Environmental Stress and Methyl Jasmonate-mediated Changes in Flavonoid Concentrations and Antioxidant Activity in Broccoli Florets and Kale Leaf Tissues

Kang Mo Ku and John A. Juvik
Department of Crop Sciences, University of Illinois at Urbana–Champaign, 307 Edgar R. Madigan Lab, 1201 West Gregory Drive, Urbana, IL 61801-3838

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Abstract. Aqueous solutions of 250 µM methyl jasmonate (MeJA) were sprayed on aerial plant surfaces 4 days before harvest at commercial maturity of five commercial broccoli (Brassica oleracea L. var. italica) hybrids, ‘Pirate’, ‘Expo’, ‘Imperial’, ‘Gypsy’, and ‘Green Magic’, and two kale cultivars, Red Winter (Brassica napus ssp. pabularia) and Dwarf Blue Curled Vates (Brassica oleracea L. var. acephala DC.) in replicated field trials over 2 years. While having no effect on broccoli floret concentrations, MeJA treatments significantly increased total phenolics in kale cultivars over two seasons by 27% and extract antioxidant activity by 31% using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Partitioning experiment-wide trait variances indicated that the variability in broccoli floret concentrations of total phenolics (74%), quercetin (24%), kaempferol (34%), and 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) (66%) and DPPH (62%) antioxidant activity was largely influenced by year-associated environmental factors. In broccoli, the differential accumulation of solar radiation among cultivars resulting from the variation in days to maturity was significantly correlated with total phenolics, ABTS, and DPPH antioxidant activity. Broccoli floret and kale total phenolic, quercetin, and kaempferol concentrations significantly correlated with DPPH and ABTS antioxidant activity. To summarize, total phenolic and flavonoid concentrations and their associated antioxidant activity in broccoli florets were unaffected by MeJA but varied significantly among cultivars and over growing seasons. Apical, compared with basal, leaves in kale were more responsive to MeJA-mediated increases in total phenolics and ABTS and DPPH antioxidant activity.

Numerous physiological and biochemical processes in the human body may produce oxygen-centered free radicals and other reactive oxygen species as byproducts of metabolism (Cai et al., 2004). Overproduction of free radicals can cause oxidative damage to biomolecules including lipids, proteins, and DNA, eventually leading to many chronic diseases such as atherosclerosis, cancer, and other degenerative diseases in humans (Cai et al., 2004; Valko et al., 2004). Dietary antioxidants including polyphenols and flavonoids protect against free radicals such as reactive oxygen species in the human body and have been associated with the prevention of cancer, Type 2 diabetes, and cardiovascular diseases (Moon et al., 2006; Poulsen et al., 1998, van Dam et al., 2013). Fruit and vegetables are good sources of natural antioxidants such as vitamins, carotenoids, flavonoids, and other phenolic compounds (Dimitrios, 2006). Broccoli (Brassica oleracea ssp. italica) and kale (Brassica napus ssp. pabularia and Brassica oleracea L. var. acephala) are frequently consumed vegetables in the United States and in other countries. They contain potential health-promoting bioactive compounds including glucosinolates and dietary antioxidants such as carotenoids, tocopherols, and flavonoids (Eberhardt et al., 2005, Velasco et al., 2007). Both vegetables are good sources of the dietary flavonoids, quercetin and kaempferol, which have been reported as potential anticancer agents (Koh et al., 2009; Moon et al., 2006). According to epidemiological and animal model studies, consumption of kaempferol and quercetin was inversely associated with cancer risk (Gates et al., 2007; Murakami et al., 2008; Neuhausser, 2004; Noldings et al., 2007). Quercetin intake has also been associated with decreasing blood pressure (Larson et al., 2012). A recent study has indicated that quercetin up-regulates low-density lipoprotein receptor gene expression, which can elicit hypolipidemic effects by improving the clearance of circulating low-density lipoprotein cholesterol levels from the blood (Moon et al., 2012). In addition to the flavonoids, both vegetables have a variety of additional polyphenol compounds such as hydroxycinnamic acid and hydroxybenzoic acid derivatives. Among them, ferulic acid and chlorogenic acid were reported to improve cardiovascular function and attenuate hypertension in hypertensive rats (Alam et al., 2013; Suzuki et al., 2006).

