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ARTICLE

Feasibility for Improving Phytonutrient Content in Vegetable Crops Using Conventional Breeding Strategies: Case Study with Carotenoids and Tocopherols in Sweet Corn and Broccoli

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Among vegetables, sweet corn (*Zea mays* L.) and broccoli (*Brassica oleracea* L. ssp. *italica*) are important sources of dietary carotenoids and tocopherols. Because medical evidence suggests that carotenoid and tocopherol health-promoting activity acts in a dose-dependent manner, conventional breeding to develop elite sweet corn and broccoli germplasm with enhanced levels of these phytochemicals will potentially promote health among the consuming public. This investigation includes the quantitative analysis of carotenoid and tocopherol contents of 41 corn and 24 broccoli genotypes grown in multiple environments (years and seasons in one location) to partition the variation into genetic, environment, and genotype by environment interaction (G×E) components and measure the phenotypic stability of genotypes for these phytochemicals. The primary carotenoids and tocopherols in corn were lutein and γ-tocopherol (65 and 73% of total carotenoid and tocopherol, respectively), whereas β-carotene and α-tocopherol were dominant in broccoli (65 and 79% of total carotenoid and tocopherol, respectively). Partitioning of the variance indicated that genetic differences among the genotypes averaged for the primary compounds in corn (lutein, zeaxanthin, and α- and γ-tocopherol) and broccoli (β-carotene, lutein, and α- and γ-tocopherol) accounted for the largest proportion of the variation (67 and 55% of total phenotypic variation averaged across the phytochemicals in sweet corn and broccoli, respectively). Stability analysis identified several corn (IL451b *sh2* and IL2027-8 *sh2*) and broccoli ('Pirate' and 'Baccus') genotypes with relatively high mean concentrations for the various carotenoids and tocopherols that were comparatively stable across seasons and years. The results of this investigation suggest that sweet corn and broccoli germplasm with enhanced concentrations of carotenoids and tocopherols can be developed using conventional breeding protocols.

KEYWORDS: *Zea mays* L.; *Brassica oleracea* L. ssp. *italica*; phytochemicals; health promotion; plant breeding; genotype by environment interaction; stability analysis

INTRODUCTION

Sweet corn is among the 10 most popular vegetables grown in the United States and is a relatively rich source of carotenoids, tocopherols, vitamin C, phenolics (ferulic acid), and fiber (*1*) in American diets. Broccoli is gaining in public acceptance, and from 1970 to 2005, U.S. per capita consumption increased 12-fold and is expected to continue to increase (*2*). Broccoli has been reported to be a rich source of phytochemicals with antioxidant activity, which following consumption putatively provide protection from cellular oxidative stress (*3*, *4*). Kurilich et al. (*5*) observed that broccoli extracts reduce cell oxidative stress in a human cell line, and the levels of extract protection from free radical oxidation vary among broccoli genotypes. The consumption of broccoli and other cruciferous vegetables has been linked to a reduced risk of cancer (*6*).

Two important classes of phytochemicals with putative health-promoting activity through their action as antioxidants are carotenoids such as lutein, zeaxanthin, and β-carotene and tocopherols such as δ-, γ-, and α-tocopherol, compounds that are relatively abundant in sweet corn and broccoli. Recent studies describing the beneficial bioactivity of carotenoids and tocopherols in human health (*7*, *8*) have led to interest in increasing dietary intake of these phytochemicals.

Among the phytochemically derived carotenoids, lutein and zeaxanthin have been found to protect against the development of cataract and age-related macular degeneration (*9*). Mechanisms suggested to explain the cancer preventive activities of carotenoids include reduced cell proliferation, antioxidant effect on mutagenesis and genotoxicity, immunomodulatory effects, increasing gap-junction cell communication, and enhancing apoptosis of cancerous cells (*10*, *11*). Elevated intake of tocopherols can protect against several degenerative diseases including cardiovascular disease, cancer, neurological disorders, and inflammatory diseases (*8*, *12*). In addition to inhibition of oxidative modification of low-density lipoprotein (LDL), tocopherols may also inhibit atherogenesis through non-antioxidant

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mechanisms at the molecular and cellular level (*13*). Much of the evidence indicates that carotenoid and tocopherol health-promoting activity is dose-dependent (*14*, *15*), suggesting that increased intake of foods rich in these compounds and increased concentrations of these compounds in popular foods are potential approaches to reduce risk associated with these degenerative diseases.

Significant qualitative and quantitative variation for carotenoid and tocopherol contents and compositions have been reported at the mature dry stage in field corn (*16*, *17*) and among sweet corn germplasm at fresh market maturity (21 days after pollination, DAP) (*18*). Dramatic variation among corn genotypes has been found for lutein, zeaxanthin, β-cryptoxanthin, and γ- and α-tocopherol contents (*18*). Kurilich et al. (*19*) studied the variation for carotene and tocopherol contents in subspecies of *Brassica oleracea* by assaying the edible portion of 65 broccoli, cabbage, kale, cauliflower, and Brussels sprout accessions and observed substantial variation both within and between the subspecies. Whereas these reports support the existence of substantial variation based on one year's evaluation, it is crucial to determine what proportion of the variation is due to genetic differences and how much is due to the influence of the environment.

Partitioning the total phenotypic variation for a trait into its component sources [genotype (G) main effect, environment (E) main effect, and G×E interaction] by analysis of variance is crucial in designing crop improvement strategies. When a high proportion of the phenotypic variance for a specific trait is due to genotypic differences, the feasibility of genetic manipulation to improve trait performance and gain from selection is enhanced (*20*). In contrast, if most of the phenotypic variance is associated with the environment, then cultural practices and crop management strategies might be employed to create growing conditions that favor improved trait performance. When a high proportion of phenotypic variance is described by G×E interaction, then the most relevant approach would require genetic selection for the trait at specific locations or growing conditions where major commercial production occurs. Partitioning of variance requires evaluating performance of genotypes in a range of environments.

Although analysis of variance can be used to partition the total variance into its components due to main effects and interactions, it is not robust in the analysis of the G×E interaction because it does not determine the pattern of response of genotypes to environments (*21*). Shukla's stability variance (*22*) uses an environmental index to measure a genotype's stability in response to the average of all genotypes grown in that environment. Shukla's stability analysis was initially developed to determine the stability of grain yield across environments, but recently the method has been applied to other crop traits including phytochemical content (*23*).

