Genotype × Environment Interactions for Potato Tuber Carotenoid Content

Kathleen G. Haynes¹

U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS), Plant Sciences Institute, Genetic Improvement of Fruits and Vegetables Laboratory, 10300 Baltimore Avenue, Building 010A, Beltsville, MD 20705

Beverly A. Clevidence and David Rao

USDA-ARS, Beltsville Human Nutrition Research Center, Food Components and Health Laboratory, Beltsville, MD 20705

Bryan T. Vinyard

USDA-ARS, Biometrical Consulting Service, Beltsville, MD 20705

J. Marion White

Mid-Florida Research and Education Center, University of Florida, Apopka, FL 32703

ADDITIONAL INDEX WORDS. *Solanum tuberosum*, yellow-flesh, zeaxanthin, antheraxanthin, violaxanthin, neoxanthin, lutein, stability

ABSTRACT. Consumption of carotenoid-containing foods can promote human health. Although yellow-fleshed potatoes (*Solanum tuberosum*) have a higher carotenoid content than white-fleshed potatoes, little is known about how growing environments may affect individual and total carotenoid content in different potato clones. The purposes of this study were to estimate the amount of genetic variability in potato for five xanthophyll carotenoids, their concentration, and to determine the stability of these carotenoids across environments. Nine white- or yellow-fleshed tetraploid clones were grown in Maine and Florida for 2 years. Carotenoids were extracted in acetone and analyzed by high-performance liquid chromatography. There were significant differences among clones for zeaxanthin, antheraxanthin, lutein, and total carotenoid content. There were significant clone × environment interactions for zeaxanthin, antheraxanthin, violaxanthin, neoxanthin, lutein, and total carotenoid. Broad-sense heritabilities (and their 95% confidence intervals) were 0.89 (0.79–0.98) for zeaxanthin, 0.93 (0.87–0.99) for antheraxanthin, 0.68 (0.14–0.92) for violaxanthin, 0.51 (0.00–0.88) for neoxanthin, 0.85 (0.70–0.97) for lutein, and 0.96 (0.89–0.99) for total carotenoid. Clonal mean total carotenoid content ranged from 101 to 511 µg/100 g fresh weight. A higher proportion of carotenoids were produced by the lycopene epsilon cyclase branch of the carotenoid biosynthetic pathway in white-fleshed than yellow-fleshed clones. Total carotenoid content in B2333-5 was significantly greater than in ‘Yukon Gold’. With genetic variation for individual and total carotenoid content in potatoes, improving the levels of carotenoids has been and should continue to be feasible; however, concentrations are likely to vary in different environments.

The presence of carotenoids in fruit, vegetables, and flowers imparts yellow, orange, or red color to them (Hari et al., 1994). Over 600 carotenoids have been identified (Pfander et al., 1987). They can be divided into two groups: 1) the carotenes, which consist of those carotenoids composed of only carbon and hydrogen; and 2) xanthophylls or oxy-carotenoids, which are composed of carbon, hydrogen, and oxygen (Delgado-Vargas et al., 2000).

Carotenoids have many different biological functions in both plants and animals; however, only microorganisms and plants are able to synthesize carotenoids de novo. Animals derive carotenoids from their dietary intake, although they can be modified during metabolism (Goodwin, 1982). Thus far, 40 to 50 carotenoids have been reported to be absorbed, metabolized, or used by humans (Khachik et al., 1991). In plants,

carotenoids serve as accessory pigments for light harvesting during the process of photosynthesis and in photo protection processes (Demming-Adams et al., 1996), and their pigmentation in flowers attracts pollinators and in fruit aids in the dispersal of seeds (Tanaka et al., 2008).

A wide variety of human health benefits of carotenoids has been reported. Some carotenoids have provitamin A activity (Institute of Medicine, 2000), all are antioxidants (Paloza and Krinsky, 1992), some enhance immune function (Lindley, 1998), have anti-inflammatory properties (Khachik et al., 1999), protect skin from ultraviolet light (Sies and Stahl, 2004), promote cell-to-cell communication (Stahl and Sies, 1998), improve mental acuity (Akbaraly et al., 2007), and may reduce the risks of cancer (Khachik et al., 1999; Machlin, 1995), cardiovascular disease (Barton-Duell, 1995; Gey, 1995), and age-related macular degeneration (Mares-Perlman et al., 1995; Seddon et al., 1994).

The carotenoid content of white-fleshed potato cultivars has been reported to be low [less than 100 µg/100 g fresh weight (FW)], whereas the carotenoid content of yellow-fleshed cultivars of the *Tuberosum* group may be as high as 560 µg/

Received for publication 3 Feb. 2010. Accepted for publication 13 Apr. 2010. Mention of a trademark, warranty, proprietary product, or vendor does not constitute a guarantee by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

¹Corresponding author. E-mail: kathleen.haynes@ars.usda.gov.

100 g FW (Hale, 2003; Iwanzik et al., 1983; Lu et al., 2001; Nesterenko and Sink, 2003; Tevini et al., 1984; Tevini and Schonecker, 1986). Native Andean cultivars of the Andigenum group have been reported to have carotenoid levels ranging from 535 to 3895 $\mu\text{g}/100$ g FW (Andre et al., 2007) and concentrations greater than 2000 $\mu\text{g}/100$ g FW have been reported in diploid *Solanum* germplasm (Andre et al., 2007; Brown et al., 1993; Lu et al., 2001). The primary tuber carotenoids are xanthophylls: zeaxanthin, antheraxanthin, violanxanthin, neoxanthin, and lutein (Andre et al., 2007; Brown et al., 1993; Lu et al., 2001).

The carotenoid biosynthetic pathway is complex and the regulation of carotenogenesis is poorly understood (Romer and Fraser, 2005). Phytoene is the first step in the carotenoid biosynthetic pathway and it is desaturated and isomerized into *all-trans* lycopene. Two possible pathways branch off from lycopene, each path governed by one of two cyclases: lycopene epsilon-cyclase (LCY-e) and lycopene beta-cyclase (LCY-b) (Diretto et al., 2006; Romer and Fraser, 2005). The major carotenoids in potato tubers are produced on both branches. In the LCY-e pathway, hydroxylation and epoxidation reactions result in the synthesis of lutein and other carotenoids. In the LCY-b pathway, the introduction of one or two beta rings results in the formation of zeaxanthin, antheraxanthin, violanxanthin, and neoxanthin.

