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# Biomass Production and Pigment Accumulation in Kale Grown Under Increasing Photoperiods

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***Abstract.*** **Consumption of fruit and vegetable crops rich in lutein and β-carotene carotenoids is associated with reduced risk of cancers and aging eye diseases. Kale (*Brassica oleracea* L. var. *acephala* D.C.) ranks highest for lutein concentrations and is an excellent source of dietary carotenoids. Kale plants were grown under varied photoperiods to determine changes in the accumulation of fresh and dry biomass, chlorophyll *a* and *b*, and lutein and β-carotene carotenoids. The plants were cultured in a controlled environment using nutrient solutions under photoperiod treatments of 6, 12, 16, or 24 hours (continuous). Fresh and dry mass production increased linearly as photoperiod increased, reaching a maximum under the 24-hour photoperiod. Maximum accumulation of lutein, β-carotene, and chlorophyll *b* occurred under the 24-h photoperiod at 13.5, 10.4, and 58.6 mg/100 g fresh mass, respectively. However, maximum chlorophyll *a* (235.1 mg/100 g fresh mass) occurred under the 12-hour photoperiod. When β-carotene and lutein were measured on a dry mass basis, the maximum accumulation was shifted to the 16-hour photoperiod. An increase in photoperiod resulted in increased pigment accumulation, but maximum concentrations of pigments were not correlated with maximum biomass production.**

The length of the photoperiod will influence a number of plant physiological factors including biomass production, bud formation, flowering, germination, leaf elongation, leaf emergence, and changes in secondary compounds (Degli Agosti et al., 1990; Densmore, 1997; Drozdova et al., 2004; Gottdenker et al., 2000; Junttila et al., 1997; Koontz and Prince, 1986; Masuda and Murage, 1998**;** Murage et al., 1997; Riihimaki and Savolainen, 2004; Taylor et al., 1994). Increases in photoperiod have consistently resulted in increased plant biomass production (Garner and Allard, 1931; Koontz and Prince, 1986; Masuda and Murage, 1998; Ohler and Mitchell, 1996). This increase in biomass result from actions related to increases in leaf area and chlorophyll content (Langton et al., 2003). Under continuous irradiance, some plants, including eggplant (*Solanum melongena* L.), potato (*Solanum tuberosum* L.), tomato (*Lycopersicon esculentum* L.), and cowpea (*Vigna unguiculata* Walp.) have responded with limited biomass production and with interveinal chlorosis and necrosis (Bradley and Janes, 1985; Murage and Masuda, 1997; Murage et al., 1997; Ohler and Mitchell, 1996; Stutte et al., 1996).

For many plant species, the increase in chlorophyll concentration as a result of increasing photoperiod is well documented. Sironval (1958) showed that changing the photoperiod of hemp (*Cannabis sativa* L.), lupin (*Lupinus albus* L.), soybean (*Glycine max* Merr.), and strawberry (*Fragaria vesca* L.) from 8 to 16 h resulted in increased leaf chlorophyll concentration. Chlorophyll concentration also increased as photoperiod increased in tomato (Hurd, 1973), geranium (*Pelargonium ×hortorum* Bailey), impatiens (*Impatiens walleriana* Hooker), pansy (*Viola ×wittrockiana* Kappert) and petunia (*Petunia ×hybrida* Vilmorin) (Langton et al., 2003). Fukuda et al. (2000) reported additions of night supplemental lighting (to establish a 24-h photoperiod) increased chlorophyll concentrations in lettuce (*Lactuca salvia* L.), pakchoi (*Brassica rapa* L. subsp. *chinensis* P. Hanelt), and tsukena (*Brassica rapa* L. subsp. *campestris* A.R. Clapham). The research into the effect of increased photoperiod on carotenoid accumulation is limited in higher plants. Arizmendi-Maldonado et al. (2003) reported adding supplemental lighting to extend the standard day to a 15-h photoperiod did not affect the accumulation of β-carotene in bermudagrass (*Cynodon dactylon* Pers.) or African stargrass (*Cynodon nlemfuensis* Vandergst).