Few studies have been performed to explore the effect of G×E interaction on carotenoid and tocopherol content in crop plants. Analysis of variance for data generated from growing 17 tropical yellow-endosperm maize genotypes at three locations for two years revealed that the genotype dominated β-carotene content, whereas G×E interaction was not significant (*24*). Galliher et al. (*16*) evaluated 100 random S_1 families from the corn synthetic RSSSC over two years and found that genetic variances (σ^2_G) were highly significant for α-tocopherol, γ-tocopherol, and total tocopherol. No studies of this nature have been conducted with sweet corn or broccoli.

The following investigation was designed to (1) partition phenotypic variance for carotenoid and tocopherol composition and content in corn and broccoli into component sources associated with genotype, environment, and G×E interaction and (2) assay sweet corn and broccoli genotypes for phytochemical stability across environments (years and seasons).

MATERIALS AND METHODS

Plant Material and Sample Preparation. Forty-one corn inbreds and genetic stocks were selected on the basis of previously published reports (*18*). Each of the genotypes selected for this survey was homozygous for either starchy (*Su1*), *sugary 1* (*su1*), *sugary enhancer1* (*su1se1*), or *shrunken 2* (*sh2*) endosperms. Seed was obtained from the University of Illinois sweet corn germplasm collection, the USDA Maize Genetics Stock Center (University of Illinois), and Iowa State University Regional Plant Introduction Station. Fifty seeds of each genotype were hand planted at the University of Illinois South Farm in a randomized complete block design with three replications over three years (May 15, 1996; May 17, 2002; and May 20, 2003) in single-row plots, 8 m rows with 75 cm between rows. The soil type was Drummer silty clay loam (Typic Haplaquoll). Fertilizer, irrigation, and pesticides were applied in each planting according to standard commercial practices (*25*). For each genotype in each replicate, 12−15 plants were self (inbreds)-pollinated or sib ($F_{2:3}$ families)-pollinated to produce ears for chemical evaluation. Ten ears were harvested from each replication for each genotype at 21 DAP. Tips and butts of each ear were removed, leaving an 8−10 cm portion of central ear that was immediately frozen in liquid nitrogen, packed in Ziploc bags, placed on ice for transport to the laboratory, and stored at −80 °C until lyophilization. After freeze-drying, 100 kernels were removed from each ear, bulked into individual samples, ground to a fine powder, and stored at −20 °C until extraction. Chemical analysis of all samples was completed within 12 weeks after tissue harvest. Previous work has shown that freeze-dried tissue samples stored at −20 °C for periods as long as 2 years displayed no significant reduction in tocopherol or carotenoid content (*18*).

A set of 24 broccoli genotypes were evaluated over two growing seasons (spring 2004 and fall 2004). Thirteen of these 24 genotypes had been grown and harvested in the fall of the previous year (fall 2003). Seeds were provided by the USDA Plant Genetic Resource Unit (Geneva, NY), Dr. Mark Farnham at the USDA Vegetable Research Center (Charleston, SC), Asgrow Seed Co., Peto Seeds, and Sakata Seeds of America, Inc. The genotypes included commercial hybrids, open-pollinated varieties, land races, inbreds, and doubled haploids. The seeds were germinated in the greenhouse and grown for 4−5 weeks with 1 additional week of cold hardening before transplanting into field plots at University of Illinois South Farms on July 3, 2003; May 17, 2004; and July 2, 2004, in a randomized complete block design with 3 replications of 10−15 plants per replication. Fertilizer, irrigation, and pesticides were applied according to standard commercial practices (*25*). The soil type was Drummer silty clay loam (Typic Haplaquoll). At commercial fresh market maturity, broccoli heads were harvested and stored on ice and immediately transported to the laboratory. Equal amounts of head tissue (100 g) were collected from eight plants in each replicate, bulked and frozen in liquid nitrogen, and stored at −80 °C prior to freeze-drying. After lyophilization, head tissue was ground into fine powder and stored at −20 °C until chemical analysis.

Carotenoid and Tocopherol Extraction and Quantification. Two sets of 600 mg (corn) and 300 mg (broccoli) of ground tissue from each bulked sample were weighed out for extraction as described by Kurilich and Juvik (*26*). The HPLC system consisted of a Waters 510 pump, a 731a autoinjector, and a 490 E multiwavelength UV−vis detector (Waters Chromatography, Milford, MA). Carotenoids were detected at 450 nm, whereas tocopherols were detected at 290 nm. Separation was performed with a YMC carotenoid C_{30} reverse phase (5 μm, 4.6 × 150 mm) (Waters Chromatography) column, protected by a (5 μm, 4.6 × 7.5 mm) guard column (Alltech Associates, Deerfield, IL). Samples were loaded into amber glass vials with 300 μL glass inserts (Alltech Associates). The mobile phase consisted of a degassed solution of (75:20:5 v/v/v) acetonitrile/methanol/methylene chloride containing 0.05% triethylamine and 0.1% BHT. Twenty microliters of each sample was injected into the HPLC at an isocratic mobile phase flow rate of 1.8 mL/min for a 40 min run. Lutein, zeaxanthin, and β-cryptoxanthin standards were obtained from Extrasynthese (Genay, France) and CaroteNature (Lupsingen, Switzerland), whereas β-carotene and all of the tocopherol isomers (α-, δ-, γ-) were purchased from Sigma Chemical Co. (St. Louis, MO). External standards

Table 1. Means and Standard Deviations (SD), Percent of Total Composition, and Genotype Range of Carotenoid and Tocopherol Content in 41 Corn Genotypes Grown over 3 Years and 24 Broccoli Genotypes Grown over 2 Seasons

compound	mean (μg/g of dry wt)	SD	% of total composition[a]	range of genotype means[b] (μg/g of dry wt)
		Corn		
carotenoids				
lutein	5.9	1.9	65	0.1−18.3
zeaxanthin	2.8	0.8	31	0.1−8.1
β-cryptoxanthin	0.4	0.2	4	0.0−1.8
total	9.1	2.6		0.2−27.8
tocopherols				
δ-tocopherol	0.9	0.2	3	0.2−2.9
γ-tocopherol	20.1	3.7	73	7.6−57.2
α-tocopherol	6.7	1.7	24	2.7−16.2
total	27.7	4.7		11.5−73.9
		Broccoli		
carotenoids				
lutein	29.8	4.1	33	16.5−50.1
zeaxanthin	0.8	0.1	1	0.5−1.3
β-cryptoxanthin	0.7	0.2	1	0.1−2.3
β-carotene	60.3	10.0	65	37.5−103.6
total	91.6	14.0		55.3−153.9
tocopherols				
δ-tocopherol	1.2	2.7	2.2	0.5−2.7
γ-tocopherol	10.4	1.5	19.3	6.2−18.1
α-tocopherol	42.4	7.5	78.5	25.9−83.4
total	54.1	8.4		35.4−98.7