Biotic and abiotic factors are associated with the biosynthesis and accumulation of phenolics and flavonoids in plant tissues. Environmental factors such as temperature, solar radiation, and rainfall can influence broccoli metabolism and resulting phytochemical composition (Byrkitman et al., 2011; Gilszczyńska-Swiglo et al., 2007; Khan et al., 2011). Also, herbivore or pathogen activity also can influence broccoli phytochemical composition (Hopkins et al., 2009; Khan et al., 2011). Recently many studies have been conducted using exogenous treatments of elicitors including jasmonic acid and salicylic acid to mimic biotic stress and increase tissue total phenolics and flavonoid concentrations. Several reports have attempted to enhance antioxidant, antiproliferative, and antiadipogenic activity by MeJA-mediated increases in flavonoids or phenolics in sweet basil, buckwheat, and blackberry, respectively (Kim et al., 2006b; Lee et al., 2013; Wang et al., 2008).

The objective of this research was to investigate how MeJA applications and year-to-year variation in environment growing conditions affect total phenolics, flavonoid concentrations, and antioxidant activity of broccoli floret and kale leaf tissues. To our knowledge, this is the first investigation of MeJA application to broccoli and kale under field conditions. To evaluate variation in phytochemical antioxidants and antioxidant activity associated with MeJA treatments, environmental effects, and genotypes, we evaluated five commercial broccoli hybrids and two distinct kale cultivars in replicated field plots over 2 years.

Materials and Methods

Broccoli and kale cultivation. The broccoli F1 hybrid cultivars used for this experiment were ‘Pirate’ (Asgrow Seed Co., Galena, MD), ‘Expo’, ‘Imperial’, ‘Gypsy’, and ‘Green Magic’ (Sakata Seed Co., Morgan Hill, CA). Kale cultivars used for this experiment were ‘Red Winter’ (Brassica napus ssp. pabularia) and ‘Dwarf Blue Curled Vates’ (Brassica oleracea L. var. acephala DC.). Seeds of each broccoli and kale genotype were germinated in 32-cell plant plug trays filled with SunGro® LC1 (Sun Gro Horticulture, Vancouver, British Columbia, Canada) professional soil mix. Seedlings were grown in a greenhouse at the University of Illinois at Champaign-Urbana under a 25/15 °C and 14-h/10-h day/night temperature regime with supplemental high-intensity discharge lighting provided from 0600 to 2000 h if light intensities fell below 2670 µ Einsteins/s/m2. Thirty days after germination, seedling trays were placed in ground beds to harden off for 1 week before
transplanting into field plots at the University of Illinois South Farm (lat. 40°04'38.89"N, long. 88°14'26.18"W). The experimental design was a split plot in randomized complete block with three replicates. The experiment was conducted by one row of guard plants to avoid a border effect. Ten broccoli or kale plants from each replicate block of each genotype were designated as control or MeJA treatment groups with each genotype. Transplanting of broccoli seedlings was conducted on 24 June 2009 and 11 June 2010. Irrigation was only applied during the first week of cultivation to establish transplanted seedlings. Broccoli heads were harvested between 23 Aug. and 18 Sept. in 2009 and from 12 Aug. to 12 Sept. in 2010. Transplanting of kale seedlings was conducted on 11 June 2010 and 13 June 2011. Harvests of leaves of both kale cultivars occurred on 25 July in 2009 and 27 July in 2010. There was substantial variation in commercial harvest maturity among broccoli hybrids and the number of days from transplant to harvest date (DTH) was calculated for each genotype (Table 1). Variation in weather factors during the growing seasons in Champaign, IL, for 2009, 2010, and 2011 including growing degree-days (GDDs) [formula = (minimum temperature + maximum temperature)/2 – 7.2 °C] (Dufault, 1997), solar radiation, and precipitation, is presented in Table 1. Weather conditions during the 2009 and 2010 growing seasons were generated from <http://www.isws.illinois.edu/warm/data/cdfs/cmiday.txt> and used to calculate these weather variables.

Treatments and yield samples preparation. An aqueous solution of 250 μM MeJA (Sigma-Aldrich, St. Louis, MO) including 0.1% Triton X-100 alone (control) was sprayed on all aerial plant tissues to the point of runoff (~200 mL for 4 d before harvest at commercial maturity. This timing of harvest and treatment concentration was based on previous studies that identified optimal timing and MeJA concentrations to maximize floret glucosinolate concentrations in broccoli cultivars (Ku and Juvik, 2012). Five broccoli heads and two leaf samples from each kale cultivar (apical: three leaves from below the meristematic growing point, at a minimum 8 cm in length; basal: three fully expanded leaves nearest the soil surface without discolored or signs of senescence or damage) were harvested and bulked from five treated and control plants of each genotype for each replicate (five heads or leaves from five plants bulked for a replicate sample). Broccoli head tissue and kale leaf samples were frozen in liquid nitrogen and stored at −20 °C before freeze-drying. Freeze-dried head and leaf tissues were ground into a fine powder using a coffee grinder and stored at −20 °C before chemical and bioactivity analyses.

