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# Measuring antioxidant activity in yellow corn (*Zea mays* L.) inbreds from three different geographic regions

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## Abstract

Corn (*Zea mays* L.) provides a major source of calories for human consumption and therefore, the nutritional components of corn have a large impact on human health. For example, corn kernels contain antioxidants, such as polyphenols (including anthocyanins and other flavonoids) and carotenoids. Such compounds represent useful targets for biofortification breeding. In this study, we used 34 corn inbred lines from three different regions (East Asia, Southern Asia, and subtropical regions) and 11 $F_1$ hybrids derived from the inbreds to investigate antioxidant activity in yellow corn. We compared different methods for measuring antioxidant activity to test their consistency and to determine whether color could be used as an indicator of antioxidant activity. We also measured carotenoid levels in yellow corn. No difference in antioxidant activity was detected between inbred corn lines from temperate vs. tropical regions. We determined that carotenoid is a major contributor to antioxidant activity in yellow corn and that kernel color, especially yellowness, could be used as an indicator of antioxidant activity in yellow corn. These findings lay the foundation for the biofortification of yellow corn by providing information about the correlations among kernel color, carotenoid contents, and antioxidant activity and by identifying an easy method to assess antioxidant activity in yellow corn.

**Keywords:** Kernel color, Antioxidant activity, Maize, Polyphenols, Carotenoid

## Introduction

Corn (maize; *Zea mays* L.) kernels contain nutritionally valuable antioxidants that benefit human health by reducing age-related disorders such as cardiovascular disease and cancer. However, much of the corn consumed worldwide lacks high levels of key antioxidants such as the dark-colored anthocyanins, because many culinary traditions have a strong preference for yellow or white corn and therefore, most of the corn used as a staple food crop worldwide is yellow or white. People in countries that use corn as a staple food can have severe nutritional deficiencies such as blindness and anemia due to the insufficient uptake of essential minerals and vitamins, many of which are important antioxidants [1, 2]. Therefore, biofortification to produce corn with improved antioxidant activities could be a solution for some severe nutrient deficiencies [3, 4]. Measuring the levels of various antioxidants in important locally adapted corn varieties provides key information for efforts to improve the antioxidant levels in corn. Moreover, establishing simple assays that correlate to antioxidant levels will facilitate these efforts [5].

Polyphenolic compounds include dark-colored pigments such as anthocyanins and are major contributors to antioxidant activity in corn kernels; indeed, many studies on antioxidant activity in corn have focused on anthocyanins, which give corn a red or purple color [6, 7].

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Hu and Xu reported that purple kernels showed higher antioxidant activity than yellow kernels, but yellow kernels also showed considerable and sometimes similar levels of antioxidant activity [8]. Another study showed that white and yellow corn kernels have considerable amounts of antioxidant activity [6]. Other polyphenols, especially ferulic acid, are thought to be major contributors to the antioxidant activity of yellow sweet corn when the corn is processed at 115 °C [9].

Carotenoids, another source of antioxidant activity in corn, provide kernels with their yellow color. Various types of carotenoids have been identified in corn kernels [10–12]. For example, β-carotene and β-cryptoxanthin have vitamin A activity, functioning as retinal pigments. Lycopene, a red pigment, has excellent health benefits by reducing coronary heart diseases. Lutein and zeaxantin also function as retinal pigments, thereby reducing blindness in elderly people [13].

In the current study, we used inbred lines from three different regions to investigate the antioxidant activity of yellow corn. The aims of this study were to (i) identify an appropriate method for measuring antioxidant activity in yellow corn kernels; (ii) determine whether antioxidant activity can be estimated based on the visual inspection of kernel color; and (iii) examine whether carotenoid is the major determinant of antioxidant activity in yellow corn.

## Materials and methods

### Plant materials

Eight Korean elite inbred lines, 16 Vietnamese elite lines, 10 CIMMYT lines (CMLs), and 11 $F_1$ hybrids (including two hybrids between Korean inbred lines and nine hybrids between KS140 and CMLs) were used for analysis. The two hybrids between Korean inbred lines Shinwangok and Hwandaok are cultivars developed by the National Institute of Crop Science, Korea [14, 15]. All kernel samples were harvested from corn grown in the field at the NICS, Suwon, Korea (37°15′47″N, 126°59′16″E) in 2019 with field preparation performed as previously described [16].

