Genetics and Breeding

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Abstract

Genetic variation within globe artichoke landraces contributes to the variable yield performance detected in cultivation. It was built up over many generations of vegetative propagation as a consequence of the limited selection applied by farmers as well as the accumulation of mutations. This chapter reports the results obtained by applying clonal selection programmes within local landraces, with the goal to identify genotypes retaining desirable traits and able to ensure a higher and stable yield. The selected high-yielding genotypes while contributing to increase productivity may also implement the conservation 'in situ' of valuable germplasm at risk of extinction. With the goal to move to breeding programme based on crossing strategies, it is pivotal the development of genetic maps with high levels of genome coverage. Genetic maps represent the first step for localizing genes or quantitative trait loci (QTL) that are associated with economically important trait and open the way for marker-assisted selection, comparative mapping between different species, a framework for anchoring physical maps and the basis for map-based cloning of genes. In this chapter, the history of the development of genetic maps in *Cynara cardunculus* is retraced, starting from the first maps based on a limited number of mostly dominant molecular markers, up to the most recently developed maps allowing the identification of genomic region influencing key agronomic traits.

6.1 Clonal Selection and Molecular Fingerprinting

The application of PCR-based markers to assess the genetic variation between and within populations of clonally propagated globe artichoke varietal types (Lanteri et al. 2001; Portis et al. 2005; Ciancolini et al. 2012) has highlighted their multi-clonal composition, which is less evident but also detectable at the phenotypic level. The latter was mainly attributed to the limited selection criteria adopted by farmers since, after some years of cultivation, plants are mown at the end of the growing season and the propagative material used for replanting is collected some months later, without any selection of the mother plants. An additional source of variation is presumably due to spontaneous mutations occurred over time after repeated cycles of cloning. These findings have stimulated the application of clonal selection programmes,

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with the goal to identify superior genotypes more productive and/or possessing high qualitative traits of the capitula.

Lanteri et al. (2004) applied a clonal selection programme within the varietal type 'Spinoso sardo' grown in Sardinia Island. A sample of 500 ovoli from as many plants was randomly collected from five populations grown in areas characterized by variable pedo-climatic conditions. The plants obtained were characterized for morphology, production and earliness over two growing seasons. A set of 24 early and more productive plants were identified and, from each of them, five plants were clonally propagated. The resulting 120 plants (24 genotypes each represented by 5 plants) were genotyped by applying 26 inter-simple sequence repeat (ISSR) primers as well as 31 amplified fragment length polymorphism (AFLP) primer combinations. The ISSR markers disclosed an extremely low level of polymorphism, while the UPGMA cluster analysis based on AFLP differentiated each selected clone and demonstrated their suitability for their fingerprinting. In the study, the AFLP markers were also used to assess genetic uniformity within each clone and, from just three plants of one clone, an electrophoretic pattern polymorphic for one or two bands was detected. Since the variant pattern was observed on the same plants of the clone with at least two primer combinations, while the other plants were 'true to clone', the authors excluded possible mis-labelling during clonal propagation or laboratory errors. Otherwise, they hypothesized that the mother plant from which the clone was obtained was a chimera not phenotypically detectable but maintained over time through vegetative propagation.

Mauro et al. (2012) performed a clonal selection within the varietal type 'Violetto di Sicilia', whose cultivation is spread in the eastern area of Sicily Island. Four populations were identified in as many locations representative of the growing area and, in each of them, propagative material was sampled from seven to ten plants. The latter were chosen on the basis of the number of floral stem ramifications (an index of yield potential), earliness as well as proper head pigmentation, shape and thickness. On the whole, 36 genotypes were selected from which 3 to 10 semi-dormant offshoots were collected. The plants obtained were characterized for morphological traits and yield during the two following growing seasons, and this led to discard 26 clones. The remaining 10, including at least one clone for each site, were further on propagated with the goal to obtain at least 20 plants per clone and then characterized for a number of head traits and number of floral stem ramifications during further two growing seasons. All traits were significantly variable and for the most part subjected to a marked seasonal influence. The mean production of the selected clones was about nine heads per plant, corresponding to a fresh weight around 1.3 kg. However, two clones produced an average of 10.6 heads, equivalent to 1.46 kg per plant over both growing season, while two produced the largest heads (on average 165 g), whose receptacle incidence reached on average 19.3 g for 100 g of fresh weight. In the study, a moderate, broad-sense heritability was detected for total yield, number of heads per plant and receptacle incidence, which means these traits represent the primary target for clonal selection within this varietal type. The ten selected clones together with ten genotypes of 'Violetto di Sicilia', which in a previous study were identified by the authors as representatives of the genetic variation within this varietal type, were genotyped by applying seven AFLP primer combinations, and just three were able to fingerprint each clone. Furthermore, three clones were found to possessed unique fragments, which the authors suggested convertible into sequence tagged site assays for their easy identification in nurseries. Interestingly, the principal component analysis based on molecular data did not group separately the ten selected clones and the other ten genotypes included in the study, demonstrating that the former are thus representative of the genetic variation present in cultivation. This result appeared to confirm that the clonal selection within 'Violetto di Sicilia' may provide higher productive genotypes without causing genetic erosion and promoting its preservation in situ. Lastly, the authors highlighted only a limited

correlation between phenotypic and the genotypic data and hypothesized that this was so because most of the AFLP loci were sited in the non-coding portion of the genome and the traits in the study were much affected by environmental conditions.

