HortScience 40(5):1230–1233. 2005.

Carotenoid and Chlorophyll Pigments in Sweet Basil Grown in the Field and Greenhouse

Dean A. Kopsell[1]
Plant Sciences Department, The University of Tennessee, Knoxville, TN 37996

David E. Kopsell[2]
Department of Plant Biology, The University of New Hampshire, Durham, NH 03824

Joanne Curran-Celentano[3]
Department of Animal and Nutritional Sciences, The University of New Hampshire, Durham, NH 03824

Additional index words. β-carotene, HPLC, human health, lutein, *Ocimum basilicum*, zeaxanthin

***Abstract*. Sweet basil (*Ocimum basilicum* L.) is a popular culinary herbal crop grown for fresh or dry leaf, essential oil, and seed markets. Recently, basil was shown to rank highest among spices and herbal crops for xanthophyll carotenoids, which are associated with decreased risks of cancer and age-related eye diseases. The research goal for the current study was to characterize the concentrations of nutritionally important carotenoid pigments in popular varieties of basil. Eight cultivars of sweet basil ('Genovese', 'Italian Large Leaf', 'Nufar', 'Red Rubin', 'Osmin Purple', 'Spicy Bush', 'Cinnamon', and 'Sweet Thai') were grown in both field and greenhouse environments and evaluated for plant pigments using HPLC methodology. Environmental and cultivar differences were observed for all of the pigments analyzed. 'Sweet Thai' accumulated the highest concentrations of lutein, zeaxanthin, and β-carotene carotenoids in the field, while 'Osmin Purple' accumulated the highest carotenoid concentrations in the greenhouse. Comparing the two environments, cultivar levels for carotenoid and chlorophyll pigments were higher in the field environment when expressed on both a fresh and dry weight basis. Exceptions were found only for the purple leaf basils ('Osmin Purple' and 'Red Rubin'). Positive correlations existed between carotenoid and chlorophyll pigments in both environments. This study demonstrates sweet basil accumulates high levels of nutritionally important carotenoids in both field and greenhouse environments.**

Herbal crops have been valued for centuries for their flavoring attributes and therapeutic properties. World-wide, sweet basil (*Ocimum basilicum* L.) is one of the most popular culinary herbal crops, and is produced for fresh or dry leaf, essential oil, and seed markets. Essential oils are the most valuable commercial forms of basil and contribute flavors and aromas to a variety of products in the food and cosmetic industries (Bagamboula et al., 2004; Putievsky and Galambosi, 1999). Basil produced for dried leaf and fresh markets rank second and third in commercial importance, respectively. The most widely used fresh market crops are the Italian basils, which are commonly used in the production of pestos. However, the flavored types are increasing in popularity due to their unique aromas and flavors, while the colored (or purple) basils are being used as decorations (Putievsky and Galambosi, 1999). Basil consumption in the U.S. increased about 8-fold during the period from 1960 to 1996 (Davidson and Johnson, 1996) and continues to increase. Currently, sweet basil is commercially produced in field, greenhouse, and hydroponic growing systems (Craker et al., 2003).

There is a large amount of morphological and biochemical diversity in the genus *Ocimum* (Lamiaceae). Sweet basil have been classified into seven different morphotypes, which include 1) tall, slender types; 2) large-leafed types ('Italian' basils); 3) dwarf types ('Bush' basils); 4) compact types ('Thai' basils); 5) purple types (with clove-like aroma); 6) *purpurascens* types (sweet purple colored basils); and 7) *citriodorum* types (flavored basils) (Darrah, 1980; Simon et al., 1999). Research also demonstrates genetic diversity in *O. basilicum* for such biochemical traits as anthocyanin pigments (Phippen and Simon, 1998; Simon et al., 1999), essential oils (Pascual-Villalobos and Ballesta-Acosta, 2003), and terpene and volatile phenylpropene compounds (Iijima et al., 2004). Genetic variation for volatile oils and flavonoid compounds is also present in tree basil (*O. gratissimum* L.) (Vieira et al., 2001).