[a] Percent proportion of the compound to total carotenoid or tocopherol content. [b] From lowest to highest genotype.

with six concentrations were run along with each run that contained 28 samples. Standard curves generated linear regressions with coefficients of determination (R^2) that were in the 99th percentile for each standard. These regression equations were used to calculate sample carotenoid and tocopherol concentrations.

Statistical Analysis. Analysis of phenotypic chemical data was performed on means from data generated from two separate analyses of each sample. Analysis of variance (ANOVA) and partitioning of variance components were conducted using the PROC GLM command in SAS 9.1 (*27*). PROC MIXED was used to analyze the effects of genotype, environment, replication, and G×E interaction on total phenotypic variation. Analysis of variance was performed using the linear model: $\chi_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \tau_{k(j)} + \varepsilon_{ijk}$, where χ_{ijk} is the kth replication of the phenotypic value of the ith genotype in year j, μ is the overall mean, α_i is the fixed effect of genotype i, β_j is the random effect of year j, $(\alpha\beta)_{ij}$ is the random interaction effect of genotype i in year j, τ_k is the nested effect of the kth block within the jth environment (year), and ε_{ijk} is the experimental error associated with χ_{ijk}. Pearson correlation coefficients were determined between the means over years and replications of individual and total carotenoids and tocopherols using PROC CORR in SAS. The stability of individual genotypes' performance to the environments was analyzed using Shukla's stability method (*22*). The equation to calculate the Shukla variance was

$$\sigma_i^2 = \frac{p}{(p-2)(q-1)}\sum_{j=1}^{p}(X_{ij} - X_{i.} - X_{.j} + X_{...})^2 - \frac{\mathrm{SSGE}}{(p-1)(p-2)(q-1)}$$

where x_{ij} = the observed mean value of genotype i (i= 1, ..., p) in environment j (j= 1, ..., q), $x_{i.}$ = means of genotype i across all environments, $x_{.j}$ = means of environment j across all genotypes, and $x_{...}$ = overall mean.

Stability variance partitions the G×E interaction component into separate stability variances for each genotype by means of linear regression. The SAS code suggested by Fernandez (*28*) was used to calculate Shukla's stability variance. Relative stability variance, which is a percentage of the proportion of G×E interaction sum of squares explained by each genotype, was calculated to allow comparisons of the relative stability among the tested genotypes by obtaining the product of each stability variance and the appropriate correction factor and then divided by the total sum of squares due to G×xE interaction as follows: relative stability variance = $[(\sigma_i^2)(g-1)(e-1)/g]$/(total sum of squares for G×E)(100), where g is the number of genotypes and e is the number of environments. The genotypes were ranked first according to their mean, their relative stability, and then according to the combined mean and stability ranking.

RESULTS

Mean Variation Carotenoid and Tocopherol Concentrations. Averaged across the 41 corn genotypes and the 3 growing years, the dominant carotenoids were lutein and zeaxanthin, whereas γ-tocopherol and α-tocopherol were the primary tocopherols found in kernels at 21 DAP. In corn, lutein and zeaxanthin accounted for 65 and 31% of the total carotenoids, respectively, whereas β-cryptoxanthin was found to be a minor component (4%) (**Table 1**). γ-Tocopherol accounted for 73% of total tocopherol, whereas α- and δ-tocopherol accounted for 24 and 3%, respectively. Averaged across the 24 broccoli genotypes, the major carotenoids were β-carotene and lutein (65 and 33% of total carotenoids, respectively). In the broccoli lines, α-tocopherol accounted for 78.5% of total tocopherols, whereas γ- and δ-tocopherol accounted for 19.3 and 2.2%, respectively (**Table 1**).

Significant variation in both carotenoid and tocopherol contents was observed among the 41 corn genotypes (**Table 2**). Dramatic differences between the means of the lowest and highest genotypes were observed for lutein (196-fold), zeaxanthin (82-fold), β-cryptoxanthin (81-fold), and total carotenoids (129-fold). Less dramatic differences among genotypes were also observed for δ-tocopherol (13-fold), γ-tocopherol (8-fold), α-tocopherol (6-fold), and total tocopherol (7-fold) (**Table 2**).

The existence of a relationship between the kernel color and carotenoid content in corn has long been known (*29*). Ia453a *sh2* (dark yellow kernels) was the genotype with the highest lutein, zeaxanthin, and total carotenoid contents, whereas genotype IL27a *su1* (light yellow kernels) was among the lowest genotypes for its contents of lutein, zeaxanthin, β-cryptoxanthin, and total carotenoids (**Table 2**). Genotypes with high oil content typically contained more tocopherol, whereas genotypes with low oil

