ARTICLE

Genetic and phenological variation of tocochromanol (vitamin E) content in wild (*Daucus carota* L. var. *carota*) and domesticated carrot (*D. carota* L. var. *sativa*)

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Carrot roots (*Daucus carota* L. var. *sativa*) produce tocochromanol compounds, collectively known as vitamin E. However, little is known about their types and amounts. Here we determined the range and variation in types and amounts of tocochromanols in a variety of cultivated carrot accessions throughout carrot postharvest storage and reproductive stages and in wild-type roots (*Daucus carota* L. var. *carota*). Of eight possible tocochromanol compounds, we detected and quantified α-, and the combined peak for β- and γ- forms of tocopherols and tocotrienols. Significant variation in amounts of tocochromanol compounds was observed across accessions and over time. Large increases in α-tocopherol were noted during both reproductive growth and the postharvest stages. The variation of tocochromanols in carrot root tissue provides useful information for future research seeking to understand the role of these compounds in carrot root tissue or to breed varieties with increased levels of these compounds.

INTRODUCTION

Vitamin E is a group of eight lipid soluble compounds (α-, β-, δ- and γ-tocopherols and tocotrienols) that are synthesized exclusively in the plastids of higher plants, algae and some cyanobacteria.[1] Tocopherols and tocotrienols both contain a chromanol head group, but differ in their isoprenoid chain.[1] Tocopherols have a saturated phytyl tail, whereas tocotrienols have a three fold unsaturated isoprenoid tail.[1] Tocochromanols are synthesized by the condensation of homogentisate and phytyl pyrophosphate derived from the shikimate pathway and the non-mevalonate pathway, respectively.[2] While all plants synthesize tocochromanols, their functions are not thoroughly understood. Generally, tocochromanol compounds are thought to protect the plant under stress conditions as antioxidants and by maintaining membrane stability, participating in intracellular signaling, and cyclic electron transport in and around photosystem II.[1] In humans, vitamin E is the major group of lipophilic antioxidants[3] and may play roles in cell membrane stability, modulation of cell signaling, regulation of gene expression and control of cell proliferation.[4] In general, seeds and nuts are recognized as rich sources of vitamin E compounds and some orange, red and leafy green vegetables have been noted to contain vitamin E.[5]

β-carotene, a pro-vitamin A compound, is responsible for the orange color of cultivated carrot roots, oranges and many other fruits and vegetables, and has been well studied.[5,6] Carotenoids and tocochromanols are biosynthetically related as they are both derived from the terpenoid family of molecules and share a common biochemical precursor, geranylgeranyl pyrophosphate.[5] Koch and Goldman[7] found a positive correlation of β-carotene and α-tocopherol compounds in the xylem tissue of carrots. Maeda *et al.*[8] found that mutants of cyanobacterium *Synechocystis* sp. deficient in tocopherol production were also susceptible to the carotenoid synthesis inhibitor, norflurazon, under stress induced by high intensity light and lipid peroxidation. This suggests that carotenoids and tocopherols may play complimentary roles in photooxidative protection in photosynthetic cells.[8] However, despite the potential biochemical and functional relationships between carotenoids and tocopherols, tocochromanol compounds have not been well classified in carrots.[7]

Carrot is a biennial crop that is harvested as a root vegetable after the first season of growth. Although domesticated carrot is the same species as wild carrot, and these plants readily intercross, the roots are distinct. Wild carrot roots are white, woody and have many lateral roots, whereas domesticated carrots have pigmented, smooth, larger and edible roots. Domesticated carrots are well known for high concentrations of pro-vitamin A carotenoids in root tissue, which is the result of human directed selection over the past 400 years. However, carrot breeding over the past 50 years has primarily focused on increasing yields, while breeding for increased nutritional content of crop plants has received less attention.[6]

Cultivated carrots are also known to contain vitamin E (tocochromanol) compounds.[7] However, little is known about the kinds and amounts of tocochromanols accumulated in carrot roots. The presence of α- and γ-tocopherol has been observed in roots of selected carrot cultivars, but other tocochromanol compounds have not been measured or were below detection limits.[5,7,9] Since no known breeding program has targeted selecting for high contents or types of tocochromanol compounds, the variation among germplasm accessions has not been studied in domesticated or wild carrots.