### Extraction of antioxidants and phenolics

Two grams of powered samples were mixed with 10 ml of 80% EtOH. The mixture was incubated at 25 °C for 24 h with 150 rpm shaking in dark. After incubation, the supernatants were collected after 10 min at 10,000 × g. The remaining sediment was mixed with another 10 ml of 80% EtOH and extracted in the same way. The supernatants were combined and filtered through Whatman No. 42 filter paper, and used for the following antioxidant activity and phenolic assays.

### Quantification of total polyphenols

The Folin-Ciocalteu method was used for quantitation of total polyphenols [17]. Sample extracts (0.5 mL volume) were mixed with 5 mL distilled water and 5 mL Folin-Ciocalteu phenol reagent for 3 min. After adding 2 mL 10% $Na_2CO_3$, the mixture was stirred in a shaker chamber at 30 °C for 1 h. Absorbance at 760 nm was measured with a spectrophotometer (U-3900 Hitachi, Tokyo, Japan). A standard curve of 1–100 ppm gallic acid (Sigma Aldrich, MO, USA) was used for quantification. The results were expressed as μg gallic acid equivalents $g^{-1}$ dry weight (DW).

### Measuring flavonoid contents

Total flavonoid contents were determined by adding 1 mL triple-distilled water and 75 μL 5% $NaNO_2$ to 250 μL extract, followed by 150 μL 10% $AlCl_3{\cdot}6H_2O$ after 5 min and 500 μL 1 N NaOH after 6 min and measuring the absorbance at 510 nm with a spectrophotometer (U-3900 Hitachi, Tokyo, Japan) [9]. A standard curve was generated using (+)-catechin (Sigma Aldrich, MO, USA). The results were presented as μg catechin equivalents $g^{-1}$ DW.

### DPPH assay

The DPPH assay was performed as described previously [18]. Each extract (0.2 mL) was mixed with 2.5 mL DPPH (Sigma Aldrich, MO, USA) solution (0.35 mM DPPH dissolved in 50% ethanol) and incubated for 10 min at room temperature. The changes in absorbance at 517 nm were measured, and antioxidant activity was calculated as the percent inhibition caused by hydrogen donor activity. The results were expressed as μmol Trolox equivalents $g^{-1}$ DW based on a standard curve generated with Trolox solution (100–1000 μM).

### ABTS assay

The reduced radical cation of 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$) was measured as previously described [8, 18]. Briefly, $ABTS^+$ was generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate. The reaction mixture was incubated in the dark at 24 °C for 1–2 days. Samples were diluted within a range of 20–80% inhibition of the blank. Each diluted extract (50 μL) was mixed with 1.9 mL diluted $ABTS^+$ solution and incubated for 6 min at 24 °C before measuring the absorbance at 734 nm. The results were expressed as μmol Trolox equivalents $g^{-1}$ DW based on a standard curve generated with Trolox solution.

### Measuring carotenoid contents

Total carotenoid content was measured according to Al-Frarsi et al. [19] with slight modifications. Freeze-dried kernels were ground to a powder with a coffee grinder and sifted through a 100-mesh screen. Extraction was performed using 2 g of sample powder and 25 mL of acetone/ethanol (1:1, v/v) containing 200 mg $L^{-1}$ butylated hydroxytoluene. The resulting extract was centrifuged for 10 min at 4 °C, 10,000×*g*. The supernatant was filtered through Whatman No. 42 filter paper and measured with a spectrophotometer (U-3900 Hitachi, Tokyo, Japan) at 470 nm. The carotenoid contents were calculated with the following formula: Total carotenoid contents (mg $g^{-1}$) = ($A_{470} \times V \times 10^6$)/($A^{1\%} \times 100 \times G$), where $A_{470}$ is the absorbance at 470 nm, $V$ is the volume of extract, $A^{1\%}$ is 2500 (the extinction coefficient for a 1% mixture of carotenoid), and $G$ is the sample weight (g).

