Chapter 6
Genome-Assisted Improvement Strategies for Climate-Resilient Carrots

Adam Bolton, Magdalena Klimek-Chodacka, Emily Martin-Millar, Dariusz Grzebelus and Philipp W. Simon

Abstract Carrot is typically categorized as a cool-season vegetable crop that is grown globally with largest per capita production in Europe, but with significant increased production in warmer regions of Asia in the last 50 years. As a high-value vegetable with relatively long postharvest storage life, combined with a high nutritional value attributable to its familiar orange carotenoid pigments, continuing adaptation of carrot to diverse climatic conditions is critical. Traits important to past success and future progress in improving climate resilience depend on the broad genetic diversity of carrot. Classical and modern approaches readily lend themselves to carrot improvement, with significant application of genome-assisted breeding tools expected to expand future prospects of success.

Keywords *Daucus carota* · Cool-season vegetable · Root crop · Climate change · Abiotic stress tolerance · Biofortification

6.1 Introduction

Plants, as sessile organisms, are at mercy of the environment in which they grow and develop. Abiotic stresses, such as heat, drought, and salinity, can result in suboptimal growing conditions for many crops, and although they can survive in environments with abiotic stress , they are likely to experience a reduction in growth and produc-

A. Bolton
Plant Breeding and Plant Genetics Program, Department of Horticulture, University of Wisconsin, Madison, WI, USA

M. Klimek-Chodacka · D. Grzebelus
Department of Plant Biology and Biotechnology, Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, Krakow, Poland

E. Martin-Millar · P. W. Simon (✉)
Vegetable Crops Research Unit, Department of Horticulture, USDA – Agricultural Research Service, University of Wisconsin, Madison, WI, USA
e-mail: philipp.simon@ars.usda.gov

C. Kole (ed.), *Genomic Designing of Climate-Smart Vegetable Crops*,
https://doi.org/10.1007/978-3-319-97415-6_6

tivity (Bray et al. 2000; Rockström and Falkenmark 2000). It has been suggested that abiotic stressors are the number one cause of crop loss and, on average, reduce yields by 50% or more (Boyer 1982). The amount of abiotic stress affected cropland is expected to increase as many climate models predict a mean global temperature increase of 1–4 °C by 2100 (Pachauri and Reisinger 2007). This increase in temperature will be accompanied by more intense heat waves, drought-like conditions, and an increase in salt accumulation in the soil (Mittler and Blumwald 2010). There is no doubt that abiotic stress is going to be an important issue facing the production of crops worldwide. The development of stress-tolerant cultivars through breeding may be one method to reduce the negative impact of abiotic stress. Until now, relatively little has been written in regards to the relationship between carrot and abiotic stress (Grzebelus 2019).

The origins of carrot were in what is now a warm, dry semiarid region. Best evidence points to Central Asia as the origin of carrot as a root crop only 1100 years ago, with Afghanistan (Mackevic 1929) and then Persia (Laufer 1919) being early sites of carrot cultivation. Molecular evidence also supports a Central Asian origin for carrot (Iorizzo et al. 2013) with a rapid spread and extensive domestication effort to the west of Central Asia into Anatolia, North Africa, and then into Europe by the 1300s (Banga et al. 1957a, b; Banga 1963). Carrot developed somewhat more slowly to the east of Central Asia with its estimated arrival time in China around 1300 (Laufer 1919).

The carrot crop today is grown on 1.2 million hectares and valued at $14 billion globally, placing it in the middle of the top 10 vegetables grown globally (FAO 2019). Global carrot production has increased steadily in the last 50 years, rising at a rate more than compensating for the increase in global population, with the most pronounced increase, greater than eightfold per capita, recorded for Asia (Simon 2019). Consequentially more of the carrot crop is grown in warmer, drier climates now than in the last several hundred years. Carrot breeders have responded to this trend by developing cultivars with improved heat tolerance. The most notable of these is “Brasilia” (Vieira et al. 1983) with not only greater tolerance to heat but also improved Alternaria leaf blight tolerance, making it better suited for climatic conditions of northeastern Brazil and accounting for a significant increase in Brazilian carrot production. Crop improvement to sustain increasing production will require much more attention to global climate trends than it has in the past.

Carrot is a diploid ($2n = 2x = 18$) outcrossing insect-pollinated crop traditionally bred for open-pollinated (OP) cultivar production until cytoplasmic male sterility was discovered in the 1940s and 1950s, when cultivar development for large-scale production shifted to hybrids, which account for the majority of large-scale carrot production today (Simon 2000).

6.2 Prioritizing Climate-Smart (CS) Traits

6.2.1 Flowering Time

Floral initiation in carrot is stimulated by exposure to cool temperature, or vernalization, and is required to trigger the transition from the vegetative crop, which is the commodity grown for commercial production, to flowering and seed production (Linke et al. 2019). Early flowering in the vegetative crop results in fibrous, woody storage roots which are unmarketable, and strong selection against early flowering ("bolting") has been exercised by European and North American breeders since carrots became popular in the 1500s. This strong selection was carried out in geographic regions where winters are too cold for production of a winter crop. In this biennial system, carrots grown as a root crop in one year are stored in root cellars until the next spring, when they are planted for seed production in the second year. Carrot cultivars with this biennial flowering behavior are referred to as "temperate." This is in contrast to carrots grown in warmer climates on an annual cycle starting with production of the vegetative crop during the winter with seed production the following summer after minimal vernalization. This second category of carrots is referred to as "subtropical." The fact that subtropical carrots flower with much less exposure to cold is critical to farmers in warm climates who produce their own seed crop since they have no extended cold season to vernalize carrots, and access to refrigerated cold storage can be limited. Consequentially they must rely on early flowering in the field to be assured of a seed crop. Given their tendency toward early flowering, subtropical carrot cultivars typically flower very readily in temperate root crop production regions and cannot be relied upon for commercial production. Similarly, when temperate carrot cultivars are grown in subtropical carrot crop production regions, access to refrigerated storage is required to be assured of a seed crop and consequentially they may not be suitable if that access is limited.

Given the independent development of temperate and subtropical carrot cultivars in the last 500 years and the role that temperature plays in differentiating them, increasing global temperatures may be expected to require a shift in production regions of temperate cultivars away from the Equator, and a concomitant expanded use of subtropical cultivars, assuming current vernalization requirements remain as they are. As new cultivars are developed, field trialing during development in targeted production regress will be more critical to be assured of reliable performance. The genetic control of vernalization requirement has been elucidated for carrot with a single gene identified by Alessandro and Galmarini (2007) and a second gene described by Wohlfeiler et al. (2019).

6.2.2 Root Characters

Genetic variation in fibrous root growth pattern has not been reported for carrot, but storage root growth, structure, and shape of cultivated carrots have received extensive attention since the storage root is the commodity of commerce. Only recently has genetic control of cultivated carrot root shape been analyzed with several quantitative trait loci (QTLs) controlling diameter, length, and shape (Macko-Podgorni et al. 2017; Turner et al 2017). The relationship between storage root shape and fibrous root growth will be of some interest.

6.2.3 Heat Tolerance

Heat stress can be defined as a rise in temperature above a specific threshold for a period long enough to cause damage to crop growth and development, with that temperature and period of time varying for each species (Wahid et al. 2007). Plant response to heat stress varies depending on the duration of stress, intensity of the temperature, and stage of development. The effects of high temperature can influence many aspects of plant physiology, including reduction of photosynthesis, oxidative stress, reduced plant growth, and inhibition of seed germination (Hasanuzzaman et al. 2013). At extreme temperatures, unrecoverable cellular injury, cell death, and collapse of crucial metabolic processes may occur within a few minutes (Schöffl et al. 1999). Although heat stress can be severely damaging, plants do have the ability to tolerate a certain level of heat stress through physiological and biochemical changes resulting from altered gene expression (Hasanuzzaman et al. 2013).

Of all the physiological aspects of plant growth, photosynthesis is one of the most profoundly affected by heat. It has been suggested that photosystem II (PSII) is the most sensitive element of the photosynthetic machinery (Berry and Bjorkman 1980) and PSII activity may be reduced or halted under heat stress (Morales et al. 2003). High temperatures also negatively affect leaf water status, stomatal conductance, and assimilation of CO_2 (Greer and Weedon 2012). It has been shown that the ability to successfully assimilate CO_2 and continue exchange of gases is directly related to whether the plant is considered heat tolerant. The reduction in CO_2 assimilation under high temperatures is likely attributed to a decrease in Rubisco activity, which is known to begin denaturing at approximately 40 °C (Feller et al. 1998). In crop plants, a reduction in photosynthetic activity reduces the amount of sequestered carbon, decreasing plant growth and adversely affecting yield.

Like Rubisco, many other enzymes important for metabolic functions are also sensitive to high temperatures. As enzymes uncouple, mechanisms normally responsible for scavenging reactive oxygen species (ROS), such as superoxide radical ($\bullet O_{2-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH), begin to degrade, causing an increase in oxidative stress (Asada 2006). These ROS can react with many biomolecules, such as proteins, pigments, lipids, and DNA, and cause a decrease in

cell membrane stability (Rodriguez and Redman 2005; Møller et al. 2007; Huang and Xu 2008). When ROS are allowed to accumulate to a high enough concentration in cells, they may even trigger programmed cell death. Examples of oxidative stress resulting from high temperatures have been demonstrated in many crops, e.g., wheat (Savicka and Shkute 2010), tobacco (Tan et al. 2011), Arabidopsis (Larkindale and Knight 2002), and maize (Gong et al. 1997). Notably, the oxidative stress is not only associated with the heat stress, but rather a general response to many abiotic stresses.

Seed germination is the first stage of plant growth affected by heat stress. The inhibition of seed germination, either complete prevention or rate reduction, typically occurs via the induction of abscisic acid (ABA), which is a known stress response hormone (Shinozaki and Yamaguchi-Shinozaki 2006). It has been suggested that as ABA increases in the seed as a response to stress, it limits the availability of energy and nutrients, thus preventing the seed from having the energy required to germinate (Garciarrubio et al. 1997). At extreme temperatures, germination may be completely inhibited due to cell death and unrecoverable embryo damage, as has been demonstrated in wheat seeds (Essemine et al. 2010).

Heat tolerance, sometimes called thermotolerance, is defined as the ability of a plant to grow under high temperatures and produce economically viable yields and is a highly complex trait that varies greatly both among and within species (Hasanuzzaman et al. 2013). Plants demonstrate different mechanisms for dealing with high temperatures depending on the duration and intensity of the heat stress. Some important mechanisms of heat tolerance include the production of antioxidants to combat oxidative stress (Maestri et al. 2002), the accumulation of compatible osmolytes to increase intracellular osmolarity (Sakamoto and Murata 2002), an increase in the chlorophyll *a:b* ratio and carotenoid content to maintain PSII function (Camejo et al. 2006), and the production of heat shock proteins (HSPs) (Bowen et al. 2002). There are multiple mechanisms by which HSPs aid in combating heat stress. HSPs help proteins normally disrupted by high temperatures maintain their shape and function, shuttle proteins aid in protein translation and translocation, reactivate denatured proteins, and protect photosystems from oxidative damage (Neta-Sharir et al. 2005; Stetler et al. 2010).

Carrot, as a cool-season crop, may be particularly sensitive to high temperatures, which is one of the major abiotic factors limiting all stages of growth (Landjeva et al. 2008). Although relatively little work has been undertaken regarding carrot thermotolerance, a few mechanisms and candidate genes have been suggested. The first, alternative oxidase (AOX), is an enzyme that is noted to relieve oxidative stress caused by the formation of ROS (Amirsadeghi et al. 2007). The carrot genome carries three *AOX* genes, one representing *AOX1* and two *AOX2* paralogs (Campos et al. 2009). The *AOX* genes might be responsible for relieving environmentally induced oxidative stress by limiting the formation of ROS in the mitochondria (Nogales et al. 2016). Indeed, the expression of carrot *AOX* was markedly affected by temperature changes, e.g., *DcAOX1* was highly upregulated when ambient temperature raised from 21 °C to 28 °C (Campos et al. 2016). Possibly, allelic variability within *DcAOX1* could have an impact on the heat stress tolerance and the gene could be a target for marker-assisted selection (Nogales et al. 2016).

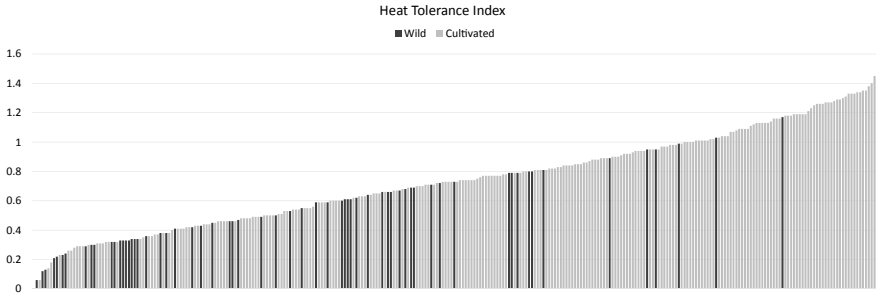

Fig. 6.1 Variation in the heat tolerance index for a collection of wild and cultivated carrot germplasm accessions. Each bar represents a different germplasm accession reported in Bolton et al. (2019)

DcHsp17.7, a carrot HSP, was reported by Malik et al. (1999) as being capable of increasing plant tolerance to high temperatures, up to 42 °C. Park et al. (2013) showed that it was rapidly synthesized in response to the heat treatment, remained abundant two days later, and subsequently decayed. Night exposure to heat showed a more pronounced effect on the accumulation of DcHsp17.7. Several other HSPs were shown to be upregulated by heat stress (Huang et al. 2015).

