Recent advance in carrot genomics

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Abstract

In recent years there has been an effort towards the development of genomic resources in carrot. The number of available sequences for carrot in public databases has increased recently. This has allowed the design of SSRs markers, COS markers and a high-throughput SNP assays for genotyping. Additional molecular tools include the first high-throughput DArT array and the full sequence of organelle genomes. These molecular resources have been successfully used to gain new insights into carrot genetic diversity, organelle genome evolution and to establish the first dense sequence-based linkage maps. Supported by a consortium of private companies, sequencing of the carrot genome is currently ongoing. Its release, will establish a solid framework for carrot genomic studies, opening a new challenging and exciting era for the carrot scientific community.

Keywords: *Daucus carota* L., genomic resource, molecular markers, genetic maps, QTLs, genome sequencing

INTRODUCTION

Carrot is among the top ten vegetable crops globally in terms of area of production and market value (http://faostat.fao.org/faostat/), the largest single source of provitamin A carotenoids in the U.S. diet (Simon et al., 2009) and among the most important root crops. Genetically carrot is a diploid species, highly heterozygous with a relatively small genome (473 Mb, Arumuganathan and Earle, 1991) that belongs to a genetically diversified genus (*Daucus*). Altogether, this makes carrot a good model to study root crop genetics and genome evolution. Despite its importance, the development of genomic tools to support carrot breeding programs and to carry on genetic studies has been very limited. The recent rapid advancement in high-throughput SNP genotyping technologies, along with next-generation sequencing (NGS) technologies, has provided essential genomic resources for accelerating the molecular understanding of biological properties and to support breeding efforts in several crops. In recent years there has been an effort towards the development of genomic resources in carrot. The number of available sequences for carrot in public databases has begun to increase, providing the opportunity to develop new genomic tools to advance carrot breeding and genetics. In this paper the status of carrot genomic resources is reviewed.

CARROT GENOME CHARACTERISTICS

Some cytological information about the structure and the content of the carrot genome has been published. Carrot is a diploid species (*2n*=*2x*=18) with nine pairs of chromosomes, an estimated genome size of 473 Mb (Arumuganathan and Earle, 1991) and 1.0 pg of DNA per 1C nucleus (reviewed by Simon et al., 2009). The genome size of carrot is substantially smaller than most dicot crops such as potato (844 Mb), tomato (900 Mb), lettuce (2.7 Gb) and sunflower (3.5 Gb). A chromosome karyotype has been established and indicates that heterochromatic domains are mainly confined to the pericentromeric regions of each chromosome (Iovene et al., 2011). Four pairs of chromosomes are metacentric and five pairs are submetacentric. Based on a DNA association curves and thermal denaturation,

the carrot genome consists of approximately 40% repetitive sequences, and the GC content is 37-38% (Owens, 1974). This basic information provides a foundation to initiate sequencing the genome.

CARROT GENOMIC RESOURCES

Generating sequence data represents the first step to develop molecular markers and to characterize the genome. Until 2008, at NCBI, there were only 600 non-plastid DNA sequences for all *Daucus* and 5,350 for the entire Apiaceae family. In the last six years the number of sequences available increased several fold, to the point where today there are 30,855 sequences available in public database for *Daucus* species and about 54,000 sequences for the *Apiaceae* family (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi). Over 50% (27,021) of these sequences are from *D. carota*. This includes 8,798 nucleotide sequences of genomic origin and 18,137 Expressed Sequence Tags (EST). The largest set of sequences in the Nucleotide database is represented by 2696 BAC-end sequences developed by Cavagnaro et al. (2009). Additionally, 57,840 assembled consensus sequences were obtained by sequencing the transcriptomes from leaf tissue and root tissue of individual plants representing a variety of pigmented carrot root colors (Iorizzo et al., 2011). These two set of sequences have been used in recent years to develop the largest set of molecular markers available today for carrot.