Carotenoids are yellow, orange, and red plant lipid-soluble pigments, produced by plants, algae and bacteria that cannot be synthesized by mammals. In plants, carotenoids are used as antenna pigments to funnel light energy to the photosynthetic reaction center. These carotenoids are in close proximity to the chlorophyll molecules and absorb energy to prevent damage to the photosynthetic system (Marschner, 1995; Miki, 1991; Taiz and Zeiger, 1998; Tracewell et al., 2001). Lutein and β-carotene carotenoids possess important human health properties. Dietary intake of foods rich in lutein and β-carotene has been associated with reduced risk of lung cancer, cataracts, and age-related macular degeneration (Ames et al., 1995; Landrum and Bone, 2001; Le Marchand et al., 1993). Kale (*Brassica oleracea* L. var. *acephala* D.C.) is an excellent source of dietary carotenoids (Holden et al., 1999; Kurilich et al., 1999; USDA, 2005). The USDA rates kale as the highest source of lutein and β-carotene of any vegetable (Holden et al. 1999; USDA, 2005).

Light is critical for plant growth and development, and the photoperiod can easily be controlled by growers in artificial growing environments. What remains unclear is the effect of photoperiod on the production of secondary plant pigments, such as carotenoids. Therefore, the goal of this study was to determine the influences of four different irradiance photoperiods on plant biomass and accumulation patterns of carotenoid pigments in the leaf tissues of kale.

## Material and Methods

*Plant culture*. 'Winterbor' kale (Johnny's Selected Seed, Winslow, Maine) was sown into 3.8-cm rockwool growing cubes (Grodan A/S, Dk-2640, Hedehusene, Denmark) and germinated in a greenhouse (22 °C day/14°C night) under natural lighting conditions (Durham, N.H., Lat. 43° 09' N) on 6 Oct. 2003, 3 Nov. 2003, and 30 Dec. 2003. Peter's 20N–6.9P–16.6K water-soluble fertilizer (Scotts Company, Marysville, Ohio) was applied at 200 mg·L$^{-1}$ every 5 d. After 2 weeks, the plants were transferred to 38-L plastic containers (Rubbermaid Inc., Wooster, Ohio). Eight plants were placed into 2 cm round holes cut at 10.6 × 9.5 cm spacing in each container lid. Four containers were placed into each of four growth chambers (E15, Conviron, Winnipeg, Manitoba). The growth chamber temperature was maintained at 20 ± 1 °C, and the photosynthetically active radiation (PAR) was measured at 500 ± 100 µmol·m$^{-2}$·s$^{-1}$. PAR was measured (Model QSO-ELEC, Apogee Instruments; Logan, Utah) at six locations, without plants, on top of each tub at the four corner plant holes and between the two side middle plant holes and averaged. PAR levels were measured at the beginning and confirmed at the end of each replication. Cool-white fluorescent (160 W) and incandescent (60W) bulbs were used during the experiment. The chambers were randomly assigned experimental treatments, and the containers within each chamber were blocked together providing 32 plants for each sample. The four chambers for photoperiod were 6, 12, 16, or 24 h. Treatments were replicated beginning on 20 Oct. 2003, 17 Nov. 2003, and 13 Jan. 2004.

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The plants were grown hydroponically in 30 L of nutrient solution (Hoagland and Arnon, 1950). Elemental concentrations of the nutrient solutions were (mg·L$^{-1}$): N (105), P (15.3), K (117), Ca (80.2), Mg (24.6), S (32.0), Fe (0.5), B (0.25), Mo (0.005), Cu (0.01), Mn (0.25), and Zn (0.025). The electrical conductivity (EC) of the starting nutrient solution was 0.7 dS·m$^{-1}$ and the pH was measured at 5.6. Solutions were aerated with an air blower (Model 25E133W222, Spencer, Winsor, Conn.) connected to air stones. Deionized water was added daily to maintain 30 L in each container. Nutrient solutions were replaced every week throughout the experiment to refresh the solution to the initial nutrient concentrations.

The plants were grown for 3 weeks. At harvest, shoot and root tissues were separated and weighed. The fourth fully expanded leaf was selected and a 4-cm$^2$ piece of the leaf was removed from each of the 32 plants in the four containers and combined to form one sample. Samples were stored at –80 °C before lyophilization. The remaining shoot material was dried at 60 °C for no less than 72 h, at which time shoot dry mass and % dry matter were determined.