With the development of carrot cultivars targeted for production in a warmer climate, the influence of elevated temperature on early crop growth has been evaluated. Nascimento et al. (2008) and Bolton et al. (2019) observed seed germination to be reduced with elevated temperatures where, relative to the control temperature of 24 °C, germination of most carrots was reduced at least 50% up to 35 °C, but several OPs evaluated exhibited no significant reduction in germination at 35 °C compared to 24 °C (Fig. 6.1). At 37.5 °C, only "Brasilia" seed germinated among the cultivars tested, but at a rate less than 10%. Temperature levels under which the carrot root crop can survive during stand establishment and crop growth beyond germination have not been reported.

Beyond production of the root crop, heat tolerance may also play an important role in carrot seed production. Broussard et al. (2017) exposed flowering carrots to "cool", "average," and "warm" greenhouse conditions and observed reduction in volatile terpenoid production and nectar quality, which was conjectured to reduce attractiveness of insect pollination. Since adequate seed production is critical to sustain crop production, expanded studies on the effects of climatic effects on the reproductive phase of the carrot life cycle will be of great importance.

6.2.4 Cold Tolerance

Climate change can include temperature fluctuations not only above recent averages, but also temperatures below recent averages. Carrot is generally regarded as cold-hardy and able to recover from cold temperatures as low as −8 °C. Beyond leaf

damage, cold temperatures cause taproot cracking in carrot. Palta and Simon (2004) observed variation among breeding stocks for leaf and root damage, and exercised selection for reduced incidence of taproot cracking. Two frost tolerant hybrid cultivars were developed and released.

6.2.5 Salinity Tolerance

There are two distinct mechanisms by which high levels of salinity impede plant growth and development. The first occurs when high levels of salt in the soil create an osmotic effect that reduces the ability of the seeds and roots to pull water from the surrounding environment and into the plant tissue, creating drought-like symptoms such as reduced cell expansion in the leaves, roots, and seeds (Munns and Tester 2008). The second mechanism is the accumulation of salts to toxic levels within the plant tissue, interfering with major biological processes critical to plant growth, and creating ionic stress that often results in tissue death. For example, the accumulation of Na^+ can reduce the functionality of chlorophylls, carotenoids, and essential photosynthetic enzymes (Davenport et al. 2005), which can result in oxidative stress caused by the formation of ROS (Apel and Hirt 2004). Mineral nutrient deficiencies can occur when Na^+ competes for transport protein sites that normally uptake critical macronutrients such as K, N, and P (Carillo et al. 2011). It was first suggested by Munns et al. (1995) that both of these effects on plant growth and survival occur in a two-phase model. In Phase 1, high levels of salts create osmotic stress that tends to decrease growth rate, followed by the toxic ionic effects of Phase 2, which are often more harmful (Carillo et al. 2011). During the second phase, ions are transported through the xylem and deposited in the leaf blade where they accumulate and can kill older leaves. These two phases of salinity stress have a greater negative effect on the shoots, which tend to be less tolerant than the roots (Munns and Tester 2008). Response to salinity varies with developmental stage, or ontogeny; the most sensitive and critical stages of the plant life cycle are typically germination, seedling establishment, and flowering (Flowers 2004). Tolerance is also dependent on other environmental conditions such as soil temperature, soil moisture, physical properties of the soil, air temperature, and humidity (Munns and James 2003). Salt-tolerant plants (halophytes) have developed mechanisms to overcome the accumulation of these toxic ions through multiple salinity tolerance mechanisms that each have been found to be under independent genetic control.

Salinity tolerance mechanisms can be broken up into three main categories: (1) tolerance to osmotic stress, (2) Na^+ exclusion from the leaves, and (3) tolerance of tissue to Na^+ accumulation (Munns and Tester 2008). Osmotic stress tolerance is typically conferred by increased water-use efficiency and/or osmotic adjustment via increased proline or soluble sugar accumulation (Munns 2005). Na^+ exclusion from the leaves starts with the selective exclusion of Na^+ over K^+ by the roots (Munns and Rawson 1999) or by efflux of Na^+ back out into the soil rather than transport into the xylem (Tester and Davenport 2003). In many species, salt exclusion is strongly

correlated with salt tolerance and has been shown to have a wide range of natural variation among species (Yeo and Flowers 1986; Munns and James 2003; Tester and Davenport 2003). Tissue tolerance of Na^+ accumulation occurs when plants are able to compartmentalize Na^+ into the vacuole to prevent reaching toxic levels in the cytoplasm. This also requires the synthesis of solutes in the cytoplasm to maintain osmotic balance with the vacuole (Tester and Davenport 2003). These solutes (e.g., proline, sucrose, glycine betaine, and mannitol) are compounds that do not interfere with normal biochemical functions (Shomer-Ilan et al. 1991). Several candidate genes related to these salinity tolerance mechanisms have been identified and could be combined to give higher levels of salinity tolerance in many crops (Yeo and Flowers 1986). Major genes have been identified that contribute to salinity tolerance, but the functions in which they are involved (ion transport, protein synthesis, hormone signaling) are complex, and consequently it is not surprising that much of adaptation to salinity stress, as well as to other abiotic stresses, is governed by quantitative variation (Sreenivasulu et al. 2007). Phenotypic parameters for screening salinity tolerance vary depending on the salinity concentration, duration of stress, and the developmental stage of the plant (Shannon 1985). The strictest measure of tolerance is whether a genotype has the ability to survive through the completion of its life cycle at high salinity levels. A genotype that can survive from seed germination, through seedling establishment, and on to flowering is considered tolerant in the most absolute sense. This level of tolerance may not be necessary for most crop species, but even relatively low levels of salinity can reduce biomass accumulation and yield significantly. For many crop species, biomass and yield reduction under salinity stress are useful criteria for quantifying tolerance but do not provide insight into the mechanisms conferring tolerance (Bado et al. 2016).

As mentioned previously, leaves are often more sensitive to salinity stress than roots, and thus have been a focus of phenotyping procedures. Leaf damage can be easily observed as necrosis or yellowing and has been successfully used to phenotype salinity stress response in wheat and barley (Richards et al. 1987), and rice (Gregorio et al. 1997). Scoring of leaf wilting, another leaf trait, has been shown to be effective in adzuki bean, *Vigna angularis* L. (Yoshida et al. 2016), but can be inaccurate due to the subjectivity of scoring.

Phenotyping at seed germination is a relatively easy and fast (7–21 days) measurement that is critical for plants in saline conditions and found to be controlled by other genes than those controlling leaf damage. The most frequently used measurement for germination tolerance is relative percent germination in salinity: percent germination under a defined salinity concentration divided by percent germination without salinity. Relative percent germination as a tolerance trait has been evaluated in many species including *Triticum durum* L. (Almansouri et al. 2001), *Arabidopsis thaliana* L. (DeRose-Wilson and Gaut 2011), *Zea mays* L. (Radić et al. 2007), and *Pisum sativum* L. (Shahid et al. 2012). Independent screening for specific traits related to all three physiological mechanisms of salt tolerance (Na^+ exclusion, K^+/Na^+ discrimination, and tissue tolerance) has been argued as the best method for maximizing the genetic improvement of salt tolerance (Noble and Rogers 1992; Munns and Rawson 1999; Munns and James 2003; Yoshida et al. 2016). Each of these traits is frequently

controlled by specific genes and therefore there is potential to pyramid these traits together to increase tolerance above what may be normally found in one genotype (Noble and Rogers 1992). Harvesting root and shoot tissue grown with and without salinity stress and analyzing it for Na^+ and K^+ concentrations allow for identification of mechanisms, whether it be salinity exclusion or tolerance, that the plant is utilizing to cope under the stress. Comparing these concentrations with percent biomass reduction under stress allows for the identification of tolerant genotypes/accessions and the mechanisms of tolerance utilized (Munns and James 2003). Quantifying the concentration of the ions can be done by studying the "Ionome" of plants (Baxter 2009).

Carrot, as a salt-sensitive glycophytic plant, has long been observed to be one of the most salt-sensitive vegetable crops (Bernstein and Ayers 1953; Maas and Hoffman 1977). Carrot yield, measured in terms of root biomass, declines approximately 14% for every unit increase in salinity past 1.0 dS m^{-1} threshold, which is much lower than the defined threshold, 4 dS m^{-1}, for a saline soil. Carrot seed germination and seedling establishment (Fig. 6.2) also suffer greatly from increased salt concentrations in the soil (Schmidhalter and Oertli 1991). Both the capacity for total seed germination and rate of germination are decreased greatly under salinity stress with these effects becoming greater as concentration of salt increases (Kahouli et al. 2014). Salinity stress has also been noted to cause reduced rates of photosynthesis and stomatal conductance in carrot (Gibberd et al. 2002).

Similar to heat stress tolerance, relatively little work has been undertaken to identify mechanisms of salt tolerance in carrot, but some have been suggested. Changes in the enzymatic and nonenzymatic antioxidant defense system of carrots under salt stress have been demonstrated by Bano et al. (2014) suggesting that increase in glycine betaine, ascorbate, and other antioxidants may place a role in salt stress tolerance. Possibly, phytoene synthase 2 (DcPSY2) may also be involved in the reaction of carrots to salinity. DcPSY2 is one of the key proteins in the carotenoid biosynthesis pathway in carrot roots (Fuentes et al. 2012; Wang et al. 2014). In turn, carotenoids are precursors of ABA. Simpson et al. (2018) showed that the salinity stress and

Fig. 6.2 Carrot plants at 42 days of growth without (left) and with (right) 150 mM NaCl added to irrigation solution

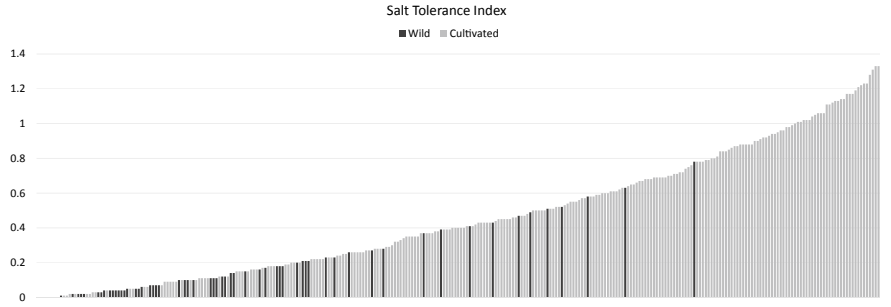

Fig. 6.3 Variation in the salt tolerance index for a collection of wild and cultivated carrot germplasm accessions. Each bar represents a different germplasm accession reported in Bolton and Simon (2019)

ABA upregulate *DcPSY2* through binding of DcAREB3 transcription factor to ABA responsive elements (A located in the promoter of *DcPSY2*).

Since carrot is irrigated in much of its global production, and rising levels of salinity is an increasing problem, an assessment of genetic diversity in carrot germplasm for salinity tolerance can provide important insights into future prospects for greater salinity tolerance in the carrot crop. Kahouli et al. (2014) evaluated 10 carrot cultivars and observed variation indicating a genetic component to carrot salinity tolerance. Bolton and Simon (2019) evaluated 294 diverse cultivated and wild carrot accessions and confirmed broad variation for tolerance to 150 mM NaCl during germination (Fig. 6.3), including breeding stocks and OPs. The observation of relatively high levels of salinity tolerance in cultivated germplasm provides an optimistic outlook for future CS carrot crop improvement.

6.2.6 Drought Stress

Reduced rainfall and changes in rainfall patterns are very dangerous for agriculture (Fahad et al. 2017). Typical symptoms of drought stress in plants are reduced leaf water potential and decreased cell growth, which adversely affect both the plant growth as well as a range of physiological or biochemical processes, including photosynthesis, nutrient metabolism, respiration, and chlorophyll synthesis (Hussain et al. 2018). Thaumatin-like proteins are included in a group of pathogenesis-related proteins. However, these proteins are also involved in response to abiotic stresses. In carrot, a *dcTLP* gene encoding a thaumatin-like protein (TLP) was reported to be upregulated upon dehydration, independently from the developmental stage and not regulated by ABA, salicylic acid or jasmonic acid. Possibly it is one of the elements conferring physiological adaptation of carrots to drought, in combination with other drought-induced genes (Jung et al. 2005). A small HSP, DcHsp17.7, referred to in the

heat stress section, was also shown to accumulate in carrots suffering from osmotic stress (Ahn and Song 2012).

Few reports of carrot growth under drought stress have been published. Sorensen et al. (1997) reported yield reduction and significant changes in sugar content and other components of nutrient composition in carrot due to drought, and they noted variation among cultivars tested to suggest a genetic component to drought tolerance in carrots. Given the recurring shortage of rainfall and dwindling access to adequate quality irrigation water in recent decades, detailed field performance information evaluating the effects of drought on carrot productivity will be valuable.

6.2.7 Disease and Pest Resistance

Several diseases challenge carrot growers (du Toit et al. 2019; LeClerc et al. 2019). The most widespread foliar disease globally is Alternaria leaf blight which especially threatens carrot production in humid climates. Genetic analyses have identified several QTLs contributing to the resistance response (LeClerc et al. 2015, 2019) and many breeding programs are selecting for improved resistance. Root-knot nematodes are another significant pest of carrot, and resistance genes to protect against *Meloidogyne incognita* and *M. javanica* have been identified (Simon et al. 2000; Parsons et al. 2015). Both Alternaria leaf blight and root-knot nematodes are widespread challenges in subtropical carrot production regions, so durable resistance is critical as climate-resilient carrots are developed. The most important postharvest disease in carrot is cavity spot, caused by several *Pythium* species. Variation in *Pythium* resistance is observed among cultivars and breeding stocks. The relatively long potential postharvest season storage is an attractive feature of carrots, but to fully realize that potential, resistant cultivars will be important. Numerous other diseases of carrots have been identified, and as production expands in subtropical carrot-growing regions, several diseases may become more important (du Toit et al. 2019).