Sample extraction. Two hundred milligrams of fine powder of each sample was extracted with 2 mL of 70% methanol at 95 °C for 10 min. After 5 min cooling on ice, the extract was centrifuged at 3000 g for 2 min. After a second round of extraction as described previously, the supernatants were pooled. Subsequently, 5 mL of the pooled supernatants was transferred to a 2-mL microcentrifuge tube (Fisher Scientific, Waltham, MA) and centrifuged at 10,000 g for 2 min. This extract was used for the ABTS and DPPH antioxidant activity assays and to quantify tissue total phenolic and flavonoid concentrations.

Determination of total phenolic content. Analysis of total phenolic content was conducted using a previously described protocol (Ku et al., 2010). Ten-microliter sample extracts were added to 0.2 N Folin-Ciocalteu’s phenol reagent (100 μL) in 96-well plates. After 3 min, 90 μL of a saturated sodium carbonate solution was added to the mixture and subsequently incubated at room temperature for 1 h. The resulting absorbance of the mixture was measured at 630 nm using a BioTek EL 808 microplate reader (Power Wave XS; Biotek Instruments Inc., Winooski, VT). The total phenolic content was calculated on the basis of a standard curve using gallic acid (concentration range 31.25 to 500 μg·mL−1). Results are expressed in milligrams of gallic acid equivalents per 100 g of dried broccoli. Three biologically replicated (block) samples were assayed with three analytical replications each.

Determination of ABTS radical scavenging activity. The ABTS assay was conducted as previously described protocol (Ku et al., 2010). Briefly, 7 mM ABTS ammonium salt was dissolved in a potassium phosphate buffer (pH 7.4) and treated with 2.45 mM potassium persulfate. The mixture was then allowed to stand at room temperature for 12 to 16 h for full color development (dark blue). The solution was then diluted with potassium phosphate buffer until absorbance reached 1.0 ± 0.02 at 630 nm using a BioTek EL 808 microplate reader (Power Wave XS; Biotek Instruments Inc.). Subsequently, 190 μL of this solution was mixed with 10 μL of sample extracts. The absorbance was recorded at room temperature after 6 min. Results were expressed as a percentage of radical scavenging activity compared with controls. Three biologically

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**Table 1. Days to harvest (DTH), growing degree-days (GDDs), solar radiation, and precipitation accumulation for the five broccoli genotypes during the 2009 and 2010 growing seasons.**

| Year | Cultivar | Treatment | DTH | GDD (°C) | Solar radiation (MJ·m⁻²) | Precipitation (mm) |
|------|----------|-----------|-----|----------|-------------------------|-------------------|
| 2009 | Expo     | Control   | 81 ± 3 | 1150 | 1705 | 296 |
|      | Expo     | MeJA      | 82 ± 3 | 1163 | 1729 | 296 |
| 2009 | Green Magic | Control | 58 ± 7 | 859 | 1257 | 223 |
|      | Green Magic | MeJA | 57 ± 7 | 846 | 1239 | 222 |
| 2009 | Gypsy    | Control   | 62 ± 5 | 906 | 1342 | 223 |
|      | Gypsy    | MeJA      | 61 ± 2 | 893 | 1318 | 223 |
| 2009 | Imperial | Control   | 60 ± 3 | 881 | 1293 | 223 |
|      | Imperial | MeJA      | 60 ± 2 | 881 | 1293 | 223 |
| 2009 | Pirate   | Control   | 77 ± 5 | 1094 | 1622 | 296 |
|      | Pirate   | MeJA      | 78 ± 5 | 1109 | 1643 | 296 |
| 2010 | Expo     | Control   | 88 ± 2 | 1531 | 2758 | 314 |
|      | Expo     | MeJA      | 93 ± 3 | 1595 | 2868 | 318 |
| 2010 | Green Magic | Control | 67 ± 6 | 1198 | 2165 | 245 |
|      | Green Magic | MeJA | 67 ± 6 | 1198 | 2165 | 245 |
| 2010 | Gypsy    | Control   | 68 ± 3 | 1215 | 2193 | 245 |
|      | Gypsy    | MeJA      | 69 ± 2 | 1231 | 2223 | 245 |
| 2010 | Imperial | Control   | 65 ± 2 | 1107 | 2100 | 245 |
|      | Imperial | MeJA      | 66 ± 4 | 1182 | 2136 | 245 |
| 2010 | Pirate   | Control   | 92 ± 6 | 1581 | 2825 | 314 |
|      | Pirate   | MeJA      | 91 ± 4 | 1570 | 2845 | 314 |

*The accumulated weather variables were calculated based on number of days from transplant to harvest. [(Minimum temperature + maximum temperature)/2 – 7.2 °C]. The results are presented as means ± sd (n = 3).
MeJA = methyl jasmonate.*
replicated (block) samples were assayed in three analytical replications.