*Carotenoid and chlorophyll determination–tissue extraction.* Frozen kale samples were lyophilized at –20 °C for a minimum of 72 h (model 6L FreeZone; LabConCo, Kansas City, Mo.). The dried tissues samples were ground with dry ice in a kitchen grinder (Handy Chopper Plus, HC 3000, Household Products Inc., Shelton, Conn.). Pigments were extracted and separated according to Kopsell et al. (2004), a procedure which is based on the method of Khachik et al. (1986). A 0.1-g subsample was placed into a Potter-Elvehjem tissue grinder tube (Kontes, Vineland, N.J.) and hydrated with 0.8 mL of deionized water. The sample was placed in a 40 °C water bath for 20 min. After hydration, 0.8 mL of the internal standard, ethyl-β-8-apo-carotenoate (Sigma Chemical Co., St. Louis, Mo.), and 2.5 mL of HPLC grade tetrahydrofuran (THF) were added to the sample. The sample was homogenized in the tube with 25 insertions with a Potter-Elvehjem tissue grinder pestle attached to a drill press at 540 rpm. The sample tube was kept immersed in ice during extraction. The tube was placed into a clinical centrifuge for 3 min at 500 $g_n$. The supernatant was removed with a Pasteur pipette, placed into a conical 15-ml test tube, capped, and held on ice. The sample pellet was re-suspended in 2.0 mL THF and homogenized with 25 insertions of the grinding pestle. The tube was centrifuged for 3 min at 500 $g_n$, and the supernatant was collected and combined with the first extracted supernatant. The extraction procedure was repeated twice more until the supernatant was colorless. The pellet was discarded and the combined 4 supernatants were placed in a 40°C water bath and reduced to 0.5 ml using nitrogen gas (model N-EVAP 111; Organomatic Inc., Berlin, Mass.). 2.5 mL of MeOH and 2.0 mL of THF were added to the sample, which was then vortexed and filtered through a 0.2 µm polytetrafluoroethylene (PTFE) filter (model Econofilter PTFE 25/20, Agilent Technologies, Wilmington, Del.) using a 5-mL syringe (Becton Dickinson, Franklin Lakes, N.J.) before high performance liquid chromatograph (HPLC) analysis.

*Carotenoid and chlorophyll determination–HPLC analysis.* A HPLC unit with photodiode array detector (Agilent 1100, Agilent Technologies, Palo Alto, Calif.) was used for pigment separation. All samples were analyzed for carotenoid compounds using a Vydac RP-C18 5.0 µm 250 × 4.6 mm column (model 201TP54; Phenomenex, Torrance, Calif.) fitted with a 4 × 3.0 mm, 7.0 µm guard column compartment. The column was maintained at 16 °C using a thermostatic column compartment. Eluents were A) 75% acetronitrile, 20% methanol, 5% hexane, 0.05% BHT, and 0.013% triethyamine (TEA) and B) 50% acetonitrile, 25% THF, 25% hexane and 0.013% TEA. The flow rate was 0.7 mL·min$^{-1}$, and the gradient was 100% eluent A for 30 min, 50% A and 50% B for 2 min, 100% B for 2 min, and 50% A and 50% B for 2 min. The eluent was returned to 100% A for 10 min before the next injection. Eluted carotenoids and chlorophyll pigments from a 20 µL injection were detected at 453 (carotenoids and internal standard), 652 (chlorophyll *a*), and 665 (chlorophyll *b*) nm, with data collected, recorded and integrated using 1100 HPLC ChemStation Software (Agilent Technologies). Internal standard % recovery ranged from 70% to 96%. Peak assignment for individual pigments were performed by comparing retention times and line spectra obtained from photodiode array detection using external standards (lutein from Carotenature, Lupsingen, Switzerland; β-carotene, Chl *a*, Chl *b* from Sigma Chemical Co.). Concentrations of external standards were determined spectrophotometrically using the following $E1\%1\text{cm}$ values: lutein, 2550 in ETOH, $\lambda_{max}$ = 445 nm; β-carotene, 2592 in hexane, $\lambda_{max}$ = 452 nm; Chl *a*, 819 in ETOH, $\lambda_{max}$ = 665 nm; and Chl *b*, 441 in ETOH, $\lambda_{max}$ = 649 nm (Davies and Köst, 1988). Standard reference material (Slurried Spinach 2385, National Institute of Science and Technology, Gaithersburg, Md.) was used for method validation.

*Statistical analysis.* Main effects were analyzed by one-way ANOVA using SPSS (Chicago, Ill.). The experiment was a randomized split plot design, consisting of four chambers being assigned one of the four treatments. Each treatment was replicated twice for a total of three runs. The relationship between experimental dependent variables and photoperiod treatments were determined by regression analysis. Orthogonal polynomials were used to study changes associated with increasing photoperiod treatments by partitioning the sum of squares into components that were associated with linear and quadratic terms (Nogueira, 2004).