With an approach analogous to the one previously described, Mauro et al. (2015) performed a clonal selection programme within another Sicilian varietal type, 'Spinoso di Palermo', which is mainly grown in the western area of Sicily Island. Within five populations grown in as many locations, from three to eight plants were selected on the basis of the number of floral stem ramifications as well as earliness in the production of heads and their number, size, colour and firmness. A set of 30 selected plants was clonally propagated and from 8 to 10 plants per clone obtained. Over the following two growing seasons, 25 clones were discarded and 5 maintained. The number of plants per selected clone was then increased to 54 for each clone (for a total of 270 plants) by transplanting their lateral offshoots, with the goal to perform a more reliable morphological characterization during two further growing seasons. A set of 54 plants of the allochthonous and early varietal type 'Violet de Provence' was also included in the study, since being characterized by high yields and long productive cycle is spreading in Southern Italy and endangers native local landraces. The morphological characterization of the whole set of plants was based on 51 traits, according to the guidelines provided by International Union for the Protection of New Varieties of Plants (UPOV). Three of the evaluated traits, e.g. days to first harvest, duration of harvest period and rate of yield (expressed as the ratio yield and harvest duration), were found significantly affected by the clone × season interaction, while the weight of main heads proved to be the most stable. The head yield and number of heads per plant were found associated with a moderate level of broad-sense heritability, suggesting that they represent key traits for primary selection criteria within this landrace. Eighteen out of the 51 scored traits did not differentiate the varietal types 'Spinoso di Palermo' and 'Violet de Provence', while other 18 were distinctive of the 'Spinoso di Palermo' clones. The latter produced an average number of heads of 13.8 per plant which corresponded to a weight of 2 kg/plant, a value higher than the one detected in 'Violet de Provence'. Two clones produced up to 2.4 kg of capitula per plant and three noticeably larger second-order heads (mean of 156 g), thus resulting suitable for the production of commercially valuable heads over a prolonged harvesting period.

Two plants for each selected clone as well as 12 genotypes previously representative of genetic variation within 'Spinoso di Palermo' and two plants of Violetto di Sicilia (used as a reference) were genotyped with seven AFLP primer combinations. The UPGMA dendrogram obtained from AFLP data clustered separately the two varietal types, and just three out of the five primer combinations were able to discriminate each clone. In this study, differently from what reported by the same authors for the varietal type 'Violetto di Sicilia' (Mauro et al. 2012), the grouping of entries generated by AFLP analyses was highly consistent with the grouping based on morphological variation, presumably as a consequence of the greater accuracy in phenotyping the material under study.

More recently, Crinò and Pagnotta (2017) performed the morphological, biochemical and molecular characterization of ten clones, result of a selection programme (Crinò et al. 2008), and representative of the 'Romanesco' varietal type which is mainly grown in Latium region of Central Italy. The ten clones were characterized by early medium and late head production. Twelve plants per clone were phenotyped, over two subsequent growing seasons, on the basis of 47 traits including globe artichoke standard UPOV descriptors and complementary Romanesco-type descriptors reported by Ciancolini et al. (2012). Morphological differences among clones were detected for 15 traits, among which shape of the head (ranging from elongated to elliptical), receptacle shape, head pigmentation (ranging from purple striped to green) and head yield, while 8 traits showed significant clone-per-year interactions highlighting their unsuitability as standard descriptors. The

similarity dendrogram based on hierarchical cluster analysis of morphological data identified three main clusters while principal component analysis highlighted that four traits (i.e. plant height, central flower-head weight, earliness and total flower-head weight) made it possible a clear grouping of the clones.

One plant of each clone was also genotyped with seven AFLP primer combinations, four ISSR and thirteen SSR markers, and 393 molecular polymorphisms were detected. Molecular assessment was allowed to discriminate each clone, and the UPGMA dendrogram based on Nei's genetic distances identified three main clusters which, however, were not consistent with the ones obtained on the basis of morphological variation.

During the first growing season, at least nine primary flower-heads from each clone were collected at the commercial stage and their polyphenol profile obtained on the basis of HPLC. The authors reported that differently from what previously found by Pandino et al. (2015) in leaves of a genotype of 'Romanesco C3', cynarin (1,3-di-O-CQA), apigenin and luteolin were not detected in any of the sample in study. On the other hand, significant differences were detected among clones with respect to the total polyphenol content as well as 1,5-O-dicaffeoylquinic acid, chlorogenic acid and cynaroside (Luteolin 7-O-glucoside).

The result of the study made it possible the identification of two clones, characterized by remarkable earliness and highly productive, which have been included with the names 'Michelangelo' and 'Raffaello' in the National Register of Varieties of the Italian Ministry of Agricultural, Food and Forestry Policies.