Carrots are often stored for months before consumption. Due to changes in plant metabolism during the storage period, the kinds and amounts of secondary compounds present in the root may change; thus, it is of interest to examine how compounds that are potentially nutritionally significant change over time.[6] Additionally, carrot roots requires a winter vernalization period in order to induce reproductive growth, which may alter the metabolism of the plant and affect accumulation of secondary compounds. Since seed production is important economically, there is interest in

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understanding plant metabolism during the reproductive stage of the crop.

In this study, we employed high-performance liquid chromatography (HPLC) with reverse phase separation and fluorescent detection methods to examine the presence and quantities of α-, β- and γ- tocopherols and tocotrienols in domesticated and wild carrot root to examine the potential for breeding for increased levels of tocochromanols and to understand the role of these compounds in root tissue. The storage and biennial nature of seed production are important characteristics of this crop, and secondary compounds like tocochromanols have not been studied over these periods. Thus, we also determined the content of tocochromanols chronologically, from harvest time in September, over the postharvest storage life and during the reproductive growth phase of carrot plants. The information may help to understand the role these compounds play in root tissue over time.

MATERIALS AND METHODS

Field, harvest and sample preparation

Twelve germplasm accessions including commercial carrot cultivars and breeding lines were grown in muck soil field conditions at Jack's Pride Farms in Randolph, Wisconsin (43.54˚N, 89.00˚W) in the summers of 2011 and 2012. Carrot accessions included: hybrid cultivars (Bastia, Brest) from Bejo seeds; open pollinated cultivars (Danvers, Scarlet Nantes, Wisconsin Dicer Synthetic Population) derived from processing carrot land races; inbred lines from University of Wisconsin-Madison (processing type carrots) and USDA-ARS (imperator type carrots) carrot breeding programs (W77C, W133B, W276B, W279B, USDA B2327B); and two inbred lines with unique traits from the University of Wisconsin-Madison (an unreleased red inbred that accumulates lycopene, and inbred W266D*rprp* containing the *rp* allele for minimal carotenoid accumulation.[10] Carrots were planted in 3.5 m rows with four rows to each 1.3 m bed and 1.2 m alleys between ranges with 3–4 cm between plants. Carrots were planted using a Planet Junior planter (Planet Junior, Tunkhannock, PA, USA) fitted with a cone seeder attachment. Experimental design was a randomized complete block design with four blocks. In 2011, carrots were planted on 27 May and harvested on 1 September. In 2012, carrots were planted 17 May and harvested on 21 August.

For each of the four blocks, 10 roots were randomly sampled for each accession immediately after harvest for analysis of tocochromanol compounds. Roots were washed and the crown was removed. A sample of root approximately 2 cm thick was then removed from the top of the root immediately after harvesting. Roots were lyophilized and ground into a powder. Samples were stored at −80 ˚C until HPLC analysis.

Postharvest storage study

In order to study the changes in tocochromanol compounds over the postharvest storage period of carrot roots, 30 additional roots from each accession in the first block were harvested for storage. These roots were packed in paper bags with wood shavings. The paper bag was then placed in a plastic bag with several holes. Roots were stored at 4 ˚C in the dark until the time of sampling. In 2011/2012, 10 roots were sampled on 28 November, 9 February and 28 March. In 2012/2013, 10 roots were sampled on 30 November, 28 January and 20 March. Due to low germination in the field and rotting during storage, the W266D*rprp* and W276B inbred lines were not included in this part of the study. At sampling, roots were processed as above.

