Development of a *C. cardunculus* Linkage Map Based on SRAP, SSR, SNP Markers and Localization of Phenotypic Traits

E. Martin[1,a], E. Acquaviva[2], V. Cravero[1], E. Portis[3], D. Scaglione[3], S. Lanteri[3] and E. Cointry[2]
[1] CONICET, Facultad de Cs. Agrarias, Universidad Nacional de Rosario, Argentina
[2] Facultad de Cs. Agrarias,Universidad Nacional de Rosario, Argentina
[3] DIVAPRA, Plant Genetics and Breeding, Università degli Studi di Torino, Italy

Keywords: artichoke, wild cardoon, molecular markers, color head, spine leaf, spine head

Abstract

In order to develop a new linkage map of *C. cardunculus* L., an F_1 mapping population was generated from an intraspecific cross between a local genotype of wild cardoon and the globe artichoke 'Estrella del Sur FCA'. The population was genotyped using a combination of molecular markers and 3 phenotypic traits. A total of 421 segregating loci were identified. The female parent map (988.09 cM) consisted in 127 loci, spread over 15 linkage groups (LGs), while the male one (578.2 cM) resulted in 112 loci, distributed in 10 major LGs. The alignment between the female and male maps, by using intercross markers, allowed to determine 17 major LGs. The phenotypic traits Color Head and Spine Leaf were included in the wild cardoon map, whereas Spine Head was positioned on the LG7 globe artichoke map. The presence of 48 loci in common between the present maps and the previously developed SSR-based linkage maps of the *Cynara cardunculus* species, allowed the alignment of the LGs. The developed maps represent important tools for the localization of interesting agronomic traits as well as for molecular-assisted breeding strategies and will be useful for the construction of an integrated map and for comparative genetics analysis.

INTRODUCTION

The genetic linkage maps represent an important tool for the localization of interesting agronomic traits as well as for molecular-assisted breeding strategies. The construction of an integrated map with genetic information from all taxa included in *C. cardunculus* L., will be useful to understand the genetics bases of important agronomic traits and for comparative genetics analysis. The high level of inbreeding depression observed in the species, does not allow the use of backcross, F_2 or recombinant inbred lines populations for mapping purposes. The haploid induction is not achievable until now, therefore is not available to generate doubled haploid populations. Thus, double pseudo-testcross approach appears as a good option for genetic mapping. In this strategy, segregating progeny is derived from a cross between two heterozygous individuals. In *C. carcundulus* L., the first genetic map was developed by Lanteri et al. (2006) analyzing a F_1 population derived from a cross between two globe artichoke types. Afterward, a number of other segregating populations have been exploited, including one generated from a hybrid between a globe artichoke and a cultivated cardoon genotype (Portis et al., 2009) and, more recently, two obtained by crossing globe artichoke with wild cardoon (Sonnante et al., 2011; Lanteri et al., 2012). The recent development of a set of gene-based microsatellites (Scaglione et al., 2009) has aided the construction of consensus genetic maps (Portis et al., 2012).

The aims of the present research, was to develop a new linkage map of *C. cardunculus* L. based on an intraspecific cross between a local genotype of wild cardoon and the globe artichoke 'Estrella del Sur FCA' and to position in this map three important phenotypic traits: Color Head, Spine Leaf and Spine Head.

[a] eamartin@unr.edu.ar

MATERIALS AND METHODS

The F_1 mapping population was generated from an intraspecific cross between a local genotype of wild cardoon (var. *sylvestris*), used as female parent, and the globe artichoke (var. *scolymus*) 'Estrella del Sur FCA', as male one, by adopting the two-way pseudo testcross strategy. The male progenitor is non-spiny varietal type and purple heads; while the female one have long sharps on its bracts and leaves and green heads. The genomics DNA from putative F_1 plants was extracted using the DNeasy Plant mini Kit (QIAGEN). The individual plants were checked for hybrid status using two informative SSR markers (Celms-30 and Celms-37) (Acquadro et al., 2009) and 81 progenies were selected for segregation analysis and map construction. The mapping population was genotyped using a combination of markers: SRAP (Sequence Related Amplified Polymorphism), SSR (Simple Sequence Repeats) and SNPs (Single Nucleotide Polymorphisms). Furthermore, three phenotypic traits (Color Head, Spine Leaf and Spine Head) were also evaluated in two seasons (2008 and 2009). Linkage analysis was performed with JoinMap4.0 (Van Ooijen, 2006). Differences between observed and expected segregation ratios were tested by χ^2, and only markers that showed Mendelian segregation ($\chi^2 \leq \chi^2_{\alpha=0.1}$) or a minor deviation ($\chi^2_{\alpha=0.1} < \chi^2 \leq \chi^2_{\alpha=0.01}$) were used for map construction and for the estimation of genetic distances. Heavily distorted loci ($\chi^2 > \chi^2_{\alpha}=0.01$) were excluded.