The therapeutic compounds in herbal crops are gaining attention since they contribute nutritionally-valuable phytochemicals to the diet. Xanthophylls, such as lutein and zeaxanthin, and carotenes, such as β-carotene and α-carotene, are examples of nutritionally important plant-derived carotenoids (Zaripheh and Erdman, 2002). A recent report ranks basil (unknown cultivar) highest among spices and aromatic herbal crops for levels of xanthophyll carotenoids (Calucci et al., 2003). Carotenoids are a class of secondary plant compounds that act as accessory photosynthetic pigments. These compounds serve many functions in plants including light harvesting, structure stabilization, and excess energy dissipation (Frank and Cogdell, 1996). Additionally, they protect plants from free radicals, such as triplet excited chlorophyll (^{3}Chl) and singlet oxygen (^{1}O), produced when light intensity exceeds photosynthetic capacity (Mortensen et al., 2001). Increased intake of lutein and β-carotene has been associated with decreased risks of cancer and other chronic diseases, especially age-related eye diseases (Sommerburg et al., 1999).

We are unaware of any studies that have examined carotenoid concentrations among cultivars of *O. basilicum*. Therefore, the objective of this study was to characterize the concentrations of nutritionally important carotenoid pigments in popular varieties of sweet basil. Commercially, sweet basil is cultivated in both field and greenhouse production systems (Putievsky and Galambosi, 1999), therefore both environments were examined for this study. Pigment data is presented on both a fresh and dry weight basis, and correlations between chlorophyll and carotenoid pigments were also calculated.

Materials and Methods

Field experiment. Eight sweet basil cultivars (Johnny's Selected Seed, Winslow, Maine) were evaluated for leaf tissue carotenoid and chlorophyll pigments in separate field and greenhouse environments. On 23 May 2003, seeds representing large-leafed Italian basil types ('Genovese', 'Italian Large Leaf', and 'Nufar'), *purpurascen* types ('Red Rubin' and 'Osmin Purple'), and specialty basils [composed of dwarf, compact, and *citriodorum* types ('Spicy Bush', 'Cinnamon', and 'Sweet Thai')] were sown into sphagnum peat moss based medium (Pro-Mix BX, Premier Horticulture, Dorval, Que.) for the field evaluation. The seeds were cultured in a greenhouse (22 °C day/14 °C night set points) for 30 d under natural photoperiods (lat. 43°09'N). Seedlings were watered as needed and fertilized with a full-strength Hoagland's nutrient solution (Hoagland and Arnon, 1950) every 7 d. On 23 June 2003, plants were transplanted into the field (Woodman Horticulture Farm, Durham, N.H.). Field plots were fertilized 2 weeks before transplanting based on recommended N–P–K rates for basil (Simon, 1985). Field

Received for publication 12 Dec. 2004. Accepted for publication 3 Mar. 2005. This paper was funded in part by a grant received by the Cooperative State Research, Education, and Extension Service, U.S. Department of Agriculture, under Agreement No. 2001-52102-11254. The authors wish to thank Sara Marcus and Joe Sheehan for their contributions to this project.

[1]Assistant professor. Corresponding author; e-mail dkopsell@utk.edu.
[2]Former postdoctoral research scientist. Current address: National Onion Labs, Inc., Collins, GA 30421.
[3]Professor.

plots consisted of 8 basil cultivars planted in single rows of 12 plants each, at a spacing of 20 cm within rows and 45 cm between rows. Cultivars were randomized within plots, and plots were replicated four times in a randomized complete block design. Irrigation was applied in split applications such that plots received 2 to 3 cm water per week. Photosynthetically active radiation (PAR) averaged 569 $\mu mol \cdot m^{-2} \cdot s^{-1}$ over the growing period (University of New Hampshire weather station data, Durham, N.H.). Basil leaf tissues were harvested from field plots on 25 July 2003. About 25 leaves were removed at random from each of ten plants per cultivar and combined to represent one sample per replication. Leaf tissues were stored at –80 °C for no less than 2 d before sample preparation. Frozen leaf tissue was lyophilized for 48 h (model 6L FreeZone; LabConCo, Kansas City, Mo.) and stored at –80 °C before extraction. Tissue moisture was calculated through the difference in leaf sample weight before and after lyophilization.

