Quantification of Carotenoid and Tocopherol Antioxidants in *Zea mays*

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Recent investigations into carotenoid and tocopherol biological activity in mammalian systems indicate that these antioxidants are associated with the prevention of degenerative diseases. Both carotenoids and tocopherols can be found in corn kernel tissue. A replicated survey of 44 sweet and dent corn lines was conducted to determine qualitative and quantitative variability of lutein, zeaxanthin, β-cryptoxanthin, α-carotene, and β-carotene, as well as the α-, δ-, and γ- forms of tocopherol. The primary carotenoids in fresh market sweet corn were found to be lutein and zeaxanthin, with the γ form dominating among the tocopherols. Mean values among the genotypes were observed to range from 0 to 20.0 and 2.4 to 63.3 μg/g dry weight for lutein and γ-tocopherol, respectively, indicating variability among genotypes in genes regulating the metabolism of these compounds. The observed genetic variability suggests profound differences in potential health promotion among genotypes and supports the feasibility of developing germplasm with enhanced levels of these antioxidant compounds at dosages that could promote health among the consuming public.

Keywords: *Zea mays; sweet corn; carotenoids; tocopherols; antioxidants*

INTRODUCTION

The composition of carotenoids and tocopherols in field corn grain has been studied extensively due to their importance in animal nutrition. Carotenoids are precursors to vitamin A and are used to enhance pigmentation of egg yolks and poultry skin (Weber, 1987). The tocopherols are important sources of vitamin E in animal diets. Diversity in carotenoid and tocopherol composition has been reported at the mature dry stage in field corn (Combs and Combs, 1985; Galliher et al., 1985; Weber, 1987). However, only a few studies with limited numbers of genotypes (3−4) have been conducted to evaluate variability in sweet corn germplasm at fresh market maturity (18−24 days after pollination, DAP). Lee et al. (1981) surveyed four commercial cultivars of sweet corn for carotenoid content, La Bonte and Juvik (1990) investigated three sweet corn inbreds for total carotenes, and Contreras-Guzman et al. (1982) assayed four varieties, including two sugary lines, for tocopherol content. There is also limited published information pertaining to the genetic control and biosynthetic interactions of these compounds. In the past, quality characteristics such as kernel sweetness and tenderness have been the focus of sweet corn varietal development. However, recent clinical and epidemiological studies highlighting the chemoprotectant properties of carotenoid and tocopherol antioxidants in human health has led to an interest in studying these compounds at the fresh market stage in sweet corn.

Oxidation occurs naturally in metabolic systems and can often lead to such conditions as cancer and cardiovascular disease (Roberfroid, 1995). Antioxidants prevent or terminate oxidation reactions by scavenging free radicals and returning them to the ground state. Carotenoids and tocopherols are two important groups of antioxidants that are relatively abundant in sweet corn. Research into the antioxidant activity of carotenoids and tocopherols has implied that a higher intake of these compounds leads to a reduced risk of cancer (Sies and Stahl, 1995; Phillips et al., 1993; Wolf, 1994).

Carotenoids are a diverse group of yellow-orange pigments found in many biological systems. They can be divided into two general classes: the carotenes and the xanthophylls (Bender, 1992; Groff et al., 1995). Carotenes are hydrocarbons, such as α- and β-carotene and lycopene, while xanthophylls are oxygenated derivatives of the carotenes and include the compounds lutein, β-cryptoxanthin, and zeaxanthin. Some carotenoids are also precursors to vitamin A. Carotenoids have been linked to inhibition of neoplastic transformation (Bertram and Bortkiewicz, 1995), photoprotection (Mathews-Roth, 1986), and immunoprotection (Bendich and Shipiro, 1986). β-Carotene has been associated with enhanced immune response by increasing the percentage of leukocytes found in peripheral blood (Bendich and Shipiro, 1986) and blocking suppression of lymphocytes and helper T lymphocytes caused by UV exposure (White et al., 1988). Lutein and zeaxanthin are found in the eye and have been associated with reduced risk of cataract development and age-related macular degeneration (Knekt et al., 1992; Seddon et al., 1994). Certain carotenoids, including β-carotene, can activate gene expression for proteins that are required in cell-to-cell communication (Bendich, 1993). The vitamin A activity of the carotenes is important for healthy skin, bones, and gastrointestinal and respiratory systems. They also play a role in achieving clear vision and preventing night blindness (Combs, 1992).

Tocopherols are vitamin E analogues including α-, β-, γ-, and δ-tocopherol. They function as the main anti-

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oxidants in biological lipid membranes, protecting against peroxyl radicals and nitrogen oxide species (Christen et al., 1997). In addition to antioxidation, tocopherols provide antiproliferative effects (Azzi et al., 1995), anticlotting activity (Dowd and Zheng, 1995), and immunoprotection (Meydani, 1995). The vitamin E activity of these compounds is important for maintaining stable cell membranes and preventing damage to tissues (Combs, 1992).

Evidence for the role of carotenoids and tocopherols in the prevention and treatment of disease is both convincing and encouraging. To investigate the potential health benefits of these antioxidants in maize, a more comprehensive set of information is needed of antioxidant form and content found within corn germplasm. Carotenoids and tocopherols typically found in corn include β-carotene, α-carotene, β-cryptoxanthin, lutein, zeaxanthin (Weber, 1987; Sies and Stahl, 1995), and α-, δ-, and γ-tocopherols (Weber, 1987; Grams et al., 1970). Elevated levels of tocopherols are found within the embryo, while carotenoids are more commonly associated with the kernel endosperm (Weber, 1987).

The following study was designed to provide a database of information on carotenoid and tocopherol variability of both sweet and dent corn germplasm at 20 days after pollination (DAP). This represents the first phase of a program to investigate the genetic control of carotenoids and tocopherols in corn. Results from this survey will be used in breeding programs to create elite genotypes containing enhanced antioxidant and vitamin levels with potential health benefits.

MATERIALS AND METHODS

Plant Material. Forty-four corn inbreds, hybrids, and genetic stocks were selected on the basis of previously published reports (Weber, 1987), variation in kernel pigmentation, which has been associated with carotenoid content (Steenbock, 1919), and differences in lipid and protein content and embryo size. The oil content in the germ has been positively correlated with tocopherol content and negatively correlated with protein content in field corn at the mature dry stage (Levy, 1973; Dudley and Lambert, 1992).

