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ORIGINAL PAPER

Molecular mapping of vernalization requirement and fertility restoration genes in carrot

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Abstract Carrot (*Daucus carota* L.) is a cool-season vegetable normally classified as a biennial species, requiring vernalization to induce flowering. Nevertheless, some cultivars adapted to warmer climates require less vernalization and can be classified as annual. Most modern carrot cultivars are hybrids which rely upon cytoplasmic male-sterility for commercial production. One major gene controlling floral initiation and several genes restoring male fertility have been reported but none have been mapped. The objective of the present work was to develop the first linkage map of carrot locating the genomic regions that control vernalization response and fertility restoration. Using an F_2 progeny, derived from the intercross between the annual cultivar 'Criolla INTA' and a petaloid male sterile biennial carrot evaluated over 2 years, both early flowering habit, which we name *Vrn1*, and restoration of petaloid cytoplasmic male sterility, which we name *Rf1*, were found to be dominant traits conditioned by single genes. On a map of 355 markers covering all 9 chromosomes with a total map length of 669 cM and an average marker-to-marker distance of 1.88 cM, *Vrn1* mapped to chromosome 2 with flanking markers at 0.70 and 0.46 cM, and *Rf1* mapped to chromosome 9 with flanking markers at 4.38 and 1.12 cM. These are the first two reproductive traits mapped in the carrot genome, and their map location and flanking markers provide valuable tools for studying traits important for carrot domestication and reproductive biology, as well as facilitating carrot breeding.

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Introduction

Carrot (*Daucus carota* L.) is a cool-season vegetable normally classified as a biennial species, requiring vernalization to induce flowering. Nevertheless, some cultivars adapted to warmer climates require less vernalization and can be classified as early flowering or annual (Dias Tagliacozzo and Valio 1994; Galmarini and Della Gaspera 1996; Rubatzky et al. 1999; Simon et al. 2008). The requirement for vernalization is an important trait because it influences not only seed production but also root development. Carrot roots quickly become very lignified after vernalization, even before the floral stalk elongates, and for that reason the initiation of flowering results in a complete loss of commercial value (Rubatzky et al. 1999). Consequently, flowering habit defines cultivar adaptation to different production areas and sowing times, depending on

whether the growers want to produce a seed or root crop (Atherton et al. 1990; Rubatzky et al. 1999).

Progeny evaluation of crosses between early- (Criolla INTA) and late-flowering (biennial lines from INTA breeding program) cultivars in previous studies demonstrated that all F_1 were annual and that the observed segregation ratios in F_2 and BC_1 families were not significantly different from segregation ratios expected under the hypothesis of a single dominant gene conditioning the annual habit (Alessandro and Galmarini 2007).

The molecular basis of vernalization has been determined in several biennial species. Among dicots, *Arabidopsis thaliana* has been used as a model plant for flowering studies. The vernalization requirement of winter annual *Arabidopsis* ecotypes is controlled by the MADS-box gene FLOWERING LOCUS C (FLC), which is a repressor of flowering that is negatively regulated by vernalization and both positively and negatively regulated by other genes of the autonomous pathway (Michaels and Amasino 1999). FLC-like genes have been identified in other taxa like *Brassica* and *Raphanus*, both within the Brassicaceae family (Ferreira et al. 1995; Osborn et al. 1997; Lan and Paterson 2000; Kole et al. 2001) and in *Beta* (Reeves et al. 2007). Among monocots, flowering in winter cereals has been extensively studied and a repressor that inhibits flowering in the fall season has been found, similar to the one seen in *Arabidopsis* (Yan et al. 2004). However, this repressor, for which the first example was the VRN2 locus cloned from diploid wheat, is a type of zinc-finger protein that is not found in the *Arabidopsis* genome (Yan et al. 2004).

Previous attempts to find the FLC gene in carrot, using BLAST method, were not successful (Alessandro 2011). High divergence within the FLC-like subfamily may explain why these and others attempts to identify FLC homologs outside Brassicaceae have failed (Reeves et al. 2007). Unlike the majority of MADS-box genes, *Arabidopsis* FLC-like genes have evolved under positive Darwinian selection (Martinez-Castilla and Alvarez-Buylla 2003), which results in rapid divergence among orthologs. In sugar beet, although an FLC-like gene was found, the difference in vernalization requirement between biennials and annuals could not be attributed to differences in expression abundance levels of this gene (Reeves et al. 2007).