Potatoes are one of the most widely consumed vegetables in the world and potato production and consumption have been increasing in developing countries for decades (Centro Internacional de la Papa, 2009). Although tuber carotenoid concentrations are low in comparison with certain raw, carotenoid-rich vegetables such as carrot [*Daucus carota* var. *sativus* (8285 μg β -carotene per 100 g FW)], tomato [*Solanum lycopersicum* (2573 μg lycopene per 100 g FW)], and spinach [*Spinacia oleracea* (12,198 μg lutein + zeaxanthin per 100 g FW)] [U.S. Department of Agriculture (USDA), 2009], potato still contributes substantially to human carotenoid consumption because of the quantities of potatoes consumed. Nine vegetables, among them potato, and five fruit were found to account for more than 96% of the carotenoid intake in one study (Granado et al., 1996).

The intensity of potato yellow flesh, as measured by colorimeter in three northeastern U.S. locations (Haynes et al., 1996), and total tuber carotenoids in two Texas environments (Reddivari et al., 2007) have been found to vary among genotypes and environments. In both studies, genotypic effects were greater than environmental or genotype $\times$ environmental effects and the genotype $\times$ environmental interactions, although significant, were relatively small. Broad-sense heritability for tuber yellow-flesh intensity was high ($H = 0.93$) (Haynes et al., 1996).

The purposes of this study were to 1) estimate broad-sense heritability for individual and total carotenoid content in potatoes; and 2) determine the stability of individual and total carotenoid content production in two widely different environments.

Materials and Methods

PLANT MATERIALS. Nine potato clones were evaluated. Two of these clones were yellow-fleshed cultivars: Yukon Gold (Johnston and Rowberry, 1981) and Peter Wilcox (USDA et al., 2007). Three of the clones were white-fleshed advanced selections from the USDA, Agricultural Research Service

Beltsville Potato Breeding Program: B0984-1, B1870-3, and B1952-2. Three clones were advanced yellow-fleshed selections: B2152-17, B2319-1, and B2333-5. The remaining clone (BTD0088-2) was a $4x-2x$ hybrid in which the female parent was the white-fleshed cultivar Atlantic and the male parent was an intensely yellow-fleshed diploid selection (BD043-3) from a *Solanum phureja-S. stenotomum* population (Haynes et al., 1995).

FIELD EXPERIMENTS. These clones were planted 28 Jan. 2004 and 26 Jan. 2005 on a Ellzey fine sand (sandy, siliceous, hyperthermic Arenic Ochraqulf) soil in Hastings, FL, and 20 May 2004 and 9 June 2005 on a Caribou gravelly loam soil (fine-loamy, mixed, frigid Typic Haplorthod) in Presque Isle, ME. The experimental design was a randomized complete block with three replications. Each plot consisted of 15 plants of one clone. In Florida, plants were spaced 20 cm within the row with 100 cm between rows and 60 cm between plots in the row. In Maine, plants were spaced 23 cm within the row with 91 cm between rows and 69 cm between plots in the row. In Florida, they were planted in the field after mowing and then plowed down sudangrass (*Sorghum bicolor* ssp. *drummondii*) grown over summer. The test location was fertilized with 188.2 $\text{kg}\cdot\text{ha}^{-1}$ nitrogen (N), 11.7 $\text{kg}\cdot\text{ha}^{-1}$ phosphorus (P), and 133.9 $\text{kg}\cdot\text{ha}^{-1}$ potassium (K) banded in row at planting and 104.7 $\text{kg}\cdot\text{ha}^{-1}$ N, 6.5 $\text{kg}\cdot\text{ha}^{-1}$ P, and 74.5 $\text{kg}\cdot\text{ha}^{-1}$ K at the 25- to 30-cm growth stage. In Maine, they were planted in the field after plowed down timothy-clover (*Phleum pretense-Trifolium* spp.) sod on site. The test location was fertilized with 168 $\text{kg}\cdot\text{ha}^{-1}$ N, 73.3 $\text{kg}\cdot\text{ha}^{-1}$ P, and 139.5 $\text{kg}\cdot\text{ha}^{-1}$ K banded in row at planting. In Florida, irrigation was by subsurface seep with an irrigation ditch every 20 rows. No irrigation was available in Maine. In both states, cultural practices were similar to those used on commercial farms in the area. Tubers were harvested into paper bags from each plot on 17 May 2004 and 23 May 2005 in Florida, and 14 Sept. 2004 and 8 Sept. 2005 in Maine. Three medium-sized tubers, free of external defects or blemishes, were selected and shipped to Beltsville, MD, where they were held at 4 °C with 95% relative humidity storage until carotenoids were extracted. Haynes et al. (1994) had previously shown an inverse relationship between yellow-flesh intensity and tuber weight when all tubers were harvested from plots; however, the plot interquartile subsample was as informative as the full sample. Therefore, medium-sized tubers were selected visually from each plot for analysis. Carotenoids were extracted from the Florida tubers in June of each year and from the Maine tubers from October to mid-November of each year.

CAROTENOID EXTRACTION AND IDENTIFICATION. Tubers were removed from cold storage and allowed to warm to room temperature (RT) for at least 24 h before carotenoid extraction. Carotenoids were extracted at RT and under yellow light. Each tuber was cut longitudinally from stem to apical end. One cut surface was blotted on a paper towel to remove excess moisture. Two 0.40- to 0.60-g samples were removed from the half-tuber midway between the stem and apical end, just inside the vascular ring, with a cork borer, one from each side of the tuber. Each sample was ground in a 3-mL ground-glass tissue grinder (tissue grind tube no. 885452-0021, tissue grind pestle no. 885451-0021; Kontes Glass, Vineland, NJ) in 0.2 mL 85% acetone (15% d water) and the resulting slurry transferred into a 1.5-mL microcentrifuge tube. The grinder was subsequently washed twice with 0.2 mL 85% acetone each time. The washes were added to the microcentrifuge tube, which was then

centrifuged at 10,600 g_n for 5 min, and the supernatant decanted into a 12 × 75-mm test tube. The pellet was resuspended in 0.2 mL 100% acetone by vortexing, again centrifuged at 10,600 g_n for 5 min, and the supernatant combined with that of the first spin. This procedure was repeated a second time. The combined extracts were dried at 35 °C for ≈30 min under N_2 after adding 400 μ L apo-8'-carotenal as an internal standard and reconstituted with 400 μ L of a mixture of acetonitrile, methylene chloride, and methanol (65:25:10) containing BHT (1 $g \cdot L^{-1}$) and N,N-di-isopropylamine (0.25 $mL \cdot L^{-1}$) to enhance the resolution. The final solution was then transferred into a high-performance liquid chromatograph (HPLC) vial containing a 200- μ L insert and tightly capped.

