild_VIII. Eight QTLs associated with weight of the main and second heads, and diameter of the main head were identified at Wild_XIV. On linkage group Est_XVII were identified 5 QTLs for weight and diameter of the main head, and for weight, length and diameter of the second heads. At Wild_IX were identified 3 QTLs for weight of the main head. Four QTLs associated with number of head per plant, weight and diameter of the main head were identified at Est_IV. Two QTLs related to diameter of the main head and weight of the second heads were localized at Wild_III and two QTLs for number of head per plant and length of the main head were identified at Wild_Vb. Only one QTL associated to number of head was mapped at Wild_Ib. The clustered QTL region at the LG XI was the only detectable in both parental maps. The other four were only detectable in one of the parental maps (Wild_VII, VIII, XIV, and Est_XVII). The percentage of phenotypic variance explained by the QTLs ranged from 15.1 to 43.8 and 27 QTLs (41%) explained more than 20% of phenotypic variance (Table 3). Only five QTLs identified from the ANOVA analysis were detectable by the SIM procedure. The SSR markers associated with a QTL were CyEM_188, CyEM_02, CELMS-02, and CELMS-57. These markers were localized at the same linkage group where QTLs were identified by SIM, but they were mapped at more than 30 cM to the

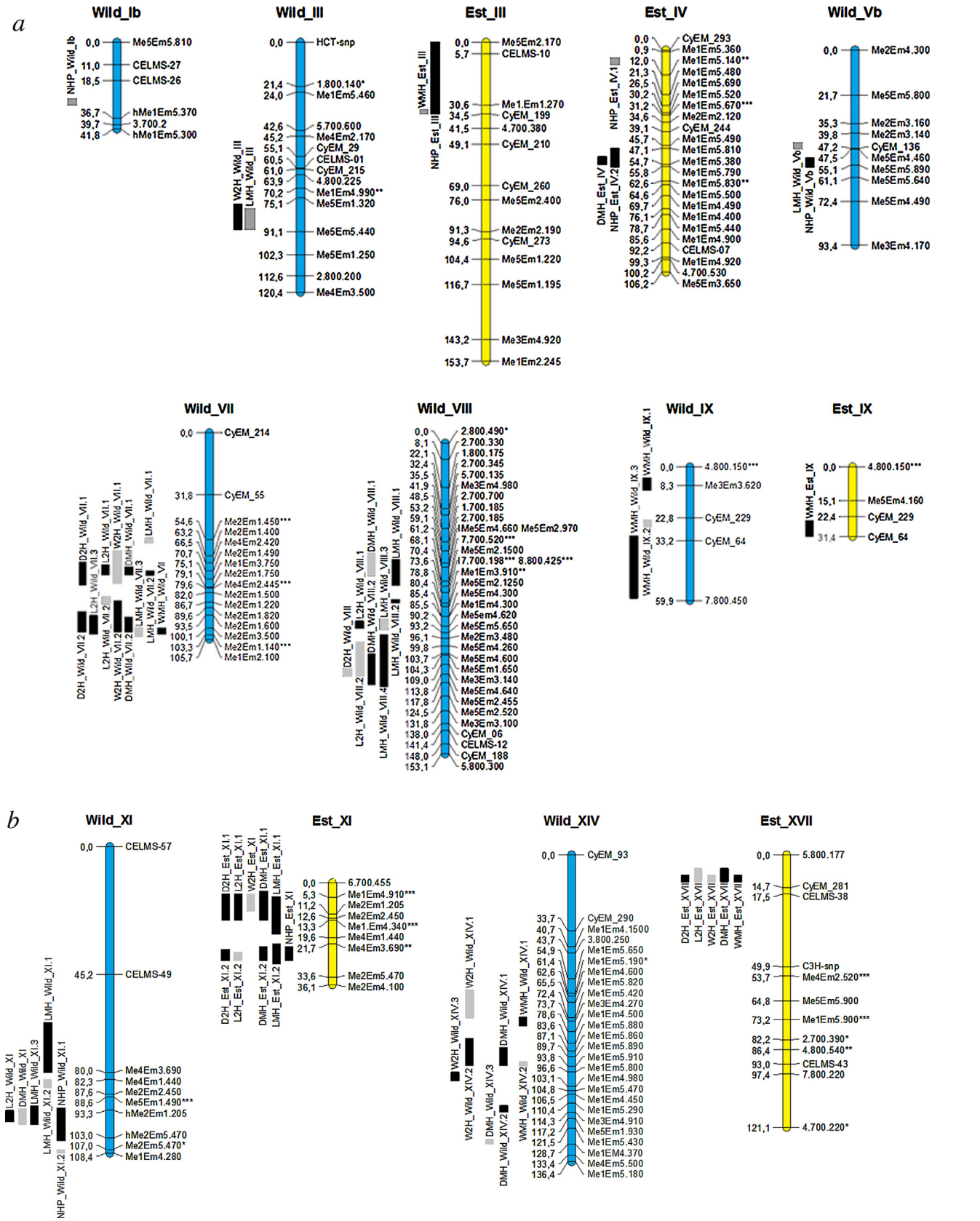

Fig. 1. *a.* Location of yield-related traits QTLs. Number of head per plant (NHP), fresh weight, diameter and length of the main head (WMH, DMH, LMH) and fresh weight, diameter and length of the second heads (W2H, D2H, L2H). Only those LGs harboring QTL are shown. Wild cardoon LGs shown in blue, "Estrella del Sur FCA" LGs in yellow. Marker showing significant levels of segregation distortion are indicated by *asterisks* (*0.1> P > 0.05, **0.05> P > 0.01, ***P < 0.01). *Bars* represent each QTL, *black bars* show QTLs detected in both seasons, while *grey bars* show QTL only expressed in the first season. b. continued. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Yield-related traits QTL detected using the WC and Est linkage maps by SIM analysis. The table indicates the position of the QTL (cM), the LOD thresholds of the interval (LOD), the proportion (%) of the total phenotypic variance explained by the QTL (PV), the closest linked markers (Locus) and their map localization (cM locus), the estimated LODs at the QTL peak (LOD locus), the proportions (%) of the total phenotypic variance explained by the marker (PV locus). The QTLs detected in both seasons are shown in **bold**.