As for organelle genomes, the full-length sequences of both plastid and mitochondrial genomes have been published (Ruhlman et al., 2006; Iorizzo et al., 2012). Comparative analysis of carrot organelle genomes provided the first evidence of a mitochondrion to plastid DNA transfer in higher plants, opening a new field of research on plant organelle genome evolution (Iorizzo et al., 2012), and demonstrating the utility of well-characterized genomes.

MOLECULAR MARKERS

During the last three decades, DNA-based molecular markers have become indispensable tools for detailed genetic analysis and molecular breeding in crop plants. DNA molecular marker systems are based on variation in nucleotide sequence detected by hybridization (RFLPs, DArT), size-based methods (SSRs, RAPD, AFLPs, Indels), or by single nucleotide polymorphisms (SNPs). Early genetic studies in carrot used primarily RAPD, RFLP, and in particular, AFLP (reviewed by Bradeen and Simon, 2007). Those markers were low throughput and provided limited sequence information. Following the development of larger sets of sequence information for carrot in the past five years, various types of molecular markers like microsatellite (SSR), Single Nucletoide Polymorphic (SNPs), Conserved Orthologous Sequence (COS) have been developed. In addition the first Diversity Arrays Technology (DArT) markers for carrot have been established. A summary of available molecular markers for carrot is reported below.

To date, both genomic and transcript sequences have been used to detect and design SSR markers in carrot. About 300 SSR markers were developed from genomic DNA sequences, including 144 SSRs detected on BAC-end sequences (BSSR, Cavagnaro et al., 2009) and 156 SSRs developed from an enriched repetitive sequence library (GSSR, Cavagnaro et al., 2011). SSR markers have also been mined from expressed sequence tags (ESSR, Iorizzo et al., 2011). A small number of additional SSR markers have been developed from various sequence resources (Niemann et al., 1997; Vivek and Simon, 1999; Rong et al., 2010). In summary there are over 9000 SSRs detected and over 400 primer pairs have been used for mapping and evaluation of genetic diversity.

The first high-throughput transcriptome data for carrot published in 2011 (Iorizzo et al., 2011) provided the opportunity to detect a massive number of SNPs and further establish the first high-throughput SNP resource. Over 20,000 SNPs were detected in 7,684 contigs with an average density of 1.36SNPs/kb. A set of 4000 SNPs has been validated using KASPar chemistry (http://www.lgcgroup.com/products/kasp-genotyping-chemistry/how-does-kasp-work/). In total 3636 markers were validated (Iorizzo et al., 2013). Sequences of all primer pairs used in this study are publicly available.

Conserved orthologous sequence (COS) markers are PCR based markers developed from a set of single copy conserved orthologous genes (Fulton et al., 2002). These markers have been largely used to elucidate phylogenies and to study comparative genomics across different species (Small et al., 2004). The first set of carrot COS markers was developed by comparing carrot ESTs sequences against *Arabidopsis*, sunflower and lettuce sequences (Arbizu et al., 2014). The markers were successfully applied to assess the taxonomic relationships among *Daucus* species. However they may also be useful for study of other Euasterid II species, given the markers were originally designed using lettuce and sunflower sequences.

Diversity arrays technology (DArT) represent a pioneering cost-effective high throughput genotyping technology that does not require prior knowledge of the genome sequence (reviewed by Kilian et al., 2012). It has been widely applied in plant science and proven to perform well for many species. Although the technology does not directly provide sequence information, it uses cloned fragments, which may be easily characterized by Sanger sequencing. In carrot, a DArT array comprising 7680 DArT clones generated from 169 diverse genotypes including wild and cultivated germplasm has been developed (Grzebelus et al., 2014). Across a diverse set of carrot germplasm, 866 markers were non-redundant, polymorphic, and present in over 95% of the samples. Seventy-nine percent of the markers were highly discriminating with PIC value above 0.25, which is comparable with similar studies carried out in other species (Xia et al., 2005; Supriya et al., 2011). The makers have been successfully applied to analyse genetic diversity, and to construct a genetic map in carrot.