6.2.8 Insect Resistance

Carrot fly (*Psila rosae*) is the most important insect pest that damages the carrot crop (Collier and Finch 2009). Partial resistance has been identified but additional sources of resistance are expected to be necessary. Carrot fly is primarily a problem in cooler growing regions of Northern Europe and Canada. If temperate carrot production moves north in these regions with the advance of warmer climates carrot fly could become more of a problem.

Insects vector several microbial diseases of carrot (Groves et al. 2019). Carrot psyllid-vectored diseases may pose especially challenging threats (Nissinen et al. 2012) and warmer climates have been projected to potentially heighten their likely impact.

6.2.9 *Antioxidants and CS Carrots—A Role in Plant Stress Tolerance and Human Health*

6.2.9.1 Antioxidant Response to Environmental Stress

While much research has been focused on the antioxidant content of carrots from a human nutritional perspective, little has been done on the antioxidant activity of carrots prior to harvest. The most prominent antioxidants in carrot are the pigments that determine the many possible colors of their roots; the carotenoids, which include alpha- and beta-carotene, lycopene, and lutein, and confer orange, red, and yellow pigmentation, respectively, and the anthocyanins, which confer purple pigmentation. These photosynthetic pigments, which only occur in the shoot in most plants, accumulate in carrot roots due to a defect in light sensing that allows the carotenoid, and possibly also the anthocyanin, metabolic pathways to be expressed in darkness (Iorizzo et al. 2016). Both carotenoids and anthocyanins function as nonenzymatic low molecular metabolites in enzymatic antioxidant systems (Gill and Tuteja 2010).

Many known antioxidants are synthesized in the carotenoid biosynthetic and its related pathways. Just upstream of carotenoid biosynthesis is the synthesis of terpenoids, which function as antioxidants (Graßmann 2005) and contribute to the distinctive flavor of carrots (Keilwagen et al. 2017). Among these, isoprene, a hemiterpene found in carrot roots (Duke 1992), is known to increase thermotolerance in kudzu (Singsaas et al. 1997), while monoterpenes, too, improve thermotolerance and protect plants against oxidative stress (Gill and Tuteja 2010). The first committed step of the carotenoid biosynthetic pathway is catalyzed by phytoene synthase, and the overexpression of its two isoforms in carrot is responsible for orange root pigmentation (Wang et al. 2014). Expression of the second isoform in carrot, *DcPSY2*, is induced by salt stress and the phytohormone ABA, which is synthesized downstream of carotenoids and plays a major role in mediating abiotic stress tolerance across plant species (Simpson et al. 2018), indicating a direct link between orange root pigmentation and abiotic stress response. ABA has also been shown to specifically enhance antioxidant response in several other diverse plant species, including intertidal seaweed species (Guajardo et al. 2016), Malabar plum (*Syzygium cumini*) (Choudhary et al. 2012), and pumpkin-grafted cucumber seedlings (Shu et al. 2016). The carotenoids synthesized in this pathway are known for protecting plants against photooxidative stress by efficiently scavenging singlet oxygen and peroxyl radicals (Stahl and Sies 2003), and among them, lycopene is the strongest antioxidant in terms of singlet oxygen quenching (Di Mascio et al. 1989). While growing carrot roots are mostly shielded from the sun, these pigments can protect plants from oxidative stress in general (Sies and Stahl 1995), which can be induced by drought, heat, and salinity stresses (Krishnamurthy and Rathinasabapathi 2013).

Anthocyanins, ubiquitous and abundant in purple carrots, are synthesized in the flavonoid pathway in response to abiotic stress as well as other stimuli. The production of anthocyanins often correlates with increased stress tolerance, and the proposed mechanisms for this include quenching of ROS, photoprotection, and stress

signaling (Kovinich et al. 2015). The accumulation of anthocyanins may also inhibit foliar senescence under nutrient deficiency (Landi et al. 2015), a condition which can be induced by salt stress (Acosta-Motos et al. 2017). In fact, salt stress has been shown to stimulate anthocyanin accumulation in higher plants (Eryılmaz 2006). In a small comparative study of black and orange carrots from Cuevas Bajas, Spain, the black carrots displayed a higher antioxidant activity than the orange, potentially due to higher total phenolic content, including anthocyanins (Algarra et al. 2014). This could also be due to higher antioxidant capacity of anthocyanins over carotenoids, or to higher total pigment content, or perhaps even to synergistic antioxidant effects of anthocyanins and carotenoids.

While there is an evident correlation between photosynthetic pigment accumulation and abiotic stress response, a causative relationship between pigment content and tolerance has not yet been determined. However, while most crops are more stress-sensitive than their wild progenitors, Bolton and Simon (2019)demonstrated that wild *D. carota*, which is predominantly white-rooted, is significantly less salt-tolerant, at least at the germination stage, than variously colored Turkish and Indian landraces of carrot, suggesting that these root pigments may directly enhance abiotic stress tolerance of carrot. This would accord with the biological principle of xenohormesis, which dictates that plants subjected to environmental stresses produce bioactive compounds that provide stress resistance to consumers (Hooper et al. 2010); carrots that thrive under abiotic stress conditions would then contain more of the antioxidant pigments that benefit human consumers, which has been the primary focus of carrot antioxidant studies.

There have been few reports of environmental effects on the accumulation of carrot carotenoids, anthocyanins, or other antioxidant compounds. Barnes (1936) reported that both root size and carotene content were higher at 17–19 °C than at either 11–13 °C or at 23–25 °C soil temperatures, and anecdotal information on carrot color intensity from season to season supports the conclusions from these early studies. Given their involvement in plant growth and response to stress and their importance in human nutrition, more information on antioxidant accumulation will be valuable.

6.2.9.2 Antioxidants and Human Health

The pigments familiar to consumers in orange carrots are provitamin A carotenoids, while lutein in yellow carrots, lycopene in red carrots, and anthocyanins in purple carrots also have important roles in human nutrition as antioxidants promoting eye health and protecting against certain forms of cancer (Simon et al. 2008; Arscott and Tanumihardjo 2010). Wide variation for pigment content and composition can be found among diverse cultivated carrots and a wide range of research has been published on the genetic control of carrot pigments (Table 6.1; reviewed by Cavagnaro and Iorizzo 2019; Simon et al 2019). Given the rise in carrot production in regions of the world with significant micronutrient deficiency and stressful climates for crop production, additional research on the antioxidants of carrot in global agricultural settings will have multiple significant implications.

Table 6.1 Mapped simply inherited traits and QTLs of carrot

Gene symbol	Trait	References
Growth and reproductive biology		
Vrn1	Vernalization	Alessandro et al. (2013)
Rf1	Nuclear restorers of CMS	Alessandro et al. (2013)
Gum1-2, Mar1-2, Gad1-2	Novel cytoplasms and sterility	Borner et al. (1995)
STS1-STS6	Petaloid male sterile and fertile cytoplasm	Nakajima et al. (1999)
14 primer pairs		Bach et al. (2002)
Phenl	Small, dark green, annual	Schulz et al. (1994)
COLA	Compressed lamina	Budahn et al. (2014)
YEL	Yellow leaf	Budahn et al. (2014)
cult	Root thickening	Macko-Podgorni et al. (2017)
5, 4, and 3 QTLs 1, 5, and 3 QTLs 6, 2, and 2 QTLs	Shoot height, biomass, area Petiole number, width, and length Root length, biomass, and area	Turner et al. (2018)
Disease and pest resistance		
3 QTLs	Alternaria leaf blight	LeClerc et al. (2019)
11 QTLs		LeClerc et al. (2015)
Mj-1	*M. javanica* root-knot nematodes	Boiteux et al. (2000, 2004)
Mj-2	*M. javanica* root-knot nematodes	Ali et al. (2013)
7 QTLs	*M. incognita* root-knot nematodes	Parsons et al. (2015)
Nutritional quality and flavor		
Y	Yellow xylem and phloem	Just et al. (2009), Iorizzo et al. (2016)
y_2	Differential orange phloem/xylem	Bradeen and Simon (1998), Just et al. (2009), Yildiz et al. (2013), Ellison et al. (2017)
16 QTLs	Carotene content	Santos and Simon (2002)
Or	Carotene content	Ellison et al. (2018)
P_1	Root anthocyanins	Vivek and Simon (1999), Yildiz et al. (2013), Cavagnaro et al. (2014)
P_3	Root and petiole anthocyanins	Cavagnaro et al. (2014)
Raa1	Acylated anthocyanins	
15 QTLs	Anthocyanin content	
30 QTLs	Volatile terpenoid content and composition	Keilwagen et al. (2017)
Rs	Reducing sugar	Vivek and Simon (1999, Yau et al. (2003, 2005)

6.3 Genetic Resources

The primary gene pool of carrots includes cultivated carrot (*Daucus carota* ssp. *sativus*) and wild carrot (*Daucus carota* ssp. *carota*). Their range of genetic and phenotypic diversity is broad, and they are freely intercrossable (Peterson and Simon 1986; Simon 2000). A secondary gene pool for carrot includes those North African and eastern Mediterranean species with the same chromosome number as carrot, 2n = 2x = 18. Interspecific crosses with species in the secondary pool have not been reported. The genus *Daucus* includes approximately 40 species (Banasiak et al. 2016; Spooner 2019) and may be considered a tertiary gene pool of carrots. A relatively extensive collection of *Daucus* germplasm has been collected (Allender 2019), but wild carrot germplasm is not well represented (Castaneda-Alvarez et al. 2016).

6.4 Classical Genetics and Breeding

6.4.1 Genetics

Carrot is not a model organism for genetic studies and genetic analysis of carrot has not been extensively pursued. Seed production requires time and experience beyond production of the root crop to vernalize plants and produce the seed crop. Furthermore, carrot flowers are very small and each flower produces a maximum of two seeds, making pollinating by hand challenging and not very rewarding. In contrast, insect pollination of carrot umbels with houseflies or blue bottle flies can yield several hundred seeds per plant.

Twenty single genes controlling phenotypic traits were reported for carrot by 1985 and no linkages had been identified. The carrot chromosome number was known but no genes were associated with chromosomes. Isozyme analysis had been used for taxonomic research but not genetic analysis (Peterson and Simon 1986). The advent of the use of biochemical and molecular markers in the 1990s stimulated more extensive carrot genetic analysis.

6.4.2 Breeding

Shorter term carrot breeding objectives focus on improving disease and pest resistance, storage root appearance, color, flavor, and population uniformity (Peterson and Simon 1986; Simon and Goldman 2007; Simon et al. 2008; Simon and Grzebelus 2019). The popularity of hybrid cultivars stems from the uniformity that they can afford, and their proprietary nature stimulated an expanded interest in initiating carrot breeding programs among seed companies (Simon 2000). Longer term carrot breeding objectives have included abiotic stress tolerance and introgression of traits

between temperate and subtropical breeding pools. Introgression of traits from wild carrot into cultivated breeding stocks can be expected to be a much longer term effort.

6.5 Diversity

6.5.1 Phenotypic Diversity

Cultivated carrot varies widely in phenotypic diversity (Fig. 6.4) as reflected in traits ranging from storage root color, shape, and flavor to leaf morphology, size, and pubescence and to umbel shape, petal color, and pollinator attractiveness. Wild carrot also varies widely in most of these traits except that roots are typically narrower, more fibrous with prominent lateral roots, and root color is white or very pale yellow. Diversity analysis of carrot has typically included an evaluation of not only phenotypic diversity but also genotypic diversity as molecular genetic markers were developed.

Fig. 6.4 Variation in carrot color attributable to carotenoid (orange, red, yellow) and anthocyanin (purple) pigments (Photo by Steve Ausmus, USDA/ARS)

6.5.2 Genotypic Diversity

Several studies have utilized diverse collections of wild and cultivated carrots to evaluate genetic diversity, geographic substructure, and patterns of domestication in carrot. Bradeen et al. (2002) utilized less than 200 molecular markers, primarily AFLPs and ISSRs, and observed clear separation between wild and cultivated carrots, but no structure among cultivated carrots evaluated based on storage root color or shape, or geographic origin. However, based on an evaluation utilizing 4,000 SNPs, Iorizzo et al. (2013) distinguished not only wild carrots from cultivated, but also found that wild carrots from Central Asia (Afghanistan, Uzbekistan) were genetically most similar to cultivated carrots, than were wild carrots from other geographic origins. This study also confirmed that cultivated carrots from east of this Central Asian center of domestication grouped separately from cultivated carrots west of Central Asia. Utilizing additional markers and diverse carrots, Ellison et al. (2018) confirmed these observations and also noted an additional cluster among cultivated carrots that included western hybrid carrots of the Imperator type. The differentiation between eastern and western geographic origins of cultivated carrots in these studies agrees with historical records indicating a separate historical development of carrot as a root crop progressing west from Central Asia around 900 through Anatolia and North Africa to southern Europe by the 1100s, while the first records of carrot in China were in the 1300s and Japan in the 1700s (Banga et al. 1957a, b; Banga 1963).