Table 2. Means (Micrograms per Gram of Dry Weight) of Carotenoid and Tocopherol Contents in Kernels of 41 Corn Genotypes at 21 DAP Grown over 3 Years

genotype	Lut[a]	Zea	β-Cryp	t Caro	δ-Toc	γ-Toc	α-Toc	t Toc
1645-121*su1*	3.7	2.7	0.2	6.2	0.5	13.1	2.8	16.5
1645-13*se1*	4.2	2.5	0.2	6.8	0.5	16.1	4.2	20.8
1645-15 *su1*	5.7	2.7	0.2	8.6	0.9	20.2	5.6	26.8
1645-165 *su1*	5.4	3.0	0.2	8.5	1.1	26.3	7.3	34.6
1645-29 *se1*	3.3	2.7	0.2	6.3	0.5	14.4	4.4	19.2
1645-32 *su1*	7.5	3.7	0.4	11.6	0.7	14.9	6.0	21.6
1645-36 *su1*	5.5	3.1	0.3	8.9	1.0	26.0	3.9	31.0
IL2022-11*sh2*	7.3	4.9	0.4	12.5	1.2	29.6	10.8	41.6
IL2027-5 *sh2*	7.9	2.1	0.8	10.8	1.3	18.1	16.0	35.3
IL2027-7 *sh2*	2.7	1.3	0.0	4.0	1.2	37.4	5.3	44.0
IL2027-8 *sh2*	9.9	4.3	0.7	14.9	0.7	13.1	9.1	22.9
A632*Su1*	1.4	3.6	0.3	5.4	0.6	11.6	6.2	18.4
B37 *Su1*	15.9	4.1	1.4	21.5	0.7	17.4	12.7	30.7
B73 *Su1*	6.0	2.4	0.3	8.8	0.8	17.1	4.9	22.8
B84 *Su1*	9.2	2.9	0.3	12.7	0.7	16.3	7.4	24.4
C68 *sh2*	10.9	5.7	0.5	17.2	0.8	15.4	8.9	25.1
IL27a *su1*	0.7	0.5	0.3	1.5	0.7	12.0	3.2	15.8
IL442a *su1*	4.1	2.7	0.4	7.1	1.1	19.1	4.2	24.4
IL451b *se1*	5.4	2.0	0.1	7.5	1.2	28.5	5.2	34.8
IL451b *su1*	10.6	3.3	0.5	14.4	1.0	22.7	5.8	29.4
IL465a *su1*	2.6	1.9	0.3	6.7	0.9	15.5	3.2	19.6
IL607a *su1*	6.3	4.5	0.5	11.3	0.9	18.8	6.6	26.2
IL618b *su1*	7.1	2.5	0.2	9.7	1.1	27.0	4.9	33.0
IL677a *se1*	1.7	2.1	0.2	4.0	1.2	21.2	5.3	27.6
IL678a *se1*	2.6	2.6	0.2	5.3	0.5	16.4	8.0	25.0
IL731a *se1*	1.6	2.2	0.3	4.1	0.9	18.1	4.3	23.3
IL747b *se1*	1.7	1.7	0.1	3.5	1.6	24.2	3.1	28.9
ILHO *Su1*	1.7	1.2	0.2	3.1	2.1	57.2	13.8	73.1
ILHP *Su1*	0.4	0.7	0.0	1.1	0.7	14.1	10.7	25.6
ILLO *Su1*	0.3	0.7	0.0	1.0	0.4	14.2	3.1	17.7
ILLP *Su1*	0.1	0.1	0.0	0.2	0.3	8.4	2.7	11.5
OH43 *su1*	6.3	2.9	0.3	9.5	0.6	19.2	8.2	28.0
R802a *Su1*	13.6	3.3	1.1	18.0	0.8	20.2	7.2	28.2
W64a *Su1*	4.9	3.8	0.5	9.3	0.6	22.8	2.8	26.2
W6786 *su1*	5.5	3.4	0.2	9.1	1.0	25.1	8.3	34.4
WC1 Su1	7.0	3.1	0.2	10.3	0.2	10.5	7.4	18.2
Y1 Su1	2.0	0.9	0.0	2.8	0.9	19.9	5.5	26.3
Y8 Su1	3.0	1.3	0.1	4.3	0.2	7.6	4.2	12.0
Ia453a *sh2*	18.3	8.1	1.4	27.8	1.8	24.2	15.1	41.1
IL451b *sh2*	17.1	5.0	1.8	23.9	1.3	21.8	8.6	31.6
IL451b *Su1*	13.3	3.7	0.4	16.4	1.2	28.4	6.6	36.2
max/min[b]	196-fold	82-fold	81-fold	129-fold	13-fold	8-fold	6-fold	7-fold
overall mean	5.9	2.8	0.4	9.1	0.9	20.1	6.7	27.7
LSD[c]	2.46	1.44	0.14	3.78	0.35	6.34	2.55	8.33

[a] Lut, lutein; Zea, zeaxanthin; β-Cryp, β-cryptoxanthin; t Caro, total carotenoids; δ-Toc, δ-tocopherol; γ-Toc, γ-tocopherol; α-Toc, α-tocopherol; t Toc, total tocopherol. [b] Means of the highest divided by the lowest genotype for each compound. [c] LSD, least significance difference at $P = 0.05$.

content had low tocopherol levels. ILHO (Illinois High Oil selection) *Su1* was the highest genotype for δ-, γ-, and α-tocopherol and total tocopherol, whereas genotypes ILLO (Illinois Low Oil selection) *Su1* and ILLP (Illinois Low Protein selection) *Su1* with low oil content had very reduced concentrations of tocopherols (**Table 2**). Genotypes IL2022-11 *sh2* and IL2027-7 *sh2* with high oil content (data not shown) also contained elevated tocopherol concentrations.

Corn genotypes were grouped according to their endosperm types (14 *Su1*, 13 *su1*, 7 *se1*, 7 *sh2*), and the contents of different carotenoids and tocopherols were averaged for each type. Means of the *sh2* endosperm mutation genotypes were significantly greater than the means of the other three endosperm types for all forms of carotenoids and tocopherols. The effect of corn endosperm type on kernel carotenoid and tocopherol content was also investigated through the evaluation and comparison of content of the four isolines of IL451b (IL451b *Su1*, IL451b *su1*, IL451b *se1*, IL451b *sh2*), and the results showed that effects associated with endosperm type on kernel carotenoid content in corn isolines were comparable with that observed among the combined genotypes (data not shown). IL451b *sh2* had the highest concentration and was significantly different from other endosperm types for all forms of carotenoids but not tocopherols.

Significant variation in both carotenoid and tocopherol contents was also observed among the 24 broccoli genotypes (**Table 3**). Moderate differences between the means of the lowest and highest genotypes were observed for carotenoids (3.0-, 2.6-, 23-, 2.8-, and 2.8-fold for lutein, zeaxanthin, β-cryptoxanthin, β-carotene, and total carotenoids, respectively) and for tocopherols (5.4-, 2.9-, 3.2-, and 2.8-fold for δ-, γ-, and α-tocopherol and total tocopherols, respectively) (**Table 3**).

