Classical and Molecular Carrot Breeding

9

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Abstract

Classical plant breeding approaches have succeeded in improving the productivity of the carrot crop for growers and the quality of the crop for consumers over the last century. A significant breeding focus has been on genetic control of male fertility to assure successful production of hybrid cultivars, with relatively little emphasis on formal studies of other reproductive traits such as seed yield and vernalization requirements, or on crop morphology. Another strong focus for carrot breeders has been selection for resistance to Alternaria leaf blight and root-knot nematodes. Future crop producers will likely face more challenging abiotic threats and additional biotic threats to the crop, and little effort has been directed to those traits. In an effort to improve carrot consumer quality, pigments and flavor compounds have received much attention by carrot breeders. With the expansion of carrot global markets, a broader range of consumer traits may require attention as carrot breeding programs move forward. The sequencing of the carrot genome provides an important foundation for a better understanding of the genetics of traits important for growers and consumers, for developing molecular tools to accelerate the breeding process, and for identifying genes of potential interest for gene editing. The breadth of genetic diversity in carrot germplasm is a valuable resource that will provide an important foundation for future carrot breeding. A better understanding of that diversity will be needed to take full advantage of it, and the carrot genome sequence will provide insights into that understanding.

9.1 Introduction

Cultivated carrot (*Daucus carota* ssp. *sativus* L.) is a diploid, outcrossing, insect-pollinated vegetable ($2n = 2x = 18$) that originated as a root crop in Central Asia around 1100 years ago. Carrot is the most widely grown member of the Apiaceae today. Storage root color and flavor were traits noted early in carrot domestication history, and root shape became an important trait to differentiate cultivars beginning around 500 years ago (see Chap. 5), but it was not until 85 years ago that the first genetic analysis of carrot was reported, describing white storage root color to be dominant over yellow, and under monogenic control (Borthwick and Emsweller 1933; Emsweller et al. 1935) (see Chap. 14).

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Given the relatively slow growth of carrots in the field and maximum of one breeding cycle per year (Simon et al. 2008), molecular markers to facilitate selection are important to assure progress in carrot breeding programs. The development of dense molecular marker-based genetic maps described in Chap. 7 combined with precise, high-throughput phenotyping technologies provides plant breeders with detailed trait maps to routinely apply marker-assisted selection (MAS) in breeding programs. These detailed trait maps will contribute to not only more efficient MAS, but also more accurate identification of candidate genes that may become targets for editing approaches for carrot improvement.

9.2 Carrot Reproductive Biology and Seed Production

Carrot is categorized as a biennial crop since the crop of commerce is harvested in the vegetative phase of its life cycle. The transition from vegetative crop to flowering varies widely in diverse carrot germplasm, and the genetic and environmental bases underlying this transition are discussed in Chap. 3. Carrot cultivars are categorized as temperate and late flowering, or subtropical and early flowering, depending on their intended area of production. Vernalization of the vegetative crop, achieved by exposing it to cold temperatures to induce floral development, is required for floral initiation in temperate carrot cultivars. In contrast, carrots developed for subtropical or tropical climates typically require little or no exposure to cold temperatures for floral induction (Simon et al. 2008). Wild carrots from many global regions will flower with no apparent vernalization required. One gene influencing floral mutation, *Vrn1*, has been reported to date (Table 9.1) (Alessandro et al. 2013). *Vrn1* was mapped to chromosome 2 in a region spanning 0.36 cM. Several additional genes controlling carrot vernalization are expected to be discovered as a broader range of germplasm is evaluated, given the wide range of variation observed for this trait.

Phenotyping of carrot germplasm for vernalization requirements is not a trivial matter. To date, phenotyping has been done in open fields, so well-characterized, reliable environmental conditions are needed for effective selection of carrot breeding stocks. As the genetic control of floral induction becomes better understood, the development of well-defined phenotyping methods will be needed to ensure progress in carrot breeding programs.

The architecture of flowering plants and seed yield varies widely in diverse carrot germplasm. While adequate seed yield is crucial for producers of commercial seed, little has been published on the genetic control of variation in carrot seed productivity.