Carrot is also an outcrossing species. Individual carrot flowers are small and flowers in the same umbel may open at different times, making hand emasculation on a large scale impractical. The discovery of brown anther genic cytoplasmic male-sterility (CMS) in carrot by Welch and Grimball (1947), the stamen petaloid male sterility discovered in a local wild carrot by H. M. Munger of Cornell University, and Wisconsin petaloid CMS by Morelock et al. (1996) made possible the commercial production of hybrid carrot seed. CMS, which is determined by plant mitochondrial genomes (Carlsson and Glimelius 2011; Eckardt 2006), is associated with the failure to produce functional pollen, and many mitochondrial genes that determine CMS can be suppressed or counteracted by the products of one or more nuclear genes known as *restorer-of-fertility* genes (Carlsson and Glimelius 2011; Eckardt 2006). Morelock (1974) proposed that dominant alleles at either of two complementary loci restored fertility to both, brown anther and petaloid, cytoplasms. The genetic analysis of Thompson (1961) with Cornell petaloid cytoplasm indicated three duplicated nuclear genes with dominant alleles necessary for maintenance of sterility and one or more epistatic restorers with dominant alleles required for fertility. Hansche and Gabelman (1963) proposed a digenic model for restoration of brown anther male sterility. Recent studies of relationships between different petaloid CMS accessions and different sources of restorer genes showed the presence of one or two genes (depending on the source of restorers) that restore fertility in all CMS (Wolyn and Chahal 1998). None of these male fertility restorer genes have been mapped.

In the same carrot populations used for mapping annual habit, evidence for a single dominant gene controlling restoration of male fertility was noted. The ability to map a male fertility restorer gene provides an additional tool to facilitate the studies pursuing the molecular basis of another important reproductive trait of carrot.

Genetic and molecular mechanisms of restoration vary among the different CMS systems studied in species like maize, rice, sunflower or *Brassicas* (reviewed by Schnable and Wise 1998; Eckardt 2006). In carrot, while several studies have associated regions of the mitochondrial genes with male sterility (Bach et al. 2002; Chahal et al. 1998; Linke et al. 2003), the molecular basis of fertility restoration is still unknown.

The complexity and variability of vernalization requirements, flowering habit, and restoration of cytoplasmic male sterility make evident the importance of evaluating traits in each species of interest, in addition to the comparative studies that could be carried out between species. To undertake such studies, the location of annual habit and restoration of male sterility genes in the carrot genetic map provide fundamental information for genetic and molecular characterization of flowering. A model for one major gene controlling floral initiation (Alessandro and Galmarini 2007) and several genes restoring male fertility to cytoplasmic male sterility have been reported, but none have been mapped. The objective of the present work was to investigate the genetic control of fertility restoration in carrot, and to develop the first linkage map of genomic regions that control vernalization response and fertility restoration in carrot.

Materials and methods

Plant materials, evaluation of flowering and DNA extraction

We evaluated an F_2 progeny, derived from a self-pollinated individual F_1 plant from the intercross between the annual cultivar 'Criolla INTA' and a petaloid male sterile biennial carrot from INTA's (Instituto Nacional de Tecnología Agropecuaria) breeding program. 'Criolla INTA' often restores fertility to cytoplasmic male-sterility (CMS) hybrids, so one of several male fertile F_1 plants was identified and an F_2 population was generated by self-pollination. Flowering and sterile:fertile plant segregation in the F_2 population were noted. Both parental stocks, as well as the F_2 population including 280 and 297 plants, were direct seeded in the field at La Consulta, Mendoza, Argentina (lat. 33°32′S, long. 69°04′W) on 4 May 2005 and 8 May 2006, respectively.

DNA, of 2006 trial, from 125 F_2 plants and 3 plants from each parent, was extracted using the CTAB and mercaptoethanol method (Briard et al. 2000). The concentration of DNA was determined using a spectrophotometer (Nanodrop ND-1000, Thermo Scientific) for SSR, RAPD and SCAR analysis and using a fluorometer (TKO 100 Mini-Fluorometer) for AFLP analysis. All samples were adjusted to a final concentration of 10 or 15 ng/µl depending on the requirements of the individual experiment, for further molecular analysis.