A small reduction in overall genetic diversity, if any, has been observed during the domestication of carrot. $H_e$in both wild and cultivated carrots was 0.32 in the Iorizzo et al. (2013) study, while genetic diversity was 3.25×10^{-5} and 3.13×10^{-5} for these respective groups in the Ellison et al. (2018) study. This may reflect the likely recurring introgression of wild carrot, thought to be widespread throughout temperate regions of Europe and Asia thousands of years ago, into cultivated carrots during domestication.

6.6 Association Mapping

Few genome-wide association studies (GWAS) have been reported for carrot (Iorizzo et al. 2019a). An evaluation of 109 SNPs distributed in 17 carotenoid biosynthesis genes in a collection of carrots varying in carotenoid-based root color by Jourdan et al. (2015) found orange color and carotenoid content to be associated with two of these genes, *ZEP* and *CRTISO*. With the availability of the carrot genome sequence (Iorizzo et al. 2016), Keilwagen et al. (2017) associated 15 volatile flavor compounds found in carrot roots with 30 QTLs. Ellison et al. (2018) detected genomic regions that differentiated wild and cultivated carrots. Three genes previously known to be associated with carotenoid accumulation and composition in orange carrots—*Y, Y_2*, and carotene hydroxylase—were included in the genomic regions mapped, as was a

candidate gene for root thickening (Macko-Podgorni et al. 2017), and a previously unidentified gene associated with carotenoid accumulation, *Or*.

The ability to detect genomic regions in GWAS depends on the occurrence of linkage disequilibrium (LD), with rapid decay expected in an outcrossing crop like carrots. In fact, Ellison et al. (2018) observed rapid decay rates, <1 kb, in wild carrots and moderate decay, <10 kb, in cultivated carrots. As in other crops, LD values vary across the genome and even slower decay is observed around genomic regions under selection during domestication in carrot. With the rapid LD decay observed in carrot, high levels of SNP coverage will benefit GWAS in carrot.

6.7 Molecular Mapping

Genetic linkage in carrot was first reported by Westphal and Wricke (1991) with four linkage groups identified mapping 12 isozyme markers. By the middle of the 2000 to 2010 decade, four additional reports mapped approximately 900 more markers, primarily RFLP, RAPD, and AFLPs, and four morphological traits (reviewed by Bradeen and Simon 2007).

Progress in molecular mapping has accelerated since 2000. QTL analysis was first reported for carrot in 2002, first extensive SSR map and FISH map in 2011, SNP map in 2013, and both DArT map and GBS map in 2014 (reviewed by Iorizzo et al. 2019a).

Early carrot genetic maps were usually derived from F_2 populations developed from unrelated parents. Mapped traits that contribute to climate resilience include floral initiation; male sterility, important for hybrid production; leaf growth, important for weed competitiveness; storage root morphology, size, and shape; leaf blight and nematode resistance; nutritional pigments; and sugars that contribute to culinary quality (6.1). As an example, the QTL map for *Meloidogyne incognita* resistance (Fig. 6.5) and table describing the contributions of those QTLs to resistance (Table 6.2) are included.

6.8 Marker-Assisted Breeding

Molecular markers have been developed for carrot root color and sugar type, and root-knot nematode resistance. Bradeen and Simon (1998) identified linkage between the Y_2 locus, which conditions carotene accumulation in the carrot xylem core, and six linked AFLP markers. A simple codominant PCR-based marker ~2 cM from Y_2 was developed. Ellison et al. (2017) refined markers for Y_2 by developing cleavage amplified polymorphic sequences <<1 cM away that were very accurate in predicting orange and non-orange phenotypes. Yau developed markers within the candidate gene for the *Rs* locus that controls the type of sugar stored in the storage root (Yau and Simon 2003) and effectively selected for sugar type of mature plants based on

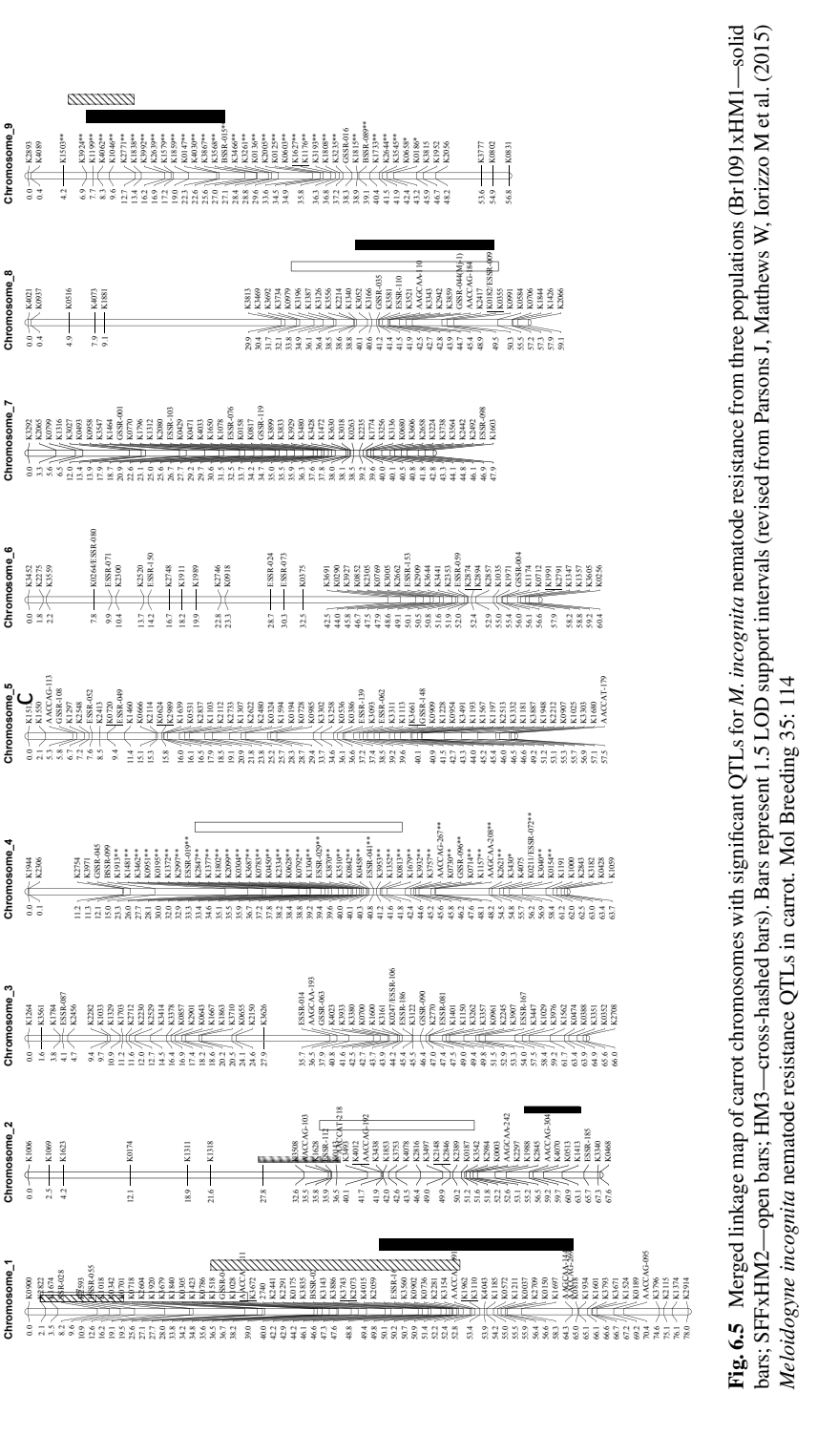

Fig. 6.5 Merged linkage map of carrot chromosomes with significant QTLs for *M. incognita* nematode resistance from three populations (Br1091xHM1—solid bars; SFFxHM2—open bars; HM3—cross-hashed bars). Bars represent 1.5 LOD support intervals (revised from Parsons J, Matthews W, Iorizzo M et al. (2015) *Meloidogyne incognita* nematode resistance QTLs in carrot. Mol Breeding 35: 114

Table 6.2 Chromosomal locations of QTL conferring *M. incognita* nematode resistance in the three carrot mapping populations and their contribution to resistance

(Mapping population) chromosome	QTL	Position (cM)	LOD	% VE[a]	Resistant parent	1.5 LOD[b]	Additive effect[c]
(Br1091× HM)							
1	*Mi-BrHM1-C1-Q3*	67.2	3.9	6.1	B1091	52–75	0.6
2	*Mi-BrHM1-C2-Q1*	63.1	17.3	34.0	HM1	61–67	1.4
8	*Mi-BrHM1-C8-Q2*	41.9	8.4	13.7	B1091	41–56	1.0
9	*Mi-BrHM1-C9-Q4*	4.2	2.6	4.1	HM1	4–22	0.6
Summed % variance explained by multi-QTL model = 55.5%							
(SFF× HM2)							
2	*Mi-SFFHM2-C2-Q3*	42.6	2.8	8.0	HM2	4–66	1.1
4	*Mi-SFFHM2-C4-Q1*	33.3	4.6	13.4	SFF	15–57	1.0
8	*Mi-SFFHM2-C8-Q2*	41.5	3.2	9.2	SFF	27–59	0.8
Summed % variance explained by multi-QTL model = 34.8%							
(HM3)							
1	*Mi-HM3-C1-Q3*	34.8	4.0	4.3	HM3	23–65	0.4
8	*Mi-HM3-C8-Q2*	41.9	13.5	15.8	HM3	41–44	0.9
9	*Mi-HM3-C9-Q1*	9.6	14.9	17.7	HM3	4–13	0.1
Summed % variance explained by multi-QTL model = 35.7%							

[a]Percentage of variation explained
[b]1.5 LOD support interval (cM)
[c]Half phenotypic difference between means of resistant and susceptible homozygous genotypes
(revised from Parsons et al. (2015) *Meloidogyne incognita* nematode resistance QTLs in carrot. Mol Breeding 35:114)

evaluations made in one-week old plants (Yau et al. 2005). Boiteux et al. (2000) mapped the *Mj-1* gene that confers resistance to *Meloidogyne javanica* root-knot nematodes, and Boiteux et al. (2004) successfully identified homozygous resistant plants in breeding populations.

6.9 Candidate Genes

6.9.1 A Candidate Gene For Root Shape

The cultivated carrot storage root is typically much wider than the taproot of wild carrots. In the evaluation of a collection of wild and cultivated carrots, a polymorphic indel on chromosome 2 was associated with root diameter and referred to as

cult. Using a mapping population developed from a cross between wild and cultivated carrots, root diameter segregated and Macko-Podgorni et al. (2017) identified *DcAHLc1* as a candidate for *cult.* The genomic region that includes *cult* was among those identified as differentiating wild and domesticated carrots in a GWAS study (Ellison et al. 2018).

6.9.2 Genes for Pigments and Color

Three genes controlling the accumulation and distribution of orange and yellow carotenoids in the carrot storage root, *Y*, Y_2, and *Or*, have been mapped in segregating populations and candidate genes identified for *Y* and *Or*. The *Y* candidate is an interesting homolog of the *Arabidopsis thaliana* gene *PSEUDO-ETIOLATION IN LIGHT*, responsible for the regulation of photomorphogenesis. Two frameshift mutations identified turn off the constitutive repression of genes downstream that usually require exposure to light to trigger plastid biogenesis (Iorizzo et al. 2016). The Y_2 and *Or* genes described above both influence plastid development. *Or* was identified in GWAS, as described above. While a definite candidate for Y_2 has not been identified, a relatively short list including transcription factors and genes involved in light signaling and carbon flux are among them (Ellison et al. 2017).

The carotene hydroxylase gene is the candidate for controlling the relatively high amount of α-carotene in carrot roots. In transgenic experiments, Arango et al. (2014) overexpressed carotene hydroxylase *CYP97A3* in orange carrots and observed that the content of α-carotene in leaves and roots was several-fold higher than in control plants. Transgenic experiments involving overexpression of *CYP97A3* lowered α-carotene content of leaves and carrots.

Three genes, *P1, P2,* and *P3*, control anthocyanin accumulation in purple carrots. *P3* controls root and petiole pigmentation and a MYB, *DcMYB7*, was identified as a candidate (Iorizzo et al. 2019b). *DcMYB7* is in a cluster of MYB genes and its identification as the candidate is based on fine mapping plus transcriptome analysis.

6.9.3 A Candidate for Sugar Type

Most carrots store a mixture of glucose and fructose but a single gene mutation, *Rs,* was discovered to condition storage roots to primarily accumulate sucrose (Freeman and Simon 1983). Yau and Simon (2003) determined that a 2.5 kb insert in the acid-soluble invertase II gene was associated with *Rs* so that roots of carrots homozygous *rs/rs* accumulate sucrose.

6.10 Genomics-Assisted Breeding and Genome Editing for CS Traits

Systematic investigations on the genetics of abiotic stress tolerance in carrot are of high significance, as they are essential for the development of new cultivars better adapted to the changing environmental conditions imposed by global warming. It can be obtained by exploring the existing genetic diversity both in the cultivated gene pool and in the wild crop relatives. For instance, wild *D. carota* and carrot landraces subspecies might be a source for increased tolerance to salinity (Kasiri et al. 2013). Kiełkowska et al. (2019) showed that increased tolerance to salinity in some Iranian landraces and their progeny was related to higher anthocyanin accumulation in petioles and increased trichome formation on leaves and petioles. While carrots have been widely cultivated in temperate climatic zones, efforts have been undertaken to breed for varieties that could be cultivated in warmer regions. In Brazil, breeding of carrot cultivars suitable for production in the subtropical climate using well-adapted local landraces of the European origin was successful. The open-pollinated cultivar "Brasilia" and its derivatives constitute the major fraction of carrot production there (Simon et al. 2008). Elucidation of major genetic determinants of adaptation to abiotic stresses and incorporation of molecular tools in breeding would certainly shorten the time required for developing and selecting plant materials showing desired characteristics, which could subsequently be introduced for production in regions suffering from malnutrition and vitamin A deficiency, supporting previous efforts implementing conventional selection methods. Application of molecular techniques (e.g., marker-assisted backcrossing) might also support more efficient transfer of abiotic stress tolerances present in the wild *D. carota* gene pool.