In contrast, since the initial discovery of cytoplasmic male sterility (CMS) in carrot in the late 1940s, the genetics and molecular basis of CMS have been extensively studied and is reviewed in Chap. 3. Most widely grown carrot cultivars in major global markets are hybrids, and reliable trait expression is mandatory for both male-sterile and male-fertile parents in the production of hybrid seed. Nuclear genes maintaining male sterility and restoring fertility in plants with male-sterile cytoplasm derived from wild and cultivated carrot have been characterized, nuclear genes controlling male sterility in plants with male-fertile cytoplasm have been reported, and their inheritance evaluated (Alessandro et al. 2013; Banga et al. 1964; Borner et al. 1995; Hansche and Gabelman 1963; Mehring-Lemper 1987; Thompson 1961) (Table 9.1). Alessandro et al. (2013) mapped *Rf1*, a nuclear restorer of cytoplasmic male sterility, to chromosome 9 within a 3.36 cM genomic region. It has been speculated that numerous additional restorers of CMS occur in carrot.

In addition to markers for nuclear restorer genes, molecular markers for the cytoplasm itself are important in breeding programs. Variation in the mitochondrial genome controls male fertility, and several studies have developed markers to differentiate male-sterile and male-fertile cytoplasms currently used in breeding programs (Bach et al. 2002; Nakajima et al. 1999)

Table 9.1 Genes of carrot: reproductive biology, morphology, and biotic stress resistance

Gene symbol (parentheses indicate suggested symbol)	Character description/trait	Mendelian inheritance reported	Placed on a linkage map
Reproductive biology			
Vrn1	Vernalization	Alessandro et al. (2013)	Alessandro et al. (2013)
Ms1-Ms-3 *Ms-4-Ms-5, ms,* *a, B, D, E,* *l, t,* *Rf1*	Nuclear restorers of CMS	Thompson (1961) Hansche and Gabelman (1963) Banga et al. (1964) Mehring-Lemper (1987) Alessandro et al. (2013)	Alessandro et al. (2013)
Gum1-2, Mar1-2, Gad1-2	Novel cytoplasms and sterility	Borner et al. (1995)	Borner et al. (1995)
STS1–STS6	Petaloid male-sterile and fertile cytoplasm		Nakajima et al. (1999)
14 primer pairs			Bach et al. (2002)
Morphology and growth			
(Cr)	Root cracking	Dickson (1966)	
Gls	Glabrous seed stalk	Morelock and Hosfield (1976)	
(sp1, sp2)	Spine formation	Nieuwhof and Garritsen (1984)	
Phenl	Small, dark green, annual	Schulz et al. (1994)	Schulz et al. (1994)
COLA	Compressed lamina	Budahn et al. (2014)	Budahn et al. (2014)
YEL	Yellow leaf	Nothnagel et al. (2005)	Budahn et al. (2014)
cult	Root thickening	Macko-Podgórni et al. (2017)	Macko-Podgórni et al. (2017)
5, 4, and 3 QTL 1, 5, and 3 QTL 6, 2, and 2 QTL	Shoot height, biomass, area Petiole number, width, and length Root length, biomass, and area	Turner et al. (2018)	Turner et al. (2018)
Disease and pest resistance			
(Ce)	Cercospora leaf spot	Angell and Gabelman (1968)	
Eh	Powdery mildew	Bonnet (1983)	
3 QTL	Alternaria leaf blight	Le Clerc et al. (2009)	Le Clerc et al. (2009)
11 QTL		Le Clerc et al. (2015)	Le Clerc et al. (2015)
(Mh-1, Mh-2)	*Meloidogyne hapla* root-knot nematodes	Wang and Goldman (1996)	
Mj-1	*M. javanica* root-knot nematodes	Simon et al. (2000)	Boiteux et al. (2000, 2004)

(continued)

Table 9.1 (continued)