Chromatography was performed using the HP 1100 LC system with Chemstation software (Agilent Technologies, Santa Clara, CA) on a reverse-phase C30 column (3 μ m, 4.6 × 250 mm) from YMC America (Cary, NC) at 20 °C with diode array detection at 450 nm (Sander et al., 1994). The analytes were separated using a gradient of methyl-tert-butyl ether (A), methanol (B), and water containing 0.05 M ammonium acetate and 0.05% triethylamine (C). C was held constant at 1%, whereas amounts of A and B changed as shown in Table 1. The injection volume was 50 μ L, and the flow rate was kept at 0.8 $mL \cdot min^{-1}$. All solvents were HPLC grade (Fisher Scientific, Pittsburgh, PA). Xanthophylls were identified by ultraviolet-vis spectra analysis and by comparing their retention times with authentic standards [lutein (Extrasyntheses, Genay, France), zeaxanthin (Endofine Chemical, Hillsborough, NJ), neoxanthin and violaxanthin (ChromaDex, Irvine, CA)], or for antheraxanthin by comparison with published retention times and absorption spectra (Yoshii et al., 2004). The concentrations of xanthophylls reported were calculated using the calibrated standard curve for lutein. We identified cis isomers of lutein according to the elution order and absorption maxima reported by Meléndez-Martínez et al. (2006), who used the same column type and elution solvents used in this study. Thus, we identified a peak that eluted at ≈10.5 min as cis-lutein (mean concentration, 10 μ g/100 g FW; range, 0 to 36 μ g/100g FW). For the purposes of this article, the amounts of cis- and trans-lutein were summed and reported as total lutein.

STATISTICAL ANALYSES. Individual and total carotenoid concentrations were analyzed by both the general linear models and mixed models procedures in SAS (Version 9.2; SAS Institute, Cary, NC) assuming all effects (environments, replication, clones) were random. Estimates of the variance components from the mixed models procedures were used to compute broad-sense heritability (H) on a clonal mean basis as:

$$H = \sigma_C^2 / (\sigma_C^2 + \sigma_{CE}^2/e + \sigma_e^2/re)$$

in which σ_C^2 = clone variance, σ_{CE}^2 = environment × clone variance, σ_e^2 = error variance, r = number of replications, and e = number of environments (Nyquist, 1991).

Mean squares from the general linear models analyses were used to calculate an upper and lower confidence interval (CI) for H (Knapp et al., 1985) as:

$$\text{Upper CI} = 1 - [(\text{MS}_C/\text{MS}_{CE})F_{(1-\alpha/2; df2, df1)}]^{-1}$$

$$\text{Lower CI} = 1 - [(\text{MS}_C/\text{MS}_{CE})F_{(\alpha/2; df2, df1)}]^{-1}$$

in which MS_C = mean squares for clone and MS_{CE} = mean squares for clone × environment.

Best linear unbiased predictors (“means”) and square roots of the estimated prediction error variance (“prediction standard errors”) of all random effects were estimated from the mixed models procedure using estimate statements (Littell et al., 2006). Broad estimates of the square roots of the estimated prediction error variance were reported to conform to the assumption that all model effects were random and not fixed.

The environment × clone interaction was partitioned into stability variance components (σ_i^2) assignable to each clone using a program written for the interactive matrix language procedure in SAS (Kang, 1989). An environmental index for individual and total carotenoids was constructed as the mean carotenoid concentration of all clones in each environment minus the mean carotenoid concentration of all clones over all four environments. Heterogeneity resulting from this environmental index was removed from the environment × clone interaction, and the remainder of the environment × clone interaction was partitioned into s_i^2 components assignable to each clone.

The carotenoids produced via the LCY-b-mediated pathway (zeaxanthin, antheraxanthin, violaxanthin, neoxanthin) were summed. The proportions of carotenoids from the LCY-e (lutein) and LCY-b-mediated pathways were subjected to cluster analysis using the unweighted pair group method by arithmetic averages (Romesberg, 1990) in SAS. A second cluster analysis was done on the proportions that each of the four carotenoids in the LCY-b-mediated pathway made to the total carotenoids from the LCY-b-mediated pathway.

Results and Discussion

There were greater temperature fluctuations during the growing seasons in Florida than there were in Maine (Fig. 1). Average monthly high temperatures were greater in Florida than in Maine and average monthly low temperatures were lower in Florida than in Maine during both growing seasons. In general, rainfall patterns were similar with adequate rainfall during most of the season, then tapering off toward the end of the season (Fig. 2). In Florida in 2004, there was little rainfall during the last 6 weeks of the growing season; however, irrigation ensured that there was adequate moisture for the crop.

There were no significant differences among environments for individual carotenoids or for the totals (Table 2). Overall, zeaxanthin, antheraxanthin, violaxanthin, neoxanthin, and lutein averaged 59, 78, 66, 55, and 59 μ g/100 g FW, respectively

Table 1. Carotenoids from potato tubers were separated by high-performance liquid chromatography using a gradient of methyl-tert-butyl ether (Meth A), methanol (B), and water containing 0.05 M ammonium acetate and 0.05% triethylamine (C) in the following proportions at the indicated time during the run.