Trait	Map	LG	QTL	cM	LOD	PV	Locus	cM locus	LOD locus	PV locus
NHP	Wild	Ib	NHP_Wild_Ib	27.4 30.5	3.1	15.7	CELMS-26	18.5	1.1	7.9
NHP	Est	III	NHP_Est_III	0.0 34.5	3.4	17.6	Me5Em2.170 CELMS-10 Me1Em1.270 CyEM_199	0.0 5.7 30.6 34.5	3.4 3.2 3.5 3.1	17.6 16.1 11.0 15.3
NHP	Est	IV	NHP_Est_IV.1	3.9 7.0	3.2	15.9	Me1Em5.360	0.9	2.7	12.8
NHP	**Est**	**IV**	**NHP_Est_IV.2**	**47.0 56.0**	**4.5**	**24.3**	**Me1Em5.810 Me1Em5.380**	**47.1 54.7**	**3.3 4.3**	**16.9 23.4**
NHP	**Wild**	**Vb**	**NHP_Wild_Vb**	**51.5 56.2**	**3.5**	**18.2**	**Me5EM5.890**	**55.1**	**3.3**	**17.1**
NHP	**Est**	**XI**	**NHP_Est_XI**	**22.6 27.7**	**4.2**	**22.6**	**Me4Em3.690**	**21.7**	**2.7**	**13.0**
NHP	**Wild**	**XI**	**NHP_Wild_XI.1**	**92.5 104.0**	**4.5**	**24.3**	**Me2Em1.205 Me2Em5.470**	**93.3 103.0**	**3.4 3.2**	**17.6 16.2**
NHP	Wild	XI	NHP_Wild_XI.2	107 108.5	3.9	20.5	Me1Em4.280	108.4	3.3	16.8
WMH	**Est**	**III**	**WMH_Est_III**	**32.6 34.0**	**3.0 3.1**	**15.6**	**CyEM_199**	**34.5**	**2.5**	**5.7**
WMH	**Wild**	**VII**	**WMH_Wild_VII**	**100.0 103.1**	**3.3**	**17.0**	**Me2Em3.500**	**100.1**	**3.0**	**11.1**
WMH	**Est**	**IX**	**WMH_Est_IX**	**24.3 31.4**	**3.4**	**17.5**	**CyEM_64**	**31.4**	**3.4**	**17.5**
WMH	**Wild**	**IX**	**WMH_Wild_IX.1**	**5.0 10.5**	**3.5**	**18.1**	**Me3EM3.620**	**8.3**	**3.5**	**18.0**
WMH	Wild	IX	WMH_Wild_IX.2	24.0 27.0	3.2	16.0	CyEM_229	23.0	2.9	13.8
WMH	**Wild**	**IX**	**WMH_Wild_IX.3**	**31.0 59.0**	**3.4**	**17.5**	**CyEM_64 7.800.450**	**33.2 59.9**	**3.4 3.2**	**17.5 16.2**
WMH	**Wild**	**XIV**	**WMH_Wild_XIV.1**	**72.0 76.0**	**3.3**	**16.8**	**Me1Em5.420 Me3Em4.270**	**72.4 73.7**	**3.3 3.3**	**16.7 16.8**
WMH	Wild	XIV	WMH_Wild_XIV.2	92.0 94.0	4.1	21.8	Me1Em5.910	93.8	3.1	15.2
WMH	**Est**	**XVII**	**WMH_Est_XVII**	**9.0 12.0**	**3.7**	**19.2**	**CyEM_281**	**14.7**	**2.2**	**9.4**
LMH	Wild	Vb	LMH_Wild_Vb	44.7 47.2	3.5	17.7	CyEM_136	47.2	3.5	3.5
LMH	Wild	VII	LMH_Wild_VII.1	54.0 56.6	3.2	15.1	Me2Em1.450	54.6	3.1	15.0
LMH	**Wild**	**VII**	**LMH_Wild_VII.2**	**70.5 73.0**	**3.2**	**16.1**	**Me2EM1.490**	**70.7**	**3.2**	**16.1**
LMH	Wild	VII	LMH_Wild_VII.3	100.0 104.3	3.6	18.5	Me2Em3.500 Me2Em1.140	100.1 103.3	3.3 3.1	16.8 12.4
LMH	**Wild**	**VIII**	**LMH_Wild_VIII.1**	**57.2 70.1**	**5.0**	**21.4**	**2.700.185 Me5Em4.660 Me5Em2.970 7.700.520**	**59.1 61.2 61.2 68.1**	**4.1 3.9 4.7 3.5**	**14.7 17.2 19.4 18.4**
LMH	**Wild**	**VIII**	**LMH_Wild_VIII.2**	**77.0 78.0**	**3.2**	**16.2**	**Me1Em3.910**	**78.8**	**3.2**	**16.2**
LMH	Wild	VIII	LMH_Wild_VIII.3	86.5 92.0	4.2	23.0	Me5Em4.620	90.2	4.0	21.7
LMH	**Wild**	**VIII**	**LMH_Wild_VIII.4**	**94.2 120**	**7.6**	**36.3**	**Me2Em3.480 Me5Em4.260 Me5Em4.600 Me5Em1.650 Me3Em3.140 Me5Em4.640 Me5Em2.455**	**96.1 99.8 103.7 104.3 109.0 113.8 117.8**	**3.6 3.2 4.0 4.4 4.7 5.2 4.0**	**12.3 22.9 27.4 29.9 31.4 34.3 21.2**
LMH	**Est**	**XI**	**LMH_Est_XI.1**	**4.9 18.3**	**6.7**	**28**	**Me1Em4.910 Me2Em1.205 Me2EM2.450 Me1Em4.340**	**5.3 11.2 12.6 13.3**	**4.3 4.1 4.0 3.9**	**23.1 28.0 21.4 10.9**
LMH	**Est**	**XI**	**LMH_Est_XI.2**	**21.7 28.7**	**5.7**	**25.6**	**Me4EM3.690**	**21.7**	**3.9**	**13.8**