Gene symbol (parentheses indicate suggested symbol)	Character description/trait	Mendelian inheritance reported	Placed on a linkage map
Mj-2	*M. javanica* root-knot nematodes	Ali et al. (2014)	Ali et al. (2014)
7 QTL	*M. incognita* root-knot nematodes	Parsons et al. (2015)	Parsons et al. (2015)
Storage root pigments, carbohydrates and terpenoids			
y	Yellow xylem and phloem	Laferriere and Gabelman (1968) Imam and Gabelman (1968) Buishand and Gabelman (1979) Simon (1996)	Just et al. (2007, 2009) Iorizzo et al. (2016)
y_1	Differential orange phloem/xylem		
y_2	Differential orange phloem/xylem		Bradeen and Simon (1998) Just et al. (2007, 2009) Yildiz et al. (2013) Ellison et al. (2017)
o, io	Orange xylem	Kust (1970)	
a, l	α-Carotene, lycopene	Umiel and Gabelman (1972)	
Rp	Reduced carotene content	Goldman and Breitbach (1996)	
16 QTL	Carotene content	Santos and Simon (2002)	Santos and Simon (2002)
Or	Carotene content	Ellison et al. (2018)	Ellison et al. (2018)
g	Petiole anthocyanins	Angell and Gabelman (1970)	
P_1	Root anthocyanins	Simon (1996) Cavagnaro et al. (2014)	Vivek and Simon (1999) Yildiz et al. (2013) Cavagnaro et al. (2014)
P_2	Node anthocyanins	Simon (1996) Cavagnaro et al. (2014)	Cavagnaro et al. (2014)
P_3	Root and petiole anthocyanins	Cavagnaro et al. (2014)	Cavagnaro et al. (2014)
Raa1	Acylated anthocyanins		
15 QTL	Anthocyanin content		
30 QTL	Volatile terpenoid content and composition	Keilwagen et al. (2017)	Keilwagen et al. (2017)
Rs	Reducing sugar	Freeman and Simon (1983) Vivek and Simon (1999) Yau and Simon (2003)	Vivek and Simon (1999) Yau and Simon (2003) Yau et al. (2005)

(Table 9.1). Additional cytoplasmic diversity has been noted in more diverse germplasm (Borner et al. 1995). Given the critical need for reliable fertility phenotypes, the extensive use of diverse carrot germplasm in carrot breeding programs that has not been characterized for CMS restorers or cytoplasms, and the fact that it can take up to one year to phenotype a plant, the development of additional molecular markers to characterize carrot male fertility restorer genes and cytoplasms is critical to progress in breeding programs.

9.3 Morphology and Growth

For carrot breeding programs that utilize both early-flowering and late-flowering germplasm in their breeding programs, a heavy emphasis is been placed upon selection for vernalization requirement to meet those production requirements in temperate or subtropical growing regions. Beyond variation in flowering tendency, carrots vary widely for growth rate and morphological characteristics, but little genetic analysis has been reported. Two carrot morphological traits—glabrous seed stalk (*gls*) (Morelock and Hosfield 1976) and tendency for root cracking (Dickson 1966)—were reported to be controlled by one gene, and spine formation (Neiuwhof and Garritsen 1984) followed a digenic pattern of inheritance (Table 9.1). None of these three traits have been placed on the carrot genetic map.

The *phen1* trait was observed in a mapping population derived from an open-pollinated cultivar (Schulz et al. 1994) (Table 9.1) where it was found to be under monogenic control, and it was the first trait mapped to the carrot genome. Two other leaf morphological traits, *COLA* (compressed leaf) first described by Nothnagel et al. (2005) on chromosome 4 and *YEL* (yellow leaf) on chromosome 1, are also monogenic traits, both discovered in crosses between cultivated and wild carrots (Budahn et al. 2014). Since MADS-box, alternative oxidase, and chalcone synthase genes had been associated with fertility and floral development in other plants, these genes were evaluated as candidates, but none colocated with *COLA* or *YEL*.

In another study that utilized a cross between cultivated and wild carrot, traits associated with domestication were evaluated. Macko-Podgórni et al. (2017) discovered a 37 kb genomic region on chromosome 2 that controlled root thickening or diameter which they evaluated with a marker referred to as *cult*. A candidate gene in that region, *DcAHLc1*, belongs to the AT-hook motif nuclear localized (AHL) family of plant regulatory genes. AHL genes are involved in the regulation of organ development, including root tissue patterning.