**Determination of the antioxidant activity by the DPPH free radical scavenging assay.** The DPPH assay was conducted as described by Ku et al. (2010) with minor modification. Reaction mixtures containing test samples (10 μL) and 190 μL of a 200 μM DPPH ethanol solution were incubated at room temperature for 30 min in 96-well plates. The absorbance of the DPPH free radical was measured at 515 nm with a BioTek EL 808 microplate reader (Power Wave XS; Biotek Instruments Inc.). Results were expressed as percentage of scavenging activity compared with control. Three biologically replicated (block) samples were assayed in three analytical replications. It is generally recommended to use at least two different types of assays for the investigation of antioxidant activities of samples because each assay has different characteristics (Moon and Shihabom, 2009). For example, the DPPH method is one of the most frequently used antioxidant assay but it is also sensitive to pH (Huang et al., 2005). To generate robust results, we used the two different scavenging radical assays.

**Statistical analysis.** JMP 10 software (SAS Institute Inc., Cary, NC) was used for statistical analysis. Analysis of variance and partitioning of variance components were conducted also using JMP 10. Treatments, genotype, and year effects were considered as fixed factors. Block was considered a random factor. Analysis of variance was performed using the linear model: \( Y_{ijklm} = \mu + G_i + Y_j + T_k + B_m + \epsilon_{ijklm} \) where \( Y_{ijklm} \) is the \( l \)th block of the \( i \)th genotype, \( j \)th treatment, \( k \)th year, \( m \)th block, and \( \epsilon_{ijklm} \) is the experimental error associated with \( Y_{ijklm} \). Correlation analysis and Student’s \( t \) test were conducted using JMP 10 software (SAS Institute Inc.). All biological sample analyses were conducted in triplicate. The results are presented as means ± sd based on the three field replicate samples.

### Results and Discussion

**Effect of MeJA treatment and variation in environmental conditions on total phenolic and flavonoid concentrations, ABTS, and DPPH antioxidant activities of broccoli floret extracts.** Treatment with 250 μM MeJA 4 d before harvest did not alter total phenolic, kaempferol, or quercetin concentrations, ABTS, or DPPH antioxidant activities in broccoli florets (Table 2). Whereas previous research has reported that MeJA treatment increased total phenolic and flavonoid content in radish and broccoli sprouts (Kim et al., 2006a; Pérez-Balibrea et al., 2011), this was not observed in this study with broccoli florets. The lack of response in our study to MeJA treatment maybe associated with the different tissues evaluated, plant developmental status, or environmental factors. Phenolic and flavonoid biosynthesis in broccoli floret tissue was apparently not influenced by exogenous MeJA application under field conditions.

In this study, year and genotype exerted a significant effect on phytochemical content and antioxidant activity among the broccoli cultivars (Table 2). Total phenolics, quercetin, and kaempferol concentrations in 2010 were 1.9-, 3.0-, and 1.7-fold higher, respectively, in controls than that observed in the 2009 control plants (Table 2). The cultivar Gypsy showed the highest quercetin concentration fold change (7.5-fold) between 2009 and 2010. Different weather conditions in 2009 and 2010 are presumed to have significantly altered the ratio of quercetin/kaempferol in ‘Pirate’, ‘Imperial’, and ‘Gypsy’. Antioxidant activity measured by the ABTS and DPPH assays was 1.5- and 2.2-fold higher, respectively, in 2010 than for broccoli harvested in 2009 (Table 2). It has been reported that solar radiation is positively correlated with flavonoid content in broccoli florets (Gliszczyńska-Swiglo et al., 2007). Increased total phenolic and flavonoid content in 2010 compared with 2009 may be explained by year-associated weather factors such as solar radiation (Pék et al., 2012). The increased quercetin/kaempferol ratio also maybe a response to increased ultraviolet-B in solar radiation (Kuhlmann and Müller, 2009). Temperature is also known to impact phytochemical content in broccoli florets (Schonhof et al., 2007). The interaction of these various environmental factors on the growth and development of broccoli cultivars likely influenced phytochemical profiles in floret tissue.