Correlation Analysis. Pearson correlation analysis was conducted on all compounds and as anticipated due to similar biosynthetic origin, high correlation coefficients were observed among all of the carotenoids for both corn and broccoli (data not shown). Lower but significant correlation coefficients were

Table 3. Means (Micrograms per Gram of Dry Weight) and LSDs for Carotenoid and Tocopherol Contents in Florets of 24 Broccoli Genotypes Grown over 2 Seasons (Spring and Fall 2004)

genotype	Lut[a]	Zea	β-Cryp	β-Caro	t Caro	δ-Toc	γ-Toc	α-Toc	t Toc
Baccus	50.1	0.9	0.5	102.5	153.9	1.3	9.5	30.8	41.6
Atlantic	45.7	1.3	0.8	103.6	151.4	1.3	8.1	45.2	54.6
BNC	41.3	1.0	2.3	94.5	139.0	0.9	18.1	27.6	46.6
SU006	39.1	0.8	1.7	86.5	128.0	1.0	10.6	55.5	67.1
Galaxy	46.6	1.1	0.8	74.3	122.9	1.5	7.7	26.2	35.4
Packman	36.8	0.6	0.1	69.6	107.2	0.7	10.2	25.9	36.8
SU003	35.8	1.1	1.3	66.8	105.0	1.0	7.6	50.0	58.6
Brigadier	31.3	0.7	1.0	69.7	102.7	0.7	8.2	43.1	52.0
VI-158	30.1	0.7	0.9	68.5	100.2	1.2	10.2	40.4	51.8
EV6-1	33.7	1.2	0.1	58.7	93.7	0.9	14.2	32.9	48.0
Pirate	29.5	0.9	0.8	60.5	91.7	0.7	10.2	59.9	70.7
Peto-7	27.4	0.9	0.6	56.1	85.0	2.1	13.2	83.4	98.7
SBC-9311	26.7	1.0	0.6	56.7	85.0	1.7	9.3	33.1	44.0
Peto-13	29.9	0.7	0.9	52.0	83.4	0.8	11.2	40.6	52.6
Mariner	27.1	0.7	0.8	52.5	81.2	1.7	7.1	33.6	42.4
Pinnacle	21.8	0.7	0.8	48.6	71.9	0.9	14.8	29.6	45.3
High Sierra	21.6	0.6	0.1	49.4	71.7	0.5	8.4	43.2	52.1
Fluorite	22.1	0.5	0.2	41.7	64.4	0.8	7.3	37.9	46.0
Majestic	21.8	0.7	0.5	39.8	62.8	1.8	9.6	46.4	57.8
EU8-1	22.9	0.8	0.1	39.0	62.7	2.0	14.3	64.4	80.7
Marathon	19.8	0.5	0.7	41.4	62.3	2.7	10.3	54.2	67.1
Zeus	21.4	0.6	0.6	38.8	61.4	0.9	12.6	27.7	41.1
Legacy	17.1	0.7	0.5	37.5	55.7	0.7	11.8	44.4	56.9
Triathlon	16.5	0.5	0.5	37.9	55.3	1.2	6.2	42.3	49.7
max/min[b]	3.0-fold	2.6-fold	23.0-fold	2.8-fold	2.8-fold	5.4-fold	2.9-fold	3.2-fold	2.8-fold
LSD[c]	7.7	0.2	0.5	20.0	26.7	0.4	3.0	13.5	15.0

[a] Lut, lutein; Zea, zeaxanthin; β-Cryp, β-cryptoxanthin; t Caro, total carotenoids; δ-Toc, δ-tocopherol; γ-Toc, γ-tocopherol; α-Toc, α-tocopherol; and t Toc, total tocopherol. [b] Means of the highest divided by the lowest genotype for each compound. [c] LSD, least significance difference at $P = 0.05$.

observed between the dominant tocopherols. Low to moderate correlation coefficients were also observed between carotenoids and tocopherols. In corn, α-tocopherol was significantly correlated with lutein, zeaxanthin, and total carotenoids. No significant negative correlations were observed.

Partitioning of Variance. In broccoli, to partition the phenotypic variation the data were divided into two different sets, one consisting of 13 genotypes grown over 3 seasons (fall 2003, spring 2004, and fall 2004) and the second consisting of 24 genotypes grown over 2 seasons in the same year (spring and fall 2004). Differences among broccoli genotypes described the greatest proportion of variation in the model in both the two- and three-environment data sets. Mean squares due to the genotypic effect was significant for most of the compounds, and genotypic sum of squares accounted for 50, 44, 49, 35, 43, 47, 32, and 30% of the total sum of squares for lutein, zeaxanthin, β-cryptoxanthin, β-carotene, total carotenoids, γ- and α-tocopherol, and total tocopherols, respectively, in the three-seasons data set, whereas higher proportions were observed in the two-seasons data set (68, 70, 60, 57, 61, 50, 44, and 39% for the same compounds, respectively) (**Table 4**).

As observed for broccoli genetic differences among corn lines described most of the variation for both carotenoid and tocopherol contents. Mean squares due to genotype effects were significant, and genotypic sums of squares accounted for 75, 60, 69, 80, 67, 78, 56, and 75% of the total sum of squares in the model for lutein, zeaxanthin, β-cryptoxanthin, total carotenoids, δ-, γ-, and α-tocopherol, and total tocopherol, respectively (**Table 4**). Similar to corn, variation due to the interaction between genotype and environment was significant and represented the second most important factor contributing to the total variation for both corn and broccoli for all carotenoids except β-carotene in the three-environments broccoli data set (**Table 4**).

Variation affiliated with environment was found to be minor, although significant, for zeaxanthin, β-cryptoxanthin, δ-tocopherol, and α-tocopherol in corn (**Table 4**). In broccoli, the environmental variation was significant for β-carotene, total carotenoid, α-tocopherol, and total tocopherol in both data sets. This source of variation was a minor contributor to the total variation except for β-carotene in the three-environments data set. Large environmental variation was observed in both broccoli data sets for α-tocopherol and total tocopherol (**Table 4**).