A recent study reported research on the genetic control of traits of interest to carrot breeders: canopy and root size and shape. Turner et al. (2018) evaluated shoot and root morphological characteristics and mapped five QTL for shoot height, seven for shoot biomass and area, seven for petiole width and length, seven for root length, and two for root biomass. Given the importance of these traits in carrot yield and cultivar classification, markers developed for these QTL may have some immediate application.

9.4 Disease and Pest Resistance

The inheritance of resistance has been reported for several carrot foliar diseases and root-knot nematodes (RKN) and discussed in Chap. 18. Monogenic resistance to two foliar diseases, Cercospora leaf spot (Angell and Gabelman 1968) and powdery mildew (Bonnet 1983), was reported, but resistance genes have not yet been mapped (Table 9.1).

Alternaria leaf blight (ALB) occurs worldwide and is regarded as the most important disease of carrots. Genetic control of resistance has been evaluated in several studies including two that mapped three and eleven QTL, respectively (Le Clerc et al. 2009, 2015) (Table 9.1), where relatively high heritability (75–78%) was reported.

Given the importance of ALB, markers to select for enhanced resistance will be of interest to breeders.

Galls formed on carrot roots caused by the attack of root-knot nematodes (RKN) disfigure them in production regions worldwide. Genetic resistance to three RKN species has been reported. Genetic resistance to *Meloidogyne hapla* has been studied and found to be controlled by two genes (Wang and Goldman 1996) which have not yet been mapped. A single dominant gene on chromosome 8, *Mj-1*, confers resistance to *M. javanica*. *Mj-1* has been mapped (Boiteux et al. 2000), and marker-assisted selection for resistance has been exercised (Boiteux et al. 2004). A second gene conferring additional *M. javanica* resistance, *Mj-2,* has also been mapped to chromosome 8 (Ali et al. 2014). The *Mj-1* gene, in addition to six additional QTL on chromosomes 1, 2, 4, and 9, confers resistance to *M. incognita* (Parsons et al. 2015), which is a common RKN species in the soils of most warmer carrot production regions of the world. Molecular markers to facilitate selection of *Mj-1* were reported (Boiteux et al. 2004).

9.5 Storage Root Quality Traits

Given their importance for human nutrition and consumer acceptance, genetic variation in the content and composition of carrot storage root pigments and flavor compounds has been studied quite extensively. Genetic control of root color due to carotenoids and anthocyanins, in particular, has been evaluated.

Relatively early studies on carotenoid-based colors of carrots discussed in Chap. 14 named the *Y, Y_1, Y_2, O, IO, A, L,* and *Rp* genes based on single gene inheritance patterns (Buishand and Gabelman 1979, 1980; Goldman and Breitbach 1996; Imam and Gabelman 1968; Laferriere and Gabelman 1968; Kust 1970; Simon 1996; Umiel and Gabelman 1972) (Table 9.1). More recently variation in the *Or* gene on chromosome 3 was discovered to be associated with orange storage root color in a diverse panel of carrots (Ellison et al. 2018). Candidate genes and/or closely linked markers developed for three of these genes (*Y, Y_2*, and *Or*) which dramatically alter the carotenoid composition and storage root color ranging from white to yellow to orange (Bradeen and Simon 1998; Ellison et al. 2017, 2018; Iorizzo et al. 2016) (Table 9.1). Candidate genes have been identified for the *Y* (Fig. 9.1) and *Or* genes, and molecular markers have been developed to facilitate breeding variation in storage root color for all three of these genes. These markers are also a valuable tool to provide insights into the domestication history of carrot (see Chap. 5). Given the important role of carrot carotenoids in human nutrition and their increasing use as a natural pigment, there has been some effort in breeding orange carrots for higher carotene content (Simon et al. 1989). In a cross between two orange carrots that had a fivefold difference in carotenoid content, 16 QTL were found to influence carotenoid content (Santos and Simon 2002).