Genetic modifications might be another method of choice, depending on the public acceptance of genetic transformation and novel, more precise techniques of gene editing. Abiotic stresses can be applied postharvest, in order to increase synthesis of valuable biologically active secondary metabolites. Carrot is highly amenable for genome engineering, using both transgenesis (Baranski 2008) and CRISPR/Cas9 genome editing (Klimek-Chodacka et al. 2018; Baranski and Lukasiewicz 2019; Xu et al. 2019). The latter technology has appeared very recently as a new possibility, and has not yet been implemented as a tool to modify the reaction of plants to abiotic stresses. However, genetic transformation has been used to improve carrot tolerance and several reports on the expression of heterologous stress-related genes in carrot have been published. Transgenic carrot plants carrying a gene coding for betaine aldehyde dehydrogenase (BADH) showed highly increased betaine content and significantly improved tolerance to salt stress (Kumar et al. 2004). Carrot transformation with mammalian 6-phosphofructo-2-kinase/fructose2,6-bisphosphatase (6-PF-2-K/Fru2,6-P2ase) gene resulted in highly increased levels of fructose 2,6-bisphosphate (Fru-2,6-P2) in roots of the transgenic plants. Under drought and cold stress, it allowed the mobilization of energy reserves by gluconeogenesis (Kovács et al. 2006). Attempts have been undertaken to use genetic engineering, which allows the introduction of specific genes, closely related to the production of compounds that

give the plant advantage in stress conditions. For this purpose, different approaches have been used: (a) the introduction of genes involved in ion and water uptake or ions transport; (b) genes encoding osmolytes, such as glycine, mannitol, or proline; (c) genes encoding transcription factors like MAPK, DREB1, and others (Parmar et al. 2017).

Among pathogenesis-related protein family PR-5, there are osmotins that have been isolated for the first time from cell cultures of tobacco (Singh et al. 1985). These proteins are usually located in the electron-dense inclusion bodies in the vacuoles. Their synthesis is regulated by various hormonal and environmental factors, including abiotic stress (salinity or desiccation). Osmotins are presumed to protect cell membranes causing membrane permeability during stress, resulting in increased tolerance in transgenic tobacco (Barthakur et al. 2001), wheat (Noori and Sokhansanj 2008), or pepper (Subramanyam et al. 2010). Callus formed from carrot hypocotyl explants was transformed with a truncated tobacco osmotin gene lacking the sequence encoding a 20-aminoacid C-terminal end (Annon et al. 2014). Removal of the C-terminal end fragment results in extracellular secretion of the protein. Transgenic lines with the overexpression of tobacco osmotin conferred tolerance to drought stress in carrot plants and exhibited faster and fuller recovery than control plants after drought treatment. Transformed plants had also higher water content, less ion leakage, lower level of lipid peroxidation, and higher relative water content. Tolerance to drought as desiccation was also the subject of research by Shiota and Kamada (2000). As a result of the research, non-embryogenic carrot cells with a high expression of C-ABI3 gene, a carrot homolog of the VPI/ABI3 gene, were obtained. This enabled tolerance of desiccation upon ABA treatment.

In plants grown in saline soil, an increased accumulation of osmoprotective compounds (glycine betaine (Gly betaine) and β-alanine betaine) is often observed, which allow plant cells to maintain homeostasis. The synthesis of Gly betaine in plants involves choline monooxygenase and BADH, which are localized in chloroplasts. Kumar et al. (2004) performed successful engineering of the carrot chloroplast genome with the vector pDD-*Dc-aadA*/*badh* by homologous recombination in the 16S-23S spacer region. Researchers observed an increase in tolerance to salinity in both cell suspension cultures and plants. Transformed cells were able to survive higher NaCl concentrations. The activity of BADH enzyme was eightfold higher in the presence of 100 mM of NaCl, and 50 times more betaine was accumulated in the transformed cells, as compared to the wild type. Transgenic plants tolerated salinity at 400 mM of NaCl, whereas non-transformed plants exhibited severe growth reduction at 200 mM of NaCl. Also, Han and Hwang (2003) performed genetic transformation of carrot to enhance salt tolerance. Researchers introduced pyrroline-5-carboxylate synthetase (P5CS) gene from moth bean which is a key gene in regulation of proline biosynthesis. Proline is known as an osmoprotectant that is accumulated in large quantities in response to environmental stresses (Ashraf and Foolad 2007). Proline is responsible for the stabilization of sub-cellular structures (e.g., membranes and proteins), scavenging free radicals, and regulating the cellular redox potential. The P5CS gene under control of P35S promoter was transferred to carrot cells via *Agrobacterium* genetic transformation. The transgenic cell lines showed six times increased

relative growth following treatment with 250 mM NaCl, as compared to wild type cells. Also, a significant, up to sixfold, increase of proline content in transgenic cells was observed.

Recent years have brought a new tool that allows even more precise modification of plant DNA: Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated protein-9 nuclease (Cas9). In this system, the Cas9 protein derived from *Streptococcus pyogenes* is engineered to target specific DNA based on Watson–Crick base complementary paring and to create double-stranded breaks. An important role is also played by the, usually 3-nucleotide, protospacer adjacent motif (PAM) located directly at the recognized DNA sequence (Mushtaq et al. 2018). The resulting breaks in the DNA are then repaired by homologous recombination (HDR) or nonhomologous end joining (NHEJ), which is often accompanied by point mutations. The CRISPR/Cas9 system was developed in model plants such as *Arabidopsis* (Jiang et al. 2013; Mao et al. 2013), rice (Feng et al. 2013), and tobacco (Li et al. 2013), but also carrot (Klimek-Chodacka et al. 2018). It has been successfully used for genome editing endogenous genes of many crop plants, mainly causing phenotypic changes such as change in the content of biochemical compounds, development of parthenocarpy, and increase in tolerance to diseases (Mushtaq et al. 2018). CRISPR technology has also been successfully used to obtain plants tolerating abiotic stresses. Osakabe and Osakabe (2017) focused on the *OPEN STOMATA 2* (*OST2*) (*AHA1*) gene encoding a plasma membrane H^+-ATPase in the stomatal response in Arabidopsis. The mutation contributed to faster stomatal closing during abiotic stress, resulting in significantly reduced water loss rates in leaves of engineered plants. CRISPR technology has also been used for editing the maize *ARGOS8* gene, a negative regulator of ethylene responses (Shi et al. 2017). It has already been demonstrated that overexpression of the *ARGOS8* gene resulted in increased grain yield under drought conditions but has no effect on yield under optimal conditions (Shi et al. 2015). The CRISPR-edited variants of the gene also enabled its overexpression and the increase of yield of drought-stressed plants.

Currently, it seems that the CRISPR technology will allow us to achieve significant progress and allow for a significant advantage of plants over abiotic stresses. Plant response to stress factors is very complex, including numerous interactions between signaling, regulatory, and metabolic pathways (Jain 2015). Often, these genes are represented by multi-gene families with functional redundancy, which are also associated with duplications present in the genome. The CRISPR system, thanks to its simplicity, is an ideal tool for simultaneous editing of a number of genes.

6.11 Bioinformatic Tools

The first carrot plastid genome (Ruhlman et al. 2006), several additional plastid and mitochondrial genomes, and two draft nuclear genomes have been published. The two available nuclear genomes include an assembly of 371.6 Mb at CarrotDB corresponding to 32 × coverage (Xu et al. 2014), and an assembly of 421.5 Mb

corresponding to 186 × coverage (Iorizzo et al. 2016). A dedicated, comprehensive bioinformatics platform for carrot and other Apiaceae called CarrotOmics is being developed (Bostan et al. 2019). Transcriptome data, linkage maps based on all marker systems, phenotypic data, and other "omics" data will be included at CarrotOmics.

6.12 Future Perspectives

Carrot production has risen in recent decades with an especially large increase in Asia. With anticipated challenges from heat, drought, and salinity arising from climate change in as soon as the next few decades, combined with much of the newer carrot production being realized in warmer climatic regions of the world, the urgency for dedicating a significant effort to improved abiotic stress tolerance by carrot breeders and other scientists involved in applied agricultural research is critical. The broad range of genetic diversity in carrot germplasm provides a strong foundation for undertaking this important effort, and the growing availability of genome-assisted breeding tools will make that task more efficient. The significant nutritional contribution that carrot can deliver to warm, dry regions of the developing world as a sustainable vitamin A source with a relatively long postharvest storage shelf life provides an additional incentive for developing nutritious CS carrots.

References

Acosta-Motos JR, Ortuño M, Bernal-Vicente A, Diaz-Vivancos P, Sanchez-Blanco M, Hernandez J (2017) Plant responses to salt stress: adaptive mechanisms. Agronomy 7:18. https://doi.org/10.3390/agronomy7010018

AhnY-J, SongN (2012) A cytosolic heat shock protein expressed in carrot (*Daucus carota* L.) enhances cell viability under oxidative and osmotic stress conditions. HortScience47:143–148

Alessandro MS, Galmarini CR (2007) Inheritance of vernalization requirement in carrot. J Amer Soc Hort Sci 132:525–529

Alessandro MS, Galmarini CR, Iorizzo M, Simon PW (2013) Molecular mapping of vernalization requirementand fertility restoration genes in carrot. Theor Appl Genet 126:415–423

Ali A, Matthews WC, Cavagnaro PF, Iorizzo M, Roberts PA, Simon PW (2013) Inheritance and mapping of *Mj-2*, a new source of root-knot nematode (*Meloidogyne javanica*) resistance in carrot. J Hered105:288–291

Algarra M, Fernandes A, Mateus N, de Freitas V, Esteves da Silva JCG, Casado J (2014) Anthocyanin profile and antioxidant capacity of black carrots (*Daucus carota* L. ssp. *sativus* var. *atrorubens* Alef.) from Cuevas Bajas. Spain. J Food Compos Anal 33:71–76. https://doi.org/10.1016/j.jfca.2013.11.005

Allender C (2019) Genetic resources for carrot improvement. In: Simon PW, Iorizzo M, Grzebelus D, Baranski R (eds) The Carrot Genome. Springer, Cham, Switzerland, pp 93–100

Almansouri M, Kinet J-M, Lutts S (2001) Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* desf.). Plant Soil 231:243–254. https://doi.org/10.1023/A:1010378409663

Amirsadeghi S, McDonald A, Vanlerberghe G (2007) A glucocorticoid-inducible gene expression system can cause growth defects in tobacco. Planta 226:453–463. https://doi.org/10.1007/s00425-007-0495-1

Annon A, Rathore K, Crosby K (2014) Overexpression of a tobacco osmotin gene in carrot (*Daucus carota* L.) enhances drought tolerance. Vitro Cell Dev Biol—Plant 50:299–306. https://doi.org/10.1007/s11627-013-9590-0

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55:373–399. https://doi.org/10.1146/annurev.arplant.55.031903.141701

Arango J, Jourdan M, Geoffriau E et al (2014) Carotenehydroxylase activity determines the levels of bothalpha-carotene and total carotenoids in orange carrots. Plant Cell 26:2223–2233

Arscott SA, Tanumihardjo SA (2010) Carrots of many colors provide basic nutrition and bioavailable phytochemicals acting as a functional food. Compr Rev Food Sci Food Saf 9:223–239

Asada K (2006) Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol 141:391. https://doi.org/10.1104/pp.106.082040

Ashraf M, Foolad MR (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Exp Bot 59:206–216. https://doi.org/10.1016/j.envexpbot.2005.12.006

Bach IC, Olesen A, Simon PW (2002) PCR-basedmarkers to differentiate the mitochondrial genome ofpetaloid and male fertile carrot (*Daucus carota* L.). Euphytica 127:353–365

Bado S, Forster B, Ghanim A, Jankowicz-Cieslak J, Berthold J, Luxiang L (2016) Protocols for pre-field screening of mutants for salt tolerance in rice, wheat and barley. Springer Internat Pub

Banasiak Ł, Wojewódzka A, Baczyński J, Reduron J-P, Piwczyński M, Kurzyna-Młynik R, Gutaker R, Czarnocka-Cieciura A, Kosmala-Grzechnik S, Spalik K (2016) Phylogeny of Apiaceae subtribe Daucinae and the taxonomic delineation of its genera. Taxon 65:563–585

Banga O (1957a) Origin of the European cultivated carrot. Euphytica 6:54–63

Banga O (1957b) The development of the original European carrot material. Euphytica 6:64–76

Banga O (1963) Main Types of the Western Carotene Carrot and Their Origin. W.E.J, Tjeenk Willink, Zwolle, The Netherlands

Bano S, Ashraf M, Akram N (2014) Salt stress regulates enzymatic and nonenzymatic antioxidative defense system in the edible part of carrot (*Daucus carota* L.). Plant-Environ Interact 9:324–329. https://doi.org/10.1080/17429145.2013.832426