Greenhouse experiment. The same sweet basil cultivars were sown into sphagnum peat moss based medium on 16 Mar. 2004 for greenhouse evaluation. Sweet basil seeds were cultured as previously described for the field experiment. On 15 Apr. 2004, seedlings were transferred to growing containers (15 cm standard round pots, Dillen Prodcuts, Middlefield, Ohio) filled with sphagnum peat moss based medium. The greenhouse evaluation was a randomized complete block design on four greenhouse benches, each bench representing a block/replication. Cultivars were randomly placed in double rows of 12 plants in each block and spaced 21 cm apart on center. Individual pots were fertilized with 100 ml of a full-strength Hoagland's nutrient solution on 15, 22, 29 Apr. and 6 May 2004. Water was applied to each pot daily at an approximate rate of 100 mL. Basil leaf tissues were harvested from greenhouse pots on 12 May 2004. Average PAR over the growing period was 515 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Leaf tissues were harvested, prepared, and stored similar to the field experiment.

Carotenoid and chlorophyll analysis. Plant pigments were extracted from freeze-dried tissues according to Kopsell et al. (2004). A 0.10 g subsample was re-hydrated with 0.8 mL of ddH_2O at 40 °C for 20 min. After incubation, 0.8 mL the internal standard ethyl-β-8-apocarotenoate (Sigma Chemical Co., St. Louis, Mo.) and 2.5 mL of tetrahydrofuran (THF) stabilized with 25 ppm 2,6-Di-*tert*-butyl-4-methoxyphenol (BHT) were added. The sample was homogenized in a Potter-Elvehjem (Kontes, Vineland, N.J.) tissue grinding tube using about 25 insertions with a pestle attached to a drill press (model Craftsman 15-inch Drill Press; Sears, Roebuck and Co., Hoffman Estates, Ill.) set at 540 rpm. During homogenation, the tube was immersed in ice to dissipate heat. The tube was then placed into a clinical centrifuge for 3 min at 500 g_n. The supernatant was removed and the sample pellet was resuspended in 2 mL THF and homogenized again with the same extraction technique. The extraction procedure was repeated two more times to obtain a colorless supernatant. The combined supernatants were reduced to 0.5 mL under a stream of nitrogen gas (N-EVAP 111; Organomation Inc., Berlin, Mass.) at 40 °C and 2.5 mL MeOH and 2 mL THF were added to the sample before HPLC analysis.

An Agilent 1100 series HPLC unit with a photo diode array detector (Agilent Technologies, Palo Alto, Calif.) was used for sample separation. All samples were analyzed using a RP C-18, 80 Å, 3.0 µm, 300 × 4.6-mm column (Adsorbosphere HS; Alltech, Deerfield, Ill.) fitted with a 7.5 × 4.0-mm 5.0 µm guard column (All Guard C-18; Alltech). The column was maintained at 16 °C using a thermostatted column compartment. Eluents were A) 75% acetonitrile, 20% methanol, 5% hexane, 0.05% BHT, 0.013% triethylamine (TEA) in water (v/v) and B) 50% acetonitrile, 25% THF, 25% hexane, 0.013% TEA in water (v/v). The flow rate was 0.7 $mL \cdot min^{-1}$ and the gradient is 100% A for 30 min, 50% A and 50% B for 2 min; 100% B for 2 min; and 50% A and 50% B for 2 min. The eluent composition was returned to 100% A and the column was equilibrated for 10 min before the next injection. Eluted compounds from a 20 µL injection were detected at 452 (carotenoids and internal standard), 652 (chlorophyll *a*), and 665 (chlorophyll *b*) nm and data was collected, recorded, and integrated using 1100 HPLC ChemStation Software (Agilent Technologies). Peak assignment was performed by comparing retention times and line spectra obtained from photodiode array detection with authentic standards (lutein from Carotenature, Lupsingen, Switzerland; β-carotene, chlorophyll *a*, chlorophyll *b* from Sigma Chemical Co.).

Data sets were analyzed by the GLM procedures of SAS (Cary, N.C.) with cultivar means separated by Duncan's multiple range test ($P = 0.05$). Data were recorded on both a fresh (mg/100 g) and dry ($mg \cdot g^{-1}$) weight basis. A separate correlation matrix for the basil cultivars under each environment was calculated for all of the variables tested.

Results and Discussion

Environmental and cultivar differences were observed for all of the pigments analyzed in fresh sweet basil tissues (Table 1). An environment × cultivar interaction was observed for the plant pigments, thus data is presented for the basil cultivars under each growing environment. Comparing the two environments, cultivar means for lutein, β-carotene, and zeaxanthin in basil leaf tissues, on both a fresh and dry weight basis, were higher in the field environment (Tables 2 and 3). Exceptions were found only for the purple leaf basils. Both 'Osmin Purple' and 'Red Rubin' had slightly higher values for the carotenoid pigments in the greenhouse environment (Tables 2 and 3).