Anthocyanin color varies widely among diverse carrot germplasm, and three genes controlling anthocyanin accumulation (*P_1, P_3*, and *Raa1*) have also been mapped (Cavagnaro et al. 2014; Yildiz et al. 2013) (Table 9.1) with candidate genes and/or closely linked markers identified for all three genes as discussed further in Chap. 15. Like carotenoids, anthocyanins also have a positive impact on human health, and they are also being extracted and extensively used as a natural food coloring. Studies evaluating the quantitative inheritance of anthocyanin content have reported 15 QTL contributing to that variation (Cavagnaro et al. 2014). As breeders incorporate genes conditioning carrot pigment content and composition, an understanding of the relative contribution of more of these genes, beyond the major genes listed above, will be important.

The two major attributes of fresh carrot flavor are sweetness and harsh flavor (Simon et al. 1980). Volatile terpenoids account for harsh flavor and Keilwagen et al. (2017) (Table 9.1)

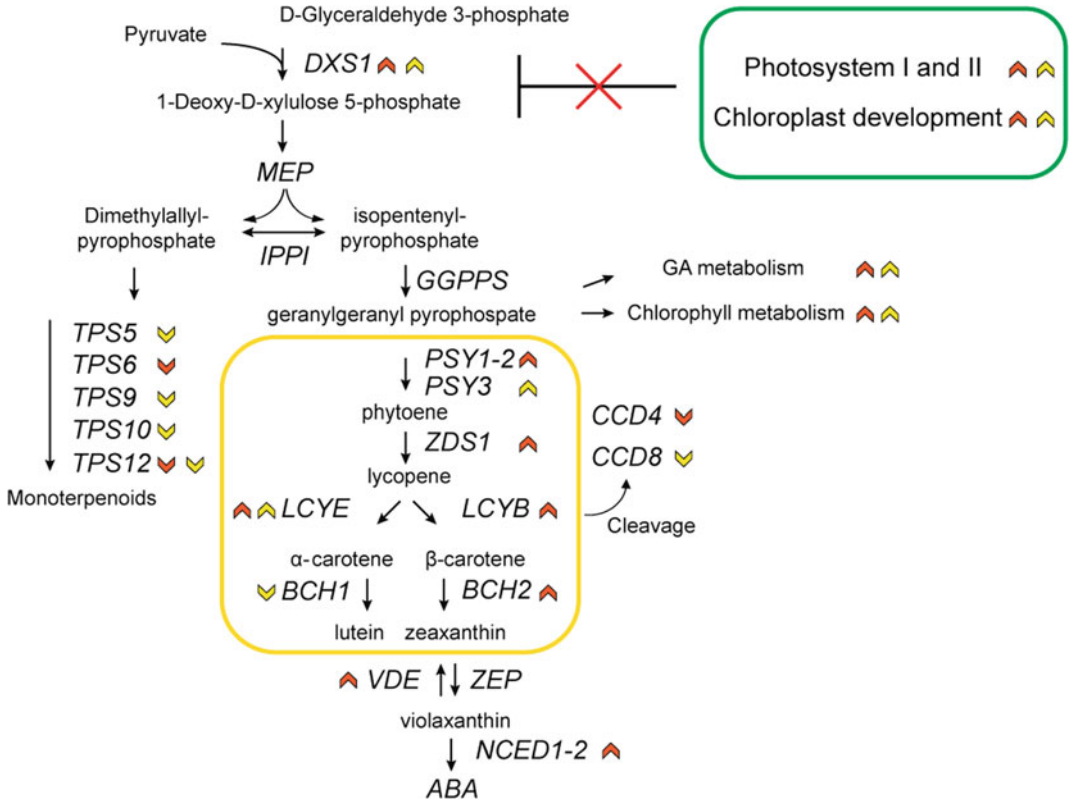

Fig. 9.1 *Y* gene of carrot that controls carotenoid accumulation in the storage root. Upward- and downward-pointing arrows indicate upregulated and downregulated genes, respectively, in the yellow versus white (yellow arrows) and dark orange versus pale orange (orange arrows) comparisons. The orange box delimits the isoprenoid biosynthetic branch that leads to the carotenoid pathway. As shown in the green box, the majority of the upregulated genes in yellow and dark orange roots are involved in the photosynthetic pathway; genes that are included are involved in the assembly and function of photosystems I and II and plastid development. We hypothesize that loss of the constitutive repression mechanisms conditioned by genes involved in de-etiolation and photomorphogenesis in non-photosynthetic tissue, such as carrot roots, induces overexpression of DXS1 and, consequently, activation of the metabolic cascade that leads to high levels of carotenoid accumulation in carrot roots. From Iorizzo et al. (2016)