LMH	**Wild**	**XI**	**LMH_Wild_XI.1**	**62.2 80.0**	**4.9**	**20.9**	**Me4Em3.690**	**80.0**	**3.8**	**13.4**
LMH	Wild	XI	LMH_Wild_XI.2	82.3 85.3	4.4	17.7	Me4Em1.440	82.3	4.2	17.7
LMH	**Wild**	**XI**	**LMH_Wild_XI.3**	**91.6 98.3**	**3.8**	**20.5**	**Me2Em1.205**	**93.3**	**3.6**	**18.9**
LMH	Wild	III	LMH_Wild_III	80.1 90.1	3.3	17.0	Me5Em5.440	91.1	2.1	6.7
DMH	**Est**	**IV**	**DMH_Est_IV**	**51.1 54.7**	**3.3**	**17**	**Me1Em5.380**	**54.7**	**3.2**	**16.7**
DMH	**Wild**	**VII**	**DMH_Wild_VII.1**	**68.5 72.7**	**4.5**	**24.4**	**Me2Em1.490**	**70.7**	**3.5**	**23.2**
DMH	**Wild**	**VII**	**DMH_Wild_VII.2**	**94.5 102.1**	**5.2**	**29.0**	**Me2Em3.500**	**100.1**	**4.7**	**25.5**
DMH	Wild	VIII	DMH_Wild_VIII.1	54.2 66.2	4.6	18.6	2.700.185 Me5Em4.660 Me5Em2.970	59.1 61.2 61.2	4.6 3.9 4.4	18.6 14.8 17.5
DMH	**Wild**	**VIII**	**DMH_Wild_VIII.2**	**103.7 118.8**	**5.6**	**24.9**	**Me5Em4.600** **Me5Em1.650** **Me3Em3.140** **Me5Em4.640** **Me5Em2.455**	**103.7 104.3** **109.0 113.8** **117.8**	**3.2 3.7 4.2 4.3** **3.0**	**16.3 19.4 22.8** **23.1 14.9**
DMH	**Est**	**XI**	**DMH_Est_XI.1**	**3.0 13.3**	**5.6**	**30.9**	**Me1Em4.910** **Me2Em1.205** **Me2Em2.450** **Me1Em4.340**	**5.3 11.2 12.6** **13.3**	**5.5 5.1 3.7 3.5**	**30.6 27.9 19.6** **18.4**
DMH	**Est**	**XI**	**DMH_Est_XI.2**	**22.7 27.7**	**4.2**	**22.8**	**Me4Em3.690**	**21.7**	**2.7**	**12.9**
DMH	Wild	XI	DMH_Wild_XI	92.6 98.3	4.7	19.9	Me2Em1.205	93.3	3.3	16.9
DMH	**DMH**	**XIV**	**DMH_Wild_XIV.1**	**85.6 93.7**	**4.3**	**17.2**	**Me1Em5.860** **Me1Em5.890**	**87.1 89.7**	**4.3 3.5**	**17.2 11.3**
DMH	**Wild**	**XIV**	**DMH_Wild_XIV.2**	**111.4 114.3**	**3.4**	**17.8**	**Me3Em4.910**	**114.3**	**3.0**	**13.6**
DMH	Wild	XIV	DMH_Wild_XIV.3	126.5 128.7	3.2	16.1	Me1Em4.370	128.7	3.1	15.5
DMH	**Est**	**XVII**	**DMH_Est_XVII**	**6.0 12.0**	**4.5**	**18.3**	**CyEM_281**	**14.7**	**2.9**	**14.2**
W2H	**Wild**	**III**	**W2H_Wild_III**	**78.1 90.1**	**3.5**	**18.4**	**Me5em5.440**	**91.1**	**2.5**	**11**
W2H	Wild	VII	W2H_Wild_VII.1	60.6 77.1	4.6	25	Me2Em1.400 Me4Em2.420 Me2Em1.490 Me1Em3.759	63.2 66.5 70.7 75.1	3.2 3.7 3.9 3.1	16.1 19.4 21.0 15.4
W2H	**Wild**	**VII**	**W2H_Wild_VII.2**	**86 102.1**	**7.2**	**43.8**	**Me2Em1.220** **Me2Em1.820** **Me2Em1.600** **Me2Em3.500**	**86.7 89.6 93.5** **100.1**	**3.8 3.7 3.7 6.5**	**26.2 14.1 19.7** **40.9**
W2H	Est	XI	W2H_Est_XI	4.0 10.3	3.7	19.4	Me1Em4.910	5.3	3.7	19
W2H	Wild	XIV	W2H_Wild_XIV.1	59.9 72.5	3.8	20.1	Me1Em5.190 Me1Em4.600 Me1Em5.820	61.4 62.6 72.4	3.3 3.5 3.1	16.8 18.3 15.9

Table 3 (*Continued*)