Reproductive life cycle

Carrot requires a vernalization period before reproductive growth. This part of the study examined the changes in tocochromanol compounds over the reproductive growth phase of the carrot life cycle. Forty additional roots were harvested from each cultivar in the second block for the vernalization study. The meristem was left intact and roots were packed according to the same protocol as the postharvest storage study roots, described above. On 2 December, 2011 and 4 December 2012, roots were taken out of cold storage and planted in 13 cm diameter pots in 1:2 Metro Mix to field soil in the greenhouse. Plants received 16 h of high-pressure sodium lighting per day with the temperature set at approximately 21 ˚C day and night. Ten plants from each cultivar (except W276B due to rotting during vernalization) were sampled at flowering when at least 50% of flowers were fully open and shedding pollen on 2 February 2012 and 30 January 2013. The remaining 10

plants were allowed to set seed and were harvested once seed was dried on 4 April 2012 and 27 March 2013. At sampling, roots were processed as above.

Wild carrot

To analyze whether tocochromanol compounds are present in wild carrot, seven accessions were obtained from Dr Phillip Simon, USDA-ARS. Accessions included: 1226-Oregon, 1237-California, 8397-Wisconsin, 280706-Chile, 9296-Tunisia, 1172-Portugal and 1221-Washington state. Seeds were planted under greenhouse conditions as described above. Plants were allowed to grow for 2 months and eight roots from each accession were then cleaned, lyophilized and analyzed using the same procedures as described for the above experiments.

Tocochromanol and β-carotene analysis

For the tocochromanol extraction process, modified from Bligh and Dyer,[11] 50 mg of lyophilized carrot tissue was added to a 2 mL Eppendorf tube with 3 (3 mm) glass beads, 400 μL of 2:1 methanol to chloroform (v/v) with 0.01% butylated hydroxytoluene, and tocol standard (rac-5,7 dimethyltocol; Matreya, Pleasant Gap, PA, USA). Samples were shaken using the Genogrinder for 3 min at a setting of 500 strokes min^{-1}. An additional 300 μL H_2O and 125 μL chloroform were then added to the homogenates, which were further shaken for 30 s at the same settings. After phase separation by centrifugation for 2 min at 21 100g, 195 μL of the organic phase was transferred to a new Eppendorf tube and dried down using a centrivap concentrator (Labconco, Kansas City, MO, USA) for 25 min at 30 ˚C (lowest temperature setting). The dried samples were then resuspended in 150 μL of MeOH with 0.05% butylated hydroxytoluene. Five microliters of each sample was injected into the HPLC (Agilent 1260; Agilent, Santa Clara, CA, USA) equipped with a Zorbax eclipse XDB-C18 reverse phase column (3 mm×150 mm, 5 μm) using a 20-min isocratic elution of 95% methanol and 5% water at a flow rate of 1.0 mL min^{-1}.[8] Tocochromanols were detected by fluorescent detection (290 nm excitation, 330 nm emission) and quantified against standard curves generated from commercially available α- and γ-tocopherol and α- and γ-tocotrienol standards (Sigma-Aldrich, St Louis. MO, USA; Cayman Chemical, Ann Arbor, MI, USA).

To determine if there is a correlation between carotenoid and tocochromanol accumulation in carrot root, β-carotene was measured at field harvest of both years. The carotenoid extraction procedure was adapted from Simon et al.[12] Three milliliters of hexane were added to 0.12 g of lyophilized carrot tissue and allowed to sit overnight at 4 ˚C. Samples were then analyzed on the spectrophotometer (Spectronic Genesys 5; Thermo Scientific, Waltham, MA, USA) at an absorbance of 450 nm. Samples were compared to a standard curve of commercially available β-carotene standard (Sigma-Aldrich).

Statistical analysis

SAS software was used for all statistical analyses. A mixed model analysis of variance was conducted on the data from the September harvest with accession as a fixed effect and all other effects and interactions treated as random. Pairwise comparisons of means were also conducted among accessions. For the postharvest storage study, accession was treated as a fixed effect and year as random. Time was treated as a regression variable in the model. An analysis of covariance was conducted to examine trends across accessions over time. For the reproductive growth study, accession and time were treated as fixed effects and year as a random effect in the model. Experiment-wide least significant difference values were calculated to compare within accession differences over time for the postharvest storage study and the reproductive growth study.[13] To examine the relationship between β-carotene and tocochromanol compounds, Pearson's correlation coefficients were calculated.