## Results

Kale shoot tissue fresh mass (FM) responded ($P \leq 0.001$) to increases in photoperiod treatments and ranged from 7.7 to 92.8 g/plant for the 6-h photoperiod to the 24-h photoperiod, respectively. Kale shoot tissue dry mass (DM) responded ($P \leq 0.001$) to increases in photoperiod treatments, and ranged from 0.6 to 10.5 g/plant for the 6 h photoperiod to the 24 h photoperiod, respectively. Kale FM and DM increased as the photoperiod treatments increased from 6 to 24 h (Table 1). Photoperiod treatment affected the percent dry matter (% DM; $P \leq 0.001$) found in the kale shoot tissues, and ranged from 8.2% to 11.4% for the 6-h photoperiod to the 24-h photoperiod, respectively (Table 1). Kale leaf tissue FM, DM, and % DM concentrations increased in response to increasing photoperiods (Table 1).

Lutein concentrations in kale leaf tissues responded to increases in photoperiod treatments ($P \leq 0.001$). Maximum lutein accumulation (13.5 mg/100 g FM) occurred under the 24-h photoperiod treatment, whereas the lowest lutein concentrations (8.8 mg/100 g FM) occurred at the 6-h photoperiod. Lutein concentrations increased in response to increasing photoperiods (Table 2). Kale leaf tissue β-carotene also responded to increases in photoperiod treatments ($P \leq 0.001$). Maximum β-carotene accumulation was 10.4 mg/100 g FM for the 24-h photoperiod treatment, whereas the lowest β-carotene accumulation (6.3 mg/100 g FM) occurred during the 6-h photoperiod treatment. β-carotene concentrations increased in response to increasing photoperiod (Table 2). The concentrations of kale leaf tissue chlorophyll *a* (Chl *a*; $P \leq 0.001$), chlorophyll *b* (Chl *b*; $P \leq 0.001$) and total chlorophyll (Total Chl; $P \leq 0.001$) pigments were influenced by increases in photoperiod treatments (Table 2). Maximum Chl *a* and Total Chl levels occurred at the 12-h photoperiod treatment, whereas maximum Chl *b* accumulation occurred at the 24-h photoperiod. Chlorophyll pigments in the kale shoot tissues increased in response to increases in photoperiod (Table 2).

The carotenoid content of vegetable crops is normally reported on a FM basis to equate to typical consumption patterns (Holden et al. 1999); however, due to the popularity of dried materials in dietary supplements as sources of antioxidants, the accumulations of lutein and β-carotene were calculated on a DM basis and on a per plant (TP) basis (Table 3). Dry mass accumulations of lutein (lutein DM; $P \leq 0.001$)

Table 1. Mean fresh and dry biomass accumulation and % dry matter in leaf tissues of 'Winterbor' kale grown under increasing photoperiod. Kale tissue fresh mass [$FM = -37.9 + 8.2(TRT) - 0.2(TRT^2)$; $R^2 = 0.92$, $P \leq 0.001$], dry mass [$DM = -2.1 + 0.4(TRT) + 0.01(TRT^2)$; $R^2 = 0.93$, $P \leq 0.001$], and % dry matter [$\%DM = 0.09 - 0.002(TRT) + 0.001(TRT^2)$; $R^2 = 0.66$, $P \leq 0.001$] responded quadratically to increasing photoperiods.

| | Plant biomass$^z$ (g/plant) | | |
|---|---|---|---|
| Photoperiod | FM | DM | % DM |
| 6 | 7.7 ± 0.4 | 0.6 ± 0.1 | 8.2 ± 0.1 |
| 12 | 41.3 ± 2.0 | 3.8 ± 0.2 | 9.1 ± 0.1 |
| 16 | 66.3 ± 3.1 | 5.7 ± 0.4 | 8.6 ± 0.1 |
| 24 | 92.8 ± 4.1 | 10.5 ± 0.4 | 11.4 ± 0.2 |
| Contrasts$^y$ | | | |
| L | *** | *** | *** |
| Q | *** | *** | *** |

$^z$Mean composition of sampled leaf tissue of 3 replications and 32 plants per replication ± standard deviation.

$^y$Significance for linear (L) and quadratic (Q) orthogonal contrasts.