Time (min)	Meth A (%)	B (%)	C (%)
0	5	94	1
5	15	84	1
10	15	84	1
15	70	29	1
20	70	29	1
22	5	94	1
30	5	94	1

(Table 3). In contrast, Haynes et al. (1996) found significant environmental effects for yellow-flesh intensity in potato tubers evaluated at three locations in the northeastern United States. Tuber carotenoid content and yellow-flesh intensity have been reported to be highly correlated ($r = 0.83$, $P < 0.01$) (Lu et al., 2001). The lack of a significant environmental effect in this study may be the result of having evaluated these clones in fewer environments than the earlier study by Haynes et al. (1996), which resulted in a less statistically powerful test. It may also be because there was greater environmental variability

in the previous study than in this one. Although the clones in this study were grown in two widely separated locations, the growing seasons were more similar, at least as far as temperature and moisture were concerned (Fig. 1), than in the earlier study in which 1 year was cool with adequate moisture and the second year was hot and dry (Haynes et al., 1996).

There were significant differences among clones for zeaxanthin, antheraxanthin, the sum of the LCY-b-mediated pathway carotenoids, lutein, and total carotenoids, but not for neoxanthin and violaxanthin (Table 2). As expected, the white-fleshed clones were significantly lower in several of the individual carotenoids and in total carotenoids than the yellow-fleshed clones. Several researchers have reported that genetic variation for carotenoid content (Hale, 2003; Iwanzik et al., 1983; Lu et al., 2001; Nesterenko and Sink, 2003; Tevini et al., 1984; Tevini and Schonecker, 1986) or yellow-flesh intensity (Haynes et al., 1994, 1996) exists in potato. The white-fleshed clones were also significantly lower in carotenoids from the LCY-b-mediated pathway than the yellow-fleshed clones (Table 3).

There were significant environment $\times$ clone interactions for each individual carotenoid and for the totals (Table 2). Four clones (B0984-1, B1952-2, B2152-17, BTD0088-2) were stable for zeaxanthin both before and after removal of environmental heterogeneity (Table 3). Five of the clones were stable for antheraxanthin both before and after removal of environmental heterogeneity (Table 3). With the exception of 'Yukon Gold', all of the clones were unstable for violaxanthin before removing environmental heterogeneity, and six clones, including 'Yukon Gold', were unstable after removing environmental heterogeneity (Table 3). The instability of violaxanthin in 'Yukon Gold' after removal of environmental heterogeneity occurred because violaxanthin was generally higher in 'Yukon Gold' than the environmental means for violaxanthin (Maine 2004—19 versus 16 $\mu\text{g}/100\text{ g FW}$; Florida 2005—137 versus 120 $\mu\text{g}/100\text{ g FW}$; Maine 2005—69 versus 66 $\mu\text{g}/100\text{ g FW}$); however, in Florida in 2004 it was lower (91 $\mu\text{g}/100\text{ g FW}$) than the environmental mean (98 $\mu\text{g}/100\text{ g FW}$). The widest fluctuations in violaxanthin among clones were observed in the Florida environments; there was a 12.5- and 8.9-fold difference between the highest and lowest clone for violaxanthin in Florida in 2004 and 2005, respectively; whereas there was only a 3.5- and 1.7-fold difference in Maine in 2004 and 2005, respectively. 'Peter Wilcox', B2333-5, and 'Yukon Gold' were stable for neoxanthin across environments both

Fig. 1. Average monthly maximum and minimum temperatures during the 2004 and 2005 potato growing season in Hastings, FL, and Presque Isle, ME.

Fig. 2. Total monthly rainfall during the 2004 and 2005 potato-growing season in Hastings, FL, and Presque Isle, ME.

Table 2. Estimates of the variance parameters from the general linear models procedure in SAS (Version 9.2; SAS Institute, Cary, NC), broad-sense heritability (H), and the 95% confidence interval (CI) about H for individual and total carotenoids for nine potato clones grown in Hastings, FL, and Presque Isle, ME, in 2004 and 2005.

Variance parameter	Zeaxanthin	Antheraxanthin	Violaxanthin	Neoxanthin	Total LCY-b ^z	Lutein	Total
Environment	703	175	2,400	5,630	2,242	296	4,258
Replication (environment)	117*	26	9	68*	16	12	14
Clone	1,811*	760*	1,117	974	23,941*	326*	29,438*
Environment $\times$ clone	535**	319**	1,950**	3,520**	3,262**	145*	4,252**
Error	1,157	580	538	549	2,689	241	3643
H	0.89	0.93	0.68	0.51	0.96	0.85	0.96
Upper 95% CI	0.98	0.99	0.92	0.88	0.99	0.99	0.99
Lower 95% CI	0.79	0.87	0.14	0.00	0.90	0.70	0.89

^zSum of zeaxanthin, antheraxanthin, violaxanthin, and neoxanthin.

*, **Significant at $P = 0.05$ or 0.01 , respectively.

LCY-b = lycopene beta-cyclase.

Table 3. Best linear unbiased predictors (means) of individual and total carotenoid concentrations in potato tubers grown in Hastings, FL, and Presque Isle, ME, in 2004 and 2005, the stability before (σ^2_i) and after (s^2_i) removing environmental heterogeneity, and the square root of the estimated prediction error variance (prediction SE) for comparisons among clones, among environments (env), and within clone $\times$ environments for individual and total carotenoids.