RESULTS

Root harvest

All eight tocochromanol compounds were present in the majority of cultivated carrot accessions harvested in September and amounts varied by accession for each compound (Figure 1). β- and γ-tocopherol eluted together as did β- and γ-tocotrienol, so accumulation of each individual compound could not be determined. Instead, the combined amount of β- and γ- tocopherol and the combined amount of β- and γ-tocotrienol were determined for each accession. α-tocopherol was the highest class of
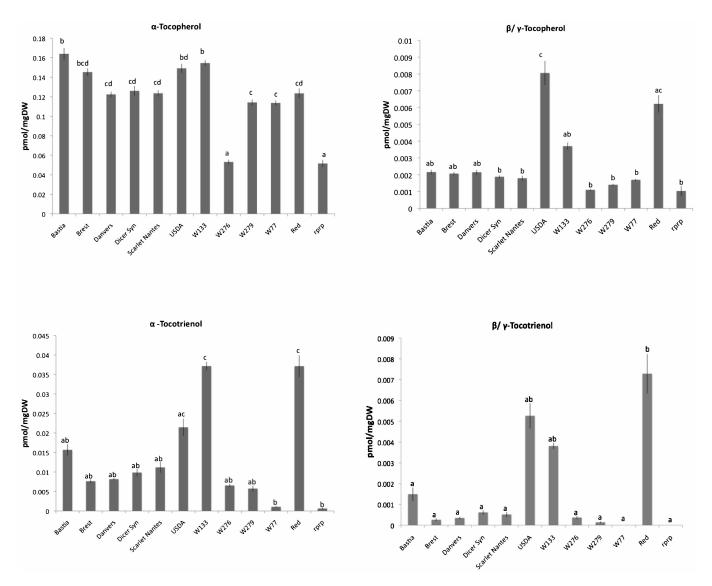
Figure 1 α-Tocopherol, β/γ-tocopherol, α-tocotrienol and β/γ-tocotrienol (pmol mgDW^{-1}) in carrot root accessions at September harvest averaged over both years. Accessions are grouped based on hybrid cultivars, open-pollinated cultivars, inbred lines and non-orange pigmented inbred lines (from left to right, respectively). Accessions with different letters represent significant differences from a pairwise comparison of means using Tukey adjusted *p* values (*p*<0.05).

tocochromanol compounds accumulated in all accessions. Bastia, Brest, USDA B2327B and W133B accumulated significantly higher amounts of α-tocopherol compared to the other accessions (Figure 1). The W266D*rprp* low carotenoid inbred and W276B inbred accumulated significantly lower amounts of α-tocopherol than the other accessions tested (Figure 1). For β/γ-tocopherol, α-tocotrienol and β/γ-tocotrienol compounds, the red, USDA B2327B and W133B inbreds accumulated significantly more of these compounds than the other accessions (Figure 1).

The representative HPLC chromatogram for several accessions shows variation in the types and amounts of accumulated tocochromanols. The Bastia hybrid contains both more types and higher levels of tocochromanol compounds compared to the W266D*rprp* low carotenoid inbred (Figure 2). There was no significant difference in tocochromanol accumulation when carrot accessions were grouped by hybrid, open pollinated and inbred categories (Figure 1). However, roots with major pigment modification had distinct tocochromanol profiles. The inbred carrying the *rprp* genotype had the lowest levels of all tocochromanol compounds and the

red inbred, which accumulates lycopene, had the most accumulation of the greatest variety of compounds (Figure 2).

A significant accession by year interaction was noted (Table 1). However, the effect was due to a change in magnitude rather than a change in rank. Thus, data from the 2 years were combined. The main effect of accession was also significant at the *p*<0.001 level for all tocochromanol compounds measured (Table 1).