Trait	Map	LG	QTL	cM	LOD	PV	Locus	cM locus	LOD locus	PV locus
W2H	**Wild**	**XIV**	**W2H_Wild_XIV.2**	**81.6 93.7**	**4.2**	**22.4 17.8**	**Me1Em5.880**	**83.6 87.1 89.7**	**3.7 4.1 3.2**	**19.4 22.3 13.7**
							Me1Em5.860			
							Me1Em5.890			
W2H	**Wild**	**XIV**	**W2H_Wild_XIV.3**	**96.6 100.6**	**3.5**		**Me1Em5.800**	**96.6**	**3.4**	**17.6**
W2H	Est	XVII	W2H_Est_XVII	9.0 12.0	3.6	19.2	CyEM_281	14.7	2.5	11.3
L2H	**Wild**	**VII**	**L2H_Wild_VII.1**	**67.5 72.7**	**3.5**	**17.9**	**Me2Em1.490**	**70.7**	**3.5**	**17.9**
L2H	Wild	VII	L2H_Wild_VII.2	84.0 87.7	3.3	17.2	Me2Em1.220	86.7	3.3	17.2
L2H	**Wild**	**VII**	**L2H_Wild_VII.3**	**93.5 103.1**	**4.7**	**25.8**	**Me2Em1.600**	**93.5 100.1**	**3.2 4.3**	**16.6 23.0**
							Me2Em3.500			
L2H	**Wild**	**VIII**	**L2H_Wild_VIII.1**	**87.5 91.2**	**3.6**	**18.8**	**Me5Em4.620**	**90.2**	**3.5**	**18.5**
L2H	Wild	VIII	L2H_Wild_VIII.2	98.1 114.8	4.7	25.8	Me5Em4.620	99.8 103.7	3.3 4.7 3.6 3.5	17.2 25.8 19.0
							Me5Em4.600	104.3 109.0	4.1	18.5 22.4
							Me5Em1.650	113.8		
							Me3Em3.140			
							Me5Em4.640			
L2H	**Est**	**XI**	**L2H_Est_XI.1**	**4.0 13.3**	**4.5**	**24.4**	**Me1Em4.910**	**5.3 11.2 12.6**	**4.2 4.3 3.4 3.2**	**23.1 23.7 17.7**
							Me2Em1.205	**13.3**		**16.7**
							Me2Em2.450			
							Me1Em4.340			
L2H	Est	XI	L2H_Est_XI.2	24.6 27.6	3.6	19.1	Me4Em3.690	21.7	1.9	7.2
L2H	**Wild**	**XI**	**L2H_Wild_XI**	**93.3 97.3**	**3.5**	**17.7**	**Me2Em1.205**	**93.3**	**3.0**	**15.0**
L2H	Est	XVII	L2H_Est_XVII	6.0 12.0	3.6	18.8	CyEM_281	14.8	2.3	10.1
D2H	**Wild**	**VII**	**D2H_Wild_VII.1**	**66.2 78.1**	**4.7**	**19.7**	**Me4Em2.420**	**66.5 70.7 75.1**	**4.1 4.7 3.9**	**15.5 19.6 14.2**
							Me2Em1.490			
							Me1Em3.750			
D2H	**Wild**	**VII**	**D2H_Wild_VII.2**	**91.6 102.1**	**6.5**	**30.4**	**Me2Em1.600**	**93.5 100.1**	**4.7 5.9**	**19.8 26.9**
							Me2Em3.500			
D2H	Wild	VIII	D2H_Wild_VIII	111.0 114.8	3.5	18.2	Me5Em4.640	113.8	3.5	17.7
D2H	**Est**	**XI**	**D2H_Est_XI.1**	**4.0 13.3**	**5.2**	**23.1**	**Me1Em4.910**	**5.3 11.2 12.6**	**5.0 5.1 4.4 4.2**	**21.7 21.9 17.3**
							Me2Em1.205	**13.3**		**16.2**
							Me2EM2.450			
							Me1Em4.340			
D2H	**Est**	**XI**	**D2H_Est_XI.2**	**23.7 27.7**	**4.0**	**21.2**	**Me4Em3.690**	**21.7**	**2.3**	**10.0**
D2H	**Est**	**XVII**	**D2H_Est_XVII**	**9.0 12.0**	**4.4**	**17.7**	**CyEM_281**	**14.7**	**2.5**	**11.2**

QTL region or even in the homologous linkage group from the other parental.

3.4. Consistency of the QTLs across two seasons

The QTLs found at the first evaluated season by SIM analysis were compared for their consistency to those observed at the second one (2014) by the same statistical procedure and parameters. Across all the traits evaluated, 42 QTLs were detected in both seasons and they were localized at clustered QTL regions (Wild_VII, VIII, XI, XIV, Est_XI, and XVII).

4. Discussion

The location of genes or genomic regions affecting economically important traits is of importance to plant and animal breeders. Many of these traits, such as yield or heading date, show a continuous range of values. This is due to the joint effects of several genetic loci (quantitative trait loci or QTLs) and the environment (Hackett, 2002). As the parental phenotypes were significantly different from one to another and the range of variation in the progeny was large for all the evaluated traits, the usefulness of these yield-related traits and the viability of this population for genetic studies to identify QTL associated to yield were confirmed.

The parent wild cardoon showed a high number of heads per plant but they were lighter and smaller than the globe artichoke "Estrella del Sur". This is related to the origin of globe artichoke, where a positive selection toward a large head size was applied during domestication, whereas the wild cardoon is considered its ancestor (Rottenberg and Zohary, 1996; Sonnante et al., 2007).

The inter-trait correlations revealed that diameter and length of the main and secondary heads were the most important factors influencing fresh weigh, with a high positive correlation between these traits in both evaluated seasons. The number of head per plant showed positive correlation values with all the evaluated traits. Similar results were reported by López Anido et al. (1998) who evaluated 27 accessions of *C. cardunculus* for yield and yield-related traits. Also, based on the correlation values the head weight depends more of its diameter than of its height.