Zeaxanthin concn ($\mu\text{g}/100 \text{ g FW}$)								
Clone	FL 2004	ME 2004	FL 2005	ME 2005	Mean	σ^2_i	s^2_i	Prediction SE
B0984-1	21	49	12	4	24	NS	NS	clone: 18.2
Peter Wilcox	88	116	101	128	105	**	**	env: 17.7
B1870-3	16	25	1	0	14	**	NS	clone $\times$ env: 10.1
B1952-2	3	57	2	1	18	NS	NS	
B2152-17	33	64	28	4	34	NS	NS	
B2319-1	60	113	32	51	64	**	**	
B2333-5	104	231	145	74	133	**	**	
BTD0088-2	54	92	63	37	61	NS	NS	
Yukon Gold	56	135	71	42	75	**	NS	
Mean	49	95	51	39	59			
Antheraxanthin concn ($\mu\text{g}/100 \text{ g FW}$)								
Clone	FL 2004	ME 2004	FL 2005	ME 2005	Mean	σ^2_i	s^2_i	Prediction SE
B0984-1	27	26	55	45	40	NS	NS	clone: 11.5
Peter Wilcox	111	95	137	171	126	**	**	env: 15.7
B1870-3	19	22	38	35	31	NS	NS	clone $\times$ env: 6.3
B1952-2	21	28	48	43	37	NS	NS	
B2152-17	34	40	65	62	51	NS	NS	
B2319-1	83	154	108	130	117	**	**	
B2333-5	133	122	169	124	135	**	**	
BTD0088-2	60	110	96	112	94	**	**	
Yukon Gold	51	82	82	87	76	NS	NS	
Mean	63	76	87	88	78			
Violaxanthin concn ($\mu\text{g}/100 \text{ g FW}$)								
Clone	FL 2004	ME 2004	FL 2005	ME 2005	Mean	σ^2_i	s^2_i	Prediction SE
B0984-1	29	8	38	24	37	**	NS	clone: 32.4
Peter Wilcox	151	28	137	40	82	**	**	env: 18.8
B1870-3	17	8	30	28	34	**	*	clone $\times$ env: 5.7
B1952-2	21	7	33	26	35	**	NS	
B2152-17	48	12	67	23	46	**	NS	
B2319-1	199	25	181	28	95	**	**	
B2333-5	178	15	200	26	93	**	*	
BTD0088-2	150	25	256	29	100	**	**	
Yukon Gold	91	19	137	31	68	NS	*	
Mean	96	21	115	31	66			
Neoxanthin concn ($\mu\text{g}/100 \text{ g FW}$)								
Clone	FL 2004	ME 2004	FL 2005	ME 2005	Mean	σ^2_i	s^2_i	Prediction SE
B0984-1	1	8	0	38	12	**	NS	clone: 46.6
Peter Wilcox	16	58	12	202	72	NS	NS	env: 23.1
B1870-3	0	3	1	28	8	**	NS	clone $\times$ env: 7.3
B1952-2	2	9	0	44	14	**	NS	
B2152-17	3	17	8	117	36	**	NS	
B2319-1	14	100	14	391	130	**	**	
B2333-5	6	24	13	192	60	NS	NS	
BTD0088-2	10	63	23	334	108	**	NS	
Yukon Gold	7	26	13	181	57	NS	NS	
Mean	10	37	12	162	55			
LCY-b pathway carotenoid concn ($\mu\text{g}/100 \text{ g FW}$) ^z								
Clone	FL 2004	ME 2004	FL 2005	ME 2005	Mean	σ^2_i	s^2_i	Prediction SE
B0984-1	76	90	105	111	101	**	NS	clone: 37.5
Peter Wilcox	363	295	389	546	394	**	**	env: 55.4

continued next page

Table 3. Continued.

Clone	Zeaxanthin concn (µg/100 g FW)							Prediction SE
	FL 2004	ME 2004	FL 2005	ME 2005	Mean	σ^2_i	s^2_i	
B1870-3	51	56	69	92	73	**	NS	clone × env: 12.3
B1952-2	44	101	82	115	91	**	*	
B2152-17	115	133	168	204	159	NS	NS	
B2319-1	355	399	335	596	416	**	**	
B2333-5	420	400	529	412	434	**	**	
BTD0088-2	274	296	438	508	375	**	*	
Yukon Gold	203	267	302	339	277	NS	*	
Mean	218	267	231	315				

Clone	Lutein concn (µg/100 g FW)							Prediction SE
	FL 2004	ME 2004	FL 2005	ME 2005	Mean	σ^2_i	s^2_i	
B0984-1	39	26	62	48	45	**	**	clone: 10.6
Peter Wilcox	59	61	51	101	67	**	**	env: 7.7
B1870-3	24	18	28	36	30	**	NS	clone × env: 4.1
B1952-2	37	33	53	58	47	**	NS	
B2152-17	38	43	61	94	59	**	NS	
B2319-1	61	83	81	132	86	**	**	
B2333-5	64	62	87	97	76	NS	NS	
BTD0088-2	38	48	77	100	65	**	*	
Yukon Gold	48	39	56	76	55	NS	NS	
Mean	46	46	62	81	59			

Clone	Total concn (µg/100 g FW) ^y							Prediction SE
	FL 2004	ME 2004	FL 2005	ME 2005	Mean	σ^2_i	s^2_i	
B0984-1	115	115	167	159	145	**	NS	clone: 46.6
Peter Wilcox	422	356	439	648	461	**	**	env: 61.6
B1870-3	75	73	98	128	101	**	NS	clone × env: 14.2
B1952-2	81	133	136	172	137	**	NS	
B2152-17	154	176	228	299	218	NS	NS	
B2319-1	415	482	416	729	504	**	**	
B2333-5	483	462	617	510	511	**	**	
BTD0088-2	312	343	515	608	440	**	**	
Yukon Gold	251	306	358	415	332	NS	NS	
Mean	262	276	329	399	316			

^zLCY-b pathway carotenoids are zeaxanthin, antheraxanthin, neoxanthin, and violaxanthin.

^yTotal carotenoids are zeaxanthin, antheraxanthin, neoxanthin, violaxanthin, and lutein.

*, **Significant at $P = 0.05$ or 0.01 , respectively; NS = nonsignificant.

FW = fresh weight; LCY-b = lycopene beta-cyclase.

before and after removal of environmental heterogeneity (Table 3). B2319-1 was the only clone unstable for neoxanthin both before and after removal of environmental heterogeneity. Neoxanthin was low in the Florida environments but fluctuated widely in the Maine environments. B2152-17 was the only clone that was stable for the sum of the LCY-b-mediated pathway carotenoids both before and after removal of environmental heterogeneity (Table 3). B2333-5 and 'Yukon Gold' were stable for lutein both before and after removal of environmental heterogeneity (Table 3). Only two clones (B2152-17 and 'Yukon Gold') were stable for total carotenoids both before and after removal of environmental heterogeneity. Based on these results, there is some variability in the stability of carotenoid concentrations among clones over environments.