The results obtained in these studies highlight that clonal selection is effective in identifying superior genotypes within the population of globe artichoke varietal types which at present are vegetatively propagated. The selected genotypes may represent the starting material for nursery activities, but undoubtedly, a limit is represented by the time needed to produce an adequate quantity of their propagative material for field cultivation. This, together with the fact that the use of the achene as reproductive unit makes it possible to treat the crop as annual and reduce the cost of planting and the diffusion of pathogens, has led to the growing in popularity of seed-propagated cultivars and F_1 hybrids. However in a recent paper, Mauromicale et al. (2018) suggested a selection scheme which combines mass phenotypic selection, self-pollination and marker-assisted selection and suggested its application for the development of open-pollinated varieties from vegetatively propagated landraces. Studies shepherded in this direction are at present very limited; however, the results reported by the authors seem very promising and demonstrate that a practical and cheap means of conducting marker-assisted breeding might be easily adopted also by small seed companies furnished with laboratories equipped for basic molecular analyses and which carry commercial activities at level of local markets. This might provide a significant contribution to their income as well as counteract the ongoing drastic genetic erosion of local germplasm characterized by peculiar qualitative and organoleptic characteristics.

6.2 Genetic Maps

Cynara cardunculus mapping populations were, at first, developed by crossing a clone of the globe artichoke varietal non-spiny-type 'Romanesco C3', used as female parent, with (i) an early-maturing varietal spiny-type 'Spinoso di Palermo', (ii) a clone of cultivated cardoon 'Altilis 41' and (iii) a clone of the wild cardoon 'Creta 4'. The three progenies obtained were highly segregating for both capitula and plant traits; furthermore, high segregation was detected for the content in phenolic esters and sesquiterpene lactones. A number of the segregants displayed aspects of morphology which might also have high potential as ornamentals, taking into consideration that any individual can be readily immortalized by vegetative propagation, as well maintained over time through 'in vitro' micropropagation (Lanteri et al. 2012).

The first genetic maps of globe artichoke were generated following genotyping of a progeny of 94 individuals, obtained by crossing the two globe artichoke clones 'Romanesco C3' and 'Spinoso di Palermo', with AFLP, M-AFLP and S-SAP markers (Lanteri et al. 2006). The female map comprised of 204 loci, spread over 18 linkage groups (LGs) 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 LGs, 1239.4 cM and 6.9 cM. The presence of 78 loci in common to both maps allowed for the alignment of 16 of the linkage groups. The maps were subsequently extended by the inclusion of a number of microsatellite loci, of which 19 were represented in both maps (Acquadro et al. 2009) and three genes involved in the synthesis of caffeoylquinic acid have also been positioned on the maps (Comino et al. 2007, 2009; Moglia et al. 2009).

A further map was developed by Portis et al. (2009a) on the progeny obtained by crossing the clone of 'Romanesco C3' with the cultivated cardoon 'Altilis 41'. The F_1 population was genotyped using a variety of PCR-based marker platforms (AFLP, S-SAP and SSR), resulting in the identification of 708 testcross markers suitable for map construction. The male map consisted of 177 loci arranged in 17 major linkage groups and spanning 1015.5 cM; while the female map was built with 326 loci arranged into 20 major linkage groups, spanning 1486.8 cM. The presence of 84 loci shared between these maps and those previously developed by Lanteri et al. (2006) allowed for map alignment and the definition of 17 homologous linkage groups, corresponding to the haploid number of the species. Furthermore, as 25 mapped markers (8%) corresponded to coding regions, this map had an additional value as functional map and represented the first genetic tool for candidate gene studies in globe artichoke. Later on, the maps have been integrated with the inclusion of all the genes involved in the synthesis of caffeoylquinic acids known in the species (Menin et al. 2010).

Later on, the same authors produced an improvement of the maps (Portis et al. 2012) based on the same progeny, following the integration of 172 microsatellite (SSR) loci derived from expressed sequence tag DNA sequence (Scaglione et al. 2009). Each of the resulting maps detected 17 major linkage groups. Their alignment was followed by the construction of a consensus map which succeeded in capturing 694 loci, 227 (217 SSRs, ten SNPs) of which involved co-dominant markers. The map generated 17 LGs with a total genetic length of 1687.6 cM and a mean inter-marker spacing of 2.5 cM. The newly developed consensus as well as the parental genetic maps was then used to elucidate the pattern of inheritance of key agronomic traits as reported in the next paragraph.

On the basis of the two-way, pseudo-testcross approach, genetic maps were also developed from F_1 progenies obtained by crossing globe artichoke with its wild progenitor, wild cardoon. Sonnante et al. (2011) developed an F_1 progeny of 192 individuals by crossing a single clone of the globe artichoke 'Mola', a non-spiny varietal type which produces green/purple flowers and used as female parent, with a single plant of the wild cardoon 'Tolfa', which produces spines on head bracts and leaves and is white-flowered. The progeny was genotyped with 149 EST-derived and genomic SSRs, AFLPs, ten genes mainly belonging to the chlorogenic acid pathway as well as phenotyped for two morphological traits: presence or absence of spines on the head bracts and flower colour. The map of the globe artichoke parent included 289 markers, ordered on 18 LGs and spanned 1486.4 cM with an average marker distance of 5.2 cM. The map of wild cardoon included 122 markers, ordered into 17 LGs and spanned 865.5 cM, with an average distance of 7.1 cM between markers. From the maternal and paternal maps, an integrated map was obtained which contained 337 molecular markers ordered in 17 LGs and the genes for the traits presence of spines on head bracts and flower colour were located, while one linkage group of globe artichoke remained as a single LG. The integrated map covered 1488.8 cM, with an average distance of 4.4 cM between markers. The integrated map was finally aligned with already existing maps for artichoke

(Lanteri et al. 2006; Acquadro et al. 2009; Portis et al. 2009a), and 17 LGs were linked via 31 bridge markers.