recently identified 30 QTL conditioning 15 mono- and sesqui-terpenoids in carrot. Only 4 of the 30 QTL comprise terpene synthase candidate genes since these genes are clustered, but 65 candidate gene models were identified.

Carrots store little starch but up to 10% free sugars which contribute to sweet flavor (Simon 2000). A single gene, *Rs*, located on chromosome 2 conditions the ratio of reducing sugars (glucose and fructose) to sucrose in storage roots (Freeman and Simon 1983; Vivek and Simon 1999; Yau and Simon 2003) (Table 9.1). Invertase isozyme II has been identified as the candidate gene controlling this trait, where the mutation is caused by a 2.5-kb insertion into an intron of this gene (Yau and Simon 2003) (Fig. 9.2). Marker-assisted selection for sugar type has been demonstrated (Yau et al. 2005). Plants with the dominant wild-type allele store primarily glucose and fructose, while *rsrs* plants store primarily sucrose. Most carrot cultivars are *RsRs*, but the *rs* allele does occur in some fresh market genetic backgrounds where it can serve as a useful marker to monitor seed purity.

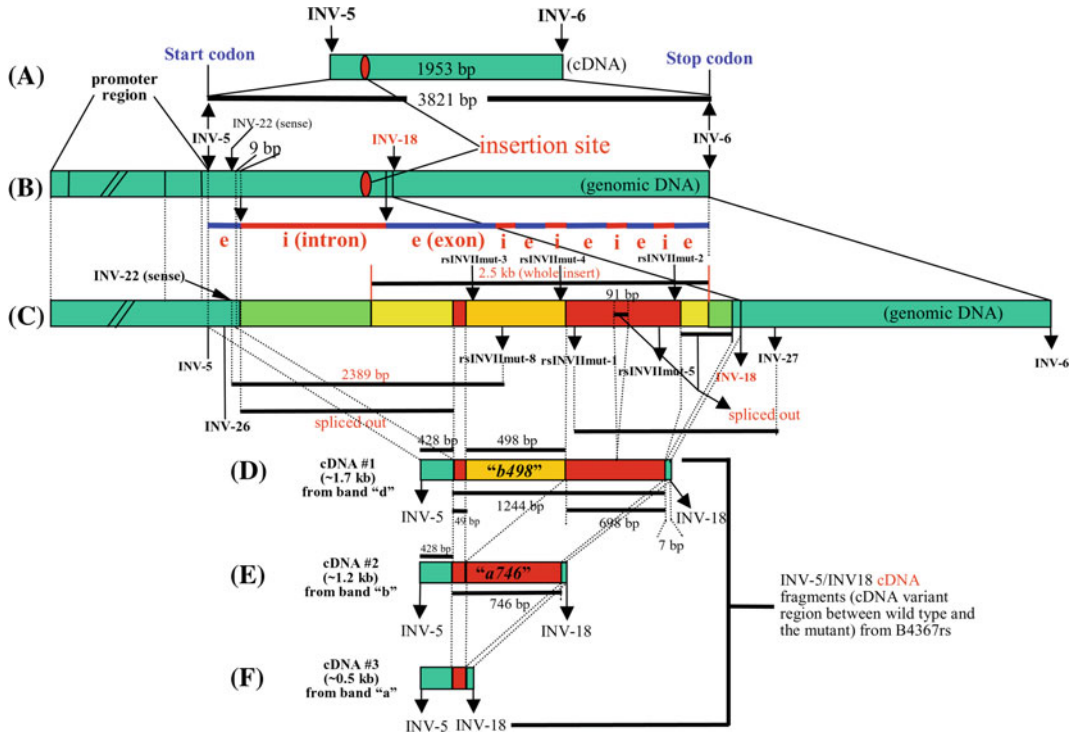

Fig. 9.2 *Rs* gene of carrot that controls sugar type in the storage root. Genomic DNA and cDNA of wild-type and mutant carrot acid soluble invertase isozyme II genes (diagrams are not drawn to scale). (A) Diagram of the cDNA of 1953 bps from the wild-type acid soluble invertase isozyme II gene from carrot line B4367Rs amplified using RT-PCR. Primers INV-5 and INV-6 contain the start and stop codons of the gene, respectively. The position of primers INV-5, INV-6, and INV-18 are indicated. (B) Diagram of genomic DNA of 3821 bps from wild-type acid soluble invertase isozyme II gene from carrot line B4367Rs. Symbols "i" and "e" stand for intron and exon, respectively. The first intron is in bright green, and the insertion site for the 2.5-kb insert is labeled with a red oval. Positions of primers INV-5, INV-6, INV-18, and INV-22 are noted. (C) Diagram of genomic DNA of the mutated acid soluble invertase isozyme II gene from carrot line B4367rs with the first intron highlighted. The green (including bright green) bars represent the wild-type carrot acid soluble invertase isozyme II gene. Bright green and yellow bars represent the sequences spliced out of the first intron during mRNA processing. Yellow, orange, and red bars comprise the 2.5-kb insert. Positions of primers INV-5, INV-6, INV-18, INV-22, INV-27, rsINVIImut-1 through rsINVIImut-5, and rsINVIImut-8 are indicated. (D–F) Diagram of cDNA fragments "a," "b," and "d" amplified using RT-PCR with primers INV-5 and INV-18 from line B4367rs. From Yau and Simon (2003)

9.6 Utilizing the Carrot Genome in Carrot Breeding