Baranski R (2008) Genetic transformation of carrot (*Daucus carota*) and other Apiaceae species. Transgen Plant J 2:18–38

Baranski R, Lukasiewicz A (2019) Genetic engineering of carrot. In: Simon PW, Iorizzo M, Grzebelus D, Baranski R (eds) The Carrot Genome. Springer, Cham, Switzerland, pp 149–186

Barnes WC (1936) Effects of some environmental factors on growth and color of carrots. NY Agri Exper Stn Ithaca Memoirs 186:1–36

Barthakur S, Babu V, Bansa KC (2001) Over-expression of osmotin induces proline accumulation and confers tolerance to osmotic stress in transgenic tobacco. J Plant BiochemBiotechnol 10:31–37. https://doi.org/10.1007/bf03263103

Baxter I (2009) Ionomics: studying the social network of mineral nutrients. Curr Opin Plant Biol 12:381–386. https://doi.org/10.1016/j.pbi.2009.05.002

Bernstein L, Ayers A (1953) Salt tolerance of five varieties of carrots. J Amer Soc Hort Sci 61:360–366

Berry J, Bjorkman O (1980) Photosynthetic response and adaptation to temperature in higher plants. Annu Rev 31:491–543. https://doi.org/10.1146/annurev.pp.31.060180.002423

Boiteux LS, Belter JG, Roberts PA, Simon PW (2000) RAPD linkage map of the genomic region encompassing the root-knot nematode (*Meloidogyne javanica*) resistance locus in carrot. Theor Appl Genet 100:439–446

Boiteux LS, Hyman JR, Bach IC, Fonseca MEN, Matthews WC, Roberts PA, Simon PW (2004) Employment of flanking codominant STS markers to estimate allelic substitution effects of a nematode resistance locus in carrot. Euphytica 136:37–44

Bolton A, Nijabat A, Mahmood-ur-Rehman M, Naveed NH, Mannan ATMM, Ali A, Rahim MA, Simon PW (2019) Variation for heat tolerance during seed germination in diverse carrot [*Daucus carota* (L.)] germplasm. HortScience 54:1470–1476. https://doi.org/10.21273/HORTSCI14144-19

Bolton A, Simon P (2019) Variation for salinity tolerance during seed germination in diverse carrot [*Daucus carota* (L.)] germplasm. HortScience 54:38–44. https://doi.org/10.21273/HORTSCI13333-18

Borner T, Linke B, Nothnagel T, Scheike R et al (1995) Inheritance of nuclear and cytoplasmic factors affectingmale sterility in *Daucus carota*. Adv Plant Breed 18:111–122

Bostan H, Senalik D, Simon PW, Iorizzo M (2019) Carrot genetics, omics and breeding toolboxes. In: Simon PW, Iorizzo M, Grzebelus D, Baranski R (eds) The Carrot Genome. Springer, Cham, Switzerland, pp 225–246

Bowen J, Lay-Yee M, Plummer KIM, Ferguson IAN (2002) The heat shock response is involved in thermotolerance in suspension-cultured apple fruit cells. J Plant Physiol 159:599–606 https://doi.org/10.1078/0176-1617-0752

Boyer JS (1982) Plant productivity and environment. Science 218:443–448. https://doi.org/10.1126/science.218.4571.443

Bradeen J, Simon P (1998) Conversion of an AFLPfragment linked to the carrot Y2 locus to a simple, codominant, PCR-based marker form. Theor ApplGenet 97:960–967

Bradeen JM, Simon PW (2007) Carrot. In: Kole C (ed) GenomeMapping and Molecular Breeding in Plants, vol 5. Vegetables. Springer, Heidelberg, pp 161–184

Bradeen JM, Bach IC, Briard M, Le Clerc V, Grzebelus D, Senalik DA Simon PW (2002) Molecular diversity analysis of cultivated carrot (*Daucus carota* L.) and wild *Daucus* populations reveals a genetically nonstructured composition. J Amer Soc Hort Sci 127:383–391

Bray E, Bailey-Serres J, Weretilnyk E (2000) Responses to abiotic stresses. Biochemistry and Molecular Biology of Plants. American Society of Plant Physiologists, Rockville, MD, pp 1158–1249

Broussard MA, Mas F, Howlett B, Pattemore D, Tylianakis JM (2017) Possible mechanisms of pollination failure in hybrid carrot seed and implications for industry in a changing climate. PLoS ONE 12:e0180215. https://doi.org/10.1371/journal.pone.0180215

Budahn H, Barański R, Grzebelus D, Kiełkowska et al(2014) Mapping genes governing flower architectureand pollen development in a double mutant populationof carrot. Front Plant Sci 5:504

Camejo D, Jiménez A, Alarcón JJ, Torres W, María Gómez J, Sevilla F (2006) Changes in photosynthetic parameters and antioxidant activities following heat-shock treatment in tomato plants.Functional Plant Biol 33:177–187 https://doi.org/10.1071/fp05067

Campos MD, Cardoso HG, Linke B, Costa JH, De Melo DF, Justo L, Frederico AMF, Arnholdt-Schmitt B (2009) Differential expression and co-regulation of carrot AOX genes (*Daucus carota*). Physiol Plant 137 doi:10.1111/j.1399-3054.2009.01282.x

Campos MD, Nogales A, Cardoso HG, Kumar SR, Nobre T, Sathishkumar R, Arnholdt-Schmitt B (2016) Stress-induced accumulation of *DcAOX1* and *DcAOX2a* transcripts coincides with critical time point for structural biomass prediction in carrot primary cultures (*Daucus carota* L.). Front Genet 7 https://doi.org/10.3389/fgene.2016.00001

Carillo P, Annunziata M, Pontecorvo G, Fuggi A, Woodrow P (2011) Salinity stress and salt tolerance. In: Shanker A, Venkateswaralu B (eds)Abiotic Stress in Plants—Mechanisms and Adaptations, 1st edn. INTECH, pp 21–38

Castaneda-Alvarez NP, Khoury CK, Achicanoy HA, Bernau V, Dempewolf H, Eastwood RJ, Guarino L, Harker RH, Jarvis A, Maxted N, Muller JV, Ramirez-Villegas J, Sosa CC, Struik PC, Vincent H, Toll J (2016) Global conservation priorities for crop wild relatives. Nat Plants 2:16022

Cavagnaro P, Iorizzo M (2019) Carrot anthocyanin diversity, genetics and genomics. In: Simon PW, Iorizzo M, Grzebelus D, Baranski R (eds) The Carrot Genome. Springer, Cham, Switzerland, pp 261–278

Cavagnaro PF, Iorizzo M, Yildiz M, Senalik D, Parsons J, Ellison S, Simon PW (2014) A gene-derived SNP-based high resolution linkage map of carrot including the location of QTL conditioning root and leaf anthocyanin pigmentation. BMC Genom 15:1118

Choudhary R, Saroha A, Swarnkar P (2012) Effect of abscisic acid and hydrogen peroxide on antioxidant enzymes in *Syzygium cumini* plant. J Food Sci Technol 49:649–652. https://doi.org/10.1007/s13197-011-0464-3

Collier R, Finch S (2009) A review of research to address carrot fly (*Psila rosae*) control in the UK. EPPO Bull 39:121–127

Costa JH, Cardoso HG, Campos MD, Zavattieri A, Frederico AM, Fernandes de Melo D, Arnholdt-Schmitt B (2009) *Daucus carota* L.—An old model for cell reprogramming gains new importance through a novel expansion pattern of alternative oxidase (*AOX*) genes. Plant Physiol Biochem 47:753–759. https://doi.org/10.1016/j.plaphy.2009.03.011%5bCorrigendum to "*Daucus carota*L.- an old model for cell reprogramming gains new importance through a novel expansion patternof alternative oxidase (*AOX*) genes. Plant Physiol Biochem 85:114." doi:https://doi.org/10.1016/j.plaphy.2014.11.013]

Davenport R, James RA, Zakrisson-Plogander A, Tester M, Munns R (2005) Control of sodium transport in Durum wheat. Plant Physiol 137:807. https://doi.org/10.1104/pp.104.057307

DeRose-Wilson L, Gaut B (2011) Mapping salinity tolerance during *Arabidopsis thaliana* germination and seedling growth. PLoS One 6 doi:10.1371/journal.pone.0022832

Di Mascio P, Kaiser S, Sies H (1989) Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Arch Biochem Biophys 274:532–538. https://doi.org/10.1016/0003-9861(89)90467-0

du Toit LJ, Le Clerc V, Briard M (2019) Genetics and genomics of carrot biotic stress. In: Simon PW, Iorizzo M, Grzebelus D, Baranski R (eds) The Carrot Genome. Springer, Cham, Switzerland, pp 317–362

Duke JA (1992) Handbook of Phytochemical Constituents of GRAS Herbs and Other Economic Plants. CRC Press, Boca Raton, FL

Ellison S, Senalik D, Bostan H, Iorizzo M, Simon P (2017) Fine mapping, transcriptome analysis, andmarker development for *Y2*, the gene that conditionsbeta-carotene accumulation in carrot (*Daucus carota*L.). G3: Genes Genom Genet7:2665–2675. https://doi.org/10.1534/g3.117.043067

Ellison S, Luby C, Corak K, Coe K et al (2018) Association analysis reveals the importance of the *Or* gene in carrot (*Daucus carota* L.) carotenoid presence and domestication. Genetics 210:1–12

Eryılmaz F (2006) The relationships between salt stress and anthocyanin content in higher plants. Biotechnol & Biotechnol Equip 20:47–52. https://doi.org/10.1080/13102818.2006.10817303

Essemine J, Ammar S, Bouzid S (2010) Impact of heat stress on germination and growth in higher plants: physiological, biochemical and molecular repercussions and mechanisms of defence. J Biol Sci 10:565–572. https://doi.org/10.3923/jbs.2010.565.572

Fahad S, Bajwa AA, Nazir U, Anjum SA, Farooq A, Zohaib A, Sadia S, Nasim W, Adkins S, Saud S, Ihsan MZ, Alharby H, Wu C, Wang D, Huang J (2017) Crop production under drought and heat stress: plant responses and management options. Front Plant Sci 8:1147. https://doi.org/10.3389/fpls.2017.01147

FAO (2019) www.fao.org/statistics

Feller U, Crafts-Brandner SJ, Salvucci ME (1998) Moderately high temperatures inhibit ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activase-mediated activation of Rubisco. Plant Physiol 116:539. https://doi.org/10.1104/pp.116.2.539

Feng Z, Zhang B, Ding W, Liu X, Yang D-L, Wei P, Cao F, Zhu S, Zhang F, Mao Y, Zhu JK (2013) Efficient genome editing in plants using a CRISPR/Cas system. Cell Res 23:1229–1232. https://doi.org/10.1038/cr.2013.114

Flowers TJ (2004) Improving crop salt tolerance. J Exp Bot 55:307–319. https://doi.org/10.1093/jxb/erh003

Freeman RE, Simon PW (1983) Evidence for simple genetic control of sugar type in carrot (*Daucus carota* L.). J Amer Soc Hort Sci 108:50–54

Fuentes P, Pizarro L, Moreno JC, Handford M, Rodriguez-Concepcion M, Stange C (2012) Light-dependent changes in plastid differentiation influence carotenoid gene expression and accumulation in carrot roots. Plant Mol Biol 79:47–59. https://doi.org/10.1007/s11103-012-9893-2

Garciarrubio A, Legaria J, Covarrubias A (1997) Abscisic acid inhibits germination of mature *Arabidopsis* seeds by limiting the availability of energy and nutrients. Planta 203:182–187. https://doi.org/10.1007/s004250050180

Gibberd MR, Turner NC, Storey R (2002) Influence of saline irrigation on growth, ion accumulation and partitioning, and leaf gas exchange of carrot (*Daucus carota* L.). Ann Bot 90:715–724. https://doi.org/10.1093/aob/mcf253

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930. https://doi.org/10.1016/j.plaphy.2010.08.016

Gong M, Chen S, Song Y, Li Z (1997) Effect of calcium and calmodulin on intrinsic heat tolerance in relation to antioxidant systems in maize seedlings. Aust J Plant Physiol 24:371–379. https://doi.org/10.1071/PP96118

Graßmann J (2005) Terpenoids as Plant Antioxidants. In: Litwack G (ed) Vitamins andHormones. Academic Press, New Yorkpp, pp 505–535

Greer D, Weedon M (2012) Modelling photosynthetic responses to temperature of grapevine (*Vitis vinifera* cv. 'Semillon') leaves on vines grown in a hot climate. Plant, Cell Environ 35:1050–1064. https://doi.org/10.1111/j.1365-3040.2011.02471.x

Gregorio G, Senadhira D, Mendoza R (1997) Screening Rice for Salinity Tolerance. IRRI, Manila, Philippines

Groves RL, Clements JR, Bradford BZ (2019) Carrot diseases resulting from phytoplasmas and viruses. In: Geoffriau E, Simon PW (eds) Carrot and Other Cultivated Apiaceae, CABI, Oxford, UK, in press

Grzebelus D (2019) Genetics and genomics of carrot abiotic stress. In: Simon PW, Iorizzo M, Grzebelus D, Baranski R (eds) The Carrot Genome. Springer, Switzerland, pp 363–372

Guajardo E, Correa J, Contreras-Porcia L (2016) Role of abscisic acid (ABA) in activating antioxidant tolerance responses to desiccation stress in intertidal seaweed species. Planta 243:767–781. https://doi.org/10.1007/s00425-015-2438-6

Han KH, Hwang CH (2003) Salt tolerance enhanced by transformation of a P5CS gene in carrot. J Plant Biotechnol 5:157–161