Broad-sense heritability for individual and total carotenoids ranged from a low of 0.51 for neoxanthin to a high of 0.96 for total LCY-b pathway carotenoids and total carotenoids (Table 2). As expected, the estimate of broad-sense heritability for total carotenoid in this study is in close agreement with the

estimate for yellow-flesh intensity ($H = 0.93$) reported by Haynes et al. (1996).

'Yukon Gold' is currently the most popular yellow-fleshed potato cultivar grown in the United States. In terms of individual and total carotenoid content, it was consistently the most stable clone evaluated in this study. However, with the exception of the yellow-fleshed clone B2152-17, individual and total carotenoids in the other five yellow-fleshed clones were sometimes significantly greater than in 'Yukon Gold': zeaxanthin in one clone (B2333-5), antheraxanthin in three clones ('Peter Wilcox', B2319-1, B2333-5), the LCY-b pathway carotenoids in three clones ('Peter Wilcox', B2319-1, B2333-5), lutein in two clones (B2319-1, B2333-5), and total carotenoid in four clones ('Peter Wilcox', B2319-1, B2333-5, BTD0088-2) (Table 3). Flesh color in B2152-17 was the palest yellow of all the yellow-fleshed clones in this study.

Brown et al. (2006) have suggested that transferring the high levels of carotenoids present in diploid papa amarilla (yellow flesh) potato germplasm may introduce a number of exotic

alleles not currently present in the North American and European tetraploid germplasm base. This study presents indirect evidence that such diploids can be used in improving carotenoid levels in tetraploid potatoes. There is a diploid yellow-fleshed grandparent in the pedigree of ‘Yukon Gold’ (Johnston and Rowberry, 1981). BTD0088-2 is the result of a 4x–2x cross involving the yellow-fleshed diploid BD001-1. B2333-5 is the result of a cross between 4x *S. tuberosum* and a 4x–2x progeny involving the diploid yellow-fleshed parent BD067-4. B2319-1 is the result of a cross involving two 4x–2x parents: the diploid from the maternal side was the yellow-fleshed clone BD016-2 and the diploid from the paternal side was BD067-4. Of the two clones consisting only of *S. tuberosum* germplasm, ‘Peter Wilcox’ had higher levels of carotenoids than ‘Yukon Gold’, but B2152-17 had lower levels of carotenoids.

Cluster analysis revealed that the proportion of carotenoids produced through the two branches on the carotenoid biosynthetic pathway differed between the white-fleshed and yellow-fleshed clones. In general, a greater proportion of the carotenoids produced in the white-fleshed clones came from the LCY-e-mediated pathway than the LCY-b mediated pathway (Table 4). As a group, 32% of the carotenoids in the white-fleshed clones were produced through the LCY-e-mediated

pathway as compared with 17% of the carotenoids in the yellow-fleshed clones. The one exception was B2152-17, a yellow-fleshed clone that clustered with the white-fleshed clones. However, as noted earlier, B2152-17 was the lightest yellow-fleshed clone of the yellow-fleshed group. Total carotenoid content in B2152-17 ranged from 114 to 293 µg/100 g FW less than in the other yellow-fleshed clones, although total carotenoid content was 73 to 117 µg/100 g FW higher than in the white-fleshed clones (Table 3).

In the LCY-b-mediated pathway, zeaxanthin, antheraxanthin, and violaxanthin are interconvertable through zeaxanthin epoxidase and violaxanthin de-epoxidase, whereas neoxanthin synthase is responsible for the isomerization of violaxanthin to neoxanthin (Diretto et al., 2006; Romer and Fraser, 2005). Cluster analysis of the proportions of zeaxanthin, antheraxanthin, violaxanthin, and neoxanthin to total carotenoids produced from the LCY-b branch of carotenogenesis revealed three distinct clusters. The clusters were largely defined by the proportion of neoxanthin (Fig. 3). One cluster consisted of two clones, BTD0088-2 and B2319-1, in which the proportion of neoxanthin from the LCY-b-mediated branch was 30% and 32%, respectively. The second cluster consisted of four clones in which the proportion of neoxanthin from the LCY-b-mediated branch was intermediate to the other two clusters:

Table 4. Proportion of total carotenoids from the carotenoid LCY-e-mediated pathway (lutein) and flesh color of nine potato clones grown in Hastings, FL, and Presque Isle, ME, in 2004 and 2005.

Clone	Proportion carotenoids from LCY-e-mediated pathway				Mean	Tuber flesh color
	FL 2004	ME 2004	FL 2005	ME 2005		
B0984-1	0.35	0.22	0.38	0.30	0.31	White
Peter Wilcox	0.14	0.17	0.11	0.16	0.14	Yellow
B1870-3	0.36	0.26	0.29	0.28	0.30	White
B1952-2	0.46	0.24	0.39	0.33	0.36	White
B2152-17	0.26	0.24	0.27	0.31	0.27	Yellow
B2319-1	0.14	0.17	0.20	0.18	0.17	Yellow
B2333-5	0.13	0.13	0.14	0.20	0.15	Yellow
BTD0088-2	0.12	0.14	0.15	0.17	0.14	Yellow
Yukon Gold	0.19	0.13	0.16	0.18	0.17	Yellow
Mean	0.24	0.19	0.23	0.23		

LCY-e = lycopene epsilon-cyclase.

Fig. 3. Proportion of zeaxanthin, antheraxanthin, violaxanthin, and neoxanthin to the total carotenoids produced by the lycopene beta-cyclase-mediated branch of carotenogenesis for each of nine potato clones grown during 2004 and 2005 in Hastings, FL, and Presque Isle, ME.

B2152-17 (22%), 'Yukon Gold' (21%), B2333-5 (14%), and 'Peter Wilcox' (19%). The last cluster consisted of the three white-fleshed clones, B0984-1, B1870-3, and B1952-2, and the proportions of neoxanthin from the LCY-b-mediated branch were 11%, 9%, and 13%, respectively.

There are two general strategies available for metabolic engineering of plant carotenoids (Giuliano et al., 2000). One strategy is to overexpress a gene encoding a rate-limiting step. Ducreux et al. (2005) produced transgenic potato plants that overexpressed phytoene synthase and thereby increased the carotenoid content of the transformed potato cultivar Désirée. In addition to generally higher levels of the usual carotenoids found in potatoes, tubers of their transgenic lines contained significant amounts of β -carotene, a carotenoid not generally reported in potato. The second strategy involves silencing a biosynthetic step downstream of the desired compound. Romer et al. (2002) were able to increase zeaxanthin in two potato cultivars up to 130-fold using antisense inactivation of zeaxanthin epoxidase and cosuppression by the corresponding sense construct.