Each of the genotypes selected for this survey was homozygous for one of four different maize endosperm carbohydrate mutations. Dent or field corn, which carries the wild-type allele at the *Sugary1* locus has low kernel sugar and high starch levels and is utilized primarily as animal feed. The *sugary1* (*su1*) mutant allele at this locus is the traditional sweet corn endosperm mutation, with a desirable creamy texture conferred by high levels of phytoglycogen. The *shrunken2* (*sh2*) mutation results in kernels with approximately 2−3 times the sugar content of *su1* but lacks phytoglycogen (Creech, 1965; Gonzales et al., 1976). *Sugary enhancer1* (*se1*) is a modifier of the *su1* mutation that, when homozygous, increases kernel sugar to levels comparable to *sh2* cultivars while maintaining a creamy texture associated with the synthesis of phytoglycogen (Ferguson et al., 1978; Gonzales et al., 1976).

Table 1 lists the genotypes surveyed and indicates the basis for their inclusion in this investigation. The dent corn inbreds A632, B37, B73, B84, R802a, R806, and W64a were selected based on published carotenoid and tocopherol levels (Weber, 1987). Total carotenes of the sweet corn inbreds IL451b *su1*, IL677a *se1*, and IL678a *se1* were reported by La Bonte and Juvik (1990).

Additional genotypes were selected for potential variability in carotenoid content based on kernel color. *White cap1 (WC1)*, *Y1*, and *Y8* are mutants of dent corn that confer a white or pale yellow endosperm (Maize Genome Database, 1995). The sweet corn inbreds IL27a, IL607a, IL677a, and IL747b were also chosen for their light yellow endosperm color. IL465a, IL442a, and IL678a have orange-colored endosperm, while C68, Ia453, IL618b, and OH43 display a dark yellow endosperm color. The near isolines A10664a (yellow endosperm) and A10664c (bronze endosperm), previously bred for early pigment development, were also chosen for comparison.

The Illinois low-oil (ILLO), Illinois high-oil (ILHO), Illinois low-protein (ILLP), and Illinois high-protein (ILHP) composite lines were selected for potential variability in tocopherol content based on oil and protein content. This material underwent 90 generations of selection for high-oil, low-oil, high-protein, or low-protein content in the Illinois Long Term Selection Experiment at the University of Illinois (Dudley and Lambert, 1992). Also selected on the basis of oil and protein content were the sweet corn genotypes IL2022-11, IL2027-5, IL2027-7, and IL2027-8, which were generated from crosses in our field plots (IL2022-11 = C68 *sh2* × ILHO; IL2027-5, IL2027-7, and IL2027-8 = IL451b *sh2* × ILHP). These lines underwent two generations of backcrossing to the *sh2* parent, then four generations of self-pollination.

Several segregating $F_{2:3}$ families, designated with the number 1645-*x*, from a previously created population (IL731a *se1* × W6786 *su1*) were also selected for evaluation. This population has previously been investigated for composition and eating quality characteristics, such as oil, protein, sucrose, starch, phytoglycogen, and tenderness (Azanza et al., 1996). The four IL451b lines, each isogenic and homozygous for either the *Su1*, *su1*, *se1*, or *sh2* alleles, were selected to assay for associations between carotenoid content and endosperm mutation.

IL731a *se1*, W6786 *su1*, and three randomly selected families from the 1645 population (1645-73, 1645-93, 1645-107) were assayed to evaluate changes in carotenoid and tocopherol physiology during kernel development. Four ears from each of two replications were harvested at 18, 21, 24, and 27 DAP for each of the five genotypes. These harvest dates were selected because most (>80%) of the dry weight accumulation in kernel development occurs between 15 and 30 DAP. Kernels of sweet corn achieve their maximum dry weight between 25 and 30 DAP (Doehlert et al., 1993).

Seed was obtained from the University of Illinois sweet corn germplasm collection and the USDA Maize Genetics Stock Center (University of Illinois). The seed was germinated in the greenhouse before seedlings were transplanted into the University of Illinois South Farm field in a randomized complete block design with three replications. Fertilizer, irrigation, and pesticides were applied according to standard commercial practices.

Six to eight ears were harvested from each replication for each genotype at 20 DAP, immediately frozen in liquid nitrogen, and placed on dry ice for transport to the laboratory. Equal numbers of kernels were removed from the center portion of each ear, bulked into sample bags, and stored at −80 °C until lyophilization. After freeze-drying, samples were ground to a fine powder using coffee grinders and stored at −20 °C.

Extraction and Analysis. The extraction procedure employed (Kurilich and Juvik, unpublished material) is a modification of a method described by Weber (1987). All sample preparations and extractions were done under gold fluorescent lights. Tissue samples of 600 mg underwent a 5 min ethanol precipitation (6 mL of ethanol containing 0.1% butylated hydroxytoluene (BHT)) in an 85°C water bath before being subjected to a 10 min saponification with 120 μL of 80% potassium hydroxide (KOH). All samples were vortexed once during saponification. Upon removal they were immediately placed in an ice bath where 3 mL of cold deionized distilled H_2O was added. Each sample then received 3 mL of hexane, was vortexed, and then was centrifuged for 10 min at 1200*g*. The upper layer was pipetted into a separate test tube, and the pellet was re-extracted twice more using hexane. The combined hexane fractions were washed with 3 mL of deionized distilled H_2O, vortexed, and centrifuged for 10 min, prior to pipetting it into another test tube. The hexane fraction was dried down in a vacuum evaporator, and samples were reconstituted in 200 μL of acetonitrile:methanol:methylene