Cultivars that accumulated the highest concentrations of lutein on a fresh weight basis in the field environment were 'Sweet Thai', 'Osmin Purple', and 'Genovese', and those that accumulated the highest concentrations in the greenhouse environment were 'Sweet Thai', 'Osmin Purple', and 'Red Rubin'. Cultivars that accumulated the highest concentrations of β-carotene on a fresh weight basis in the field environment were 'Sweet Thai', 'Genovese', and 'Cinnamon', and those that accumulated the highest concentrations in the greenhouse environment were 'Osmin Purple' and 'Red Rubin'. Cultivars that accumulated the highest concentrations of zeaxanthin on a fresh weight basis in the field environment were 'Sweet Thai' and 'Genovese', and those that accumulated the highest concentrations in the greenhouse environment were 'Osmin Purple' and 'Red Rubin' (Table 2). The U.S. Department of Agriculture Nutrient Database lists carotenoid values in sweet basil (unknown cultivar) to be 5.6 mg/100 g and 3.1 mg/100 g for lutein/zeaxanthin and β-carotene, respectively (U.S. Dept. of Agriculture, 2004). Carotenoid concentrations for the current study correspond to, or slightly exceed, these values.

Since basil has value as a dried herb in culinary industries, the carotenoid concentrations in the sweet basil cultivars were also calculated on a dry weight basis. Cultivars that accumulated the highest concentrations of lutein on a dry weight basis in the field environment were 'Sweet Thai' and 'Osmin Purple', and those that accumulated the highest concentrations in the greenhouse environment were 'Osmin Purple' and 'Red Rubin'. Cultivars that accumulated the highest concentrations of β-carotene on a dry weight basis in the field environment were 'Sweet Thai' and 'Genovese', and those that accumulated the highest concentrations in the greenhouse environment were 'Osmin Purple' and 'Red Rubin'. Cultivars that accumulated the highest concentrations of zeaxanthin on a dry weight basis in the field environment were 'Sweet Thai' and 'Osmin Purple', and those that accumulated the highest concentrations in the greenhouse environment were 'Osmin

Table 1. Analysis of variance of a randomized complete block design for carotenoid and chlorophyll pigments in fresh basil[z] leaf tissue.

		Lutein		β-Carotene		Zeaxanthin		Chlorophyll *a*		Chlorophyll *b*		Total chlorophyll	
Source	df	MS	$P > F^y$	MS	$P > F$	MS	$P > F$	MS	$P > F$	MS	$P > F$	MS	$P > F$
Environment (E)	1	44.2	***	40.4	***	0.34	***	52141.4	***	1630.4	***	72211.8	***
Cultivar (C)	7	6.2	***	2.3	***	0.13	***	1291.1	***	96.1	***	1996.8	***
E × C	7	3.7	***	7.2	***	0.17	***	387707	***	147.7	***	5518.1	***
Error	48	0.23		0.24		0.001		125.9		7.0		186.8	

[z]Eight cultivars grown in both greenhouse and field environments.
[***]Significant level of F statistic at $P < 0.001$.

Purple' and 'Red Rubin' (Table 3). Previously, concentrations of lutein and zeaxanthin in basil (cultivar not identified) were reported to be 0.24 and 0.02 mg·g^{-1}, respectively (Calucci et al., 2003).

Compared to the carotenoid pigments, basil cultivars had much higher concentrations of chlorophyll pigments (on a fresh weight basis) in the leaf tissues. Across the two environments, average chlorophyll pigment values were higher in the field than in the greenhouse (Table 4). Cultivars that accumulated the highest concentrations of chlorophyll *a*, chlorophyll *b*, and total chlorophyll in the field environment were 'Sweet Thai' and 'Cinnamon'. Cultivars that accumulated the highest concentrations of chlorophyll *a*, chlorophyll *b*, and total chlorophyll in the greenhouse environment were 'Osmin Purple' and 'Red Rubin' (Table 4).

Table 2. Mean values[z] for carotenoid pigments (mg/100 g fresh weight) for eight basil cultivars grown under field or greenhouse (GH) environments.