Postharvest storage study
For α-tocopherol, time postharvest, year and accession by time were all significant effects in the model for the post-harvest storage study at the *p*<0.05 level (Table 1). The significant effect of year was due to a change in magnitude, rather than a change in rank. Thus, data from the 2 years were again combined. Many of the effects in the analysis of variance were significant for the other compounds (Table 1). However, there was no trend across accessions over time for α-tocotrienol, β/γ-tocotrienol or β/γ-tocopherol, so amounts are not reported for these compounds. For all accessions, the amount of α-tocopherol nearly doubled from September (month 0) to
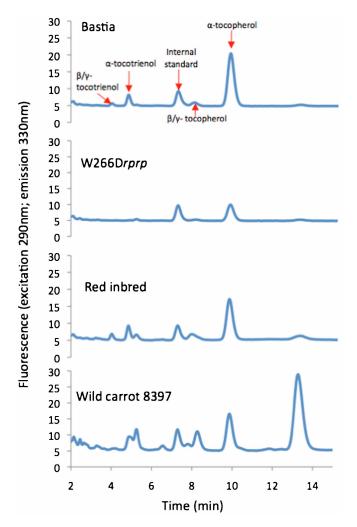
Figure 2 HPLC chromatograms of tocochromanol compounds in Bastia, W266D*rprp*, red inbred (all from 2012 September harvest) and wild carrot root (accession 8397-Wisconsin). Internal standard used was rac-5,7-dimethyltocol.

March (month 6), indicating that the levels of α-tocopherol increase over the postharvest storage life of the carrot roots (Table 2).

Reproductive life cycle study
All effects in the α-tocopherol model for the reproductive life cycle analysis were significant at the $p<0.05$ level except for the accession by year interaction (Table 1). The significant year effect was due to a change in magnitude, rather than a change in rank so data from the 2 years were combined. The majority of effects in the analysis of variance for the models of the other compounds were also significant (Table 1). However, like during postharvest storage, there were no consistent trends across accessions over time. Much like in the storage study, the amounts of α-tocopherol for all accessions increased from the initial harvest during flowering. However, the amounts decreased slightly when measured at seed set (Table 2). Differences between all sampling times were significant ($p<0.05$).

Wild carrot
To investigate the levels and variation of tocochromanol content in wild carrots, seven wild carrot accessions were analyzed for tocochromanol compounds. α-, β-, γ- and δ-forms of tocopherols and tocotrienols were present in wild carrot roots and there was variation among accessions (Table 3). The types and amounts of compounds accumulating in wild carrot roots were quite different compared to domesticated carrot (Figure 2) and there was wide variation in the types and amounts of tocochromanol compounds (Table 3). In addition to the eight tocochromanol compounds, there were several compounds detected in the chromatogram of the wild carrot that were not seen on the chromatogram of cultivated carrot. Although these peaks had the same fluorescence emission spectra as the eight tocochromanols, this study did not seek to determine the identity of these compounds.

Carotenoids
While some have noted biochemical relationships between tocopherol and carotenoid compounds,[5,7,9] we did not find a significant correlation between β-carotene and tocochromanol accumulation across the accessions in this study. β-carotene levels varied from 0–594 mg kg^{-1} in this study. There was not a strong or significant

Table 1 Type III mean squares from the analysis of variance for tocochromanol accumulation at root harvest in September, over the postharvest storage period of carrot roots and over the reproductive growth phase of the carrot life cycle. All 12 accessions and both years were included in the model. Significance based on: NS, no significance; $p<0.1$; *$p<0.05$; **$p<0.01$; ***$p<0.001$

Effect	df	α-tocopherol	β/γ-tocopherol	α-tocotrienol	β/γ-tocotrienol
		Root harvest			
Accession	11	***	***	***	***
Year	1	***	***	***	***
Block (year)	6	*	NS	*	NS
Accession*year	11	***	***	***	***
Accession*block (year)	62	***	***	***	***
		Postharvest storage			
Accession	9	.	***	***	***
Year	1	*	NS	***	***
Time	1	***	***	NS	***
Accession*year	9	NS	***	***	***
Accession*time	9	**	***	NS	***
		Reproductive growth			
Accession	10	***	***	***	***
Year	1	***	NS	***	***
Time	2	***	***	*	***
Accession*year	10	NS	***	***	***
Accession*time	10	***	***	***	***

Table 2 Table of combined year mean accumulation of α-tocopherol (pmol mgDW^{-1}) for each accession over the postharvest storage period and the reproductive growth phase of carrot. Experiment-wide LSD tests were computed between means for within each cultivar for postharvest storage and reproductive growth