Phenotypic traits

Two phenotypic traits were considered: the vernalization requirement scored by annual (flowering) or biennial (not flowering) plants which has been demonstrated to be a monogenic trait (Alessandro and Galmarini 2007), and the fertility restoration scored by the presence (male fertile) or absence of anthers (petaloid). Individual plants were evaluated weekly and scored as being vegetative until the first floral internode elongated (Alessandro and Galmarini 2007). Flowering plants were also scored for fertility/sterility, defining qualitative classes as stamen (with anther-visible pollen or anther-no visible pollen) and petaloid-stamen (petaloid/carpeloid or spoon-shaped structures) (Wolyn and Chahal 1998).

SSR analysis

335 primer pairs were evaluated. For the initial screening of primers parental DNA samples and a set of 8–16 F_2 individual DNAs were used. Primer pairs generating polymorphic PCR products between samples were subsequently used to evaluate the complete F_2 population.

304 primer pairs, develop from BAC library and genome sequences (Cavagnaro et al. 2011), were evaluated in a 15 µl volume PCR containing 30 ng of carrot genomic DNA, 5 µM of forward and reverse primers, 2.4 µl of 25 mM $MgCl_2$, 1.2 µl of 2.5 mM dNTP (dATP, dCTP, dGTP, dTTP), 1.5 µl of 10× PCR buffer and 0.5 U of *Taq* DNA polymerase from Promega (Madison, WI, USA). PCR amplicons were generated using the following PCR conditions: (1) initial denaturation at 94 °C for 5 min, (2) 40 cycles of 94 °C for 20 s, 50–60 °C for 1 min, 71 °C for 1 min, (3) final step at 72 °C for 5 min. Amplicons were resolved by 2 % high-resolution agarose (SFR) gel electrophoresis in 1× TAE buffer for variable time at 190 V, stained with ethidium bromide and photographed under UV.

31 EST-SSR primer pairs (Iorizzo et al. 2011) were tested using a fluorescent method (Schuelke 2000). PCR was performed in a 20 µl final volume including 50 ng (2 µl) of carrot genomic DNA, 5 µM of reverse primer, 2.5 µM of M13-tailed forward primer, 5 µM of M13 labelled either with 6-FAM, HEX or NED fluorochromes, 0.8 µl of 2.5 mM dNTPs (dATP, dCTP, dGTP, dTTP), 2 µl of 10× PCR buffer, 12.5 µl water and 0.5 U of *Taq* polymerase (MBI, Fermentas, USA). The amplification conditions were: (1) 94 °C for 2 min; (2) 10 cycles of 94 °C for 40 s, 60 °C for 1 min with a reduction of 0.5 °C each cycle, 72 °C for 1 min; (3) 40 cycles of 94 °C for 45 s, 60 °C for 1.0 min, 72 °C for 1.0 min and 20 s, and (3) a final step at 72 °C for 10.0 min. Amplicon lengths were estimated using an ABI 3730xl capillary sequencer available at the University of Wisconsin Biotechnology Center and analyzed with GeneMarker software version 1.8 (SoftGenetics, State College, Pennsylvania).

Polymorphic amplicons were identified with the SSR letters and primer pair number, preceded by letters B, G or E depending if the SSR was developed from the BAC library, genome sequences or ESTs (e.g. GSSR111; Cavagnaro et al. 2011; Iorizzo et al. 2011).

RAPD analysis

193 randomly selected 10-mer primers were evaluated. Numbers 1–20 of series E, F, AA, AB and AU, plus series G numbers 1–7 and 19, H 1 and H 20, I 9, M 1–14, O 12, T 18, U 15, W 7, AC 1–5, AE 1–14, AQ 1–13, AT 3–15, AV 1–6 and AX 1–12 from Operon Technologies were used. Primers from the University of British Columbia were also evaluated, including numbers 231, 242, 252, 257, 299, 306, 318, 388, 450, 469, 523, 551, 592, 600, 617, 618, 641, 642 and 652. For the initial screening of primers parental DNA and a set of 8–16 F_2 individual DNAs were used. Primers generating polymorphic PCR products between sample individuals were subsequently used to

evaluate the complete F_2 population. All PCRs were performed in a 15 µl volume containing 30 ng of carrot genomic DNA, 5 µM of primer, 1.8 µl of 25 mM $MgCl_2$, 1.2 µl of 2.5 mM dNTP, 1.5 µl of 10× PCR buffer and 0.5 U of *Taq* DNA polymerase from Promega (Madison, WI, USA). The following cycling profile was employed: (1) initial denaturation at 94 °C for 2 min; (2) 49 cycles of 94 °C for 30 s, 36 °C for 1 min, 72 °C for 90 s; (3) a 68 °C for 7 min final extension and soak.