Table 1. List of Corn Genotypes Included in This Study and Basis for Selection

genotype	endosperm	reason for inclusion	source of information
A632	*Su1*	low carotenoid	Weber, 1987
B37	*Su1*	high carotenoid; low tocopherol	Weber, 1987
B73	*Su1*	low carotenoid	Weber, 1987
B84	*Su1*	low carotenoid; low tocopherol	Weber, 1987
R802a	Su1	high carotenoid	Weber, 1987
R806	*Su1*	high carotenoid	Weber, 1987
W64a	*Su1*	medium carotenoid	Weber, 1987
C68	*sh2*	dark yellow kernel color	personal observation
Ia453	*sh2*	dark yellow kernel color	personal observation
IL618b	*su1*	dark yellow kernel color	personal observation
OH43	*su1*	dark yellow kernel color	personal observation
IL27a	*su1*	light yellow kernel color	personal observation
IL607a	*su1*	light yellow kernel color	personal observation
IL747b	*se1*	light yellow kernel color	personal observation
IL677a	*se1*	light yellow kernel color	personal observation
IL465a	*su1*	orange kernel color	personal observation
IL442a	*su1*	orange kernel color	personal observation
IL678a	*se1*	orange kernel color	personal observation
WC1	*Su1*	pale yellow kernel color	Maize Genome Database, 1995
Y1	*Su1*	pale yellow kernel color	Maize Genome Database, 1995
Y8	*Su1*	pale yellow kernel color	Maize Genome Database, 1995
A10664A	*su1*	early pigment development	unpublished data, Dr. J. Juvik
A10664C	*su1*	early pigment development	unpublished data, Dr. J. Juvik
IL2022-11	*su2*	high oil	unpublished data, Dr. J. Juvik
IL2027-5	*sh2*	low oil	unpublished data, Dr. J. Juvik
IL2027-7	*sh2*	high oil	unpublished data, Dr. J. Juvik
IL2027-8	*sh2*	low oil	unpublished data, Dr. J. Juvik
ILHO	*Su1*	high oil	Dudley and Lambert, 1992
ILLO	*Su1*	low oil	Dudley and Lambert, 1992
ILHP	*Su1*	high protein	Dudley and Lambert, 1992
ILLP	*Su1*	low protein	Dudley and Lambert, 1992
IL731a	*se1*	parent of $f_{2:3}$ population	Azanza et al., 1996
W6786	*su1*	parent of $F_{2:3}$ population	Azanza et al., 1996
1645-13	*se1*	$F_{2:3}$ family segregating for *se1*	Azanza et al., 1996
1645-15	*su1*	$F_{2:3}$ family segregating for *se1*	Azanza et al., 1996
1645-29	*se1*	$F_{2:3}$ family segregating for *se1*	Azanza et al., 1996
1645-32	*su1*	$F_{2:3}$ family segregating for *se1*	Azanza et al., 1996
1645-36	*su1*	$F_{2:3}$ family segregating for *se1*	Azanza et al., 1996
1645-121	*su1*	$F_{2:3}$ family segregating for *se1*	Azanza et al., 1996
1645-165	*su1*	$F_{2:3}$ family segregating for *se1*	Azanza et al., 1996
IL451b	*se1*	isoline; endosperm mutation effect	
IL451b	*su1*	isoline; endosperm mutation effect	
IL451b	*sh2*	isoline; endosperm mutation effect	
IL451b	*Su1*	isoline; endosperm mutation effect	

chloride (45:20:35 v/v/v) prior to injection into the HPLC. Recovery rates of corn samples spiked with purified standards would suggest the extraction procedure is effective and that there was no appreciable thermic isomerization of β-carotene or thermic degradation of lutein (Kurilich and Juvik, unpublished material).

Carotenoids and tocopherols were separated and quantified by high-performance liquid chromatography (HPLC) using UV−vis detection. The columns consisted of a Vydac 201TP54, C_{18} reverse phase, 5 μm, 4.6 × 150 mm column (Separations Group, Hesperia, CA) connected to a Waters Nova-Pak C_{18} reverse phase, 4 μm, 3.9 × 150 mm column (Waters Chromatography, Milford, MA). The columns were protected by an Adsorbosphere C_{18} reverse phase, 5 μm, 4.6 × 7.5 mm guard column (Alltech Assoc., Deerfield, IL). The HPLC system consisted of an ERMA Optima LTD ERC 3510 degasser (Anspec Co., Ann Arbor, MI), a Waters 510 pump, 731a autoinjector, and a 490E multiwavelength UV−vis detector (Waters Chromatography, Milford, MA). Data were collected and processed using Waters Millenium 2010 software (Waters Chromatography, Milford, MA). The mobile phase consisted of acetonitrile:methanol:methylene chloride (75:20:5 v/v/v), containing 0.05% triethylamine (TEA) and 0.1% BHT (Hart and Scott, 1995). The flow rate was 1.8 mL/min at ambient temperature. To maximize detection, absorbance was measured at 450 and 290 nm for carotenoids and tocopherols, respectively.

Three sets of working standards (lutein and zeaxanthin; β-cryptoxanthin, α- and β-carotene; α-, δ-, and γ-tocopherol) at five concentrations were prepared, dried down in a vacuum concentrator, and reconstituted in acetonitrile:methanol:methylene chloride (45:20:35 v/v/v). Concentration ranges for carotenoid standards in sweet corn analyses were lutein = 0.116−150 μg/mL; zeaxanthin = 0.069−90 μg/mL; β-cryptoxanthin = 0.022−14 μg/mL; α-carotene = 0.013−8 μg/mL; and β-carotene = 0.016−10 μg/mL. Ranges of tocopherol standards were α- and δ-tocopherol = 0.4−50 μg/mL; γ-tocopherol = 1.44−180 μg/mL. Retention times for the various compounds were as follows: 3.1 min = lutein; 3.3 min = zeaxanthin; 5.2 min = δ-tocopherol; 5.9 min = γ-tocopherol; 6.8 min = α-tocopherol; 7.1 min = β-cryptoxanthin; 15.7 min = α-carotene; and 17.2 min = β-carotene.

Statistical Analysis. Analysis of variance (ANOVA) was performed for each compound in 20 DAP kernels using the PROC GLM routine of SAS (SAS Institute, 1988). Compound concentrations were considered to be significantly different between genotypes when $p < 0.05$. Pearson correlation analyses were run between all compounds using the PROC CORR routine of SAS (SAS Institute, 1988). Compounds were considered correlated when $p \leq 0.01$. The PROC GLM routine with mean separations was also used to compare compound variation among the groups of endosperm mutations. ANOVAs and T-tests were also used to determine significant differences in carotenoid and tocopherol concentrations at the various kernel developmental stages.