Stability Analysis. Stability analysis identified several corn genotypes with relatively high carotenoid and tocopherol concentrations and relatively high stability (**Table 5**), suggesting that the concentration of these compounds in specific genotypes is consistent across years. Although IL451b *sh2* was the second highest genotype for carotenoids, it accounted for only 0.2 and 1.0% of the G×E sum of squares for lutein and total carotenoid content compared to 22 and 13% for Ia453a *sh2*, the genotype with the highest concentration for both compounds. IL451b *sh2*, B37 *Su1*, and R802a *Su1* had high lutein with good stability (3.7 and 3.3% of the G×E sum of squares, respectively). IL2022-11 *sh2* and IL607a *su1* showed high zeaxanthin contents, whereas maintaining good stability (only 4.0%, and 1.0% respectively of GxE sum of squares). For tocopherol, genotypes IL2027-7 *sh2*, IL2022-11 *sh2* and IL451b *Su1* were among the highest genotypes in γ-tocopherol and total tocopherol contents with stability variance estimates representing only 0.16, 1.6, and 0.22%, respectively, for γ-tocopherol and 0.35, 1.4, and 1.1% (as % of G×E sum of squares), respectively, for total tocopherol. Kernels of IL2027-5 *sh2* had the highest α-tocopherol content while contributing only 3.5% of the G×E sum of squares.

Stability analysis also identified several broccoli genotypes with relatively high carotenoid and tocopherol concentrations that displayed high stability across seasons and years (**Table 6**).

Table 4. Percentages of Total Phenotypic Variation and Significance Level in Carotenoid and Tocopherol Concentrations Associated with Genotype, Environment, and Genotype × Environment Interaction for 41 Corn Genotypes Grown over 3 Years and 13 and 24 Broccoli Genotypes Grown over 3 (Fall 2003 and Spring and Fall 2004) or 2 (Spring and Fall, 2004) Seasons, Respectively

source of variation		Lut[a]	Zea	β-Cryp	β-Caro	t Caro	δ-Toc	γ-Toc	α-Toc	t Toc
genotype (G)	corn	75.0	59.8	68.8		79.7	67.4	77.6	55.7	75.2
		<0.0001	<0.0001	<0.0001		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	broccoli	49.8	44.2	48.5	34.6	43.4	48.0	46.8	32.0	30.0
	(3 environments)	0.0177	0.0554	0.0076	0.0429	0.0130	0.0459	0.0563	ns	ns
	broccoli	68.1	70.4	59.7	57.1	60.1	47.4	50.2	44.1	38.9
	(2 environments)	0.0053	0.0007	ns	ns	0.0354	ns	ns	ns	ns
environment (E)	corn	1.8	7.7	14.2		0.3	7.0	6.4	11.8	2.1
		ns	0.0490	<0.0001		ns	0.0078	ns	0.0008	ns
	broccoli	6.0	10.1	13.1	33.0	22.9	8.7	7.3	30.1	28.6
	(3 environments)	ns	ns	ns	0.0003	0.0037	ns	ns	0.0019	0.0032
	broccoli	7.3	9.7	1.9	9.8	9.1	14.6	7.5	22.5	23.7
	(2 environments)	0.0283	0.0162	ns	0.0186	0.0204	0.0064	ns	0.0011	0.0016
G×E	corn	21.1	28.3	16.6		17.1	23.8	19.7	30.7	19.5
		<0.0001	<0.0001	<0.0001		<0.0001	0.0001	0.0002	<0.0001	0.0014
	broccoli	36.6	41.5	30.5	30.6	30.1	43.1	44.1	34.2	37.8
	(3 environments)	<0.0001	<0.0001	0.0001	0.0005	0.0003	<0.0001	<0.0001	<0.0001	<0.0001
	broccoli	22.6	17.3	33.9	31.5	28.2	37.6	39.8	31.8	35.6
	(2 environments)	<0.0001	0.0002	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
R^2 [b] described by the model	corn	0.87	0.72	0.94		0.83	0.78	0.76	0.80	0.74
	broccoli (3 environments)	0.77	0.79	0.77	0.75	0.76	0.84	0.77	0.81	0.80
	broccoli (2 environments)	0.82	0.80	0.80	0.78	0.80	0.89	0.79	0.82	0.83

[a] Lut, lutein; Zea, zeaxanthin; β-Cryp, β-cryptoxanthin; β-Caro, β-carotene; t Caro, total carotenoids; δ-Toc, δ-tocopherol; γ-Toc, γ-tocopherol; α-Toc, α-tocopherol; t Toc, total tocopherol. [b] R^2 = coefficient of determination.

'Baccus', 'Pirate', and 'Packman' were among the most stable genotypes with high carotenoid content. Other stable genotypes were 'Baccus' and 'Pirate' for lutein, 'Pirate' for zeaxanthin, BNC for β-cryptoxanthin, and 'Pirate' and Peto-13 for β-carotene in both the two- and three-environment data sets. For tocopherols 'Pirate', 'Marathon', and Peto-7 were the most stable for α-tocopherol and total tocopherols, whereas BNC and 'Marathon' were the most stable for γ-tocopherol.

DISCUSSION

Both sweet corn and broccoli are known to be relatively rich sources of carotenoids and tocopherols (*18*, *19*). The level of protection against degenerative diseases provided by a particular phytochemical is related to the bioavailability of the compound following ingestion and the resultant levels observed in human blood serum. Epidemiological studies have shown that plasma levels of β-carotene of >0.4 μmol/L (equivalent to an intake of 2−4 mg/day) and α-tocopherol levels of >30 μmol/L (about 15−30 mg/day) have been associated with decreased risk of degenerative diseases (*30*). Accordingly, it is possible to estimate from our data that daily consumption of approximately 100 g of broccoli could provide a level of β-carotene recommended for a protective effect. Available medical evidence suggests that daily consumption of at least 6 mg of lutein and zeaxanthin from fruits and vegetables may decrease the risk of age-related macular degeneration (AMD) (*31*). Consequently, daily consumption of approximately 150 g (one medium-sized ear) of a sweet corn genotype that is rich in lutein and zeaxanthin (Ia453a *sh2*) will provide the recommended dose for decreasing the risk of AMD. This is without consideration of concurrent intake of carotenoids and tocopherols in the daily diet from other foods.