An F_1 mapping population from the cross between an accession of wild cardoon and a single plant belonging to the open-pollinated Argentinian globe artichoke variety 'Estrella del Sur FCA' was bred by Martin et al. (2013) as well. On the basis of a prior study (Cravero et al. 2007), most of the genotypic data needed for linkage map construction were derived from SRAP markers, which constituted the major backbone of the two parental maps; however, other PCR-based markers were included in the map and made it possible to allow cross-referencing with the SSR-based consensus map previously produced by Portis et al. (2009b). The 1465.5 cM map based on the segregation of alleles present in the wild cardoon parent comprised of 214 loci distributed across 16 LGs, while the 910.1 cM globe artichoke-based map featured 141 loci falling into 12 LGs. Three morphological traits (head spininess, leaf spininess and head colour) for which the parents contrasted were found to be inherited monogenetically, and the genes conditioning these traits were also mapped. On the basis of 48 co-dominant markers, an alignment was possible with the Portis et al. (2009b) SSR-based consensus map.

Interestingly, a comparison of heterozygosity between the mapping parents used by Portis et al. (2009a) showed that fewer loci were informative in the seed-propagated variety of cultivated cardoon than in the vegetatively propagated globe artichoke varietal types. This highlighted that clonal propagation allows the maintenance of high heterozygosity while seed propagation introduced an element of purifying selection to stabilize production and reduce the global level of heterozygosity. Martin et al. (2013) reported that the map of the wild cardoon was constituted by a higher number of loci than the one of the globe artichoke (214 vs. 141 markers) and was some 50% longer (1465.5 vs. 910.1 cM). In their work, both progenitors were seed-propagated and it must be kept in mind that the globe artichoke genotype underwent over time some phenotypic selection on the basis of key commercial traits which reduced its global level of heterozygosity, while the cardoon genotype was collected from the wild as a result of open-pollination without any anthropic selection.

With the goal to increase the number of markers available and contribute to the development denser *C. cardunculus* genetic maps, Scaglione et al. (2012) combined the restriction-site-associated DNA (RAD) approach with the Illumina DNA sequencing platform to affect the rapid and mass discovery of SNP markers. RAD tags were sequenced from the genomic DNA of the three parents (globe artichoke 'Romanesco C3', cultivated cardoon 'Altilis 41' and the wild cardoon accession 'Creta 4') of the mapping population previously described. A sample of heterozygous SNP loci was mapped by CAPS assays, and the CAPS-derived genotypic data were incorporated into a pre-existing data set of molecular loci already used to generate the cultivated cardoon genetic map (Portis et al. 2012).

Recently, two high-density genetic maps have been developed through the re-sequencing of 'Romanesco C3' and 'Altilis 41' genotypes at a depth of $\sim 30\times$, together with a $\sim 1\times$ genotyping-by-sequencing of the F_1 mapping population. This enabled linkage analysis to be carried out, facilitated by the use of the SOILoCo pipeline (Scaglione et al. 2016, https://bitbucket.org/dscaglione/soiloco). The maps, each comprised 17 LGs, harbouring 1157 (C3) and 1497 (Altilis 41) independently segregant genetic bins and enclosing respectively 23,366 and 15,227 haplotype markers. The overall length of the C3 map was 1459.6 cM, with an average genome-wide inter-locus distance of 1.33 cM. Assuming the physical size of each chromosome to be as reported by Scaglione et al. (2016), the global mean ratio of physical to genetic distance was 356.4 kbp per cM. The length of the 'Altilis 41' map was 1748.2 cM, and its mean inter-locus distance was 1.23 cM. The global ratio between the physical and genetic distance was 300.7 kbp per cM. The 312 markers on shared scaffolds between the two maps allowed the two sets of LGs to be inter-aligned, and homologous

genomic regions with the LGs to be identified (Portis et al. 2018) (Fig. 6.1). The high-density genetic maps were then used for the mapping of genomic regions encoding biomass-related traits as described in the next paragraph.

6.3 QTL Analysis

The availability of genetic maps is essential for the dissection of quantitative trait loci (QTL), which underline the inheritance of many key