$^{***}$Significance at $P \leq 0.001$.

Table 2. Mean pigments concentrations expressed on a fresh mass basis in the leaf tissues of 'Winterbor' kale grown under increasing photoperiod. Kale tissue lutein [$\text{lutein} = 3.6 + 1.0(\text{TRT}) – 0.03(\text{TRT}^2)$; $R^2 = 0.51$, $P \leq 0.001$], β-carotene [$\beta\text{-carotene} = 3.0 + 0.6(\text{TRT}) - 0.01(\text{TRT}^2)$; $R^2 = 0.54$, $P \leq 0.001$], chlorophyll *a* [$\text{Chl } a = 118.0 + 12.9(\text{TRT}) – 0.3(\text{TRT}^2)$; $R^2 = 0.33$, $P \leq 0.001$], chlorophyll *b* [$\text{Chl } b = 21.4 + 4.1(\text{TRT}) – 0.1(\text{TRT}^2)$; $R^2 = 0.58$, $P \leq 0.001$], and total chlorophyll [$\text{total Chl} = 139.4 + 17.0(\text{TRT}) – 0.4(\text{TRT}^2)$; $R^2 = 0.39$, $P \leq 0.001$] pigments responded quadratically to increasing photoperiods.

| | Pigment concn[z] (mg/100 g fresh mass) | | | | |
|---|---|---|---|---|---|
| Photoperiod | Lutein | β-carotene | Chl *a*[y] | Chl *b*[y] | Total Chl[y] |
| 6 | 8.8 ± 0.4 | 6.3 ± 0.2 | 179.3 ± 5.0 | 41.2 ± 1.1 | 220.5 ± 5.9 |
| 12 | 12.8 ± 0.3 | 8.3 ± 0.3 | 235.1 ± 6.3 | 58.1 ± 1.4 | 293.3 ± 7.5 |
| 16 | 13.1 ± 0.8 | 9.7 ± 0.6 | 223.4 ± 11.7 | 56.7 ± 2.3 | 280.0 ± 13.7 |
| 24 | 13.5 ± 0.6 | 10.4 ± 0.4 | 228.7 ± 8.3 | 58.6 ± 1.6 | 287.2 ± 9.4 |
| Contrasts[y] | | | | | |
| L | *** | *** | ** | *** | *** |
| Q | *** | *** | *** | *** | *** |

[z]Mean composition of sampled leaf tissue of 3 replications and 32 plants ± standard deviation. Abbreviations: Chl *a* = chlorophyll *a*; Chl *b* = chlorophyll *b*, Chl = chlorophyll.

[y]Significance for linear (L) and quadratic (Q) orthogonal contrasts.

[**,***]Significance at $P \leq 0.01$, or $P \leq 0.001$, respectively.

Table 3. Mean pigment concentrations as a function of dry mass and total plant pigment in the leaf tissues of 'Winterbor' kale grown under increasing photoperiod. Dry mass accumulations of kale tissue lutein [$\text{L DM} = 0.36 – 0.09(\text{TRT}) - 0.003(\text{TRT}^2)$; $R^2 = 0.41$, $P \leq 0.001$] and β-carotene [$\beta\text{ DM} = 0.27 + 0.06(\text{TRT}) – 0.002(\text{TRT}^2)$; $R^2 = 0.25$, $P = 0.002$] responded quadratically to increasing photoperiods. Lutein [$\text{L TP} = –5.5 + 1.1(\text{TRT}) – 0.01(\text{TRT}^2)$; $R^2 = 0.87$, $P \leq 0.001$] and β-carotene [$\beta\text{ TP} = –3.5 + 0.69(\text{TRT}) – 0.006(\text{TRT}^2)$; $R^2 = 0.89$, $P \leq 0.001$] expressed on a total plant basis responded quadratically to increasing photoperiods.

| | Pigment[z] (mg·g⁻¹ dry mass) | | Total pigment[z] (mg/plant) | |
|---|---|---|---|---|
| Photoperiod | Lutein | β-carotene | Lutein | β-carotene |
| 6 | 0.77 ± 0.04 | 0.54 ± 0.02 | 0.7 ± 0.1 | 0.5 ± 0.1 |
| 12 | 0.94 ± 0.02 | 0.62 ± 0.03 | 5.3 ± 0.3 | 3.5 ± 0.2 |
| 16 | 0.96 ± 0.07 | 0.71 ± 0.06 | 8.7 ± 0.6 | 6.4 ± 0.4 |
| 24 | 0.67 ± 0.03 | 0.51 ± 0.02 | 12.5 ± 0.7 | 9.6 ± 0.5 |
| Contrasts[y] | | | | |
| L | NS | NS | *** | *** |
| Q | *** | ** | *** | *** |

[z]Mean composition of sampled leaf tissue of 3 replications and 32 plants ± standard deviation.