**Correlation between weather conditions and phytochemical change.** From the correlation of extract phytochemical compound concentrations with antioxidant activity and with weather-related environmental growing conditions for each cultivar over 2 years, several meaningful relationships were observed (Table 3). Accumulated GDD, precipitation, and solar radiation for each genotype were associated with DTH. There was a significantly positive correlation between GDD \( (r = 0.703, P < 0.001) \) and solar radiation \( (r = 0.796, P < 0.001) \) with total phenolic concentrations. There were highly significant correlations between tissue ABTS antioxidant

### Table 2. Total phenolic and flavonoid concentrations and antioxidant activity of untreated and MeJA-treated broccoli florets over two seasons.¹

| Source of variation | Treatment/season | Total phenolics | Quercetin | Kaempferol | Q/K ratio | ABTS | DPPH |
|---------------------|------------------|----------------|-----------|------------|-----------|------|------|
| Treatment           |                  |                |           |            |           |      |      |
| Control             | Control          | 668 ± 244      | 112 ± 107 | 63 ± 26    | 1.55 ± 0.76 | 36.0 ± 9.8 | 30.1 ± 14.8 |
| MeJA                | Control          | 671 ± 252      | 117 ± 92  | 67 ± 23    | 1.59 ± 0.79 | 32.7 ± 9.6 | 25.8 ± 13.3 |
| Year                |                  |                |           |            |           |      |      |
| 2009                | Control          | 463 ± 108      | 57 ± 36   | 46 ± 14    | 1.17 ± 0.45 | 28.2 ± 5.5 | 18.9 ± 6.2 |
| 2010                | Control          | 873 ± 147*     | 167 ± 126*| 80 ± 25*   | 1.93 ± 0.82* | 43.7 ± 6.4* | 41.3 ± 12.1* |
| Genotype            |                  |                |           |            |           |      |      |
| Pirate              | 2009             | 386 ± 45       | 51 ± 7    | 36 ± 3     | 1.44 ± 0.18 | 25.4 ± 3.2 | 18.2 ± 5.2 |
|                     | 2010             | 942 ± 3*       | 139 ± 27* | 62 ± 10*   | 2.25 ± 0.22* | 40.0 ± 3.3* | 39.5 ± 1.4* |
| Expo                | 2009             | 524 ± 28       | 37 ± 23   | 43 ± 10    | 0.83 ± 0.35 | 30.7 ± 1.4 | 21.1 ± 1.9 |
|                     | 2010             | 800 ± 42*      | 81 ± 9*   | 61 ± 7     | 1.34 ± 0.16 | 45.0 ± 4.0* | 37.4 ± 1.2* |
| Green Magic         | 2009             | 631 ± 71       | 115 ± 34  | 69 ± 13    | 1.65 ± 0.19 | 34.9 ± 3.8 | 25.5 ± 5.5 |
|                     | 2010             | 820 ± 42*      | 133 ± 5   | 87 ± 4     | 1.52 ± 0.02 | 38.0 ± 3.3 | 33.5 ± 2.5 |
| Imperial            | 2009             | 391 ± 13       | 25 ± 12   | 40 ± 2     | 0.62 ± 0.30 | 23.3 ± 5.7 | 11.2 ± 6.3 |
|                     | 2010             | 701 ± 40*      | 78 ± 3*   | 66 ± 10*   | 1.20 ± 0.13* | 42.0 ± 6.5* | 32.2 ± 1.3* |
| Gypsy               | 2009             | 385 ± 17       | 54 ± 10   | 41 ± 7     | 1.31 ± 0.16 | 26.9 ± 5.4 | 18.6 ± 3.4 |
|                     | 2010             | 1104 ± 55*     | 405 ± 8*  | 122 ± 2*   | 3.32 ± 0.12* | 52.7 ± 3.1* | 63.9 ± 2.6* |

¹The results are presented as means ± sd (n = 3). Three analytical replications were conducted for each biological sample. Student’s t-tests were conducted to determine significance at \( P \leq 0.05 \). MeJA treatment groups are not presented here because they were not significantly different from control groups.

²Total phenolic was measured by spectrophotometry using Folin-Ciocalteu reagent and expressed as milligrams of gallic acid equivalent concentration in 100 g of freeze-dried broccoli powder.

³Flavonoids were measured by high-performance liquid chromatography and expressed as μmol/100 g of freeze-dried broccoli powder.

⁴Ratio of quercetin to kaempferol concentrations.

⁵Antioxidant activities are presented as Trolox equivalent concentration (mmol/100 g of freeze-dried broccoli powder).