This investigation uncovered substantial phenotypic variability for all of the carotenoids and tocopherols assayed among the 41 corn and 24 broccoli genotypes. The concentrations for corn calculated in this study are similar to those reported by Lee et al. (*32*) and by Kurilich and Juvik (*18*) in sweet corn (21 DAP). In this investigation, whereas dramatic variation among corn genotypes was observed, variation among broccoli genotypes was not as pronounced, which may reflect the narrow genetic variability found among this recently domesticated and less cosmopolitan crop species. In addition, the range of variation in carotenoid and tocopherol concentrations may be more restricted in green tissues such as broccoli florets due to the role they play in protection from radical oxygen species generated via photosynthesis versus non-photosynthesizing seeds. Broccoli concentrations calculated in this investigation tend to agree with the values previously reported in the literature (*19*).

This investigation found that the phenotypic variation existing among maize and broccoli genotypes for both carotenoid and tocopherol contents is primarily under genetic control, which suggests that genetic manipulation and selection can be conducted to modify levels of these phytochemicals and consequently the putative health promotion associated with consumption of these vegetables. The observation that the greater proportion of the phenotypic variation is associated with differences among genotypes tends to agree with previous work concerning the regulation of carotenoids and tocopherol in maize (*17*, *24*) and

Table 5. Means (Micrograms per Gram of Dry Weight) and Relative Stability Variance[a] of Different Carotenoids and Tocopherols in Kernels at 21 DAP of 41 Corn Genotypes Grown over 3 Years

	total carotenoid					total tocopherol				
genotype	mean	mean rank[b]	stability	stability rank[c]	overall rank[d]	mean	mean rank	stability	stability rank	overall rank
1645-121*su1*	6.2	28	0.18	12	17	16.5	37	2.10	29	39
1645-13*se1*	6.8	25	0.10	9	8	20.8	32	2.92	33	37
1645-15 *su1*	8.6	21	0.36	13	9	26.8	19	1.92	25	24
1645-165 *su1*	8.5	22	0.85	18	18	34.6	8	1.63	19	6
1645-29 *se1*	6.3	27	0.00	1	4	19.2	34	0.79	11	26
1645-32 *su1*	11.6	11	3.67	34	29	21.6	31	0.23	5	14
1645-36 *su1*	8.9	19	1.16	24	24	31.0	12	4.65	35	30
IL2022-11*sh2*	12.5	10	9.58	38	31	**41.6**[e]	**3**	**1.41**	**18**	**3**
IL2027-5 *sh2*	10.8	13	14.41	41	36	35.3	6	1.65	20	5
IL2027-7 *sh2*	4.0	33	1.12	23	38	**44.0**	**2**	**0.35**	**7**	**1**
IL2027-8 *sh2*	14.9	7	12.23	39	30	22.9	29	1.29	17	27
A632*Su1*	5.4	29	0.02	4	6	18.4	35	2.42	30	38
B37 *Su1*	21.5	3	5.81	36	16	30.7	13	2.01	27	18
B73 *Su1*	8.8	20	0.65	16	12	22.8	30	0.15	2	10
B84 *Su1*	12.7	9	1.66	27	13	24.4	26	1.79	24	32
C68 *sh2*	17.2	5	3.66	33	14	25.1	24	0.45	8	11
IL27a *su1*	1.5	38	0.38	14	33	15.8	39	0.15	3	21
IL442a *su1*	7.1	24	2.30	30	37	24.4	27	0.96	13	19
IL451b *se1*	7.5	23	0.93	20	25	34.8	7	2.68	32	17
IL451b *su1*	14.4	8	4.61	35	26	29.4	14	1.15	16	7
IL465a *su1*	6.7	26	0.01	3	5	19.6	33	0.06	1	12
IL607a *su1*	11.3	12	1.61	26	15	26.2	21	0.75	10	8
IL618b *su1*	**9.7**	**15**	**0.05**	**6**	**1**	33.0	10	5.5	36	28
IL677a *se1*	4.0	34	0.09	8	22	27.6	18	6.14	38	34
IL678a *se1*	5.3	30	0.15	11	19	25.0	25	5.71	37	36
IL731a *se1*	4.1	32	1.01	21	35	23.3	28	0.91	12	20
IL747b *se1*	3.5	35	0.69	17	34	28.9	15	1.72	22	15
ILHO *Su1*	3.1	36	3.62	32	40	73.1	1	13.16	41	22
ILHP *Su1*	1.1	39	0.02	5	28	25.6	23	1.08	14	16
ILLO *Su1*	1.0	40	0.12	10	32	17.7	41	2.62	31	23
ILLP *Su1*	0.2	41	0.00	2	27	11.5	37	0.29	6	41
OH43 *su1*	9.5	16	0.87	19	11	28.0	17	0.16	4	4
R802a *Su1*	18.0	4	5.92	37	20	28.2	16	4.34	34	33
W64a *Su1*	9.3	17	1.21	25	23	26.2	22	0.59	9	9
W6786 *su1*	9.1	18	0.62	15	7	34.4	9	8.62	39	31
WC1 Su1	**10.3**	**14**	**0.05**	**7**	**2**	18.2	36	1.69	21	35
Y1 Su1	2.8	37	2.69	31	41	26.3	20	1.96	26	29
Y8 Su1	4.3	31	2.27	29	39	12.0	40	2.03	28	40
Ia453a *sh2*	27.8	1	12.68	40	21	41.1	4	9.17	40	25
IL451b *sh2*	**23.9**	**2**	**1.05**	**22**	**3**	31.6	11	1.74	23	13
IL451b *Su1*	16.4	6	1.73	28	10	**36.2**	**5**	**1.14**	**15**	**2**

[a] Relative stability variance = $[(\sigma_i^2)(g-1)(e-1)/g]$/(total sum of squares for G×E)(100). [b] Rank of the genotypes according to their mean. [c] Rank of the genotypes according to their relative stability. [d] Rank of the genotypes according to their combined mean and stability rank (mean rank + stability rank = overall rank). [e] Numbers in bold display high overall rank.

other crops (*33*, *34*). Selection for these compounds in corn kernels and broccoli floret tissue should effectively enhance the content of these antioxidants in a breeding program.