For most of the traits of importance to carrot breeders discussed above, biparental mapping populations were used to map genes controlling important traits, followed by the development of molecular markers linked to those genes to track them using MAS in breeding programs. For relatively simply inherited traits, this application of genomic tools will continue to be critically important, since breeding programs usually exercise selection in biparental populations. But those same approaches can be applied in genome-wide association studies (GWAS) to evaluate variation in broad-based germplasm collections, rather than biparental populations, especially when genetic control of the trait of interest in complex. Ellison et al. (2018) discovered the *Or* gene on chromosome 3 using GWAS, and the association between terpenoid

biosynthetic genes and volatile terpenoid flavor components was accomplished using GWAS (Keilwagen et al. 2017). As discussed in Chap. 14, GWAS have also been used to evaluate the association between carotenoid pathway genes and carotenoid color (Jourdan et al. 2015). It is expected that the use of GWAS for molecular genetic mapping will be broadly applied by plant breeders in the future (Myles et al. 2009; Yu et al. 2006), and with the sequencing of the carrot genome, carrot will be no exception.

Regardless of whether candidate genes are identified with GWAS in diverse germplasm collections or in biparental populations, accurate phenotyping is essential to success in identifying candidate genes. To address that requirement, machine phenotyping has recently been developed to gather digital images for evaluating carrot top size and root shape (Turner et al. 2017, 2018). Both of these traits are difficult to phenotype accurately by manual analysis, but with the detail of digital imaging, extensive genetic data was collected and QTL mapped. This study sets the stage for fine-mapping of these traits and for identifying candidate genes. In these studies, the machine data was collected after harvest, which can be useful to evaluate storage root and canopy traits during storage for vernalization. But for many field traits such as biotic and abiotic stress, collection of machine data in the field, and with minimal human attendance, will be the intended approach for traits of interest for carrot breeders, as it is for other plant breeders.

The identification of candidate genes not only identifies the best genomic region in which to develop molecular markers to track a trait, but it also provides a breeding program able to utilize gene editing with the basic information to edit. Genome editing has been demonstrated to be successfully accomplished in carrot as described in Chap. 10, and as editing may well become a standard plant breeding technique, the carrot genome will play a critical role in the application of this technology.

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