RESULTS AND DISCUSSION

A total of 25 SRAP primers combination (Cravero et al., 2007), 279 SSR (Acquadro et al., 2003, 2005a,b, 2009; Scaglione et al., 2009) and 10 SNPs (Comino et al., 2007, 2009; Moglia et al., 2009; Menin et al., 2010) were used to genotyped the mapping population.

From the different molecular markers, 421 segregating loci were identified (336 SRAPs, 77 SSRs, 5 SNPs and 3 phenotypic traits) and a set of 304 loci, which showed a Mendelian segregation ratio ($\chi^2 \leq \chi^2_{\alpha=0.01}$), were used for map construction and genetic distances estimation.

The female parent map (988.09 cM) consisted in 127 loci, spread over 15 linkage groups (LGs) with a mean inter-marker distance of 7.78 cM, while the male one (578.2 cM) resulted in 112 loci, distributed in 10 major LGs with a mean inter-marker distance of 5.16 cM (Fig. 1). The alignment between the female and male maps, by using intercross markers, allowed to determine 17 major LGs (with more than 4 markers), corresponding to the haploid number of the species.

The phenotypic traits Color Head and Spine Leaf were included in the wild cardoon map; Color Head was located on the LG 14, between C_Me5Em3.01 (SRAP) and C_CyEM_86 (SSR) markers, whereas Spine Leaf was included in LG 7 between two SRAPs. The trait Spine Head was positioned between SNP Acyltrans_1 and the microsatellite CELMS-07, on the globe artichoke LG 7.

The presence of 48 loci (44 SSRs and four SNPs) in common between the present maps and the previously developed SSR-based linkage maps (Portis et al., 2009b; Sonnante et al., 2011), allowed the alignment of the LGs; indicated that a set of 14 SSR markers was here positioned, for the first time, in a *C. cardunculus* map.

The genetics linkage maps represent important tools for the localization of interesting agronomic traits as well as for molecular-assisted breeding strategies and will be useful for the construction of an integrated map and for comparative genetics analysis.

Literature Cited

Acquadro, A., Portis, E. and Lanteri, S. 2003. Isolation of microsatellite loci in artichoke (*Cynara cardunculus* L. var. *scolymus*). Mol. Ecol. Notes 3:37-39.

Acquadro, A., Portis, E., Lee, D., Donini, P. and Lanteri, S. 2005a. Development and characterization of microsatellite markers in *Cynara cardunculus* L. Genome 48:217-225.

Acquadro, A., Portis, E., Albertini, E. and Lanteri, S. 2005b. M-AFLP-based protocol for

microsatellite loci isolation in *Cynara cardunculus* L. (*Asteraceae*). Mol. Ecol. Notes 5:272-274.
Acquadro, A., Lanteri, S., Scaglione, D., Arens, P., Vosman, B. and Portis, E. 2009. Genetic mapping and annotation of genomic microsatellites isolated from globe artichoke. Theor. Appl. Genet. 118:1573-1587.
Comino, C., Lanteri, S., Portis, E., Acquadro, A., Romani, A., Hehn, A., Larbat, R. and Bourgaud, F. 2007. Isolation and functional characterization of a cDNA coding a hydroxycinnamoyltransferase involved in phenylpropanoid biosynthesis in *Cynara cardunculus* L. BMC Plant Biol. 7:14.
Comino, C., Hehn, A., Moglia, A., Menin, B., Bourgaud, F., Lanteri, S. and Portis, E. 2009. The isolation and mapping of a novel hydroxycinnamoyltransferase in the globe artichoke chlorogenic acid pathway. BMC Plant Biol. 9:30.
Cravero, V., Martin, E. and Cointry, E. 2007. Genetic diversity in *Cynara cardunculus* determined by sequence-related amplified polymorphism markers. J. Amer. Soc. Hort. Sci. 132(2):1-5.
Lanteri, S., Acquadro, A., Comino, C., Mauro, R., Mauromicale, G. and Portis, E. 2006. A first linkage map of globe artichoke (*Cynara cardunculus* var. *scolymus* L.) based on AFLP, S-SAP, M-AFLP and microsatellite markers. Theor. Appl. Genet. 112:1532-1542.
Lanteri, S., Portis, E., Acquadro, A., Mauro, R.P. and Mauromicale, G. 2012. Morphology and SSR fingerprinting of newly developed *Cynara cardunculus* genotypes exploitable as ornamentals. Euphytica 184(3):311-321.
Menin, B., Comino, C., Moglia, A., Dolzhenko, Y., Portis, E. and Lanteri, S. 2010. Identification and mapping of genes related to caffeoylquinic acid synthesis in *Cynara cardunculus* L. Plant Science 179:338-347.
Moglia, A., Comino, C., Portis, E., Acquadro, A., De Vos, R., Beekwilder, J. and Lanteri, S. 2009. Isolation and mapping of a C3'H gene (CYP98A49) from globe artichoke, and its expression upon UV-C stress. Plant Cell Reports 28:963-974.
Portis, E., Mauromicale, G., Mauro, R., Acquadro, A., Scaglione, D. and Lanteri, S. 2009. Construction of a reference molecular linkage map of globe artichoke (*Cynara cardunculus* var. *scolymus*). Theor. Appl. Genet. 120:59-70.
Portis, E., Scaglione, D., Acquadro, A., Mauromicale, G., Mauro, R., Knapp, S.J. and Lanteri, S. 2012. Genetic mapping and identification of QTL for earliness in the globe artichoke / cultivated cardoon complex. BMC Research Notes 5:252.
Scaglione, D., Acquadro, A., Portis, E., Taylor, C., Lanteri, S. and Knapp, S. 2009. Ontology and diversity of transcript-associated microsatellites mined from a globe artichoke EST database. BMC Genomics 10:454.
Sonnante, G., Gatto, A., Morgese, A., Montemurro, F., Sarli, G., Blanco, E. and Pignone, D. 2011. Genetic map of artichoke × wild cardoon: toward a consensus map for *Cynara cardunculus*. Theor. Appl. Genet. 123(7):1215-1229.
Van Ooijen, J. 2006. JoinMap 4: software for the calculation of genetic linkage maps in experimental populations. Kyazma B.V., Wageningen.