Additional markers linked to specific genes includes anthocyanin and carotenoid biosynthesis genes (Just et al., 2007; Yildiz et al., 2013), Centromeric Histone H3 (CENH3, Dunemann et al., 2014), alternative oxidase gene (AOX2a, Guerra Cardoso et al., 2009) and markers linked to phenotypic loci such as β-carotene accumulation (Bradeen and Simon, 1998) and resistance to *Meloidogyne javanica* (Boiteux et al., 2004).

CARROT GENETIC MAPS

Construction of linkage maps represents a prerequisite to determine the genomic location of loci controlling traits important to horticulture and quality. Sequence based markers are the only category of markers directly transferrable from genetic linkage maps and genomic sequence resources, and allow analyses such as study of candidate genes controlling important traits, and to establish comparative genome studies. A summary of the genetic maps and traits mapped in carrot is reported in Table 1. To date, fourteen mapping populations, (mainly F_2 segregating populations) have been used for genetic mapping in carrot. The first linkage map integrating biochemical (isozymes) and DNA based=markers (RFLP and RAPD) was developed by Schultz et al., 1994. Until 2004 the number of sequence-based co-dominant markers was very limited. Just et al. (2007) integrated the first set of SNP markers into the B493xQAL AFLP linkage map, previously developed by Santos and Simon (2002). The SNPs markers anchored 22 genes related to the carotenoid pathway. Cavagnaro et al. (2011) further integrated 49 SSRs markers, making this the densest sequence based marker map for carrot, and it opened the opportunity to integration of additional data. Iovene et al. (2011) anchored this linkage map to the corresponding pachytene chromosomes by FISH mapping of 17 map-anchored BACs. This work established a landmark to further anchor genetic maps to carrot chromosomes.

Advent of DArT and SNPs molecular markers for carrot has brought first high-throughput class of genetic markers for mapping. Grzebelus et al. (2014) developed the first linkage map based on DArT markers which span 419.1 cM and included 431 un-redundant markers across nine LGs. Recently, Parsons et al. (2015) built three linkage maps including over 550 SNP markers. To support the carrot genome initiative, efforts are currently ongoing to generate a high-density integrated linkage map that will be used to anchor the carrot genome.

Table 1. Summary of genetic mapping studies in carrot.

Mapping population	Generation	Type of markers	No. of mapped markers		Mapped traits/genes	Number & name of gene/QTL	References
			Dominant	Codominant			
$I_{0/1}$ lines	F_2	Isozyme, RFLP, RAPD	55	-	-	-	Schulz et al., 1994
B9304xYC7262	F_2	AFLP, SNP	6	1	Carotenoid accumulation	*Y2*	Bradeen and Simon, 1998
B9304xYC7262	F_2	RFLP, RAPD, AFLP, SSR	99	10	Anthocyanin, carotenoid and sugar accumulation	*P1*, *Y2*, *Rs*	Vivek and Simon, 1999
Brasilia-1252xB6274	F_2	RAPD	4	0	*Meloidogyne javanica* resistance	*Mj*1	Boiteux et al., 2000
BrasiliaxHCM	F_2	AFLP	164	0	Carotenoid accumulation	16 QTLs	Santos and Simon, 2002
B493xQAL	F_2	AFLP	141	0	Carotenoid accumulation		Santos and Simon, 2002
		Integration of SNPs	22	22	-		Just et al., 2007
		Integration of DcMTD	51	0	-		Grzebelus et al., 2007
		Integration of SSR	-	55	-		Cavagnaro et al., 2011
		FISH, 17 BAC clones mapped	-	-	-		Iovene et al., 2011
S269xR268	F_2	AFLP, ISSR	139	0	Resistance to *Alternaria dauci*	3 QTLs	Le Clerc et al., 2009
BiennialxCriolla INTA 220132013	F_2	AFLP, SSR	355	23	Vernalization and male fertility restoration loci	*Vrn*, *Rf*1	Alessandro et al., 2013
B1896xB7261	F_2	AFLP, SSR, SNPs	279	38	Anthocyanin, carotenoid accumulation	*P1*, *Y2*	Yildiz et al., 2013
70349	F_2	DArT			Markers associated to domestication	2 markers	Grzebelus et al., 2014
PI 652188 × B7262	F_3	SSRs, SNPs	0	8	Resistance to *Meloidogyne javanica*	*Mj*-2	Ali et al., 2014
YellowxCola	F_2	AFLP, RAPD, DcMTD, SSR, SNPs		4	Centromeric Histone H3	CENH3	Dunemann et al., 2014
Br1091xHM1	F_2	SNPs	-	389	Resistance to *Meloidogyne incognita*	4 QTLs	Parsons et al., 2014
SFFxHM2	F_2	AFLP, SSRs, SNPs	20	118	Resistance to *Meloidogyne incognita*	3 QTLs	Parsons et al., 2014
HM3	F_5	SSRs, SNPs	-	70	Resistance to *Meloidogyne incognita*	3 QTLs	Parsons et al., 2014