⁶Values presented for individual genotype are untreated controls. Asterisks indicate means that are significantly different based on the Student’s t-test between treatments or different years within same genotypes (\( P \leq 0.05 \)).

MeJA = methyl jasmonate; ABTS = 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; DPPH = 2,2-diphenyl-1-picrylhydrazyl.
activity with total phenolics ($r = 0.927$, $P < 0.001$), quercetin ($r = 0.728$, $P < 0.001$), and kaempferol ($r = 0.785$, $P < 0.001$) concentrations (Table 3). There were also highly significant correlations between tissue DPPH antioxidant activity with total phenolics ($r = 0.934$, $P < 0.001$), quercetin ($r = 0.860$, $P < 0.001$), and kaempferol ($r = 0.830$, $P < 0.001$). Because ABTS, DPPH, and total phenolics are based on an electron transfer antioxidant assay, linear correlations are often observed between these antioxidant assays and total phenolics (Huang et al., 2005). As was previously observed by Pék et al. (2013), total phenolic content was negatively correlated with solar radiation but was not significant ($r = 0.120$; kaempferol, $P = 0.078$). Unlike previous reports, there was only a weak correlation between solar radiation and flavonoid (quercetin $r = 0.360$, $P = 0.120$; kaempferol $r = 0.420$, $P = 0.065$) concentrations (Gliszczynska-Swiglo et al., 2007). In addition, the ratio of quercetin to kaempferol tended to weakly correlate with solar radiation but was not significant ($r = 0.403$, $P = 0.078$).

### Table 3. Correlation between accumulated weather factors and phytochemicals and antioxidant activities.

| DTH | GDD | Precipitation/DTH | Solar radiation | TPC | ABTS | DPPH | Quercetin | Kaempferol |
|-----|-----|-------------------|-----------------|-----|------|------|----------|------------|
| DTH | 1.000 | 0.893*** | 1.000 | 0.773*** | 0.974*** | –0.620** | 0.796*** | 1.000 |
| GDD | 0.893*** | 1.000 | 0.774*** | 1.000 | 0.796*** | 1.000 | 0.796*** | 1.000 |
| Solar radiation | –0.701*** | –0.800*** | 1.000 | 0.344 | 0.644*** | –0.518** | 0.747*** | 1.000 |
| TPC | 0.773*** | 0.974*** | –0.620** | 0.344 | 0.644*** | –0.518** | 0.747*** | 1.000 |
| ABTS | 0.344 | 0.644*** | –0.518** | 0.344 | 0.644*** | –0.518** | 0.747*** | 1.000 |
| DPPH | 0.344 | 0.644*** | –0.518** | 0.344 | 0.644*** | –0.518** | 0.747*** | 1.000 |
| Quercetin | 0.019 | 0.260 | –0.261 | 0.019 | 0.260 | –0.261 | 0.360 | 0.769*** |
| Kaempferol | 0.002 | 0.304 | –0.197 | 0.002 | 0.304 | –0.197 | 0.360 | 0.769*** |
| Q/K ratio | 0.067 | 0.322 | –0.327 | 0.067 | 0.322 | –0.327 | 0.360 | 0.769*** |

*Table 3. Correlation between accumulated weather factors and phytochemicals and antioxidant activities.**

**Pearson correlation coefficients were calculated based on mean values of all pair variables.

***Indicates factor that describes a significant proportion of the total variance using analysis of variance at $P < 0.001$.

### Table 4. Percentages of total variance described by main factors (genotype, treatment, year) and factor interactions for broccoli floret phytochemical concentrations and bioactivities.

| Total phenolics | Quercetin | Kaempferol | ABTS | DPPH |
|-----------------|----------|-----------|------|------|
| Genotype (G)    | 6.5***   | 14.9***   | 33.6*** | 6.9*** |
| Treatment (T)   | 0.0      | 0.9      | 0.1   | 2.9   |
| Year (Y)        | 74.0***  | 23.9***  | 33.5*** | 64.8*** |
| G × T           | 0.4      | 4.8      | 0.3   | 0.6   |
| G × Y           | 12.2***  | 36.5***  | 28.4*** | 9.5*** |
| T × X           | 0.0      | 0.9      | 0.0   | 0.0   |
| G × T × X       | 2.5      | 4.3      | 1.1   | 4.0   |
| Block (year)    | 0.4      | 0.5      | 0.2   | 1.1   |
| Residual        | 4.0      | 13.3     | 2.8   | 10.0  |
| $R^2$           | 0.96     | 0.97     | 0.86  | 0.90  |

*Total phenolics were measured by spectrophotometer using Folin-Ciocalteu reagent and expressed as milligrams of gallic acid equivalent concentration in 100 g of freeze-dried broccoli powder.