[y]Significance for linear (L) and quadratic (Q) orthogonal contrasts.

[NS,**,***]Nonsignificant or significance at $P \leq 0.01$, or $P \leq 0.001$, respectively.

and β-carotene (β-carotene DM; $P \leq 0.001$), and lutein (lutein TP; $P \leq 0.001$) and β-carotene (β-carotene TP; $P \leq 0.001$) expressed on a total plant basis responded to increases in photoperiod treatments. Lutein DM and β-carotene DM concentrations initially increased, then decreased quadratically with increasing photoperiods. Lutein TP and β-carotene TP concentrations increased with increased photoperiod in a quadratic response (Table 3).

Ratios of the carotenoid and chlorophyll pigments were calculated (Table 4). All of the pigment ratios, except for Chl *b* to lutein, responded to increases in photoperiod treatments. Decreasing quadratic trends occurred for Chl *a*:l, Chl *a*:β, Chl *b*:β, Chl:l, Chl:β, and Chl *a*:*b*. Increasing, then decreasing quadratic trends occurred for Chl *b*:β and TChl:β (See Table 4 for pigment abbreviations). Maximum Chl *a*:l, Chl *a*:β, and Chl *a*:*b* ratios occurred with the 6-h photoperiod, whereas maximum Chl:β and Chl *b*:β occurred at the 12-h photoperiod.

## Discussion

The 24-h photoperiod (continuous irradiance) resulted in the largest FM, DM, lutein, β-carotene and Chl *b* accumulation, whereas increasing the photoperiod from 6 to 16 h resulted in an increase in Total Chl of 27%. Hurd (1973) showed similar results with tomato, where changing the photoperiod from 8 to 16 h increased the chlorophyll concentration of the leaves by 25% to 34%. Increasing photoperiod has also increased the chlorophyll concentration in several bedding plants, lettuce, pakchoi and tsukena (Fukuda et al., 2000; Langton et al., 2003).

In our study of kale, β-carotene increased 65% and lutein increased 64% from the 6-h to 24-h photoperiod treatment. However, β-carotene only increased 17% when the photoperiod was changed from 12 to 16 h, and no change was measured for lutein. This increase in the carotenoid accumulation is different from the results of Arizmendi-Maldonado et al. (2003) who found that increasing the photoperiod to 15 h did not affect the accumulation of β-carotene in bermudagrass or stargrass. Nicklisch and Woitke (1999) found that half of the algae species tested had a significant change in lutein or β-carotene if the photoperiod was changed from 6 to 12 h. However, one algae species, a diatom (*Synedra acus* Kütz), showed increases in β-carotene as the photoperiod increased, and no species has been reported that had an increase in lutein.

Interestingly, different trends resulted when the kale carotenoid pigments were calculated on a DM basis (Table 3). Kale tissue % DM was influenced by photoperiod, with the greatest % DM occurring during the 24-h photoperiod. The lutein and β-carotene concentrations on a DM basis had peak accumulations during the 16-h photoperiod. Measuring the carotenoid accumulation on a total plant basis resulted in maximum accumulation occurring at the 24-h photoperiod.

Positive correlations between chlorophyll and carotenoid pigments exist in kale (Kopsell et al., 2004), Swiss chard (*Beta vulgaris* L.; Ihl et al., 1994), and lettuce (Mou, 2005). Measurements of chlorophyll concentrations, or green colorations, are more efficient and cost effective when compared to carotenoid pigment analysis. The high correlations between chlorophyll and carotenoid pigments suggest that selection for chlorophyll concentrations would be an effective way for breeders to select for higher carotenoid levels in leafy