RESULTS AND DISCUSSION

Variation in Antioxidant Content. Forty-four sweet and dent corn genotypes were surveyed for variability in α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin, α-tocopherol, δ-tocopherol, and γ-tocopherol content at 20 DAP to obtain an accurate profile of

Table 2. Mean and Standard Deviation (μg/g Dry Weight) of Carotenoids in Corn Genotypes[a]

genotype	endo	α-carotene	β-carotene	β-cryptoxanthin	lutein	zeaxanthin	total
A632	*Su1*	0.03 ± 0.00	0.36 ± 0.01	0.59 ± 0.04	0.89 ± 0.13	3.95 ± 0.24	5.82 ± 0.42
B37	*Su1*	0.57 ± 0.39	1.71 ± 0.38	2.00 ± 0.15	19.81 ± 1.43	4.22 ± 0.46	28.30 ± 2.79
B73	*Su1*	0.27 ± 0.08	0.13 ± 0.02	0.48 ± 0.08	5.00 ± 2.35	1.85 ± 0.92	7.73 ± 3.45
B84	*Su1*	0.05 ± 0.01	0.32 ± 0.10	0.46 ± 0.04	12.81 ± 0.47	3.06 ± 0.24	16.70 ± 0.86
R802a	*Su1*	1.21 ± 0.31	5.59 ± 1.09	2.10 ± 0.27	18.44 ± 2.41	4.04 ± 0.59	31.37 ± 4.67
R806	*Su1*	0.11 ± 0.03	0.59 ± 0.10	1.47 ± 0.03	10.69 ± 2.72	4.06 ± 0.77	16.92 ± 3.65
W64a	*Su1*	0.07 ± 0.01	0.27 ± 0.04	0.88 ± 0.22	3.25 ± 1.71	3.13 ± 1.89	7.60 ± 3.87
C68	*sh2*	0.30 ± 0.03	7.64 ± 0.51	0.85 ± 0.05	13.34 ± 2.22	7.73 ± 2.19	29.87 ± 5.00
Ia453	*sh2*	0.81 ± 0.06	0.70 ± 0.04	2.40 ± 0.19	27.59 ± 1.56	1.61 ± 0.12	33.11 ± 1.97
IL618b	*su1*	0.14 ± 0.09	1.46 ± 1.09	0.29 ± 0.04	7.37 ± 2.28	2.23 ± 0.82	11.48 ± 4.33
OH43	*su1*	0.14 ± 0.07	0.30 ± 0.22	0.51 ± 0.11	4.61 ± 0.48	2.45 ± 0.10	8.00 ± 0.97
IL27a	*su1*	0.03 ± 0.00	0.14 ± 0.02	0.08 ± 0.01	0.11 ± 0.04	0.09 ± 0.01	0.45 ± 0.07
IL607a	*su1*	0.70 ± 0.25	0.59 ± 0.13	1.05 ± 0.22	4.66 ± 0.97	2.76 ± 0.66	9.76 ± 1.54
IL747b	*se1*	0.04 ± 0.01	0.23 ± 0.16	0.21 ± 0.08	0.98 ± 0.13	0.85 ± 0.10	2.31 ± 0.50
IL677a	*se1*	0.10 ± 0.02	0.39 ± 0.07	0.34 ± 0.07	1.61 ± 0.05	1.77 ± 0.08	4.20 ± 0.29
IL465a	su1	0.09 ± 0.05	0.44 ± 0.15	0.51 ± 0.15	2.30 ± 0.15	1.67 ± 0.21	5.00 ± 0.71
IL442a	*su1*	0.09 ± 0.01	0.41 ± 0.16	0.62 ± 0.05	2.00 ± 0.11	1.61 ± 0.04	4.73 ± 0.37
IL678a	*se1*	0.09 ± 0.01	0.33 ± 0.01	0.46 ± 0.02	2.39 ± 0.33	1.82 ± 0.10	5.10 ± 0.47
WC1	*Su1*	0.17 ± 0.05	0.13 ± 0.01	0.47 ± 0.08	7.28 ± 0.35	3.26 ± 0.10	11.30 ± 0.59
Y1	*Su1*	ND	0.11 ± 0.01	0.08 ± 0.01	0.02 ± 0.03	0.19 ± 0.02	0.40 ± 0.07
Y8	*Su1*	0.02 ± 0.00	0.18 ± 0.09	0.15 ± 0.08	0.47 ± 0.44	0.30 ± 0.28	1.11 ± 0.90
A10664A	su1	0.05 ± 0.01	0.28 ± 0.17	0.35 ± 0.12	2.56 ± 0.56	2.78 ± 1.05	6.02 ± 1.91
A10664C	*su1*	0.06 ± 0.01	0.44 ± 0.01	0.54 ± 0.11	1.39 ± 0.08	2.81 ± 0.56	5.24 ± 0.76
IL2022-11	*sh2*	0.03 ± 0.00	0.80 ± 0.62	0.41 ± 0.31	2.31 ± 1.72	2.34 ± 1.71	5.89 ± 4.36
IL2027-5	*sh2*	0.27 ± 0.03	1.06 ± 0.21	1.20 ± 0.59	17.11 ± 2.38	2.74 ± 0.07	22.38 ± 3.26
IL2027-7	*sh2*	ND	0.19 ± 0.03	0.09 ± 0.03	1.59 ± 0.41	0.04 ± 0.01	1.90 ± 0.49
IL2027-8	*sh2*	0.21 ± 0.12	0.57 ± 0.21	1.15 ± 0.20	16.63 ± 7.52	5.40 ± 1.52	23.97 ± 9.57
ILHO	*Su1*	0.03 ± 0.00	0.14 ± 0.00	0.10 ± 0.01	0.13 ± 0.15	0.38 ± 0.09	0.77 ± 0.25
ILLO	*Su1*	ND	0.09 ± 0.02	0.07 ± 0.01	0.07 ± 0.11	0.22 ± 0.16	0.46 ± 0.30
ILHP	Su1	0.03 ± 0.00	0.16 ± 0.07	0.12 ± 0.03	0.16 ± 0.16	0.60 ± 0.37	1.07 ± 0.63
ILLP	*Su1*	ND	0.07 ± 0.02	0.07 ± 0.01	ND	0.01 ± 0.00	0.15 ± 0.03
IL731a	*se1*	0.06 ± 0.02	0.22 ± 0.06	0.21 ± 0.09	0.92 ± 0.08	0.80 ± 0.11	2.22 ± 0.37
W6786	*su1*	0.12 ± 0.03	0.24 ± 0.09	0.45 ± 0.15	5.18 ± 1.37	2.16 ± 0.76	8.14 ± 2.39
1645-13	*se1*	0.09 ± 0.01	0.18 ± 0.05	0.32 ± 0.04	5.79 ± 2.29	1.23 ± 0.15	7.60 ± 2.54
1645-15	*su1*	0.12 ± 0.01	0.21 ± 0.02	0.44 ± 0.12	7.77 ± 1.88	2.49 ± 0.47	11.03 ± 2.49
1645-29	*se1*	0.07 ± 0.01	0.16 ± 0.01	0.33 ± 0.03	5.79 ± 0.88	1.25 ± 0.80	7.59 ± 1.72
1645-32	*su1*	0.11 ± 0.00	0.29 ± 0.02	0.80 ± 0.07	14.09 ± 0.92	2.07 ± 0.19	17.35 ± 1.21
1645-36	*su1*	0.10 ± 0.00	0.26 ± 0.02	0.55 ± 0.02	7.94 ± 1.09	2.01 ± 1.04	10.85 ± 2.17
1645-121	*su1*	0.08 ± 0.00	0.20 ± 0.01	0.34 ± 0.00	5.40 ± 0.33	2.16 ± 0.01	8.17 ± 0.35
1645-165	*su1*	0.10 ± 0.00	0.23 ± 0.01	0.41 ± 0.04	8.09 ± 0.78	1.77 ± 1.32	10.60 ± 2.16
IL451b	*se1*	0.05 ± 0.01	0.28 ± 0.10	0.25 ± 0.10	6.48 ± 0.84	2.12 ± 0.49	9.17 ± 1.54
IL451b	*su1*	0.19 ± 0.02	0.47 ± 0.03	0.84 ± 0.16	10.65 ± 0.93	2.34 ± 1.88	14.49 ± 3.03
IL451b	*sh2*	0.66 ± 0.17	0.45 ± 0.03	2.45 ± 0.25	18.30 ± 0.28	1.41 ± 0.14	23.28 ± 0.87
IL451b	*Su1*	0.05 ± 0.01	0.57 ± 0.04	0.81 ± 0.22	14.26 ± 3.13	2.66 ± 2.16	18.35 ± 5.56
LSD		0.19	0.56	0.24	2.57	1.38	
mean		0.16	0.68	0.55	5.95	2.16	10.41
range		0.00−0.70	0.07−7.64	0.07−2.40	0.00−27.59	0.01−7.73	0.15−33.11