Although several reports in the literature have indicated that the environment can play a major role in determining carotenoid (*35*) and tocopherol (*36*) concentrations in plants, our data suggest that environmental effects are not key in the regulation of carotenoid and tocopherol content in maize. To our knowledge this is the first study to evaluate the G×E interaction and genetic stability for these phytochemicals in kernels of corn genotypes at 21 DAP. Significant environmental variation for carotenoid and tocopherol concentrations was observed among broccoli lines evaluated in the fall of 2003 and 2004 and between spring and fall plantings in 2004. Genotypes in the spring (harvested in the summer) and the fall evaluation would experience significantly different temperature regimens, light intensity and quality, day length, and rainfall, environmental factors that all have been reported to influence carotenoid and tocopherol content in plants (*35*−*37*). Although this study took place in one location, the genotypes experienced substantially different growing conditions across the growing environments. Lower than optimal temperatures at spring transplanting and higher than optimal temperature at maturity and harvest provide an environment distinct from that of a summer planting and fall harvest. Biotic stresses would also be unique to plants grown in each season. All of these factors may have contributed individually or collectively to explain the greater contribution of the environment in determining carotenoid and tocopherol contents among broccoli accessions. The greater contribution from environmental variation is also a function of the fact that total phenotypic variation in these compounds was less than that observed for corn.

Potential factors explaining the genetic and G×E interaction components of variation in these phytochemical concentrations are that environmental stimuli are both up- and down-regulating known genes associated with carotenoid and tocopherol

Table 6. Means (Micrograms per Gram of Dry Weight) and Relative Stability Variance [a] of Total Carotenoid and Tocopherol Concentrations in 24 Broccoli Genotypes Grown over 2 (Spring and Fall 2004) or 3 (Fall 2003 and Spring and Fall 2004) Seasons

	total carotenoid					total tocopherol				
genotype	mean	mean rank[b]	stability	stability rank[c]	overall rank[d]	mean	mean rank	stability	stability rank	overall rank
Florette	64.4	17	5.3	15	19	46.0	17	6.5	18	24
Mariner	81.2	14	0.1	2	5	42.4	20	0.0	1	13
Triathlon	55.3	24	1.1	11	23	49.7	14	3.1	12	18
Galaxy	122.9	5	5.4	16	12	35.4	24	0.1	2	19
SBC9311	85.0	12	0.2	3	4	44.0	19	0.0	1	11
Atlantic	151.4	2	4.9	14	6	54.6	9	2.4	10	9
Pinnacle	71.9	15	1.7	13	16	45.3	18	1.0	4	15
SU006	128.0	4	10.8	19	14	67.1	4	3.3	13	6
Packman	**107.2**	**6**[e]	**0.7**	**7**	**3**	36.8	23	1.3	6	21
EV6-1	93.7	10	26.7	22	20	48.0	15	4.9	16	23
Zeus	61.4	21	1.6	12	22	41.1	22	0.2	3	17
VI-158	100.2	9	0.8	9	7	51.8	13	1.2	5	8
SU003	105.0	7	11.0	20	15	58.6	6	3.7	14	12
EU8-1	62.7	20	9.7	18	24	80.7	2	53.7	19	14
BNC	139.0	3	6.7	17	10	46.6	16	0.0	1	7
Peto-13	83.4	13	0.5	6	8	**52.6**	**10**	**0.0**	**1**	**3**
High Sierra	71.7	16	0.3	5	13	52.1	11	0.0	1	4
Majestic	62.8	19	0.0	1	11	57.8	7	4.6	15	16
Legacy	55.7	23	0.7	8	18	56.9	8	2.5	11	10
Brigadier	102.7	8	11.3	21	17	52.0	12	6.4	17	22
Baccus	**153.9**	**1**	**0.2**	**4**	**1**	41.6	21	1.4	7	20
Pirate	**91.7**	**11**	**0.0**	**1**	**2**	**70.7**	**3**	**0.1**	**2**	**1**
Marathon	63.3	18	0.0	1	9	67.1	5	2.1	9	5
Peto-7	56.1	22	0.9	10	21	**98.7**	**1**	**1.5**	**8**	**2**

[a] Relative stability variance = $[(\sigma_i^2)(g-1)(e-1)/g]$/(total sum of squares for G×E)(100). [b] Rank of the genotypes according to their mean. [c] Rank of the genotypes according to their relative stability. [d] Rank of the genotypes according to their combined mean and stability rank. [e] Numbers in bold display high overall rank.

biosynthesis. The mRNA levels of *PSY* (gene encoding phytoene synthase) increased under high light intensity in both mustard and *Arabidopsis* (*38*) and *ctr-b* and *ctr-e* (genes that encode lycopene cyclase) in *Arabidopsis* and tomato (*39*). The environmental effect was a relatively unimportant source of variation (except for β-carotene and α-tocopherol in broccoli).

Correlation analysis revealed significant positive associations between concentrations of various carotenoids and tocopherols in corn kernels and broccoli floret tissue. This suggests that the development of maize and broccoli genotypes with improved content of these phytochemicals is a feasible breeding objective and that selection for increased carotenoid and tocopherol contents can be simultaneously conducted within segregating populations.

The stability analysis identified several genotypes with relatively high carotenoid and tocopherol contents and comparatively good stability across the growing environments. These lines may possess broad-range tolerance to both biotic and abiotic environmental stresses. IL451b *sh2*, C68 *sh2*, and B37 *Su1* are potential donors of genes to enhance carotenoid levels in immature corn kernels, whereas germplasm that can be used to enhance tocopherol levels include IL2027-7 *sh2*, IL2022-11 *sh2*, and IL451b *Su1*. Commercial genotypes such as 'Pirate', 'Marathon', and 'Baccus' showed high stability for most compounds across environments, making them potential parents in any breeding program aiming to enhance carotenoid and tocopherol levels in broccoli.

The quantitative differences in carotenoid and tocopherol contents detected in this investigation may reflect allelic variation of gene loci regulating the biosynthesis of these compounds. The results also suggest that currently available commercial and public germplasm can be utilized in breeding programs to develop new sweet corn and broccoli germplasm with elevated carotenoid and tocopherol contents. Stability differences detected among genotypes tested in this study emphasize the need to survey multiple environments. In conclusion, our results support the feasibility of conventional breeding to increase both carotenoid and tocopherol contents in corn and broccoli. Heritability studies on carotenoid and tocopherol contents in two sweet corn and broccoli populations are underway to substantiate these results and estimate trait gain over cycles of selection. Extrapolation of the results obtained in this study to other corn and broccoli genotypes and growing environments is constrained by the fact that this investigation was conducted in a single location over multiple years.

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