This study and others suggest that sufficient heritable genetic variation exists in *Solanum* germplasm for progress to be made through plant breeding and it is likely that new alleles will be uncovered. In addition, our understanding of and ability to manipulate key steps in carotenogenesis through genetic engineering is increasing. Thus, several mechanisms exist to improve the carotenoid content and hence the nutritional value of potatoes.

Literature Cited

- Akbaraly, N.T., H. Faure, V. Gourlet, A. Favier, and C. Berr. 2007. Plasma carotenoid levels and cognitive performance in an elderly population: Results of the EVA study. *J. Gerontol. A Biol. Sci. Med. Sci.* 62:308–316.
- Andre, C.M., M. Oufir, C. Guignard, L. Hoffmann, J.-F. Hausman, D. Evers, and Y. Larondelle. 2007. Antioxidant profiling of native Andean potato tubers (*Solanum tuberosum* L.) reveals cultivars with high levels of β -carotene, α -tocopherol, chlorogenic acid, and petanin. *J. Agr. Food Chem.* 55:10839–10849.
- Barton-Duelli, P. 1995. The role of dietary antioxidants in prevention of atherosclerosis. *Endocrinologist* 5:347–356.
- Brown, C.R., C.G. Edwards, C.-P. Yang, and B.B. Dean. 1993. Orange flesh trait in potato: Inheritance and carotenoid content. *J. Amer. Soc. Hort. Sci.* 118:145–150.
- Brown, C.R., T.S. Kim, Z. Ganga, K. Haynes, D. De Jong, M. Jahn, I. Paran, and W. De Jong. 2006. Segregation of total carotenoid in high level potato germplasm and its relationship to beta-carotene hydroxylase polymorphism. *Amer. J. Potato Res.* 83:365–372.
- Centro Internacional de la Papa. 2009. Potato/CIP potato facts: Potato: Growth in production accelerates. 30 Oct. 2009. <<http://www.cipotato.org/potato/facts/growth.asp>>.
- Delgado-Vargas, F., A.R. Jiménez, and O. Paredes-López. 2000. Natural pigments: Carotenoids, anthocyanins, and betalains—Characteristics, biosynthesis, processing, and stability. *Crit. Rev. Food Sci. Nutr.* 40:173–289.
- Demming-Adams, B., A.M. Gilmore, and W.W. Adams. 1996. In vivo functions of carotenoids in higher plants. *Federation Amer. Soc. Expt. Biol. J.* 10:403–412.
- Diretto, G., R. Tavazza, R. Welsch, D. Pizzichini, F. Mourgues, V. Papacchioli, P. Beyer, and G. Giuliano. 2006. Metabolic engineering of potato tuber carotenoids through tuber-specific silencing of lycopene epsilon cyclase. *BMC Plant Biol.* 6:13.
- Ducreux, L.J.M., W.L. Morris, P.E. Hedley, T. Shephert, H.V. Davies, S. Millam, and M.A. Taylor. 2005. Metabolic engineering of high carotenoid potato tubers containing enhanced levels of β -carotene and lutein. *J. Expt. Bot.* 56:81–89.
- Gey, K.F. 1995. Ten-year retrospective on the antioxidant hypothesis of arteriosclerosis: Threshold plasma levels of antioxidant micro-nutrients related to minimum cardiovascular risk. *Nutr. Biochem.* 6:206–236.
- Giuliano, G., R. Aquilani, and S. Dharmapuri. 2000. Metabolic engineering of plant carotenoids. *Trends Plant Sci.* 5:406–409.
- Goodwin, T.W. 1982. Biochemistry of the carotenoids. Vol. 1: Plants. 2nd Ed. Chapman and Hall, New York, NY.
- Granado, R., B. Olmedilla, I. Blanco, and E. Rojas-Hidalgo. 1996. Major fruit and vegetable contributors to the main serum carotenoids in the Spanish diet. *Eur. J. Clin. Nutr.* 50:246–250.
- Hale, A.L. 2003. Screening potato genotypes for antioxidant activity, identification of the responsible compounds, and differentiating Russet Norkotah strains using AFLP and microsatellite marker analysis. PhD Diss., Texas A&M Univ., College Station, TX.
- Hari, R.K., T.R. Patel, and A.M. Martin. 1994. An overview of pigment production in biological systems: Functions, biosynthesis, and applications in food industry. *Food Rev. Int.* 10:49–70.
- Haynes, K.G., W.E. Potts, J.L. Chittams, and D.L. Fleck. 1994. Determining yellow-flesh intensity in potatoes. *J. Amer. Soc. Hort. Sci.* 119:1057–1059.
- Haynes, K.G., J.B. Sieczka, M.R. Henninger, and D.L. Fleck. 1996. Clone $\times$ environment interactions for yellow-flesh intensity in tetraploid potatoes. *J. Amer. Soc. Hort. Sci.* 121:175–177.
- Haynes, K.G., D.R. Wilson, and M.S. Kang. 1995. Genotype $\times$ environment interactions for specific gravity in diploid potatoes. *Crop Sci.* 35:977–981.
- Institute of Medicine. 2000. Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids. National Academy Press, Washington, DC.
- Iwanzik, W., M. Tevini, R. Stute, and R. Hilbert. 1983. Carotinoidgehalt undzusammensetzung verschiedener deutscher Kartoffelsorten und deren Bedeutung für die Fleischfarbe der Knolle. *Potato Res.* 26:149–162.
- Johnston, G.R. and R.G. Rowberry. 1981. Yukon Gold: A new yellow-fleshed, medium-early, high quality table and French fry cultivar. *Amer. Potato J.* 58:241–244.
- Kang, M.S. 1989. A new SAS program for calculating stability–variance parameters. *J. Hered.* 80:415.
- Khachik, F., G.R. Beecher, M.B. Goli, and W.R. Lusby. 1991. Separation, identification, and quantification of carotenoids in fruits, vegetables and human plasma by high performance liquid chromatography. *Pure Appl. Chem.* 63:71–80.
- Khachik, F., J.S. Bertram, M.-T. Huang, J.W. Fahey, and P. Talalay. 1999. Dietary carotenoids and their metabolites as potentially useful chemoprotective agents against cancer, p. 203–229. In: L. Packer, M. Hiramatsu, and T. Yoshikawa (eds.). Antioxidant food supplements in human health. Academic Press, San Diego, CA.
- Knapp, S.J., W.W. Stroup, and W.M. Ross. 1985. Exact confidence intervals for heritability on a progeny mean basis. *Crop Sci.* 25:192–194.
- Lindley, M.G. 1998. The impact of food processing on antioxidants in vegetable oils, fruits and vegetables. *Trends Food Sci. Technol.* 9:336–341.
- Littell, R.C., G.A. Milliken, W.W. Stroup, R.D. Wolfinger, and O. Schabenberger. 2006. SAS for mixed models. 2nd Ed. SAS Institute, Cary, NC.
- Lu, W., K. Haynes, E. Wiley, and B. Clevidence. 2001. Carotenoid content and color in diploid potatoes. *J. Amer. Soc. Hort. Sci.* 126:722–726.
- Machlin, L.J. 1995. Critical assessment of the epidemiologic data concerning the impact of antioxidant nutrients on cancer and cardiovascular diseases. *Crit. Rev. Food Sci. Nutr.* 35:41–50.
- Mares-Perlman, J.A., W.E. Brady, R. Klein, B.E.K. Klein, P. Bowen, M. Stacewiczapuntsakis, and M. Palta. 1995. Serum antioxidants and age-related macular degeneration in a population-based case–control study. *Arch. Ophthalmol.* 113:1518–1523.