[a] ND = not detected; endo = endosperm mutation. LSD = least significant difference between means using T tests where $p = 0.05$.

antioxidant variability in corn germplasm at typical sweet corn fresh market harvest. Significant differences in the germplasm would determine the feasibility for breeding elite genotypes containing enhanced levels of these antioxidants. The biological activity of individual antioxidants can be estimated by a combination of their relative oxidant quenching ability and concentrations in the kernel. On the basis of reported rate constants of quenching (k_q) singlet oxygen (Di Mascio et al., 1991), α-carotene has the greatest quenching activity (1.9×10^{10} k_q), followed by β-carotene (1.4×10^{10} k_q), zeaxanthin (1.0×10^{10} k_q), lutein (8×10^9 k_q), and β-cryptoxanthin (cryptoxanthin = 6×10^9 k_q). Among the tocopherols, α-tocopherol appears to be a more efficient antioxidant when reacting with peroxyl radicals and singlet oxygen (Sies and Stahl, 1995) while γ-tocopherol is more effective at protecting against nitrogen oxide species (Christen et al., 1994). Rate constants of singlet oxygen quenching in the tocopherols are α-tocopherol (2.8×10^8 k_q), γ-tocopherol (2.3×10^8 k_q), and δ-tocopherol (1.6×10^8 k_q) (Di Mascio et al., 1991). On a concentration basis, the dominant carotenoids were lutein and zeaxanthin while the dominant tocopherols were the α and γ forms (Tables 2 and 3). On the basis of the quenching ability and concentration, these four compounds should be considered as the most important antioxidants when planning a breeding strategy for corn.

Tables 2 and 3 show the mean concentration and standard deviation of carotenoids and tocopherols over the three replicates of each genotype for each antioxidant compound. Total carotenoids and tocopherols are also listed for each genotype. Substantial variability exists in all compounds between genotypes, as indicated by the range in values. The ranges shown here are similar to those reported by Lee et al. (1981) for α-carotene, β-carotene, and β-cryptoxanthin concentrations in sweet corn.

Analysis of the variance revealed significant differences among the genotypes for all compounds. The greater the variability for an individual trait, the greater the opportunity for genetic improvement by plant breeders. Results from this survey indicate that all of these antioxidant compounds have significant potential for enhancement via genetic manipulation. The ANOVA also revealed that for all the compounds between 88%