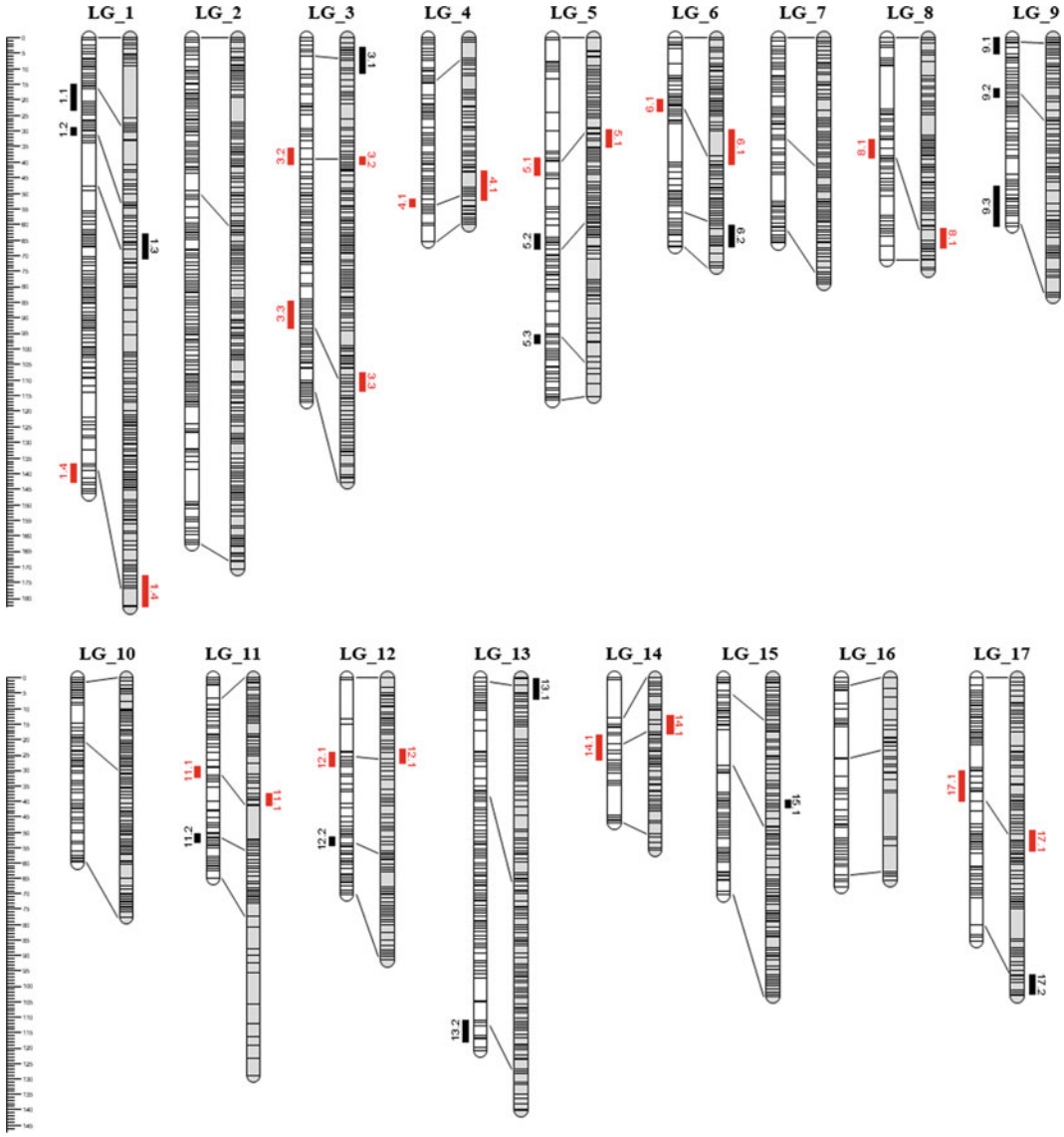

Fig. 6.1 High-density linkage maps derived from the female parent 'Romanesco C3' (white LGs) and the male parent 'Altilis 41' (grey LGs), showing the location of biomass-related QTL. Bars represent merged confidence intervals for each QTL. QTL-harbouring regions are numbered progressively along each LG, and homologous regions are shown highlighted in red. Only the scaffolds shared by the parental maps lying at the top, middle and bottom of each LG are shown, as well as those lying within a QGR. The scale to the left indicates lengths in cM. Adapted from Portis et al. (2018)

agronomic characters. A few studies have been carried out, at present, in *C. cardunculus* on the identification of genomic region influencing QTL, most of them by using the previously described map obtained from the F_1 progeny 'Romanesco C3' × 'Altilis 41'.

Portis et al. (2012) elucidate the pattern of the key commercial trait 'head production earliness'. Indeed, shortening the life cycle is seen as an important breeding goal in terms of both globe artichoke's economic value but can also increase the exploitability of cultivated cardoon as an energy crop. Earliness in both parents and F_1 progeny was assessed over two seasons and scored either as the number of days between transplanting (first season) or awakening (second season) and harvesting of both the main (eMH trait) and first- and second-order heads, obtained from the ramification of the main stem (eFOH and eSOH traits). An evaluation of the variance for the three earliness-related traits established significant genotypic differences between the two mapping parents. All three traits were found to vary continuously among the F_1 progeny, although no progeny was as early flowering as 'Romanesco C3', while a few were later flowering than 'Altilis 41', due to transgressive segregation. Across all three traits, a total of 25 QTLs were detected, of which 19 were stable across both growing seasons, with the other six expressed only during the second season. In the study, a broad-sense heritability for eMH of 0.76 was detected, which indicates the trait to be predominantly under genetic control, but rather lower heritabilities were found for the traits eFOH and eSOH, suggesting that the environment is quite influential in their determination. At any rate, the identified QTL accounted for up to 74% of the overall phenotypic variance and a cluster of large-effect QTL residing on the homologous LG 1 of globe artichoke and LG 2 of cultivated cardoon were claimed to represent a reasonable candidate for marker-assisted breeding and include two SSR loci which might serve as an indirect selection criterion for earliness.