Amplified products were separated by electrophoresis in 3 % agarose gels with 1× TAE for variable time at 190 V, stained with ethidium bromide and photographed under UV. Polymorphic amplicons were identified with the primer name and the number of fragment, if there were more than one, preceded by the letter R (e.g. Rg21, corresponding to primer from Operon Technology G2, number of band 1).

SCAR analysis

6 primer pairs from previously mapped genes were evaluated. For the initial screening of primers, parents and a set of 8–16 F_2 individual DNAs were used. Primer pairs generating polymorphic PCR products between sample individuals were subsequently used to evaluate the complete F_2 population. For each primer pair, a specific PCR and cycling profile was used, as previously described for Y2 (Bradeen and Simon 1998), Q1/850 and Q6-500 (Boiteux 2000), Scar-AFLP3 (Santos and Simon 2002) and PSY1 (Just et al. 2007). In addition, Q1/850Flanking marker (primer pair: 5′-GAG CGC CTT GAT TGA TGC TGT GTT GCC-3′ and 5′-GAG CGC CTT GGC AGC ATC GAT TAG TAG-3′) was used with the following PCR and conditions: 30 ng of carrot genomic DNA, 5 µM of forward and reverse primers, 2.4 µl of 25 mM $MgCl_2$, 1.2 µl of 2.5 mM dNTP, 1.5 µl of 10× PCR buffer, and 0.5 U of *Taq* DNA polymerase; PCR conditions: (1) initial denaturation at 94 °C for 5 min, (2) 40 cycles of 94 °C for 20 s, 51 °C for 1 min, 71 °C for 1 min , and (3) final step at 72 °C for 5 min.

Amplified products were separated by electrophoresis in 3 % agarose gels with 1× TAE at 190 V, stained with ethidium bromide and photographed under UV. Polymorphic markers were identified with the primer name.

AFLP analysis

The AFLP reaction was performed as described by Vos et al. (1995) but with highly reduced quantities of enzymes and primers provided in the manufacturer's (GIBCO-BRL, Life Technologies) kit (Briard et al. 2000). 19 AFLP primer combinations were evaluated (EcoRI-MseI combinations, Table 1 of Supplementary material) using polyacrylamide gels and P33 labeling. Polymorphic markers were visually identified and serially numbered from 1 to 100, beginning with the largest fragment, preceded by the primer combination (e.g. acacat19, corresponding to the primer combination eaca and mcat, band number 19).

Data scoring, linkage analysis and map construction

Linkage analysis and map construction were conducted using JoinMap version 3.0 (Van Ooijen and Voorrips 2001). A marker scoring matrix was created in Excel and the data were formatted for F_2 mapping. Deviations of marker locus frequencies from expected segregation ratios for an F_2 generation were calculated by $\chi 2$ analysis implemented in JoinMap®.

Linked loci were grouped using a LOD threshold of 4–7 and a maximum recombination fraction of 0.4. Map distances in centi-Morgans (cM) were calculated using the Kosambi mapping function.

Most of the parental allelic phase was ambiguous or unknown, so in a first approach all dominant markers were scored as coming from the same parent and codominant markers were scored as two dominant markers arbitrarily named A and B (e.g. gssr46A and gssr46B). This analysis gave us pairs of linkage groups, with each pair identified by their codominant markers. For a given pair, the parental allelic phase was determined for each group of markers and codominant markers. This new scoring matrix was analyzed with JoinMap® for final map construction.

Graphical presentation of linkage map was done using Mapchart version 2.2 software (Voorrips 2002).

Results

Trait and marker segregation

All of the 'Criolla INTA' plants were male fertile, whereas all of the biennial maternal parental plants were petaloid male sterile over 2 years of field evaluation. Percentages of male fertile F_2 plants were 70.2 and 69.8 % in 2005 and 2006, respectively. Segregation ratios in F_2 families, which we report here for the first time, were not significantly different from the expected ratio under the hypothesis of a single dominant gene conditioning restoration of petaloid cytoplasmic male sterility, which we name *Rf1* (Table 1). As previously reported (Alessandro and Galmarini 2007), early flowering habit was evaluated over 2 years and behaved as a monogenic dominant trait, which we name *Vrn1*.