Accession	Months postharvest storage				Reproductive growth phase		
	0	2	4	6	Harvest	Flowering	Seed set
Bastia	0.182	0.215	0.262	0.274[a]	0.162	0.287	0.268[b]
Brest	0.129	0.200	0.216	0.229[a]	0.160	0.268	0.234[b]
Danvers	0.133	0.209	0.229	0.241[a]	0.122	0.252	0.191[b]
WI dicer synthetic	0.127	0.220	0.198	0.242[a]	0.117	0.245	0.209[b]
Scarlet Nantes	0.112	0.188	0.226	0.226[a]	0.129	0.260	0.204[b]
USDA B2327B	0.134	0.246	0.354	0.368[a]	0.149	0.425	0.485[b]
W133B	0.151	0.245	0.216	0.275[a]	0.159	0.243	0.244[b]
W279B	0.098	0.204	0.229	0.227[a]	0.118	0.216	0.194[b]
W77C	0.107	0.203	0.207	0.217[a]	0.125	0.214	0.169[b]
Red inbred	0.143	0.238	0.293	0.250[a]	0.109	0.241	0.242[b]
W266D*rprp*	—	—	—	—	0.057	0.194	0.150[b]

[a] Indicates significant difference across postharvest storage periods within accession from 0–6 months at $p<0.05$ based on experiment-wide LSD (0.01).
[b] Indicates significant difference across reproductive growth phase within accession from harvest to seed set at $p<0.05$ based on experiment-wide LSD (0.01).

correlation between levels of β-carotene and any of the tocochromanol compounds measured.

DISCUSSION

Breeding for increased pro-vitamin A carotenoids has been successful in carrot and concentrations have increased significantly over the past 50 years.[12,14,15] One goal of this study was to determine if there was significant variation in tocochromanol accumulation across accessions and whether this would be a practical application of breeding to increase the nutrient content in carrot. This study found that both cultivated and wild carrot roots accumulate all eight tocochromanol compounds and that their amounts varied across accessions. This significant variation across accessions for all compounds measured may likely be attributed to a genetic component of tocochromanol accumulation.

Types and amounts of each compound also changed over the postharvest storage and reproductive life cycle stages of the crop. While several studies have noted the presence of α− and γ-tocopherol in carrot root, none examined the full tocochromanol spectrum.[5,7,9] This is the first study to report the presence of β-tocopherol and α-, β-, δ- and γ-tocotrienol in carrot root tissue and also to determine tocochromanol levels over the storage and seed production stages of the crop. We also report the presence of tocochromanols in wild carrot root tissue.

Unlike in most seed oils,[16] α-tocopherol, the most bioactive and beneficial to humans of the tocochromanols, is the most abundant of the tocochromanol compounds found in carrot roots. The amounts of α-tocopherol present in hybrid carrot roots from this study (0.12 µg gFW^{-1}) are comparable to levels found in other vegetable crops such as lettuce (*Lactuca sativa* L.) (0.1 µg gFW^{-1}), green pepper (*Capsicum annuum* L.) (0.1 µg gFW^{-1}) and red and yellow pepper (*Capsicum annuum* L.) (both 0.6 µg gFW^{-1}).[5]

Table 3 Mean accumulation of tocochromanol compounds (pmol mgDW^{-1}) in wild carrot accessions

Accession	α-tocopherol	β/γ-tocopherol	α-tocotrienol	β/γ-tocotrienol
1226-Oregon	5.62×10^{-2}	9.53×10^{-4}	9.71×10^{-3}	2.60×10^{-4}
1237-California	9.79×10^{-2}	1.06×10^{-3}	1.17×10^{-2}	5.57×10^{-4}
8397-Wisconsin	1.03×10^{-1}	1.59×10^{-3}	2.23×10^{-2}	5.56×10^{-4}
280706-Chile	9.67×10^{-2}	1.02×10^{-3}	7.69×10^{-3}	8.30×10^{-4}
9296-Tunisia	6.07×10^{-2}	8.56×10^{-4}	7.00×10^{-3}	1.46×10^{-4}
1172-Portugal	5.78×10^{-2}	5.80×10^{-4}	1.33×10^{-2}	7.43×10^{-4}
1221-Washington	8.18×10^{-2}	1.13×10^{-3}	1.32×10^{-2}	5.05×10^{-4}