In further work (Portis et al. 2014), the mapping population was phenotyped in order to elucidate the inheritance of seven main and first-order capitulum traits over two growing seasons. This study represented the first report on yield traits' QTL in globe artichoke. Capitula were harvested when they had reached a marketable size (prior to bract divergence), and measured with respect to their number per plant (*nc*), length (*cl*) diameter (*cd*), shape index (*si*) [cl:cd ratio] fresh weight (*fw*), receptacle diameter (*rd*) and receptacle height (*rh*). The cl, cd, si, fw, rd and rh traits were scored for both the main head (MH traits) and the first-order head (FOH traits) developed on the ramifications of the main stem. As previously highlighted for earliness, all the assessed yield-related traits were under polygenic control as were found to be normally distributed among the mapping progeny. A total of 100 QTLs associated with the seven capitulum traits were mapped to 23 chromosomal regions, scattered over 12 of the 17 linkage groups. Among these, 73 were expressed in both growing seasons, while the others were only detected in one season. As reported by the authors, the effect of a QTL has been shown to vary over time in perennial plants, like *C. cardunculus*, with changing biotic and abiotic factors (Brown et al. 2003); furthermore, since the two seasons were not identical in terms of temperature, rainfall, etc., their results seem to suggest that the species is adaptable to yearly variations in climate resulting in a significant degree of genotype × environment interaction.

The capitulum size and weight were found to be genetically determined by multiple QTL, which proved to be located in 18 distinct genomic regions. The number of QTL per trait ranged from five to nine (mean 7.2), and the only traits for which more than one QTL mapped to a single LG were fw and rd. Major QTLs, responsible for some 20% of the phenotypic variation, were detected for capitulum length, diameter, shape index and fresh weight. In the study, both head and receptacle size were found to be positively correlated with their fresh weight, as was the number of capitula with both capitulum length and diameter, and QTL for correlated traits frequently co-localized, presumably due to pleiotropy. In a previous work, Mauro et al. (2009) highlighted that the economic yield of globe

artichoke is positively correlated with the number and weight of the secondary capitula (Mauro et al. 2009), while clonal selection within a specific globe artichoke varietal type has shown that although yield was strongly dependent on the number of capitula, yet there was no significant relationship between yield and mean capitulum weight, (Mauro et al. 2012, 2015). A positive correlation between capitulum diameter and weight was previously been reported by Lopez Anido et al. (1998) and as pointed out by Lanteri et al. (2005), in the spiny elongated capitulum type, the heaviest capitula tend to be those having the greatest length and diameter. The correlations among different traits as well as their co-localization indicate the magnitude and direction of correlated response to selection as well as the relative efficiency of indirect selection (Holland 2006). As suggested by Carter et al. (2011), when traits are highly correlated, plant breeders can select for the trait with higher heritability and simultaneously indirectly select for the other trait, thus maximizing genetic gain for both traits in segregating populations. The results of Portis et al. (2014) suggest that the strong correlation between receptacle height and heads' size and weight might facilitate the indirect selection of the incidence of the edible head fraction, which is desirable for both fresh consumption and processing. Interestingly, the capitulum weight and size of the majority of the mapping progeny individuals were below the mid-parent value, which might be explained from exerting of a degree of semi-dominance of cultivated cardoon alleles over those from globe artichoke.

Following phenotyping of the same progeny, Portis et al. (2015) identified QTL for the traits' bract pigmentation and fleshy thorns. There is considerable variation in cultivated germplasm for the intensity of capitulum pigmentation, a character which is also very sensitive to temperature; however, varieties are conventionally classified as either violet or green. Cravero et al. (2005) have proposed that capitulum colour is genetically determined as follows: *P*—allows anthocyanin production, resulting in purple bracts, while *pp* inhibits anthocyanin production resulting in green bracts; *U*—results in an uneven distribution of anthocyanin pigments encoded by *P*, while *uu* results in an even distribution of pigment in the presence of *P*. This proposed model is presumably sufficient for breeders seeking to enhance colour; however, it was supposed that other modifier genes or multiple alleles might be involved (Pochard et al. 1969). In the work of Portis et al. (2015) by extending the classification of bract pigmentation to seven classes (one green; two green with purple hue; three green-purple; four purple-green; five purple with green hue; six purple; seven dark purple), the authors were able to identify a continuous like distributions appropriate for a QTL analysis. Three QTL regions, each stable across the two seasons, were identified. One was represented in both parents (homologous QTL), one only in globe artichoke and one only in cultivated cardoon. The largest effect QTL (PVE of 66–69%) mapped to globe artichoke LG 5 and cultivated cardoon LG 1 coincident with *P*. The globe artichoke QTL (PVE 16–17%) was located on LG 13 and cultivated cardoon QTL (PVE of 21–23%) on LG 11 confirm the presence of modifier loci involved in the genetic control of anthocyanin distribution, and whose effect might be variable in different progenies. The genetic basis of spine formation relates to the allelic constitution at the *Sp* locus, with the spiny "thistle-like" phenotype determined by the recessive allele *Sp* (Basnitzki and Zohary 1994), and the map location of *Sp* has been reliably established with a few mapping exercises (Lanteri et al. 2006; Sonnante et al. 2011; Martin et al. 2013). In their mapping population, Portis et al. (2015) by scoring the phenotypes on a 1–5 scale, identified a QTL justifying 68–73% of the PVE plus a second locus (PVE of 11–12%) exclusive of cultivated cardoon and possibly related to modifier genes.