Transgressive segregation was observed for number of head per plant where a high number of F_1 individuals (~33% in 2013 and ~20% in 2014) showed higher values than their wild parent; but no progeny produced head as heavy as those produced by "Estrella del Sur". Portis et al. (2014) observed similar results in a cross between a domesticated cardoon (ALT) and globe artichoke (C3). Transgressive segregation was also observed by López Anido et al. (2010) when two F_2 populations from reciprocal crosses of artichoke were evaluated.

A total of 70 SSR markers were polymorphic among parents and segregated in the F_1 population. Sixty-eight of these SSRs (97%) presented a fully consistent monogenic Mendelian segregation ($\chi^2 \leq \chi^2_{\alpha=0.1}$) and it were used for QTL-marker association. This single-marker analysis is useful to start an evaluation of the genetics of quantitative traits by testing for associations between the trait values and marker genotypes (Hackett, 2002). In total, 22 QTLs were identify and linked to eleven SSR markers. The highest number of QTL-marker associations was observed for diameter of the second heads, with a total of eight QTLs detected. Moreover, three SSRs were associated with fresh weight of the mean head, three with diameter of the main head, five with fresh weight of the second heads and three with length of the second heads.

The proportion of total phenotypic variation explained by an individual locus is probably the most important obstacle to the implementation of marker-assisted selection (Vasconcelos Cavalcanti et al., 2010). In general terms, an individual QTL may be described as "major" or "minor", based on the proportion of the phenotypic variation that it explain (R^2 value). Major QTL will account for a relatively large amount of variation (>10%) and minor QTLs will usually account for <10% (Collard et al., 2005). In this work, the percentages of phenotypic variation explained by the associations between the SSR locus and the QTL ranged from 6 to 18%. Of these, 36.4% reveal major effects (R^2 ranged from 0.10 to 0.18) in one or few loci, while most QTL explained less than 10% of the variation (minor-effect QTL). Similar results were observed in sunflower, an *Asteraceae* member closely related to globe artichoke, where the percentage of phenotypic variance explained by QTL for yield-related traits ranged from 4% to 40% (Poormohammad Kiani et al., 2009). Rachid et al. (2004), in a population of 77 sunflower RILs, reported that the effect of each QTL related to several agronomic traits (plant height, stem diameter, head diameter, grain weight per plant, 1,000 grain weight and percentage of oil in grains) ranged from 7% to 37%. Similar results have been reported in studies in other crops (Vasconcelos Cavalcanti et al., 2010; Pereira da Costa et al., 2014). Sometimes, major QTLs may refer to QTLs that are stable across environments whereas minor QTLs may refer to those that may be environmentally sensitive (Collard et al., 2005). Several works related to the identification of major and minor QTLs highly influenced by the environment were reported in rice (Fan et al., 2006; Chen et al., 2014; Xu et al., 2015), barley (Xue et al., 2010), and cotton (Shen et al., 2007).

In a simple interval mapping, the traits values are related to the genotype of a putative QTL at different locations along each linkage group or chromosome. In this procedure, the information from a linkage map of molecular makers is used to infer the probability of each QTL genotype (Hackett, 2002). The QTL mapping analysis was based on molecular linkage map previously developed by Martin et al. (2013) between a local genotype of wild cardoon and the commercial variety "Estrella del Sur FCA" of globe artichoke. A total of 66 QTLs ($LOD_{min} = 3.0$) were detected across both parental linkage maps (31 QTLs at $LOD_{min} = 4.0$), 55 of them were located in six linkage groups establishing clustered QTL regions. A clustered QTL region at LG XI was identified in both parental maps, showed that the included loci were heterozygous in both progenitors, whereas the other four clustered QTL regions were heterozygous in only one of the parents. The number of QTL detected per trait (mean 9.4) and the percentage of phenotypic variance explained by each QTL were superior to the values reporter by Portis et al. (2014). Based on the QTL classification by Collard et al. (2005), all the QTLs detected in this work by the SIM analysis were major QTL (>10%) and 27 of these explained up to 20% of the phenotypic variance.

The genetic location of some of the QTLs agree with those assigned by Portis et al. (2014) to related-yield QTL, particularly those detected in Wild VII, VIII, IX, and XIV in our work with C3_7/Alt_5, C3_8/Alt_10, C3_9/Alt_17, and C3_14. Only five QTLs identified from the ANOVA analysis were detectable by the SIM procedure. Usually, a one-way ANOVA is applied to identify molecular marker linked to agronomic traits when the development of a linkage map is not possible (Collard et al., 2005; Pereira da Costa et al., 2014). According to our results, most of the linked SSR detected by the ANOVA analysis were false positives. Bernardo (2004) reported that for a trait controlled by few genes, like yield, the proportion of false QTL can be 10–30 times higher than the comparison-wise significance level used. Since the identification of a QTL is a statistical procedure always carries some risk that such identification is false and this can result in wasted resources, as well as confuse the QTL literature and databases.