Hasanuzzaman M, Nahar K, Alam MM, Roychowdhury R, Fujita M (2013) Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. Intl J Mol Sci 14:9643–9684. https://doi.org/10.3390/ijms14059643

Hooper PL, Hooper PL, Tytell M, Vígh L (2010) Xenohormesis: health benefits from an eon of plant stress response evolution. Cell Stress Chaperones 15:761–770. https://doi.org/10.1007/s12192-010-0206-x

Huang B, Xu C (2008) Identification and characterization of proteins associated with plant tolerance to heat stress. J Integr Plant Biol 50:1230–1237. https://doi.org/10.1111/j.1744-7909.2008.00735.x

Huang Y, Li M-Y, Wang F, Xu Z-S, Huang W, Wang G-L, Ma J, Xiong A-S (2015) Heat shock factors in carrot: genome-wide identification, classification, and expression profiles response to abiotic stress. Mol Biol Rep 42:893–905. https://doi.org/10.1007/s11033-014-3826-x

Hussain HA, Hussain S, Khaliq A, Ashraf U, Anjum SA, Men S, Wang L (2018) Chilling and drought stresses in crop plants: implications, cross talk, and potential management opportunities. Front Plant Sci 9:393. https://doi.org/10.3389/fpls.2018.00393

Iorizzo M, Senalik DA, Ellison SL, Grzebelus D, Cavagnaro PF, Allender C, Brunet J, Spooner DM, Van Deynze A, Simon PW (2013) Genetic structure and domestication of carrot (*Daucus carota* L. subsp. *sativus* L.) (Apiaceae). Amer J Bot 100:930–938

Iorizzo M, Ellison S, Senalik D, Zeng P, Satapoomin P, Huang J, Bowman M, Iovene M, Sanseverino W, Cavagnaro P, Yildiz M, Macko-Podgórni A, Moranska E, Grzebelus E, Grzebelus D, Ashrafi

H, Zheng Z, Cheng S, Spooner D, Van Deynze A, Simon P (2016) A high-quality carrot genome assembly provides new insights into carotenoid accumulation and asterid genome evolution. Nat Genet 48:657–666. https://doi.org/10.1038/ng.3565

Iorizzo M, Ellison S, Pottorff M, Cavagnaro P (2019a) Carrot molecular genetics and mapping. In: Simon PW, Iorizzo M, Grzebelus D, Baranski R (eds) The Carrot Genome. Springer Nature, Cham, Switzerland, pp 101–118

Iorizzo M, Cavagnaro P, Bostan A, Zhao Y, Zhang J, Simon PW (2019b) A cluster of MYB transcription factors regulates anthocyanin biosynthesis in carrot (*Daucus carota* L.) root and petiole. Front Plant Sci 9:1927

Jain M (2015) Function genomics of abiotic stress tolerance in plants: a CRISPR approach. Front Plant Sci 6. https://doi.org/10.3389/fpls.2015.00375

Jiang W, Zhou H, Bi H, Fromm M, Yang B, Weeks DP (2013) Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in *Arabidopsis*, tobacco, sorghum and rice Nucl Acids Res 41:e188–e188. https://doi.org/10.1093/nar/gkt780

Jourdan M, Gagne S, Dubois-Laurent C et al (2015) Carotenoid content and root color of cultivated carrot: a candidate-gene association study using an originalbroad unstructured population. PLoS ONE 10:e0116674

Jung YC, Lee HJ, Yum SS, Soh WY, Cho DY, Auh CK, Lee TK, Soh HC, Kim YS, Lee SC (2005) Drought-inducible—but ABA-independent—thaumatin-like protein from carrot (*Daucus carota* L.). Plant Cell Rep 24:366–373

Just BJ, Santos CA, Yandell BS, Simon PW (2009) Major QTL for carrot color are positionally associatedwith carotenoid biosynthetic genes and interactepistatically in a domesticated x wild carrot cross.Theor Appl Genet 119:1155–1169

Kahouli B, Borgi Z, Hannachi C (2014) Effect of sodium chloride on the germination of the seeds of a collection of carrot accessions (*Daucus carota* L.) cultivated in the region of Sidi Bouzid. J Stress Physiol Biochem 10:28–36

Kasiri MR, Hassandokht MR, Kashi A, Shahi-Gharahlar A (2013) Evaluation of genetic diversity in Iranian yellow carrot accessions (*Daucus carota* var. *sativus*), an exposed to extinction rooty vegetable, using morphological characters. Intl J Agri Crop Sci 6:151–156

Keilwagen J, Lehnert H, Berner T, Budahn H, Nothnagel T, Ulrich D, Dunemann F (2017) The terpene synthase gene family of carrot (*Daucus carota* L.): Identification of QTLs and candidate genes associated with terpenoid volatile compounds. Front Plant Sci 8:1930. https://doi.org/10.3389/fpls.2017.01930

Kiełkowska A, Grzebelus E, Lis-Krzyścin A, Maćkowska K (2019) In vitro selection in protoplast cultures of the carrot (*Daucus carota* L.) and evaluation of the response of regenerants to soil salinity. Plant Cell Tiss Organ Cult 137:379–395

Klimek-Chodacka M, Oleszkiewicz T, Lowder LG, Qi Y, Baranski R (2018) Efficient CRISPR/Cas9-based genome editing in carrot cells. Plant Cell Rep 37:575–586. https://doi.org/10.1007/s00299-018-2252-2

Kovács G, Sorvari S, Scott P, Toldi O (2006) Pyrophosphate: fructose 6-phosphate 1-phosphotransferase operates in net gluconeogenic direction in taproots of cold and drought stressed carrot plants. Acta Biol 50:25–30. https://doi.org/10.1556/AAgr.55.2007.1.8

Kovinich N, Kayanja G, Chanoca A, Otegui M, Grotewold E (2015) Abiotic stresses induce different localizations of anthocyanins in *Arabidopsis*. Plant Signal Behav 10. https://doi.org/10.1080/15592324.2015.1027850

Krishnamurthy A, Rathinasabapathi B (2013) Oxidative stress tolerance in plants: novel interplay between auxin and reactive oxygen species signaling. Plant Signal Behav 8. https://doi.org/10.4161/psb.25761

Kumar S, Dhingra A, Daniell H (2004) Plastid-expressed betaine aldehyde dehydrogenase gene in carrot cultured cells, roots, and leaves confers enhanced salt tolerance. Plant Physiol 136:2843–2854. https://doi.org/10.1104/pp.104.045187

Landi M, Tattini M, Gould KS (2015) Multiple functional roles of anthocyanins in plant-environment interactions. Funct Roles Second Metab Plant-Environ Interact 119:4–17. https://doi.org/10.1016/j.envexpbot.2015.05.012

Landjeva S, Neumann K, Lohwasser U, Börner A (2008) Molecular mapping of genomic regions associated with wheat seedling growth under osmotic stress. Biol Plant 52:259–266. https://doi.org/10.1007/s10535-008-0056-x

Larkindale J, Knight MR (2002) Protection against heat stress-induced oxidative damage in *Arabidopsis* involves calcium, abscisic acid, ethylene, and salicylic acid. Plant Physiol 128:682. https://doi.org/10.1104/pp.010320

Laufer B (1919) Sino-Iranica. Chicago, Field Museum of Natural Hist. Pub. 201; Anthropot Ser 15:451–454

Le Clerc V, Briard M (2019) Carrot disease management. In: Geoffriau E, Simon PW (eds) Carrot and other cultivated Apiaceae, CABI, Oxford, UK, in press

Le Clerc V, Pawelec A, Birolleau-Touchard C, Suel A, Briard M (2009) Genetic architecture of factors underlying partial resistance to Alternaria leaf blight in carrot. Theor Appl Genet 118:1251–1259

Le Clerc V, Marques S, Suel A, Huet S, Hamama L, Voisine L, Auperpin E, Jourdan M, Barrot L, Prieur R (2015) QTL mapping of carrot resistance to leaf blight with connected populations: stability across years and consequences for breeding. Theor Appl Genet 128:2177–2187

Li J-F, Norville JE, Aach J, McCormack M, Zhang D, Bush J, Church GM, Sheen J (2013) Multiplex and homologous recombination–mediated genome editing in *Arabidopsis* and *Nicotianabenthamiana* using guide RNA and Cas9. Nat Biotechnol 31:688–691. https://doi.org/10.1038/nbt.2654

Linke B, Alessandro MS, Galmarini C, Nothnagel T (2019) Carrot floral development and reproductive biology. In: Simon PW, Iorizzo M, Grzebelus D, Baranski R (eds) The Carrot Genome. Springer, Cham, Switzerland, pp 27–58

Maas E, Hoffman G (1977) Crop salt tolerance—current assessment. J Irrig Drain Div 103:115–134

Mackevic VI (1929) The carrot of Afghanistan. Bul Appl Bot Genet Plant Breeding 20:517–562

Macko-Podgórni A, Machaj G, Stelmach K, Senalik D et al (2017) Characterization of a genomic region under selection in cultivated carrot (*Daucus carota* subsp. *sativus*) reveals a candidate domestication gene. Front Plant Sci 8:12

Maestri E, Klueva N, Perrotta C, Gulli M, Nguyen H, Marmiroli N (2002) Molecular genetics of heat tolerance and heat shock proteins in cereals. Plant Mol Biol 48:667–681. https://doi.org/10.1023/A:1014826730024

Malik MK, Slovin JP, Hwang CH, Zimmerman JL (1999) Modified expression of a carrot small heat shock protein gene *Hsp17.7*, results in increased or decreased thermotolerance. Plant J 20:89–99

Mao Y, Zhang H, Xu N, Zhang B, Gou F, Zhu J-K (2013) Application of the CRISPR–Cas system for efficient genome engineering in plants. Mol Plant 6:2008–2011. https://doi.org/10.1093/mp/sst121

Mittler R, Blumwald E (2010) Genetic engineering for modern agriculture: challenges and perspectives. Annu Rev Plant Biol 61:443–462. https://doi.org/10.1146/annurev-arplant-042809-112116

Møller I, Jensen P, Hansson A (2007) Oxidative modifications to cellular components in plants. Annu Rev Plant Biol 58:459–481. https://doi.org/10.1146/annurev.arplant.58.032806.103946

Morales D, Rodríguez P, Dell'Amico J, Nicolás E, Torrecillas A, Sánchez-Blanco M (2003) High-temperature preconditioning and thermal shock imposition affects water relations, gas exchange and root hydraulic conductivity in tomato. Biol Plant 47:203. https://doi.org/10.1023/B:BIOP.0000022252.70836.fc

Munns R (2005) Genes and salt tolerance: bringing them together. New Phytol 167:645–663. https://doi.org/10.1111/j.1469-8137.2005.01487.x

Munns R, Schachtman D, Condon A (1995) The significance of a two-phase growth response to salinity in wheat and barley. Aust J Plant Physiol 22:561–569. https://doi.org/10.1071/PP9950561

Munns R, Rawson H (1999) Effect of salinity on salt accumulation and reproductive development in the apical meristem of wheat and barley. Funct Plant Biol 26:459–464. https://doi.org/10.1071/PP99049

Munns R, James R (2003) Screening methods for salinity tolerance: a case study with tetraploid wheat. Plant Soil 253:201–218. https://doi.org/10.1023/A:1024553303144

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911

Mushtaq M, Bhat JA, Mir ZA, Sakina A, Ali S, Singh AK, Tyagi A, Salgotra RK, Dar AA, Bhat R (2018) CRISPR/Cas approach: A new way of looking at plant-abiotic interactions. J Plant Physiol 224–225:156–162. https://doi.org/10.1016/j.jplph.2018.04.001

Nakajima Y, Yamamoto T, Muranaka T, Oeda K (1999) Genetic variation of petaloid male-sterile cytoplasm ofcarrots revealed by sequence-tagged sites (STSs). Theor Appl Genet 99:837–843

Nascimento WM, Vieira JV, Silva GO, Reitsma KR, Cantliffe DJ (2008) Carrot seed germination at high temperature: effect of genotype and association with ethylene production. HortScience 43:1538–1543

Neta-Sharir I, Isaacson T, Lurie S, Weiss D (2005) Dual role for tomato heat shock protein 21: protecting photosystem II from oxidative stress and promoting color changes during fruit maturation. Plant Cell 17:1829. https://doi.org/10.1105/tpc.105.031914

Nissinen AI, Lemmetty A, Pihlava J-M, Jauhiainen L, Munyaneza JE, Vanhala P (2012) Effects of carrot psyllid (*Trioza apicalis*) feeding on carrot yield and content of sugars and phenolic compounds. Ann Appl Biol 161:68–80

Noble C, Rogers M (1992) Arguments for the use of physiological criteria for improving the salt tolerance in crops. Plant Soil 146:99–107. https://doi.org/10.1007/BF00012001

Nogales A, Nobre T, Cardoso HG, Muñoz-Sanhueza L, Valadas V, Campos MD, Arnholdt-Schmitt B (2016) Allelic variation on *DcAOX1* gene in carrot (*Daucus carota* L.): An interesting simple sequence repeat in a highly variable intron. Plant Gene 5:49–55. https://doi.org/10.1016/j.plgene.2015.11.001

Noori SAS, Sokhansanj A (2008) Wheat plants containing an osmotin gene show enhanced ability to produce roots at high NaCl concentration. Russ J Plant Physiol 55:256–258. https://doi.org/10.1134/s1021443708020143