	Lutein[y]		β-carotene		Zeaxanthin	
Cultivar	Field	GH	Field	GH	Field	GH
Cinnamon	6.10 c	4.27 d	6.97 b	4.16 b	0.44 c	0.29 cd
Genovese	6.99 b	4.24 d	7.33 ab	3.58 b	0.50 b	0.26 de
Italian Large Leaf	6.20 c	3.65 d	5.39 cd	3.37 b	0.40 cd	0.23 e
Nufar	6.07 c	4.09 d	5.68 c	3.78 b	0.43 cd	0.25 de
Osmin Purple	7.01 b	7.78 a	4.78 d	6.23 a	0.42 cd	0.42 a
Red Rubin	6.22 c	6.24 b	5.04 cd	5.83 a	0.37 cd	0.37 b
Spicy Bush	6.33 c	4.48 cd	5.19 cd	4.22 b	0.39 cd	0.29 cd
Sweet Thai	8.27 a	5.15 c	7.70 a	4.19 b	0.62 a	0.30 c
Mean	6.64	4.99	6.01	4.42	0.45	0.30

[z]Composition of leaf samples from 4 replications, 10 plants each.
[y]Mean separation within columns by Duncan's multiple range test, $P = 0.05$.

Table 3. Mean values[z] for carotenoid pigments (mg·g^{-1} dry weight) for eight basil cultivars grown under field or greenhouse (GH) environments.

	Lutein[y]		β-carotene		Zeaxanthin	
Cultivar	Field	GH	Field	GH	Field	GH
Cinnamon	0.60 d	0.39 cd	0.57 ba	0.38 bc	0.04 c	0.03 bc
Genovese	0.68 bc	0.38 cd	0.59 a	0.32 bc	0.04 bc	0.02 d
Italian Large Leaf	0.58 d	0.34 d	0.50 bc	0.31 c	0.03 bc	0.02 d
Nufar	0.60 d	0.39 cd	0.51 bc	0.36 bc	0.04 bc	0.02 cd
Osmin Purple	0.72 b	0.76 a	0.49 c	0.61 a	0.04 ab	0.04 a
Red Rubin	0.64 cd	0.61 b	0.52 bc	0.57 a	0.03 bc	0.04 a
Spicy Bush	0.60 d	0.43 c	0.49 c	0.41 b	0.04 bc	0.03 b
Sweet Thai	0.80 a	0.46 c	0.59 a	0.38 bc	0.05 a	0.03 bc
Mean	0.65	0.47	0.53	0.42	0.04	0.03

[z]Composition of leaf samples from 4 replications, 10 plants.
[y]Mean separation within columns by Duncan's multiple range test, $P = 0.05$.

Table 4. Mean values[z] for chlorophyll (Chl) pigments (mg/100 g fresh weight) for 8 basil cultivars grown in field or greenhouse (GH) environments.

	Chl *a*[y]		Chl *b*		Total Chl	
Cultivar	Field	GH	Field	GH	Field	GH
Cinnamon	166.4 b	81.3 c	31.3 b	15.6 c	197.7 b	96.9 c
Genovese	179.4 ab	75.8 c	34.4 ab	14.9 c	213.8 ab	90.7 c
Italian Large Leaf	137.2 dc	70.1 c	25.3 c	13.5 c	162.6 dc	85.6 c
Nufar	146.2 c	79.2 c	27.0 c	14.2 c	173.2 c	93.4 c
Osmin Purple	124.7 d	140.0 a	27.8 c	32.0 a	152.5 d	172.0 a
Red Rubin	126.6 d	117.0 b	26.4 c	26.5 b	153.0 d	143.5 b
Spicy Bush	122.8 d	89.1 c	25.9 c	17.7 c	148.7 d	106.8 c
Sweet Thai	192.8 a	86.9 c	37.2 a	18.4 c	229.9 a	105.2 c
Mean	149.5	93.8	29.4	19.1	179.0	110.0

[z]Composition of leaf samples from 4 replications, 10 plants each.
[y]Mean separation within columns by Duncan's multiple range test, $P = 0.05$.

Table 5. Correlation coefficients[z] (r) between carotenoid and chlorophyll[z] pigments in basil averaged across eight cultivars over two growing environments.