Table 6.1 documents the total number of QTL detected using the 'Romanesco C3' and 'Altilis 41' maps by Portis et al. (2012, 2014, 2015), their distribution over years, homologies between maps as well as the maximum values estimated for LOD and the proportion (%) of the total phenotypic variance (PV).

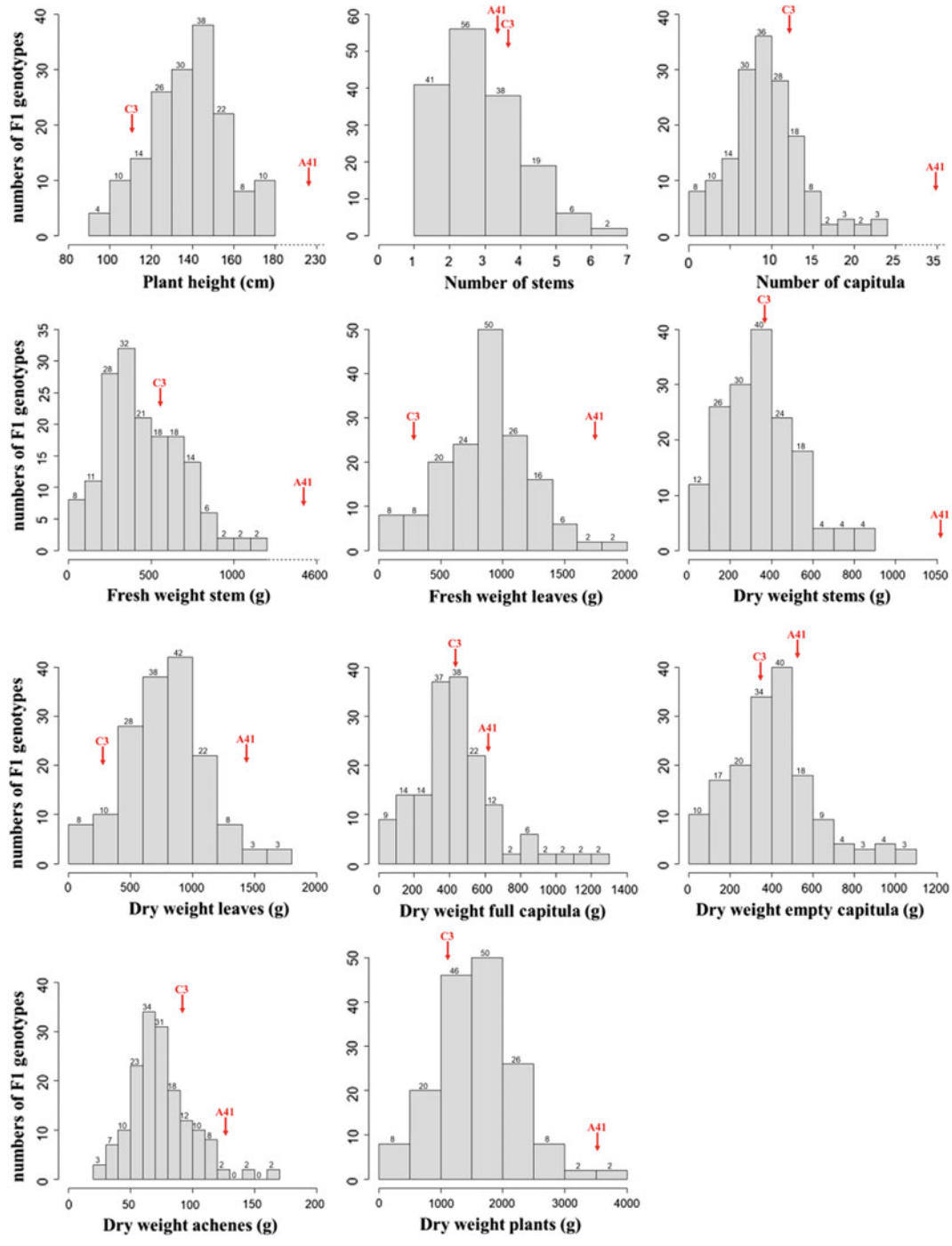

Fig. 6.2 Distribution of phenotypes for the eleven biomass-related traits across the mapping population. Traits segregating for the set of 162 F_1 individuals scored in one season are reported. The parental mean performances are indicated by arrows. Adapted from Portis et al. (2018)

Table 6.1 Total number and characteristics of the main head (MH), first-order head (FOH) and second-order head (SOH) QTL detected using the C3 and Altilis 41 maps by Portis et al. (2012)