Interestingly, during both the storage and seed production stages of the crop, amounts of α-tocopherol nearly doubled (Table 2). Since carrot can be stored for up to 6 months before consumption, changes in the presence and abundance of secondary compounds are of interest for human nutrition. Unlike pro-vitamin A carotenoids that tend to degrade over storage,[17,18] this study found that α-tocopherol increases over storage and during the reproductive life cycle stage. As carrots are often stored before consumption, increases in α-tocopherol during storage may provide some enhanced nutritional quality to carrots.[3,19] Changes in carrot plant metabolism during storage and vernalization periods are not well studied and the biochemical mechanism underlying substantial increase in α-tocopherol is currently not known and could be an area of future research.

While vegetables, including carrot, have been thought to contribute to the recommended daily intake of vitamin E,[20] this study found that levels present in carrot root are likely not high enough to be nutritionally significant. The recommended daily intake of vitamin E in adults is 15 mg,[21] whereas combined vitamin E is only 17 µg in a 128 g serving of carrot according to the current study (average of hybrid carrot varieties measured, adjusted to 80% moisture level). Thus carrot root does not appear to be a significant source of dietary vitamin E as a serving only provides about 0.1% of the recommended daily intake. This suggests the levels may not be nutritionally significant and attempting to breed for increased levels would not be a fruitful direction to pursue.

Contrary to the suggestion in the USDA database[20] that carrots contribute a nutritionally significant portion of vitamin E to the human diet, this study found that, while vitamin E compounds are present, amounts are very low and are not a nutritionally significant source of vitamin E. The current survey of wild carrot germplasm suggests that there is not much genetic potential to drastically increase the level of vitamin E by introgression of wild germplasm into cultivated lines. However, given that carrots started from almost no carotenoid in wild carrot and now contain high levels in current cultivars, there may be potential to increase the level of similar isoprenoids, such as tocochromanols, to a much higher level, if the tocochromanol level is followed during future breeding efforts. An alternative strategy could be to obtain mutations that would induce tocochromanol accumulation in roots by carefully following the tocochromanol levels, as was done to increase carotenoids. The current results provide critical baseline information of the type and amount of tocochromanols present under a variety of conditions in both domesticated and wild carrots

and provide guidance on how to potentially improve essential nutrients in this widely consumed vegetable crop.

This study did not find a significant correlation between levels of tocochromanols and levels of carotenoids in the accessions studied, despite the biochemical relationship of these two classes of compounds. Even though there was no trend across accessions, the reduced carotenoid mutant, W266D*rprp*, also contained the lowest amount of all tocochromanol compounds. Thus, while there may not be a close correlation, the mutation that reduces production of carotenoids in carrot roots may also inhibit tocochromanol accumulation.

The role of tocochromanol compounds in carrot root tissue is unclear. This study noted the presence and variation of all eight tocochromanol compounds in both cultivated and wild carrot root tissue. Tocochromanol compounds have been conserved across all photosynthetic organisms and there appears to be genetic potential for accumulation in non-photosynthetic tissues. The presence of tocochromanol compounds in wild carrot root suggests that the role of these compounds may be different from that of carotenoids in cultivated carrot root tissues, which are present in root tissues primarily as the result of human directed selection. Few studies have examined the role of tocochromanol compounds in non-photosynthetic tissues and this question remains to be investigated. Future studies could further examine the evolutionary role of these compounds in carrot root tissue and the metabolic changes that trigger tocochromanol accumulation over the storage and life cycle periods of carrot roots.

CONFLICTS OF INTEREST

Author ILG has an interest in Phyto Colorants LLC, which licenses table beet germplasm from the Wisconsin Alumni Research Foundation to the food industry. There is no conflict of interest associated with this study; however, we would prefer to disclose this and allow the Editor to make a determination as to whether it should be listed. Authors CHL and HAM declare no conflicts of interest.

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