Variable[y]	Lut	BC	Zea	Chl *a*	Chl *b*	Total Chl
Field						
Lut	---	0.32^{NS}	0.76^{***}	0.51^{**}	0.68^{***}	0.54^{***}
BC		---	0.73^{***}	0.92^{***}	0.81^{***}	0.91^{***}
Zea			---	0.86^{***}	0.91^{***}	0.87^{***}
Chl *a*				---	0.91^{***}	0.99^{***}
Chl *b*					---	0.93^{***}
Total Chl						---
Greenhouse						
Lut	---	0.94^{***}	0.93^{***}	0.95^{***}	0.92^{***}	0.96^{***}
BC		---	0.93^{***}	0.97^{***}	0.94^{***}	0.97^{***}
Zea			---	0.91^{***}	0.86^{***}	0.91^{***}
Chl *a*				---	0.94^{***}	0.99^{***}
Chl *b*					---	0.94^{***}
Total Chl						---

[z]Lut = lutein; BC = β-carotene; Zea = zeaxanthin; Chl = chlorophyll.
[NS,**,***]Nonsignificant and significant at $P \leq 0.01$ or 0.001, respectively.

Carotenoids provide photoprotective functions in photosynthesis (Demmig-Adams et al., 1996). The light intensity (average PAR) and day length (hours of light) were higher in the field environment. Thus, we believe that the higher basil leaf carotenoid values found in the field environment may be due to the increases in solar radiation intensity and duration. Kurasova et al. (2000) reported increases in both carotenoid and chlorophyll concentrations in barley (*Hordeum vulgare* L.) with increased irradiance, while Behera and Choudhury (2003) demonstrated similar increases in carotenoid and chlorophyll pigments in wheat (*Triticum aestivum* L.) under increased irradiance levels.

Carotenoid concentrations for the *purpurascen* basils were higher in the greenhouse environment, whereas carotenoid concentrations were much lower in the other basil cultivars when grown in the greenhouse (Table 2). The exact cause for this is unknown. The dark purple leaf color in these cultivars is caused by accumulations of anthocyanin pigments. Anthocyanins are flavonoids that reduce photooxidative injury in leaves by dissipating excess high-energy light and scavenging oxygen free radicals (Neill and Gould, 2003). Lutein and β-carotene increased 11% and 30%, respectively, in 'Osmin Purple' and 0.3% and 16%, respectively, in 'Red Rubin' when comparing greenhouse to field culture. Data suggests that greenhouse production would be better suited to maximize carotenoid concentrations in the *purpurascen* basils.

Correlation coefficients were calculated for all plant pigments among the cultivars over the two growing environments. Positive correlations between and among the carotenoid and chlorophyll pigments were observed in the greenhouse environment (Table 5). In the field environment, carotenoid and chlorophyll pigments were also positive and highly correlated (Table 5). There are previous reports of highly positive correlations between carotenoid and chlorophyll pigments in other leafy crops species, such as swiss chard (*Beta vulgaris* L.) (Ihl et al., 1994), and kale (*Brassica oleracea* L. var. Acephala) (Kopsell et al., 2004). This suggests that it may be possible to use chlorophyll content, or degree of green coloration, to estimate relative values of important xanthophyll carotenoids in basil.

This is the first attempt to characterize the concentrations of nutritionally important carotenoid compounds within cultivars of sweet basil. Previous research shows sweet basil to contain high levels of anthocyanins, essential oils, phenolic, and flavonoid compounds, which express antioxidant, antimicrobial and anticarcinogenic properties (Iijima et al., 2004; Pascual-Villalobos and Ballesta-Acosta, 2003; Phippen and Simon, 1998; Vieira et al., 2001). The current study demonstrates that sweet

basil has high concentrations of xanthophyll and carotene carotenoids. Increases in dietary xanthophylls and carotene carotenoids convey both antioxidant and photo-protective functions in the macular region of the eye (Johnson, et al., 2000; Khachik et al., 1997). Moreover, consumption of vegetable crops providing a mixture of carotenoids was more strongly associated with decreased risks of cancer and eye diseases, when compared to ingestions of monomolecular carotenoid supplements (Johnson, et al., 2000; Le Marchand et al., 1993). Sweet basil can accumulate high levels of nutritionally important carotenoids, with concentrations affected by both genetic and environmental factors. Identifying basil cultivars with high values of important dietary carotenoids may have important health implications for consumers.

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