Figures

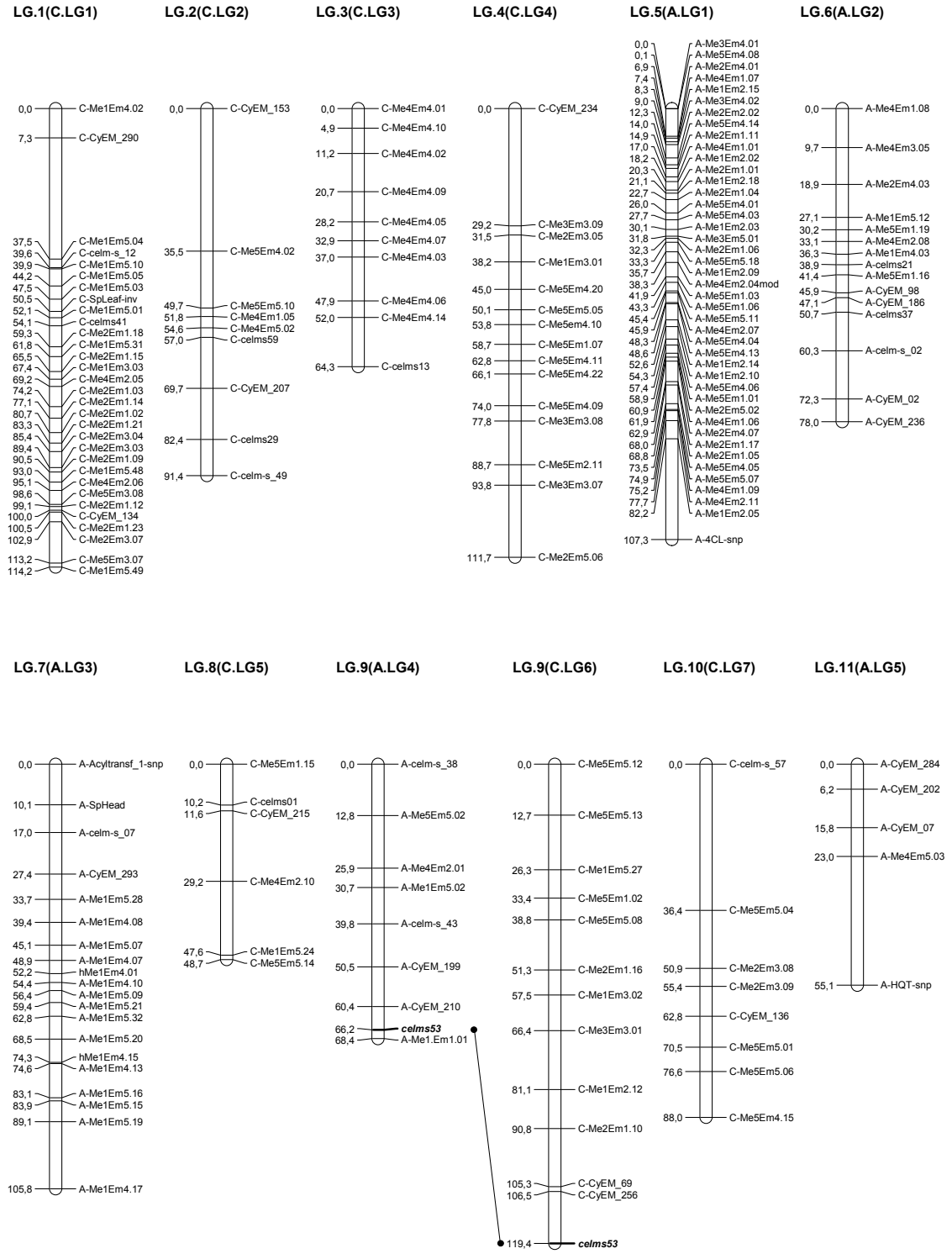

Fig. 1. Genetic linkage map of *Cynara cardunculus* L., derived from the cross between wild cardoon × 'Estrella del Sur FCA'. LG derived from the wild cardoon were identified with C