TRAIT MAPPING

To date six phenotypic loci segregating and 29 QTLs across 11 mapping populations have been mapped for carrot (Table 1). Particular emphases have been given to mapping loci and QTLs controlling pigments accumulation and disease resistance. In total 16 QTLs and a phenotypic locus *Y2,* controlling carotenoid accumulation in carrot roots have been mapped across five populations. The integration of common markers in 10117 and 493xQAL maps, indicated that the *Y2* locus controlling beta-carotene accumulation, is consistently mapped at the end of Ch7. A PCR based marker closely linked to *Y2* named y2mark, have been developed (Bradeen and Simon, 1998) opening the opportunity for fine mapping studies to identify the gene underlying this trait. The locus *P1* controlling anthocyanin accumulation in carrot roots, was mapped to Ch3 of the 10117-linkage map (Yildiz et al., 2013), and LGA of the B9304xYC7262 map (Vivek and Simon, 1999). The lack of common molecular markers in the two maps did not allow a direct comparison between the two locations.

Multiple studies have focused on nematode resistance in carrot. QTLs and two loci named *Mj1* and *Mj2* controlling resistance to *Meloidogine incognita* and *M. javanica* have been mapped. Comparative mapping analysis revealed overlapping QTLs for both nematode resistance loci in Ch8, suggesting possible interaction between the different sources of resistance. PCR based markers linked to all these loci are available, providing a tool for breeding and genetic studies on nematode resistance. Additional mapping efforts included the localization of a CMS restoration locus (*Rf1*), vernalization requirement (*Vrn*, Alessandro et al., 2013), QTLs for resistance to *Alternaria dauci* (Le Clerc et al., 2009), DArT markers associated with domestication (Grzebelus et al., 2014) and the localization of the Centromeric Histone H3 (*CENH3*, Dunemann et al., 2014).

CONCLUSIONS

A significant amount of genomic resources and tools has been available in carrot though carrot genomics research is behind that of other major vegetable crops such as tomato, potato and cucumber. These resources and tools have facilitated identifying and mapping genes and QTLs of importance to carrot. Nevertheless, many efforts are needed to further develop the resources and to make the tools readily usable in applications in order to fully and effectively use them in carrot genetic improvement and biology research. To advance the carrot research at genome wide level an initiative is ongoing to sequence the carrot genome. A consortium of 8 private companies supported the project. Sequencing efforts have focused on a dihaploid orange genotype (DH1) with an estimated genome size of 473 Mbp (Kmer spectrum analysis). The overall goal of this project is to leverage the current genetic and genomic resources in carrot to develop an integrated genomic sequence of carrot including physical map, genetic maps, transcriptomes and SNP database. An integrated and annotated genomic sequence can significantly advance our understanding of agricultural traits, increase genetic gain by making informed selections and shorten breeding cycles by incorporating marker-assisted and genomic selection strategies for breeding carrot and close relatives. It is expected that the carrot genome will be available soon. This initiative is going to significantly change the nature of research in carrot biology. A primary focus of research might shift more towards extensive genetic screens for genome-wide association analysis and functional genomics.

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