- Meléndez-Martínez, A.J., G. Britton, I.M. Vicario, and F.J. Heredia. 2006. HPLC analysis of geometrical isomers of lutein epoxide isolated from dandelion (*Taraxacum officinale* F. Weber ex Wiggers). *Phytochemistry* 67:771–777.
- Nesterenko, S. and K.C. Sink. 2003. Carotenoid profiles of potato breeding lines and selected cultivars. *HortScience* 38:1173–1177.
- Nyquist, W.E. 1991. Estimation of heritability and prediction of selection response in plant populations. *Crit. Rev. Plant Sci.* 10:235–322.
- Palozza, P. and N.I. Krinsky. 1992. Antioxidant effects of carotenoids in vivo and in vitro: An overview. *Methods Enzymol.* 213:403–420.
- Pfander, H., M. Gerspacher, M. Rychener, and R. Schwabe. 1987. Key to carotenoids. Birkhäuser, Basel, Switzerland.
- Reddivari, L., A.L. Hale, and J.C. Miller, Jr. 2007. Genotype, location, and year influence antioxidant activity, carotenoid content, phenolic content, and composition in specialty potatoes. *J. Agr. Food Chem.* 55:8073–8079.
- Romer, S. and P.D. Fraser. 2005. Recent advances in carotenoid biosynthesis, regulation and manipulation. *Planta* 221:305–308.
- Romer, S., J. Lubeck, F. Kauder, S. Steiger, C. Adomat, and G. Sandmann. 2002. Genetic engineering of a zeaxanthin-rich potato by antisense inactivation and co-suppression of carotenoid epoxidation. *Metab. Eng.* 4:263–272.
- Romesberg, H.C. 1990. Cluster analysis for researchers. Robert E. Krieger, Malabar, FL.
- Sander, L.C., K.E. Shapless, N.E. Craft, and S.A. Wise. 1994. Development of engineered stationary phases for the separation of carotenoid isomers. *Anal. Chem.* 66:1667–1674.
- Seddon, J.M., U.A. Ajani, R.D. Speruto, R. Hiller, N. Blair, T.C. Burton, M.D. Farber, E.S. Gragoudas, J. Haller, D.T. Miller, L.A. Yannuzzi, and W. Willett. 1994. Dietary carotenoids, vitamin A, vitamin C and vitamin E and advanced age-related macular degeneration. *J. Amer. Medical Assn.* 272:1413–1420.
- Sies, H. and W. Stahl. 2004. Nutritional protection against skin damage from sunlight. *Annu. Rev. Nutr.* 24:173–200.
- Stahl, W. and H. Sies. 1998. The role of carotenoids and retinoids in gap junctional communication. *Int. J. Vitam. Nutr. Res.* 68:354–359.
- Tanaka, Y., N. Sasaki, and A. Ohmiya. 2008. Biosynthesis of plant pigments: Anthocyanins, betalains and carotenoids. *Plant J.* 54:733–749.
- Tevini, M., W. Iwanzik, and G. Schonecker. 1984. Analyse vorkommen und nerhalten von carotinoiden in kartoffeln und kartoffelprodukten. *Jahrbuch Forschungskreis Ernährungsindustrie* 5:36–53.
- Tevini, M. and G. Schonecker. 1986. Occurrence, properties, and characterization of potato carotenoids. *Potato Res.* 29:265.
- U.S. Department of Agriculture. 2009. USDA national nutrient database for standard reference, release 22. 2 Nov. 2009. <<http://www.ars.usda.gov/Services/docs.htm?docid=8964>>.
- U.S. Department of Agriculture, Agricultural Research Service; North Carolina Agricultural Research Service; New Jersey Agricultural Experiment Station; Pennsylvania Agricultural Experiment Station; Maine Agricultural and Forestry Experiment Station; Florida Agricultural Experiment Station; and New York Agricultural Experiment Station. 2007. Naming and release of the Peter Wilcox potato variety. Official release notice. U.S. Dept. Agr., Washington, DC.
- Yoshii, Y., T. Hanyuda, I. Wakana, K. Miyaji, S. Arai, K. Ueda, and I. Inouye. 2004. Carotenoid composition of *Cladophora* balls (*Aegagropila linnaei*) and some members of the cladophorales (Ulvophyceae, chlorophyta): Their taxonomic and evolutionary implications. *J. Phycol.* 40:1170–1177.