### Colorimetric analysis

Kernel color was determined with an *L*, *a*, and *b* colorimeter (CR-200 Minolta, Tokyo, Japan). The colorimeter was calibrated using the manufacturer's standard white plate. The center of the abgerminal side of the kernel was measured. Six kernels per genotype were measured, and the mean values were used for correlation analysis. The colors are represented as *L*, *a*, and *b* values, i.e., lightness, red-green, and blue-yellow, respectively.

### Statistical analysis

All measurements were conducted in triplicate from three different ear samples. The data obtained from these assays were analyzed for correlation coefficient and ANOVA followed by post hoc analysis using Excel (Microsoft Office 2016, USA) and SAS software version 9.3 for Windows (SAS Institute Inc., USA), respectively. A web-based analysis program (www.metaboanalyst.ca) was used for PCA.

## Results and discussion

### Color properties of corn kernels

To broadly sample corn varieties from different geographic and climatic regions [East Asia, Southern Asia, and subtropical regions (Central America)] and examine the behavior of antioxidant-related traits in hybrids, we examined 8 elite Korean inbred lines, 16 elite Vietnamese lines, 10 CIMMYT (Centro Internacional de Mejoramiento de Maíz y Trigo, Mexico) lines (CMLs), and 11 $F_1$ hybrids. The corn lines used in this study typically have yellow or white kernels (Fig. 1). We characterized kernel color by recording *L* (lightness), *a* (red-green), and *b* (blue-yellow) values with a colorimeter. The *L*, *a*, and *b* values ranged from 63.10 to 83.07, 0.45 to 20.22, and 22.90 to 58.71, respectively. *L* and *a* values showed a strong negative correlation ($r^2 = 0.865$; Fig. 1F), whereas *a* and *b* values showed a positive correlation ($r^2 = 0.206$; Fig. 1E). These values reflected the color characteristics of the corn materials used in this study.

When we compared color values in corn from different geographic locations, the Korean lines and Vietnamese lines shared similar color ranges, whereas the CIMMYT lines (CMLs) tended to have lighter kernels, as the CMLs had higher *L* values and lower *a* and *b* values than the other corn lines ($p < 0$ 0.01; Fig. 1B–D). Kernel color has been used to estimate carotenoid contents in previous studies [20, 21]. Analysis of the variation in color values of the samples and their clear correlations reveal the consistency among the color values of the corn samples.

### Antioxidant activities of yellow corn

We examined antioxidant activity in kernels by three methods: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, and measurement of polyphenol contents. In addition, we measured total flavonoid contents in the kernels (Table 1). The values from all samples were compared via principal component analysis (PCA) (Fig. 2). Flavonoid and polyphenol contents, and ABTS and DPPH activities showed similar directions in the analysis, whereas carotenoid contents showed a different direction in the PCA (Fig. 2B). The first two components of the PCA explained 79.3% of the total variance of the dataset (Fig. 2A). The inbred lines from three different locations were randomly dispersed in the score plot, indicating that geographic origin had little effect on antioxidant activity. Most of the inbreds showed consistency among biological replicates. 14K28 and CML177 showed the highest antioxidant activity among Vietnamese lines and CMLs, respectively (Fig. 2A).

Vietnamese lines are generally more diverse than the Korean lines and CMLs, based on their positions in the score plot. 14K9 and CL17 showed variation among individuals in the direction of carotenoid content. Korean lines were located close to the center, and KS showed less genetic variation in terms of antioxidant activities and carotenoid contents. The CMLs showed less variation among individuals than the Vietnamese lines (Fig. 2A).

### Effect of hybridization on antioxidant activities

We obtained 11 $F_1$ hybrids, including 2 from crosses between KS lines and 9 from crosses between KS140 and CMLs. Both Korean $F_1$ hybrids tended to have reduced antioxidant activity compared to their parents. Most hybrids between KS140 and the CMLs also showed a