The QTLs identified in both seasons were compared for their consistency. A total of 42 QTLs were validated among both seasons by SIM. Moreover, some of these were mapped at clustered QTL regions coincided with those reported by Portis et al. (2014). The linkage group XI, shown several yield-related QTLs co-localized at

the same region in both parents over the two seasons, suggesting that this region will be target in breeding programs related to improve the yield in the species.

5. Conclusion

This work represents an initial platform for the dissection of a complex trait, such as yield in *C. cardunculus* L. We have detected 66 new QTLs controlling yield-related traits, providing evidence that there are several loci controlling globe artichoke yield. The purpose of most of the globe artichoke breeding programs is to increase the number of head per plant without reduce weight and dimension of the heads. According to this, plants should be selected with the presence of markers linked to QTL with positive phenotypic effects for number of heads per plant (QTL mapped in wild cardoon parental) in combination with high values for weight and dimension of the heads (QTLs identifies at globe artichoke parental). Even if the QTLs were confirmed in a second year evaluation, the QTL identified in this work should be validated using other genetic backgrounds and environments. This QTLs and their molecular marker associated will increase the selection efficiency, and elite plants with higher yields and quality production will be selected in an early development stage.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.scienta.2016.02.033.

References

Acquadro, A., Lanteri, S., Scaglione, D., Arens, P., Vosman, B., Portis, E., 2009. Genetic mapping and annotation of genomic microsatellites isolated from globe artichoke. Theor. Appl. Genet. 118 (8), 1573–1587.

Balzarini, M., Di Rienzo, J., 2004. Info-Gen: Software para análisis estadístico de datos genéticos. Facultad de Ciencia Agropecuarias. Universidad Nacional de Córdoba, Argentina.

Bernardo, R., 2004. What proportion of declared QTL in plants are false? Theor. Appl. Genet. 109, 419–424.

Chen, J., Guo, L., Chen, Y., Zhang, H., Ying, J., Zhuang, J., 2014. Fine mapping of qHd1, a minor heading date QTL with pleiotropism for yield traits in rice (*Oriza sativa* L.). Theor. Appl. Genet. 127 (11), 2515–2524.

Collard, B.C.Y., Jahufer, M.Z.Z., Brouwer, J.B., Pang, E.C.K., 2005. An introdution to markers, quantitative trait loci (QTL) mapping and marker-assited selection for crop improvement: the basic consepts. Euphytica 142, 169–196.

Cravero, V., Picardi, L., Cointry, E., 2005. An approach for understanding the heredity of two quality traits (head color and tightness) in globe artichoke (*Cynara scolymus* L.). Genet. Mol. Biol. 28, 431–434.

Cravero, V., Martin, E., López Anido, F., Cointry, E., 2010. Stability through years in a non-balanced trial of globe artichoke varietal types. Sci. Hortic. 126, 73–79.

Fan, C., Xing, Y., Mao, H., Lu T. m Han, B., Xu, C., Li, X., Zhang, Q., 2006. GS3, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encondes a putative transmembrane protein. Theor. Appl. Genet. 112 (6), 1164–1171.

FAO. 2013. Food and Agriculture Organization of the United Nations. Available online: http://fao.org.

Hackett, C.A., 2002. Statistical methods for QTL mapping cereals. Plant Mol. Biol. 48, 585–599.

López Anido, F., Firpo, I., Garcia, S., Cointry, E., 1998. Estimation of genetic parameters for yield traits in globe artichoke (*Cynara scolymus* L.). Euphytica 103, 61–66.

López Anido, F.S., Cravero, V.P., Martin, E.A., Crippa, I., Cointry, E.L., 2010. Differential expression of the genetic variance in F2 populations of reciprocal crosses of artichoke. Span. J. Agric. Res. 8 (3), 679–685.

Martin, E., Cravero, V., Esposito, M., Lopez anido, F., Milanesi, L., Cointry, E., 2008. Identification of markers linked to agronomic traits in globe artichoke. Aust. J. Crop Sci. 1 (2), 43–46.

Martin, E., Cravero, V., Portis, E., Scaglione, D., Acquaviva, E., Cointry, E., 2013. New genetic maps for globe artichoke and wild cardoon and their alignment with an SSR-based consensus map. Mol. Breed. 32, 177–187.