Osakabe Y, Osakabe K (2017) Genome editing to improve abiotic stress responses in plants. Prog in Mol Biol and Transl Sci, 99–109 doi:10.1016/bs.pmbts.2017.03.007

Pachauri R, Reisinger A (2007) Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland

Park H, Ko E, Jang E, Park S, Lee J, Ahn Y-J (2013) Expression of *DcHsp17.7*, a small heat shock protein gene in carrot (*Daucus carota* L.) Hort Environ Biotechnol 54:121–127. https://doi.org/10.1016/j.nbt.2011.04.002

Parmar N, Singh KH, Sharma D, Singh L, Kumar P, Nanjundan J, Khan YJ, Chauhan DK, Thakur AK (2017) Genetic engineering strategies for biotic and abiotic stress tolerance and quality enhancement in horticultural crops: a comprehensive review. 3Biotechnol 7:239. https://doi.org/10.1007/s13205-017-0870-y

Palta J, Simon G (2004) Developing and successfully implementing a strategy for breeding frost-hardy carrots. HortScience 39:880

Parsons J, Matthews W, Iorizzo M et al (2015) *Meloidogyne incognita* nematode resistance QTL in carrot. Mol Breed 35:114

Peterson CE, Simon PW (1986) Carrot breeding. In: Vegetable Breeding (ed) Bassett MJ. Crops.AVI, Westport, CN, pp 321–356

Radić V, Beatović D, Mrđa J (2007) Salt tolerance of corn genotypes (*Zea mays* L.) during germination and later growth. J Agri Sci 52:115–120. https://doi.org/10.2298/JAS0702115R

Richards RA, Dennett CW, Qualset CO, Epstein E, Norlyn JD, Winslow MD (1987) Variation in yield of grain and biomass in wheat, barley, and triticale in a salt-affected field. Field Crops Res 15:277–287. https://doi.org/10.1016/0378-4290(87)90017-7

Rockström J, Falkenmark M (2000) Semiarid crop production from a hydrological perspective: gap between potential and actual yields. Crit Rev Plant Sci 19:319–346. https://doi.org/10.1080/07352680091139259

Rodriguez R, Redman R (2005) Balancing the generation and elimination of reactive oxygen species. Proc Natl Acad Sci USA 102:3175. https://doi.org/10.1073/pnas.0500367102

Ruhlman T, Lee S-B, Jansen RK, Hostetler JB, Tallon LJ, Town CD, Daniell H (2006) Complete plastid genome sequence of *Daucus carota*: Implications for biotechnology and phylogeny of angiosperms. BMC Genom 7:222

Sakamoto A, Murata N (2002) The role of glycine betaine in the protection of plants from stress: clues from transgenic plants. Plant, Cell Environ 25:163–171. https://doi.org/10.1046/j.0016-8025.2001.00790.x

Santos CAF, Simon PW (2002) QTL analyses reveal clustered loci for accumulation of major provitamin A carotenes and lycopene in carrot roots. Mol Genet Genom 268:122–129

Savicka M, Shkute N (2010) Effects of high temperature on malondialdehyde content, superoxide production and growth changes in wheat seedlings (*Triticum aestivum* L.). Ekologija 56: https://doi.org/10.2478/v10055-010-0004-x

Schmidhalter U, Oertli J (1991) Transpiration/biomass ratio for carrots as affected by salinity, nutrient supply and soil aeration. Plant Soil 135:125–132

Schöffl F, Prändl R, Reindl A (1999) Molecular responses to heat stress. In: Shinozaki K, Yamaguchi-Shinozaki K (eds) Molecular Responses to Cold, Drought, Heat and Salt Stress in Higher Plants. Landes Co, Austin, TX, pp 81–98

Schulz B, Westphal L, Wricke G (1994) Linkage groupsof isozymes, RFLP and RAPD markers in carrot(*Daucus carota* L. *sativus*). Euphytica 74:67–76

Shahid M, Balal R, Pervez M, Abbas T, Ashfaq M, Ghazanfar U, Afzal M, Rashid A, Garcia-Sanchez F, Mattson N (2012) Differential response of pea (*Pisum sativum* L.) genotypes to salt stress in relation to the growth, physiological attributes antioxidant activity and organic solutes. Aust J Crop Sci 6:828–838

Shannon M (1985) Principles and strategies in breeding for higher salt tolerance. Plant Soil 89:227–241. https://doi.org/10.1007/BF02182244

Shi J, Gao H, Wang H, Lafitte HR, Archibald RL, Yang M, Hakimi SM, Mo H, Habben JE (2017) ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. Plant Biotechnol J 15:207–216. https://doi.org/10.1111/pbi.12603

Shi J, Habben JE, Archibald RL, Drummond BJ, Chamberlin MA, Williams RW, Lafitte HR, Weers BP (2015) Overexpression of ARGOS genes modifies plant sensitivity to ethylene, leading to improved drought tolerance in both arabidopsis and maize. Plant Physiol 169:266–282. https://doi.org/10.1104/pp.15.00780

Shinozaki K, Yamaguchi-Shinozaki K (2006) Gene networks involved in drought stress response and tolerance. J Exp Bot 58:221–227. https://doi.org/10.1093/jxb/erl164

Shiota H, Kamada H (2000) Acquisition of desiccation tolerance by cultured carrot cells upon ectopic expression of C-ABI3, a carrot homolog of ABI3. J Plant Physiol 156:510–515. https://doi.org/10.1016/s0176-1617(00)80166-2

Shomer-Ilan A, Jones G, Paleg L (1991) In vitro thermal and salt stability of pyruvate kinase are increased by proline analogues and trigonelline. Funct Plant Biol 18:279–286. https://doi.org/10.1071/PP9910279

Shu S, Gao P, Li L, Yuan Y, Sun J, Guo S (2016) Abscisic acid-induced H_2O_2 accumulation enhances antioxidant capacity in pumpkin-grafted cucumber leaves under $Ca(NO_3)_2$ stress. Front Plant Sci 7:1489. https://doi.org/10.3389/fpls.2016.01489

Sies H, Stahl W (1995) Vitamins E and C, beta-carotene, and other carotenoids as antioxidants. Am J Clin Nutr 62:1315S–1321S. https://doi.org/10.1093/ajcn/62.6.1315S

Simon PW (2000) Domestication, historical development, and modern breeding of carrot. Plant Breed Rev 19:157–190

Simon PW (2019) Economic and academic importance. In: Simon PW, Iorizzo M, Grzebelus D, Baranski R (eds) The Carrot Genome. Springer, Cham, Switzerland, pp 1–8

Simon PW, Goldman IL (2007) Carrot. In: Singh RJ (ed) Genetic Resources, chromosome Engineering, and Crop Improvement Series, vol 3. CRC Press. Boca Raton, FL, pp 497–517
Simon PW, Grzebelus D (2019) Carrot genetics and breeding. In: Geoffriau E, Simon PW (eds) Carrot and other cultivated Apiaceae, CABI, Oxford, UK, in press
Simon PW, Matthews WC, Roberts PA (2000) Evidence for simply inherited dominant resistance to *Meloidogyne javanica* in carrot. Theor Appl Genet 100:735–742
Simon PW, Freeman RE, Vieira JV, Boiteux LS, Briard M, Nothnagel T, Michalik B, Kwon YS (2008) Carrot. In: Prohens J, Nuez F (eds) Handbook of Plant Breeding: Vegetables II: Fabaceae, Liliaceae, Solanaceae, and Umbelliferae. Springer, New York, pp 327–357
Simon PW, Geoffriau E, Ellison S, Iorizzo M (2019) Carrot carotenoid genetics and genomics. In: Simon PW, Iorizzo M, Grzebelus D, Baranski R (eds) The Carrot Genome. Springer, Cham, Switzerland, pp 247–260
Simpson K, Fuentes P, Quiroz-Iturra LF, Flores-Ortiz C, Contreras R, Handford M, Stange C (2018) Unraveling the induction of phytoene synthase 2 expression by salt stress and abscisic acid in *Daucus carota*. J Exp Bot 69:4113–4126. https://doi.org/10.1093/jxb/ery207
Singh NK, Handa AK, Hasegawa PM, Bressan RA (1985) Proteins associated with adaptation of cultured tobacco cells to NaCl. Plant Physiol 79:126–137. https://doi.org/10.1104/pp.79.1.126
Singsaas EL, Lerdau M, Winter K, Sharkey TD (1997) Isoprene increases thermotolerance of isoprene-emitting species. Plant Physiol 115:1413. https://doi.org/10.1104/pp.115.4.1413
Sorensen JN, Jorgensen U, Kuhn BF (1997) Drought effects on the marketable and nutritional quality of carrots. J Sci Food Agri 74:379–391
Spooner DM (2019) *Daucus*: Taxonomy, phylogeny, distribution. In: Simon PW, Iorizzo M, Grzebelus D, Baranski R (eds) The Carrot Genome. Springer, Switzerland, pp 9–26
Sreenivasulu N, Sopory S, Kishor P (2007) Deciphering the regulatory mechanisms of abiotic stress tolerance in plants by genomic approaches. Gene 388:1–13. https://doi.org/10.1016/j.gene.2006.10.009
Stahl W, Sies H (2003) Antioxidant activity of carotenoids. Fat Soluble Vitam Old Mol Nov Prop 24:345–351. https://doi.org/10.1016/S0098-2997(03)00030-X
Stetler RA, Gan Y, Zhang W, Liou AK, Gao Y, Cao G, Chen J (2010) Heat shock proteins: cellular and molecular mechanisms in the central nervous system. Prog Neurobiol 92:184–211. https://doi.org/10.1016/j.pneurobio.2010.05.002
Subramanyam K, Sailaja KV, Subramanyam K, Muralidhara Rao D, Lakshmidevi K (2010) Ectopic expression of an osmotin gene leads to enhanced salt tolerance in transgenic chilli pepper (*Capsicum annum* L.). Plant Cell Tiss Org Cult105(2), 181–192. https://doi.org/10.1007/s11240-010-9850-1
Tan W, Meng Q wei, Brestic M, Olsovska K, Yang X (2011) Photosynthesis is improved by exogenous calcium in heat-stressed tobacco plants. J Plant Physiol 168:2063–2071. https://doi.org/10.1016/j.jplph.2011.06.009
Tester M, Davenport R (2003) Na^+ tolerance and Na^+ transport in higher plants. Ann Bot 91:503–527. https://doi.org/10.1093/aob/mcg058
Turner S, Ellison S, Senalik DA, Simon PW et al (2018) An automated, high-throughput image analysis pipeline enables genetic studies of shoot and root morphology in carrot (*Daucus carota* L.). Front Plant Sci 9:1703
Turner SD, Maurizio PL, Valdar W, Yandell BS, Simon PW (2017) Dissecting the genetic architecture of shoot growth in carrot (*Daucus carota* L.) using a diallel mating design. Genes Genom Genet 8:411–426
Vieira JV, Della Vecchia P, Ikuta H (1983) Cenoura 'Brasilia'. Hort Bras 1:42
Vivek BS, Simon PW (1999) Linkage relationshipsamong molecular markers and storage root traits ofcarrot (*Daucus carota L.* ssp. *sativus*). Theor ApplGenet 99:58–64
Wahid A, Gelani S, Ashraf M, Foolad MR (2007) Heat tolerance in plants: an overview. Environ Exp Bot 61:199–223. https://doi.org/10.1016/j.envexpbot.2007.05.011

Wang H, Ou C-G, Zhuang F-Y, Ma Z-G (2014) The dual role of phytoene synthase genes in carotenogenesis in carrot roots and leaves. Mol Breed 34:2065–2079. https://doi.org/10.1007/s11032-014-0163-7

Westphal L, Wricke G (1991) Genetic and linkage analysis of isozyme loci in *Daucus carota* L. Euphytica 56:259–267

Wohlfeiler J, Alessandro MS, Galmarini CR (2019) Multiallelic digenic control of vernalization requirement in carrot (*Daucus carota* L.). Euphytica 215:1–10

Xu Z-S, Tan HW, Wang F, Hou XL, Xiong AS (2014) CarrotDB: a genomic and transcriptomic database for carrot. Database (Oxford) 2014 doi:10.1093/database/bau096

Xu Z-S, Feng K, Xiong A-S (2019) CRISPR/Cas9-mediated multiply targeted mutagenesis in orange and purple carrot plants. Mol Biotechnol 61:191–199

Yau Y, Simon PW (2003) A 2.5-kb insert eliminates acid soluble invertase isozyme II transcript in carrot (*Daucus carota* L.) roots, causing high sucrose accumulation. Plant Mol Biol 53:151–162

Yau YY, Santos K, Simon PW (2005) Molecular tagging and selection for sugar type in carrot roots with codominant, PCR-based markers. Mol Breed 16:1–10

Yeo A, Flowers T (1986) Salinity resistance in rice (*Oryza sativa* L.) and a pyramiding approach to breeding varieties for saline soils. Aust J Plant Physiol 13:161–173. https://doi.org/10.1071/PP9860161

Yildiz M, Willis DK, Cavagnaro PF, Iorizzo M, Abak K, Simon PW (2013) Expression and mapping of anthocyaninbiosynthesis genes in carrot. Theor Appl Genet 126:1689–1702

Yoshida Y, Marubodee R, Ogiso-Tanaka E, Iseki K, Isemura T, Takahashi Y, Muto C, Naito K, Kaga A, Okuno K, Ehara H, Tomooka N (2016) Salt tolerance in wild relatives of adzuki bean, *Vigna angularis* (Willd.) Ohwi et Ohashi. Genet Resour Crop Evol 63:627–637. https://doi.org/10.1007/s10722-015-0272-0