Table 3. Mean and Standard Deviation (μg/g Dry Weight) of Tocopherols in Corn Genotypes

genotype	endo	δ-tocopherol	γ-tocopherol	α-tocopherol	total
A632	*Su1*	1.26 ± 0.06	17.24 ± 2.70	9.40 ± 1.25	27.90 ± 4.01
B37	*Su1*	0.87 ± 0.18	19.47 ± 1.28	15.52 ± 1.32	35.86 ± 2.79
B73	*Su1*	0.99 ± 0.26	17.95 ± 7.31	5.93 ± 1.60	24.87 ± 9.17
B84	*Su1*	0.94 ± 0.03	19.98 ± 3.16	9.32 ± 1.57	30.24 ± 4.75
R802a	*Su1*	1.13 ± 0.06	28.56 ± 6.01	10.34 ± 1.77	40.03 ± 7.84
R806	*Su1*	1.93 ± 0.41	31.14 ± 4.72	13.82 ± 2.90	46.88 ± 8.02
W64a	*Su1*	0.61 ± 0.34	22.60 ± 10.54	1.43 ± 0.37	24.64 ± 11.25
C68	*sh2*	0.71 ± 0.25	11.75 ± 5.03	12.42 ± 2.00	24.88 ± 7.29
Ia453	*sh2*	2.45 ± 0.55	19.73 ± 0.73	32.54 ± 2.91	54.71 ± 4.19
IL618b	*su1*	1.73 ± 0.49	34.48 ± 9.55	6.55 ± 2.52	42.76 ± 12.56
OH43	*su1*	0.91 ± 0.04	19.87 ± 0.20	10.74 ± 3.71	31.51 ± 3.95
IL27a	*su1*	0.79 ± 0.10	12.54 ± 0.12	3.07 ± 1.23	16.41 ± 1.45
IL607a	*su1*	0.91 ± 0.16	12.85 ± 0.60	10.50 ± 1.57	24.25 ± 2.33
IL747b	*se1*	1.79 ± 0.59	20.28 ± 7.43	3.26 ± 1.80	25.33 ± 9.83
IL677a	*se1*	1.29 ± 0.42	19.29 ± 2.08	5.78 ± 0.90	26.36 ± 3.40
IL465a	*su1*	1.04 ± 0.11	18.02 ± 3.73	4.17 ± 1.13	23.23 ± 4.96
IL442a	*su1*	1.33 ± 0.31	18.35 ± 6.50	5.13 ± 1.88	24.81 ± 8.70
IL678a	*se1*	1.11 ± 0.04	24.51 ± 0.97	12.41 ± 0.86	38.04 ± 1.87
WC1	*Su1*	0.29 ± 0.14	14.44 ± 3.03	11.80 ± 0.91	26.52 ± 4.07
Y1	*Su1*	1.19 ± 0.33	25.36 ± 7.63	7.97 ± 2.80	34.53 ± 10.76
Y8	*Su1*	0.37 ± 0.06	2.37 ± 0.83	4.50 ± 1.15	7.24 ± 2.04
A10664A	*su1*	1.07 ± 0.22	22.53 ± 6.62	4.42 ± 2.34	28.03 ± 9.19
A10664C	*su1*	1.74 ± 0.13	25.65 ± 4.33	5.39 ± 1.10	32.78 ± 5.56
IL2022-11	*sh2*	1.17 ± 0.40	31.41 ± 12.44	14.86 ± 4.29	47.44 ± 17.14
IL2027-5	*sh2*	0.86 ± 0.26	8.30 ± 7.85	22.09 ± 1.82	31.25 ± 9.93
IL2027-7	*sh2*	0.96 ± 0.15	38.76 ± 3.97	8.92 ± 0.16	48.65 ± 4.27
IL2027-8	*sh2*	1.06 ± 0.16	3.29 ± 0.19	16.01 ± 1.83	20.36 ± 2.18
ILHO	*Su1*	4.27 ± 1.29	63.28 ± 5.58	18.07 ± 3.42	85.62 ± 10.29
ILLO	*Su1*	0.49 ± 0.25	9.87 ± 5.74	1.78 ± 0.77	12.14 ± 6.75
ILHP	*Su1*	1.19 ± 0.01	18.00 ± 0.31	13.29 ± 4.60	32.47 ± 4.92
ILLP	*Su1*	0.20 ± 0.07	7.41 ± 1.36	3.12 ± 0.62	10.74 ± 2.05
IL731a	*se1*	1.09 ± 0.24	17.10 ± 1.06	4.09 ± 0.46	22.27 ± 1.77
W6786	*su1*	1.34 ± 0.14	19.56 ± 1.84	7.56 ± 1.35	28.46 ± 3.32
1645-13	*se1*	0.71 ± 0.21	10.59 ± 3.81	3.59 ± 1.28	14.88 ± 5.30
1645-15	*su1*	0.86 ± 0.32	15.77 ± 2.75	5.69 ± 2.24	22.32 ± 5.30
1645-29	*se1*	0.71 ± 0.18	12.51 ± 1.41	3.89 ± 0.76	7.11 ± 2.36
1645-32	*su1*	1.04 ± 0.26	13.79 ± 1.50	6.42 ± 0.22	21.25 ± 1.98
1645-36	*su1*	0.93 ± 0.33	18.19 ± 7.21	3.50 ± 1.12	22.63 ± 8.66
1645-121	*su1*	0.50 ± 0.11	9.31 ± 1.31	1.82 ± 0.05	11.63 ± 1.48
1645-165	*su1*	1.19 ± 0.32	24.06 ± 5.60	6.58 ± 0.89	31.84 ± 6.81
IL451b	*se1*	1.54 ± 0.19	34.00 ± 1.48	6.84 ± 0.46	42.38 ± 2.13
IL451b	*su1*	0.91 ± 0.36	26.98 ± 4.76	8.46 ± 3.51	36.35 ± 8.63
IL451b	*sh2*	1.51 ± 0.03	24.32 ± 0.76	10.93 ± 1.62	36.76 ± 2.40
IL451b	*Su1*	1.12 ± 0.1	28.41 ± 0.66	6.36 ± 0.23	35.89 ± 0.98
LSD		0.90	8.54	4.01	
mean		1.10	20.03	8.06	30.10
range		0.20−4.27	2.37−63.28	1.43−32.54	7.24−85.62

[a] ND = not detected; endo = endosperm mutation. LSD = least significant difference between means using T tests where $p = 0.05$.

(α-carotene) to 97% (β-carotene) of the total variation found was due to differences between the genotypes, with the remainder due to sample variability among replicates. In the single year and location tested, environmental variation associated with field replicates was quite low.

Xanthophyll concentrations for A632, B37, B73, B84, R802a, R806, and W64a were generally lower those reported by Weber (1987) and Wong et al. (1998), while carotene levels were approximately comparable. Weber and Wong analyzed these genotypes at the mature dry stage (>45 DAP), suggesting that concentrations are dependent on kernel maturity. This hypothesis is supported by results from the kernel developmental series, which show that β-cryptoxanthin and lutein levels tend to increase as kernels mature (Table 4) with no consistent changes in α-carotene, β-carotene, or zeaxanthin content. The sweet corn inbreds IL677a *se1*, IL 678a *se1*, and IL451b *su1*, analyzed at 21 DAP by La Bonte and Juvik (1990), contained comparable total carotene levels to those observed in this study (Table 2).