**Flavonoids were measured by high-performance liquid chromatography and expressed as mmol/100 g of freeze-dried broccoli powder.

***Antioxidant activities are presented as Trolox equivalent concentration (mmol/100 g of freeze-dried broccoli powder).

### Conclusion

These results suggest that appropriate cultivar selection or breeding for adaptation to certain environmental conditions can maximize phenolics and antioxidant bioactivity of broccoli florets. With appropriate parental material and selection schemes, it may be feasible to achieve high flavonoid and phenolic content in shorter maturing varieties as was previously reported for the development of higher glucoraphanin content in early-maturing broccoli germplasm (Farnham et al., 2004).

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Endogenous jasmonic acid has been observed in lettuce (Oh et al., 2010). Reported that water stress increased phenolic content in rapeseed with increased phenolic content in rapeseed (Ko et al., 2013; Lee et al., 2013; Wang et al., 2011). Research reported that MeJA treatment increased levels of antioxidant phytochemicals and antioxidant activity in kale leaf tissue, respectively (Table 6), whereas no significant MeJA effect was observed on phytochemical content and antioxidant activity in broccoli floret tissue (Table 4). Flavonoids were measured by high-performance liquid chromatography and expressed as μmol/100 g of freeze-dried kale powder. Antioxidant activities are presented as Trolox equivalent concentration (mmol/100 g of freeze-dried kale powder).

### Table 5. Total phenolic and flavonoid concentrations and antioxidant activity of untreated and MeJA-treated kale leaf samples over two seasons.

| Source of variation | Treatment/season | Total phenolics (mg/100 g) | Quercetin (mg/100 g) | Kaempferol (mg/100 g) | ABTS (μmol/100 g) | DPPH (μmol/100 g) |
|---------------------|-----------------|---------------------------|---------------------|----------------------|----------------|----------------|
| **Genotype**        |                 |                           |                     |                      |                 |                 |
| Dwarf Blue Curled Vates | Control      | 1653 ± 225                | 120 ± 72            | 177 ± 24             | 5130 ± 406     | 3476 ± 1167   |
|                     | MeJA          | 2188 ± 132***             | 241 ± 147           | 169 ± 29             | 5778 ± 155**   | 4229 ± 711    |
| Red Winter          | Control      | 1438 ± 165                | 186 ± 102           | 131 ± 61             | 4734 ± 393     | 4148 ± 1682   |
|                     | MeJA          | 1735 ± 185*               | 232 ± 145           | 182 ± 45             | 5365 ± 265**   | 5741 ± 1232   |
| **Year**            |               |                           |                     |                      |                 |                 |
| 2010                | Control      | 1846 ± 113                | 184 ± 34            | 165 ± 25             | 5488 ± 68      | 4533 ± 214    |
|                     | MeJA          | 2298 ± 50**               | 373 ± 27**          | 152 ± 11             | 5911 ± 56**    | 4819 ± 359    |
| 2011                | Control      | 1461 ± 48                 | 58.2 ± 7.7          | 190 ± 17             | 4770 ± 144     | 2419 ± 77     |
|                     | MeJA          | 2079 ± 73***              | 109 ± 26*           | 186 ± 33             | 5645 ± 64***   | 3639 ± 100*** |

*The results are presented as means ± SD (n = 3). Three analytical replications were conducted for each biological sample. Student’s t-tests were conducted to determine significance at *; P < 0.05, **; P < 0.01, and ***; P < 0.001. Total phenolics were measured by spectrophotometry following Folin-Ciocalteu reagent and expressed as mg of gallic acid equivalent concentrations in 100 g of freeze-dried kale powder.

Although the physiological relationship between antioxidant compounds and human health-promoting activity has not been thoroughly established, consumers have indicated their willingness to pay a premium for produce with high nutritional value and antioxidant activity (i.e., melons with 25% more vitamin C) (Bond et al., 2008). Previous research reported that MeJA treatment increased the dietary intake of various crops on quinine reductase, antioxidant, antiproliferative, and antiadipogenic activity as measured by the effect of extracts on cultured mammalian cells (Kim et al., 2006b; Ku et al., 2013; Lee et al., 2013; Wang et al., 2008). This study showed that MeJA could enhance levels of antioxidant phytochemicals and antioxidant activity in kale leaf tissues. It may be feasible to develop brassica vegetables with enhanced consumer health-promoting properties, but the magnitude of this effect may be attenuated by interaction with biotic and abiotic stress conditions in the growing environment (Mewis et al., 2012).
Table 6. Percentages of total phenolics described by main factors (genotype, treatment, year) and factor interactions for kale leaves phytochemical concentrations and antioxidant activities.