and LG from artichoke were identified with A. The common intercross loci are shown in bold, and their positions are connected with a line to identified the homologous LG.

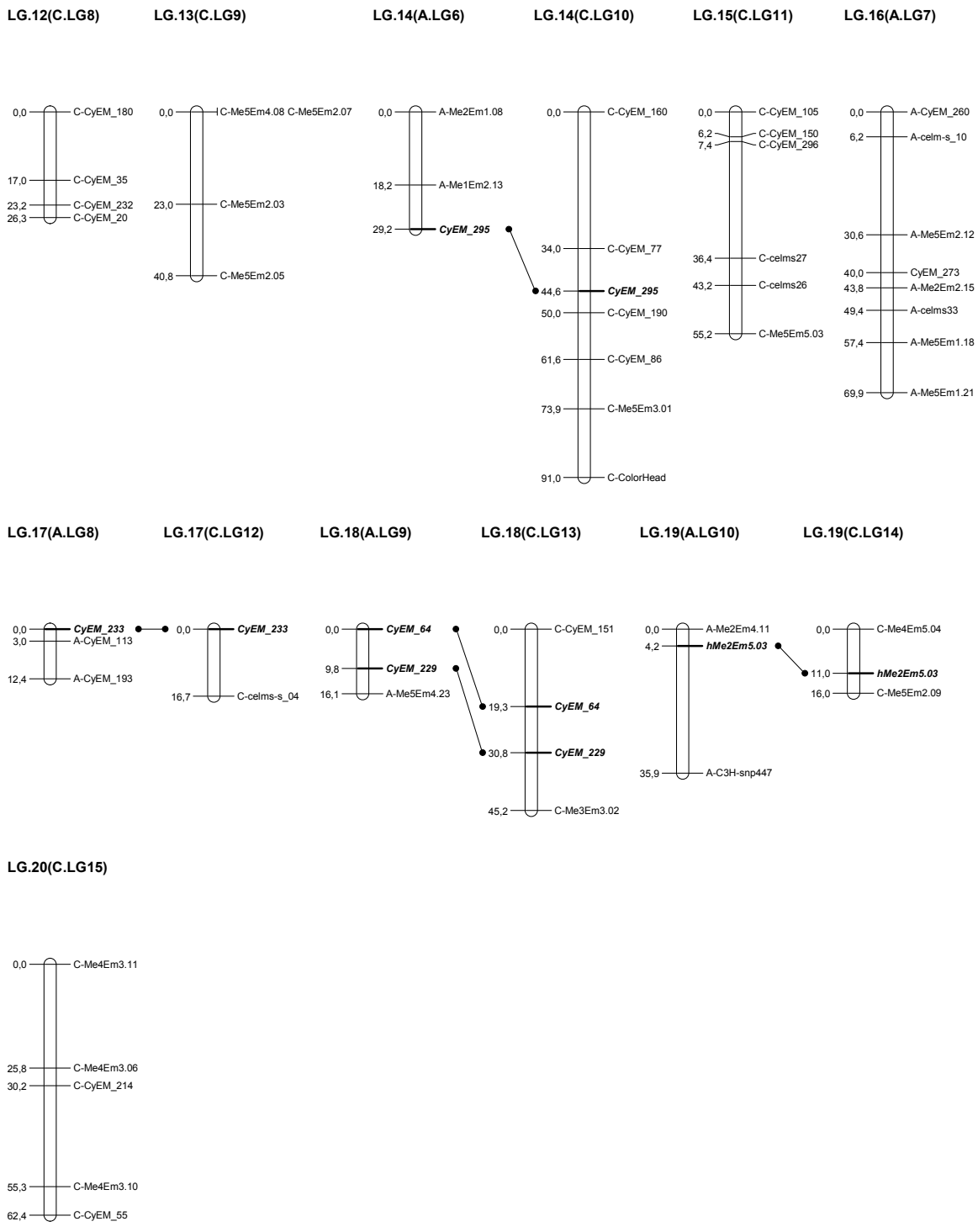

Fig. 1. Continued.