Table 4. Mean values for pigment ratios in the leaf tissues of 'Winterbor' kale grown under increasing photoperiod. The pigment ratios of Chl *a*:l [$\text{Chl } a\text{:l} = 24.9 - 0.1(\text{TRT}) + 0.02(\text{TRT}^2)$; $R^2 = 0.25$, $P \leq 0.001$], Chl *a*:β [$\text{Chl } a{:}\beta = 31.8 - 0.5(\text{TRT}) – 0.002(\text{TRT}^2)$; $R^2 = 0.44$, $P \leq 0.001$], TChl:l [$\text{total Chl:l} = 30.1 - 0.86(\text{TRT}) + 0.02(\text{TRT}^2)$; $R^2 = 0.22$, $P = 0.004$], and Chl *a*:*b* [$\text{Chl } a{:}b = 4.8 – 0.09(\text{TRT}) + 0.002(\text{TRT}^2)$; $R^2 = 0.28$, $P \leq 0.001$] decreased quadratically in response to increasing photoperiods. The pigment ratios of Chl *b*:β [$\text{Chl } b{:}\beta = 66 + 0.04(\text{TRT}) – 0.003(\text{TRT}^2)$; $R^2 = 0.19$, $P = 0.003$] and TChl:β [$\text{total Chl:}\beta = 38.5 – 0.41(\text{TRT}) – 0.002(\text{TRT}^2)$; $R^2 = 0.40$, $P \leq 0.001$] increased, then decreased quadratically in response to increasing photoperiods.

| | Pigment ratios[z] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Photoperiod | Chl *a*:l | Chl *b*:l | Chl *a*:β | Chl *b*:β | TChl:l | TChl:β | Chl *a*:*b* | l:β |
| 6 | 20.9 ± 1.2 | 4.8 ± 0.3 | 28.7 ± 0.4 | 6.6 ± 0.1 | 25.6 ± 1.4 | 35.3 ± 0.5 | 4.35 ± 0.07 | 1.42 ± 0.07 |
| 12 | 18.4 ± 0.2 | 4.6 ± 0.1 | 28.4 ± 0.6 | 7.1 ± 0.3 | 22.9 ± 0.2 | 35.5 ± 0.9 | 4.05 ± 0.06 | 1.55 ± 0.04 |
| 16 | 17.2 ± 0.8 | 4.4 ± 0.2 | 23.5 ± 1.1 | 6.0 ± 0.3 | 21.6 ± 0.9 | 29.5 ± 1.4 | 3.92 ± 0.11 | 1.37 ± 0.04 |
| 24 | 17.1 ± 0.7 | 4.4 ± 0.2 | 22.3 ± 0.9 | 5.7 ± 0.2 | 21.6 ± 0.8 | 28.0 ± 1.1 | 3.91 ± 0.10 | 1.32 ± 0.06 |
| Contrasts[y] | | | | | | | | |
| L | ** | NS | *** | ** | ** | *** | *** | NS |
| Q | *** | NS | *** | ** | ** | *** | *** | NS |

[z]Mean composition of sampled leaf tissue of 3 replications and 32 plants ± standard deviation. Abbreviations: Chl = Chlorophyll; Chl *a*:l = Chl *a* to lutein; Chl *b*:l = Chl *b* to lutein; Chl *a*:β = Chl *a* to β-carotene; Chl *b*:β = Chl *b* to β-carotene; TChl:l = Total Chl to lutein; TChl:β = Total Chl to β-carotene; Chl *a*:*b* = Chl *a* to *b*, l:β = lutein to β-carotene.

[y]Significance for linear (L) and quadratic (Q) orthogonal contrasts.

[NS,**,***]Nonsignificant or significance at $P \leq 0.01$ or $P \leq 0.001$, respectively.

vegetable crops (Kopsell et al., 2004; Mou, 2005). Decreasing chlorophyll to carotenoid pigment ratios in the current study demonstrate that carotenoid pigments in kale increase relative to chlorophyll as the photoperiod increases. Decreasing quadratic trends show an initial drop in pigment ratios from the 6-h to the 12-h photoperiod. Stabilization in the pigment ratios from the 12-h to 24-h photoperiod would support previous suggestions for the indirect selection of carotenoid pigments in leafy vegetable crops using chlorophyll concentrations.

The largest FM, DM and fresh basis lutein, β-carotene, and Chl *b* accumulation occurred at the 24-h photoperiod, with the maximum Chl *a* occurring during the 12-h photoperiod. The largest accumulation of lutein DM and β-carotene DM occurred during the 16-h photoperiod. Increased photoperiod resulted in increases in pigment accumulation but maximum concentrations of pigments were not required for maximum biomass production. Controlling the photoperiod allows plants to be grown for optimization of fresh mass, pigment accumulation or both.

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