The carotenoid content of the genotypes selected on the basis of color showed that dark yellow kernels have the greatest total carotenoid levels followed by light yellow, orange, and pale yellow. Genotypes with dark yellow kernel color were also generally high in β-carotene content (Table 2). The two lines selected for early pigment development (A10664A and A10664C) were comparable to the orange-colored lines in total carotenoid content. Pigmentation differences in these lines do not appear to be affiliated with carotenoid levels.

Tocopherol levels among the genotypes analyzed by Weber (1987) and Wong et al. (1998) were higher than those observed in this study. Again, these differences appear to be due to differences in kernel maturity since our results show substantial increases in γ-tocopherol content as kernels mature. There were no consistent changes in δ- or α-tocopherol levels from 18 to 27 DAP (Table 4).

Genotypes with higher oil content (IL2027-5, IL2027-7, and ILHO) typically contained more total tocopherols, while genotypes with low oil content (IL2022-11, IL2027-8, and ILLO) had low tocopherol levels (Table 3). This suggests that tocopherol and oil content may be physiologically associated. Further investigations are required to establish the nature of this relationship.

Table 4. Kernel Carotenoid and Tocopherol Concentrations (μg/g Dry Weight) of Five Sweet Corn Genotypes at 18, 21, 24, and 27 Days after Pollination[a]

DAP	α-carotene	β-carotene	β-cryptoxanthin	lutein	zeaxanthin	δ-tocopherol	γ-tocopherol	α-tocopherol
				IL731a *se*				
18	0.04 a	0.10 a	0.16 a	7.30 a	1.10 a	1.66 a	16.00 a	11.87 a
21	0.08 a	0.20 a	0.27 ab	9.60 a	2.06 b	1.16 a	22.74 ab	4.80 a
24	0.13 a	0.17 a	0.41 ab	11.93 a	2.54 b	4.27 b	28.66 b	8.42 a
27	0.09 a	0.37 a	0.54 b	13.75 a	2.18 b	1.99 a	37.10 c	5.53 a
				W6786				
18	0.12 a	0.28 a	0.34 a	12.25 a	2.35 a	1.97 b	28.35 a	12.68 a
21	0.11 a	0.19 a	0.44 b	16.89 ab	3.05 a	1.57 ab	36.77 ab	10.79 a
24	0.17 a	0.25 a	0.58 c	21.25 b	3.27 a	2.39 c	44.18 bc	13.65 a
27	0.16 a	0.22 a	0.60 c	18.67 ab	2.95 a	1.70 ab	53.78 c	11.03 a
				1645-73				
18	0.16 a	0.37 a	0.85 a	12.35 a	2.59 a	0.87 a	16.20 a	8.58 a
21	0.14 a	0.38 a	1.51 a	25.83 a	3.75 b	1.92 b	37.05 ab	8.42 a
24	0.13 a	0.41 a	1.70 a	22.76 a	3.64 ab	1.60 b	31.68 ab	8.92 a
27	0.15 a	0.34 a	1.43 a	25.61 a	3.24 ab	2.03 b	52.27 b	9.26 a
				1645-93				
18	0.06 a	0.19 a	0.75 a	15.46 a	2.74 a	1.51 a	17.57 a	7.97 a
21	0.14 a	0.21 a	0.65 a	15.74 a	2.68 a	1.41 a	28.06 ab	12.17 a
24	0.16 a	0.27 a	0.72 a	14.67 a	1.99 a	1.42 a	39.93 ab	9.70 a
27	0.08 a	0.20 a	0.92 a	18.25 a	2.10 a	1.85 a	47.79 b	12.86 a
				1645−107				
18	0.15 a	0.39 a	0.72 a	24.96 a	2.81 a	1.48 a	25.51 a	6.45 a
21	0.10 a	0.44 a	0.85 a	22.63 a	3.02 a	1.90 ab	32.42 a	7.34 a
24	0.11 a	0.29 a	1.16 a	27.93 a	3.30 a	1.39 ab	31.87 a	8.95 a
27	0.15 a	0.35 a	1.24 a	23.19 a	2.62 a	2.16 b	54.78 a	7.42 a

[a] Means with different letters within each genotype indicate significant differences at $p \leq 0.05$.

Lutein, β-cryptoxanthin, and α-tocopherol concentrations were significantly different between IL731a *se1* and W6786 *su1*. Significant differences were also observed among families within the IL731a *se1* × W6786 *su1* population (genotypes 1645-*x*, Tables 2 and 3). Four of the seven families showed higher total carotenoid levels and either higher or lower total tocopherol levels than either of the parents, indicative of transgressive segregation for these traits in this population.

Comparison of Genotypes. The pale yellow endosperm mutant lines, *Y1* and *Y8* contained considerably less total carotenoid content compared to the mean of all lines (0.4, 1.1, and 10.4 μg/g, respectively) (Table 2). In contrast, the total carotenoid content of *WC1* (11.3 μg/g) was slightly higher than the overall mean (10.4 μg/g) and approximately one-third that of the highest lines.

The oil lines conformed to the notion that oil is positively associated with tocopherol content. ILHO contained the highest level of total tocopherols (85.6 μg/g), which was 50% greater than the second highest line, Ia453 *sh2* (54.7 μg/g), and almost 12 times greater than the lowest line *Y8* (7.2 μg/g) (Table 3). ILLO with 12.1 μg/g contained one-half the total tocopherol content of the overall mean (30.1 μg/g). Tocopherol levels in the high- and low-protein lines were significantly different, but this appears to be an artifact of these two lines. A recent study conducted in this laboratory involving the IL451b *se1* × W6786 *su1* population found that protein levels were not correlated with δ- or γ-tocopherol; however, there was a significant, though relatively low, correlation between α-tocopherol and protein levels ($R^2 = 0.19$) (Kurilich and Juvik, unpublished data).