| Apical tissue | Total phenolics* | Quercetin* | Kaempferol* | ABTS* | DPPH* |
|---------------|------------------|------------|-------------|-------|-------|
| Genotype (G)  | 27.5***          | 1.4        | 3.7         | 17.8*** | 9.7   |
| Treatment (T) | 17.5***          | 76.2***    | 16.8        | 28.7*** | 41.0*** |
| Year (Y)      | 42.5***          | 12.1***    | 6.2         | 44.6*** | 11.7   |
| G × T         | 0.3              | 0.3        | 0.5         | 0.0    |
| G × Y         | 1.1              | 5.2***     | 5.7         | 4.4*** | 12.1   |
| T × Y         | 3.5              | 2.4***     | 12.0        | 0.0    |
| G × T × Y     | 0.1              | 0.4        | 3.8         | 0.1    |
| Block (year)  | 2.0              | 1.1        | 1.4         | 2.1    |
| Residual      | 5.5              | 1.0        | 50.0        | 3.0    |
| $R^2$         | 0.94             | 0.99       | 0.50        | 0.97   |

| Basal tissue | Total phenolics* | Quercetin* | Kaempferol* | ABTS* | DPPH* |
|--------------|------------------|------------|-------------|-------|-------|
| Genotype (G) | 0.9              | 0.3        | 18.5        | 11.3  | 47.0*** |
| Treatment (T)| 69.3***          | 47.8***    | 15.2        | 35.8*** | 16.4   |
| Year (Y)     | 10.3***          | 0.8        | 1.3         | 29.6*** | 4.1    |
| G × T        | 4.7              | 1.6        | 5.7         | 4.5    | 0.5    |
| G × Y        | 0.9              | 1.4        | 2.6         | 0.0    | 1.2    |
| T × Y        | 0.7              | 14.7       | 0.0         | 0.6    | 0.0    |
| G × T × Y    | 3.0              | 14.7       | 15.8        | 0.3    | 7.9    |
| Block (year) | 0.7              | 0.8        | 2.0         | 0.8    |
| Residual     | 9.5              | 18.0       | 39.0        | 17.0   |
| $R^2$        | 0.90             | 0.82       | 0.61        | 0.83   |

Seasonal differences in environmental conditions between 2010 and 2011 explained a significant proportion of the variance for total phenolics, quercetin, and ABTS antioxidant activity in kale apical tissue and basal tissue, but the portion was smaller in basal leaf tissue.

MeJA treatment of broccoli inflorescence and kale leaf tissue appears to be a tissue-specific response. Kim and Juvik (2011) reported MeJA treatment under greenhouse conditions significantly increased glucosinolate concentrations in broccoli florets but had no effect on phenolic and flavonoid concentrations. Under biotic or abiotic stress, plants tend to accumulate defense compounds in vulnerable tissues such as young leaves or florets (Dam et al., 1996; Zangerl and Bazzaz, 1992). Accumulation of different defense compounds in plants is tissue-specific and preferentially allocated to plant parts that promote plant fitness and survival that are at risk of attack from herbivores (Zangerl and Bazzaz, 1992). Because the photosynthetic capacity of leaves declines with age, young leaf tissue plays a critical role in survival and thus would show a more dramatic response to MeJA treatment. Previous studies have reported that the youngest leaves of the rosette plants of *Cynoglossum officinale* contain 50 to 190 times higher concentrations of pyrrolizidine alkaloid than old leaves and that the compound acts as a defense against generalist herbivores (Dam et al., 1996). In kale, MeJA treatment increased not only flavonoids and phenolics, but also certain glucosinolates including glucocanasturtiin, glucobrassicin, and neoglucobrassicin (data not shown). MeJA application to kale plants may be a commercially viable protocol to potentially enhance health-promoting activity to consumers.

In conclusion, unlike previous studies on several plants, MeJA treatment did not significantly influence total phenolic and flavonoid concentrations or antioxidant bioactivity in broccoli florets. Treatment increased those variables in kale leaves. MeJA treatments appear to interact with tissue type, age of leaves, and environmental conditions. Selection of appropriate genotypes with manipulation of environmental conditions can increase total phenolic and flavonoid concentrations in broccoli florets resulting in elevated antioxidant activity and potential health promotion.

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