Since F_2 plants were derived from a single F_1 plant, the χ^2 goodness-of-fit tests were performed for expected F_2 ratios of 3:1 for dominant and 1:2:1 for codominant markers. Neither restoration of petaloid cytoplasmic male

Table 1 Observed and expected segregation ratios for male fertility restoration in a carrot population under the hypothesis of a single dominant gene conditioning restoration of petaloid cytoplasmic male sterility, Chi-squared value (χ^2), and probability (*P*), from segregating generations derived from the cross Criolla INTA × L2 when plants where grown in the field in La Consulta, Mendoza, Argentina

Generation	Observed segregation ratio		Expected ratio	χ^2	*P*
	Male fertile	Petaloid			
F_2^a	125 (70.2 %)	53 (29.7 %)	3:1 (133.5:44.5)	0.82	0.67
F_2^b	155 (69.8 %)	67 (30 %)	3:1 (166.5:55.5)	1.18	0.56

Sowing dates: [a]4 May 2005 and [b]8 May 2006

sterility nor early flowering habit deviated significantly from the expected ratio of 3:1 at the 5 % level.

Amplified products of 33 SSR primer pairs (9.3 %) were polymorphic and with the expected segregation ratios for an F_2 generation: 28 were codominant and 5 were dominant. Only 12 amplified products from RAPD markers (6.2 %) segregated with the expected segregation ratio. Among SCAR markers, Q1/850Flanking was polymorphic and codominant, with the expected segregation ratio for an F_2 population. Using the AFLP technique, 19 primer combinations amplified 867 bands, with an average of 46 total bands for each primers combination (Supplementary material, Table 1). Of these, 342 (39.8 %) were polymorphic and fit expected segregation ratios in the F_2 population.

Linkage analysis

A map of 355 markers was grouped in 9 linkage groups with LOD scores between 4 and 7. The total map length was 669 cM with an average marker-to-marker distance of 1.88 cM (Table 2). The number of markers per linkage group ranged from 26 to 51 with an average of 39 markers per linkage group. AFLP markers were the most abundant (319) and distributed in all linkage groups, whereas 24 SSR, 9 RAPDs and 1 SCAR were also mapped (Table 2). Linkage groups were ordered and named according to their corresponding physical chromosomes (chr) (Cavagnaro et al. 2011; Iovene et al. 2011; unpublished data).

Rf1 mapped to chromosome 9 with 25 molecular markers, including 1 codominant SSR (Table 2). Total size of the group was 73 cM and flanking markers to *Rf1*, both AFLP markers, were 4.38 and 1.12 cM away (Fig. 1). The *Vrn1* locus was mapped to chromosome 2 with 47 molecular markers, including 2 codominant SSRs (Table 2). The total size of the group was 64 cM and flanking markers to *Vrn1*, both AFLP markers, were at 0.70 and 0.46 cM (Fig. 1).

Discussion

This study reports a restorer of cytoplasmic male sterility in carrot and mapped two genes that exercise control over reproductive traits of carrot that are of significant importance to agriculture and great interest for fundamental molecular and ecological studies of *Daucus carota* L. Several studies have reported multiple genes controlling restoration of male fertility in CMS carrots (Thompson 1961; Hansche and Gabelman 1963; Wolyn and Chahal 1998) and multiple genes controlling initiation of flowering have been observed in other plant species. Consequently,

Table 2 LOD scores and marker distribution on carrot linkage groups in a population mapping *Vrn1* and *Rf1* genes

Group	LOD	Number of markers							Length (cM)	Spacing (cM)
		Phenotypic	RAPD	SSR	AFLP	SCAR	Total	Codominants		
1	7.0	0	0	3	27	0	30	3	51.687	1.72
2	7.0	1	2	2	43	0	48	2	63.644	1.32
3	6.0	0	0	3	48	0	51	3	75.906	1.48
4	6.0	0	0	3	39	0	42	3	57.058	1.36
5	6.0	0	2	4	30	0	36	3	99.102	2.75
6	5.0	0	2	3	31	0	36	3	88.398	2.45
7	6.0	0	0	2	40	0	42	2	96.464	2.30
8	7.0	0	0	2	41	1	44	3	63.448	1.44
9	4.0	1	3	2	20	0	26	1	72.986	2.81
Total		2	9	24	319	1	355	23	668.693	1.88