Germplasm that has been identified as a genetic source for enhanced levels of carotenoids include Ia453 *sh2*, R802a *Su1*, C68 *sh2*, B37 *Su1*, IL2027-8 *sh2*, IL451b *sh2*, and IL2027-5 *sh2*. Genotypes containing elevated levels of tocopherols include ILHO *Su1*, Ia453 *sh2*, IL2022-11 *sh2*, R806 *Su1*, IL618b *su1*, Il451b *se1*, and R802a *Su1*.

Potential Antioxidant Protection from Corn. 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 α-carotene and β-carotene > 0.4 μmol/L (equivalent to an intake of 2−4 mg/day) and α-tocopherol > 30 μmol/L (about 15−30 mg/day) have been associated with decreased risk of degenerative diseases (Stahl and Sies, 1996). Unfortunately, no dosage information for the prevention or treatment of disease is available for the major antioxidant components in corn, lutein, and γ-tocopherol. However, it is possible to estimate from our data that daily consumption of approximately 250 g or two ears of a genotype such as C68 *sh2* would provide a level of β-carotene required for a protective effect. This is without considering the health benefits afforded by the other carotenoids and tocopherols concurrently ingested or the rate and efficiency at which these compounds are absorbed.

Correlations among Compounds. To optimize the concentration of these antioxidants for improving the health benefits derived from sweet corn, some understanding of the biosynthetic interactions between these compounds is of value. To investigate these biosynthetic interactions, Pearson correlation coefficients using genotype means for each compound were calculated. All the carotenoids are formed from one biosynthetic pathway (Armstrong et al., 1996), as are all the tocopherols (Mann, 1995). If no genetic variability exists in genes regulating the biosynthesis of these antioxidants among the genotypes tested, then highly significant correlations between these compounds would be anticipated. If there is genetic diversity, relative concentrations of these compounds could be influenced at every step in their biosynthetic pathways. In this study, the only signifi-

Table 5. Mean Carotenoid and Tocopherol Concentrations (μg/g Dry Weight) of the Genotypes Grouped by Endosperm Mutation and in the IL451b Isolines[a]

endosperm	α-carotene	β-carotene	β-cryptoxanthin	lutein	zeaxanthin	δ-tocopherol	γ-tocopherol	α-tocopherol
All Genotypes Grouped by Endosperm Mutation								
sh2	0.38 a	1.63 a	1.22 a	13.84 a	3.04 a	1.24 a	19.65 a	16.82 a
su1	0.16 ab	0.48 a	0.52 b	4.08 b	2.09 a	1.18 a	21.08 a	6.60 b
se1	0.07 b	0.29 a	0.29 b	2.48 b	1.47 a	1.37 a	23.03 a	6.48 b
Su1	0.22 ab	0.69 a	0.66 ab	6.65 b	2.13 a	1.12 a	21.74 a	8.84 b
LSD	0.29	1.52	0.63	6.59	1.68	0.73	11.74	5.27
IL451b Isolines								
sh2	0.66 a	0.45 b	2.45 a	18.30 a	1.41 a	1.51 a	24.32 c	10.93 a
su1	0.19 b	0.47 b	0.84 b	10.65 c	2.34 a	0.91 b	26.98 bc	8.46 ab
se1	0.05 c	0.28 c	0.25 c	6.48 d	2.12 a	1.54 a	34.00 a	6.84 b
Su1	0.05 c	0.57 a	0.81 b	14.26 b	2.66 a	1.12 b	28.41 b	6.36 b
LSD	0.11	0.08	0.26	2.25	0.94	0.28	3.38	2.59

[a] Means with different letters indicate significance at $p < 0.05$. LSD = least significant difference between means using T tests where $p = 0.05$.

cant correlations observed were between β-carotene and lutein ($r = 0.607$, $p = 0.01$) and between δ-tocopherol and γ-tocopherol ($r = 0.621$, $p = 0.005$). This suggests that for these lines there is considerable allelic variation among the genes controlling the biosynthesis of these compounds. All other correlations including those between the carotenoids and tocopherols were nonsignificant, suggesting that concentrations of these compounds are under independent regulation. This indicates that increased levels of the various antioxidants can be bred for simultaneously.

Endosperm Mutation Effect on Antioxidant Content. Mean separation analysis of genotypes grouped by endosperm mutation for individual carotenoids and tocopherols indicated that lutein and α-tocopherol were significantly higher in the *sh2* endosperm mutation compared to *Su1*, *su1*, and *se1* mutations (Table 5). When the same analyses were performed on the IL451b isolines (*Su1*, *su1*, *se1*, *sh2*), concentrations of α-carotene, β-cryptoxanthin, and lutein were significantly greater in kernels with the *sh2* endosperm mutation, γ-tocopherol significantly higher in *se1*, and the *Su1* isoline most abundant in β-carotene. The *se1* endosperm lines were consistently lower for all carotenoids, although the differences were not always significant. Genetically, these isolines are presumed to differ at only one locus (*se1* on chromosome 2) (Tadmor et al., 1995), while all other loci are fixed. Results from the IL451b isoline comparisons suggest that endosperm mutations may differentially interact with carotenoid and tocopherol biosynthesis. Further and more extensive investigations are required to obtain a more accurate understanding of this effect.

This survey was the first phase of a program designed to enhance the potential health benefits derived from the consumption of sweet corn. On the basis of these results, the next phase will focus on creating new germplasm with increased antioxidant concentrations. Potential parents for use in breeding programs will be chosen from the surveyed material, and segregating populations will be generated to investigate the genes that regulate the form and concentration of these compounds. As shown in Tables 2 and 3, significant variability was observed in the IL731a *se1* × W6786 *su1* population, suggesting gene segregation. One hundred and sixty-four $F_{2:3}$ families from this population have been assayed for carotenoid and tocopherol content (Kurilich, 1998). A saturated linkage map containing 93 probes previously constructed for this population (Tadmor et al., 1995) was used to identify molecular markers linked to the genes controlling the concentration of these compounds (Kurilich and Juvik, unpublished). These molecular markers can assist in future breeding programs to create germplasm with increased levels of these antioxidants with potentially enhanced nutritional quality.

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