Mauromicale, G., Morello, N., Santoiemma, G., Ierna, A., 2000. Nuove varieta' per migliorare la cinaricoltura siciliana. Inf. Agr. 26, 47–51.

Moglia, A., Lanteri, S., Comino, C., Acquadro, A., De Vos, R., Beekwilder, J., 2008. Stress-induced biosynthesis of dicaffeoylquinic acids in globe artichoke. J. Agric. Food Chem. 56, 8641–8649.

Pandino, G., Lombardo, S., Mauromicale, G., Williamson, G., 2011. Phenolic acids and flavonoids in leaf stem of cultivated and wild *Cynara cardunculus* L. genotypes. Food Chem. 126, 417–422.

Pereira da Costa, J.H., Rodríguez, G.R., Pratta, G.R., Picardi, L.A., Zorzoli, R., 2014. QTLs detection for fruit shelf life and quality traits across segregating populations of tomato. Sci. Hortic. 156, 47–53.

Poormohammad Kiani, S., Maury, P., Nouri, L., Ykhlef, N., Grieu, P., Sarrafi, A., 2009. QTL analysis ofuield-related traits in sunflower under different water treatments. Plant Breed. 128 (4), 363–373.

Portis, E., Scaglione, D., Acquadro, A., Mauromicale, G., Mauro, R., Knapp, S., Lanteri, S., 2012. Genetic mapping and identification of QTL for earliness in the globe artichoke/cultivated cardoon complex. BMC Res. Notes 5, 252.

Portis, E., Mauro, R.P., Barchi, L., Acquadro, A., Mauromicale, G., Lanteri, S., 2014. Mapping yield-associated QTL in globe artichoke. Mol. Breed. 34, 615–630.

Rachid, G., Al-Chaarani, L., Gentzbittel, X., Huang, Q., Sarrafi, A., 2004. Genotypic variation and identification of QTLs for agronomic traits, using AFLP and SSR markers in RILs of sunflower (*Helianthus annuus* L.). Theor. Appl. Genet. 109 (7), 1353–1360.

Rottenberg, A., Zohary, D., 1996. The wild ancestry of the cultivated artichoke. Genet. Resour. Crop Evol. 43, 53–58.

Scaglione, D., Acquadro, A., Portis, E., Taylor, C.A., Lanteri, S., Knapp, S.J., 2009. Ontology and diversity of transcript-ssociated microsatellites mined from globe artichoke EST database. BMC Genom. 10, 454.

Shapiro, S.S., Wilk, M.B., 1965. An analysis of variance test for normality (complete samples). Biometrika 52, 591–611.

Shen, X., Guo, W., Lu Zhu, X., Yuan, Y., Zhang, T., 2007. Genetic mapping of quantitative trait loci for fiber quality and yield trait by RIL approach in Upland cotton. Euphytica 155, 371–380.

Shinohara, T., Shinsuke, A., Kil, S.Y., Leskovar, D.I., 2011. Irrigation and nitrogen management of artichoke: yield, head quality, and phenolic content. HortScience 46 (3), 377–386.

Snedecor, G., 1964. Métodos Estadísticos, quinta ed. Compañía Editorial, México D.F.631.

Sonnante, G., Pignone, D., Hammer, K., 2007. The domestication of artichoke and cardoon: from roman times to the genomic age. Ann. Bot. 100 (5), 1095–1100.

Xu, F., Sun, X., Chen, Y., Huang, Y., Tong, C., Bao, J., 2015. Rapid identification of major QTLs associated with rice grain weight and their utilization. PLoS One 10 (3), e0122206, http://dx.doi.org/10.1371/journal.pne.0122206.

Xue, D., Zhou, M., Zhang, X., Chen, S., Weu, K., Zeng, F., Mao, Y., Wu, F., Zhang, G., 2010. Identification of QTLs for yield and yield components of barley under different growth conditions. J. Zhejiang Univ.-Sci. B 11 (3), 169–176.

Van Ooijen J.W. 2006. JoinMap® v.4, software for the calculation of genetic linkage maps in experimental populations. Kyazma BV, Wageningen, Netherlands.

Van Ooijen J.W. 2009. MapQTL® v.6, Software for the mapping of quantitative trait loci in experimental populations of diploid species. Kyazma BV, Wageningen, Netherlands.

Vasconcelos Cavalcanti, J.J., Costa dos Santos, F.H., Pereira da Silva, F., Pinheiro, C.R., 2010. QTL detection of yield-related traits of cashew. Crop Breed. Appl. Biotechnol. 12, 60–66.

Voorrips, R., 2002. MapChart: software for the graphical presentation of linkage maps and QTLs. J. Hered. 93 (1), 77–78.