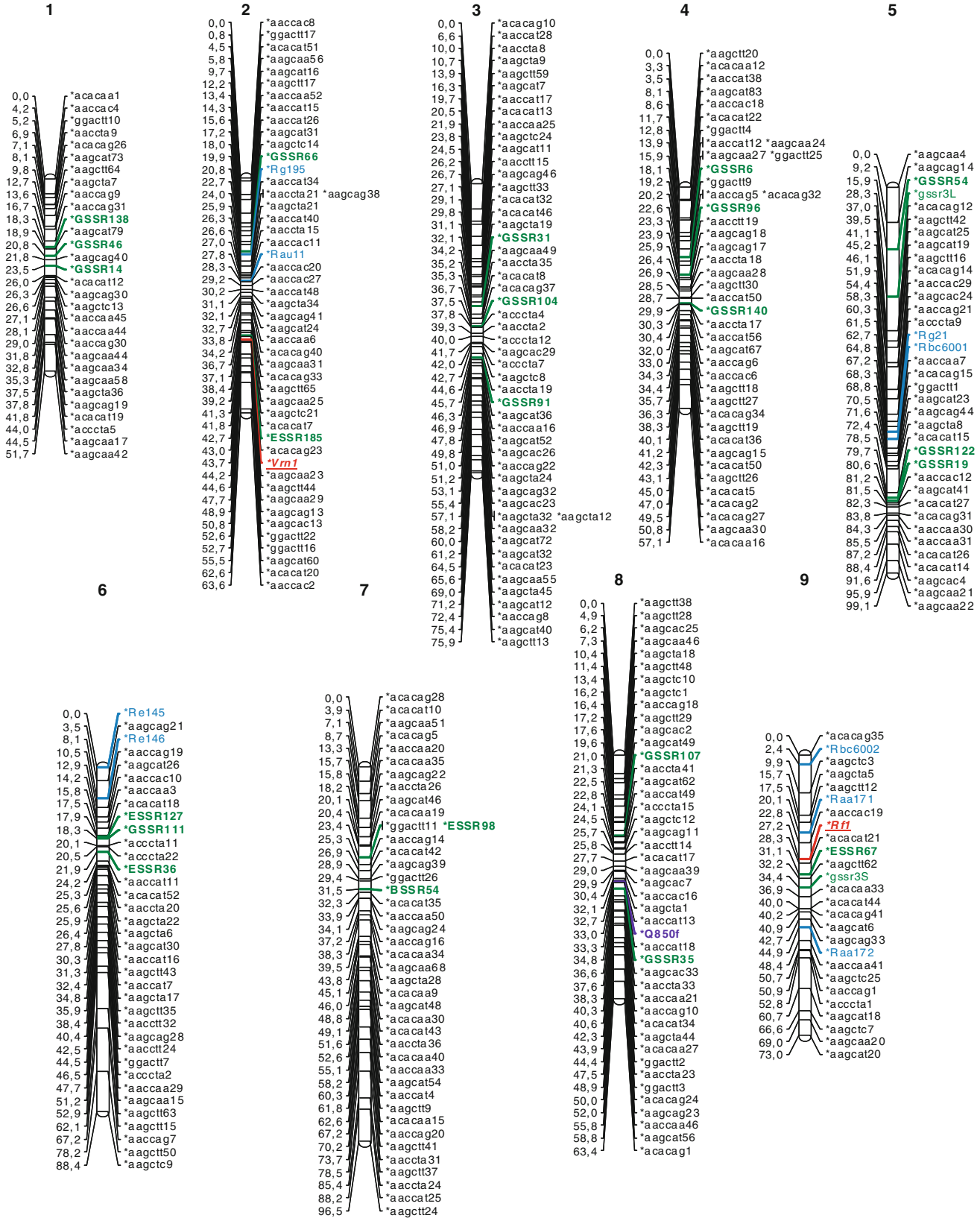

Fig. 1 Carrot linkage map for *Vrn1* and *Rf1* (loci in *red*). Microsatellites mapped in this work are denoted in *green*. RAPDs and SCARs are denoted in *blue* and *purple*, respectively (color figure online)

we should expect future studies to reveal additional genes controlling these important traits. Unfortunately, none of the carrot genetic stocks previously reported to restore cytoplasmic male sterility have been maintained; so, tests to determine alleleism of *Rf1* by intercrossing with other carrot lines restoring CMS are not possible, but other segregating families (F_2, F_3, BC) from the intercross between 'Criolla INTA' and the petaloid male sterile biennial carrot are available, and sibling Criolla INTA lines are under development.