Trait	Head	Code	Homolog. QTL	QTL in C3 map			QTL in A41 map			Max LOD	Max PV (%)
				1st season	2nd season	Both seasons	1st season	2nd season	Both seasons		
Earliness	MH	eMH	2	4	5	4	3	4	3	14.7	47.9
	FOH	eFOH	3	3	4	3	4	5	4	8.9	31.0
	SOH	eSOH	3	2	3	2	3	4	3	7.1	32.2
Capitulum length	MH	clMH	2	6	5	5	4	4	4	6.0	21.5
	FOH	clFOH	1	2	1	1	2	2	2	4.4	15.6
Capitulum diameter	MH	cdMH	2	6	5	5	3	2	2	5.9	20.3
	FOH	cdFOH	1	5	4	3	3	3	3	3.7	15.8
Shape index	MH	siMH	0	3	3	3	4	4	4	8.6	29.5
	FOH	siFOH	0	2	2	2	1	1	1	7.9	29.2
Fresh weight	MH	fwMH	3	4	3	3	6	5	5	5.1	19.9
	FOH	fwSOH	1	5	5	5	5	5	5	3.8	13.8
Receptacle diameter	MH	rdMH	2	4	3	2	5	4	3	4.4	18.8
	FOH	rdFOH	1	2	3	1	2	3	1	3.9	16.0
Receptacle height	MH	rhMH	1	5	4	4	1	1	1	4.8	11.3
	FOH	rhFOH	0	4	4	3	0	1	0	3.5	9.6
Number of *capitula*	All	nc	3	4	3	3	3	4	3	3.9	12.6
Bracts colour	All	bc	1	2	2	2	2	2	2	20.6	69.3
Bract thorniness	All	bt	1	1	1	1	2	2	2	14.0	73.2

The 'C3 x Altilis' F_1 population was, finally, phenotyped over two seasons by Portis et al. (2018) with respect to the following biomass-related traits: plant height (PH), number of stems (NS), number of capitula (NC), fresh weight of leaves (FWL), fresh weight of stems (FWS); then the material were dried and evaluated for dry weight plant (DWP), dry weight of leaves (DWL), dry weight of stems (DWS), dry weight of full capitula (DWFC), dry weight of empty capitula (DWEC) after achenes removal, and dry weight of achenes (DWA). In a preliminary study, Martin et al. (2016a) characterized the segregation of five biomass-related traits in a population bred from a cross between var. *sylvestris* and var. *scolymus* taxa, but the scope of the study was limited by its being based on data from a single growing season and its reliance on simple interval mapping for QTL identification. The research of Portis et al. (2018) therefore represents the most exhaustive attempt so far to identify the genetic basis of biomass accumulation in *C. cardunculus.*

The phenotypic data were combined with the high-density linkage maps previously described to identify 81 quantitative trait loci, of which 50 were placed on the C3 map and 31 on the Altilis map. The loci were scattered over 13 linkage groups and were clustered within 27 genomic regions, 22 of which harboured two or more QTL. Ten of these regions were specific to the C3 map and six to the Altilis map, while the other eleven were represented on both maps. The number of QTL per trait ranged from four to twelve (mean 7.4); for the traits FWL, DWL, DWFC, DWEC and DWP, more QTL located in

a single LG. The QTL allele effects were largely consistent with the phenotypic difference between parents, reinforcing the robustness of the mapping. There was a marked degree of transgressive segregation towards C3 for the traits DWS, DWA and NC: non-parental allele combinations are generally invoked to explain this phenomenon (Tanksley and McCouch 1997), but given that the majority of the progeny performed below the mid-parent value for these traits (Fig. 6.2), it is reasonable to suggest instead that a degree of semi-dominance was exerted by the globe artichoke over the cultivated cardoon alleles.

The co-localization of many of the QTL was inferred from the overlap of 1-LOD support intervals, a measure used to provide an approximate confidence interval for a QTL's true location. Only a small number of the QTL-genomic regions harboured QTL influencing the variation for a single trait. In contrast, five regions each harboured at least five QTL influencing a variety of traits—these were the genomic regions named 1.1 (seven traits), 3.3 (nine traits) and 9.1, 17.1 and 17.2 (five traits) in Fig. 6.1. The high inter-trait correlations established between traits for which the controlling QTL were frequently clustered imply that the clusters mostly likely did not comprise a group of linked loci, but rather were the expression of a single pleiotropic locus. The map location of some of the QTL was consistent with the outcomes reported by Portis et al. (2014) and Martin et al. (2016a, b). In particular, the location of NC QTL to LGs 01, 03, 05 and 09 reiterates the conclusions drawn from AFLP/SSR-based maps developed from the same population but phenotyped independently (Portis et al. 2012), as well as those obtained by mapping of a population derived from the var. *sylvestris* and var. *scolymus* cross-analysed by Martin et al. (2016b). The much higher saturation of the high-density maps has allowed further some additional NC QTL (on LGs 06, 08, 13 and 14) to be detected. At least some of the QTL influencing main capitulum fresh weight which have been mapped to LGs 01, 06, 08, 09, 12 and 13 (Portis et al. 2014) and to LGs 03 and 09 (Martin et al. 2016a) may be identical to the DWFC and DWEC QTL reported here.

Based on available genomic information, a total of 80 scaffolds covering each of the 27 QTL-genomic regions were derived and characterized. The length of the scaffolds ranged from 15,521 to 1,019,252 nucleotides (average 326,566 nt), and specified an estimated 1960 genes (24.5 genes per scaffold); 83% of these genes have been functionally annotated. An enrichment for certain gene ontology terms was noted for the gene content of the genomic regions harbouring loci influencing seed yield and the number/weight of stems.

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