The carrot linkage map in this study has more markers (355) than most of the previous studies (Schulz et al. 1993; Westphal and Wricke 1997; Vivek and Simon 1999; Santos and Simon 2004; Cavagnaro et al. 2011) with one linkage group for each carrot chromosome ($n = 9$). Total map size, 669 cM, was similar to that found by Schulz et al. (1993), but smaller than the size reported by Santos and Simon (2004) and Cavagnaro et al. (2011). The average marker spacing was 1.88 cM, reflecting a higher map coverage for carrot than previous studies (Vivek and Simon 1999; Santos and Simon 2004).

Approximately 90 % of the markers on the map consisted of AFLPs, 7 % were SSRs, 2.5 % RAPDs and 0.3 % SCARs. Of the 355 mapped markers, 332 (93.5 %) were dominant, while 23 (6.5 %) of the markers were codominant. Polymorphic variation of AFLP markers that fit expected segregation ratios was 40 % (342 amplicons), of which 319 amplicons (92 %) were mapped. The rate of polymorphism of SCARs, SSRs, and RAPDs was much lower (16, 9, and 6 %, respectively), but all polymorphic SCARs and most of SSRs were mapped to linkage groups. Only 42 % of the polymorphic RAPDs were on linkage groups. Similar values were reported by Vivek and Simon (1999), Boiteux et al. (2000) and Santos and Simon (2002). These results can be attributed to the inefficiency generally associated with dominant markers to detect linkages.

In recent studies (Iovene et al. 2011), linkage groups (LGs) were associated with actual chromosomes by means of fluorescent in situ hybridization (FISH) mapping of BAC clones anchored by LG-specific markers. SCAR and SSR markers of the present map provide the possibility of comparing linkage groups with previous maps and giving the physical position through recently developed FISH mapping in carrot (Cavagnaro et al. 2011; Iovene et al. 2011; unpublished data). The LGs in Fig. 2 were named, ordered and oriented according to the corresponding chromosomes.

The map information generated and the linked markers identified in the present study will be very useful in the development of PCR-based markers for marker-assisted selection (MAS). Since flowering and restoration of fertility are important adaptative characteristics that are difficult and time-consuming to score in many environments, MAS for these traits will be very valuable for breeding programs. The identification of annual or biennial plants and the presence of fertility restorer genes in early stages of plant growth, independent of environment, will elevate the efficiency of the breeding process.

The majority of the worldwide carrot breeding effort has focused on temperate, or late flowering types because of their larger market share and crop value (Rubatzky et al. 1999); but there is a current need for selection of improved carrot types adapted to tropical lowland environments (Bradeen and Simon 2007). MAS will facilitate the introgression of genes between annual and biennial forms, which also will broaden the genetic base of each group, allowing the exploitation of complementary and heterotic effects that might occur.

The molecular genetic basis of CMS and fertility restoration are of interest from several perspectives. From a practical standpoint, they are used for the commercial-scale production of F_1 hybrid seed in many crops and modern carrot breeding primarily focuses on development of hybrids. Furthermore, CMS systems provide valuable insights into fundamental ecological and molecular questions that stem from the interaction between nuclear and cytoplasmic factors.

Vrn1 mapped to chromosome 2 along with numerous AFLPs and 2 SSRs, while *Rf1* was mapped to chromosome 9 along with AFLPs and 1 codominant SSR. The close linkage of SSR and AFLP markers with *Vrn1* will allow fine mapping and positional cloning of the trait, however, markers are needed closer to *Rf1*. Recent development and characterization of a deep-coverage carrot BAC library (Cavagnaro et al. 2009) and ESTs collection (Iorizzo et al. 2011) can serve this purpose, using the chromosome walking technique (Bradeen et al. 2003). In the near future, sequencing of the genomic regions that control vernalization requirement and fertility restoration in carrot will also provide both additional markers and a better understanding of the molecular bases of these strategic traits.

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