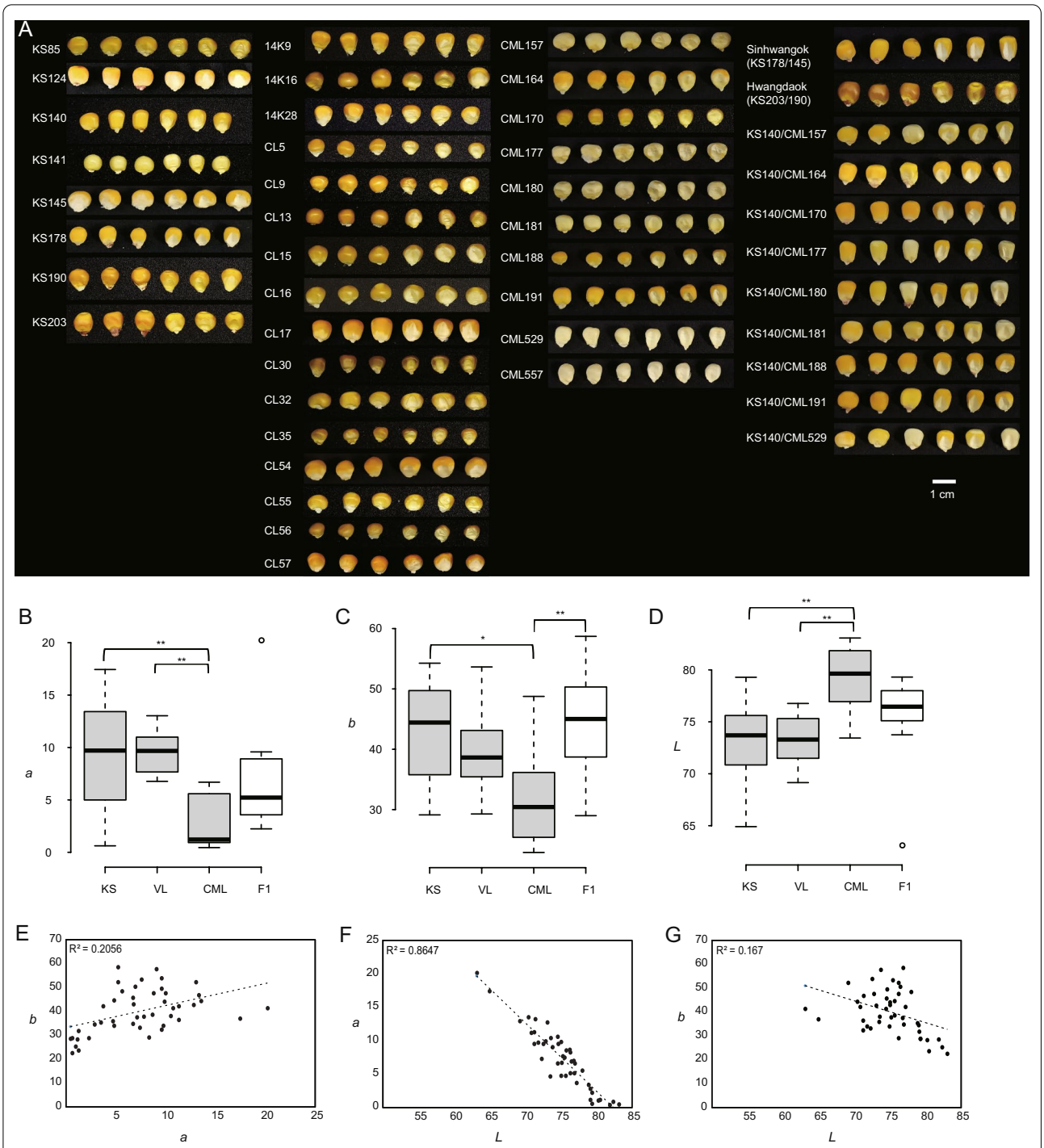


**Fig. 1** Color phenotypes of kernels of the corn lines used in this study. **A** Three abgerminal and three germinal sides of representative kernels are shown in the same rows. Bar = 1 cm. **B**–**D** Boxplots of *a*, *b*, and *L* values of the corn samples. Significant differences among samples are indicated by $*p < 0.01$ and $**p < 0.005$. **E**, **F** Scatter plots among *a*, *b*, and *L* values and their correlation coefficients

clear tendency to have reduced antioxidant activity based on their placement in the PCA score plot (Fig. 3). The five components showed similar patterns in the loading plots, in which carotenoid loaded in a different direction than the others (Fig. 3B). The tendency for a general reduction in antioxidant activity among hybrids suggested that the yellow inbreds examined lack dominant useful alleles for higher antioxidant activity. However, these observations

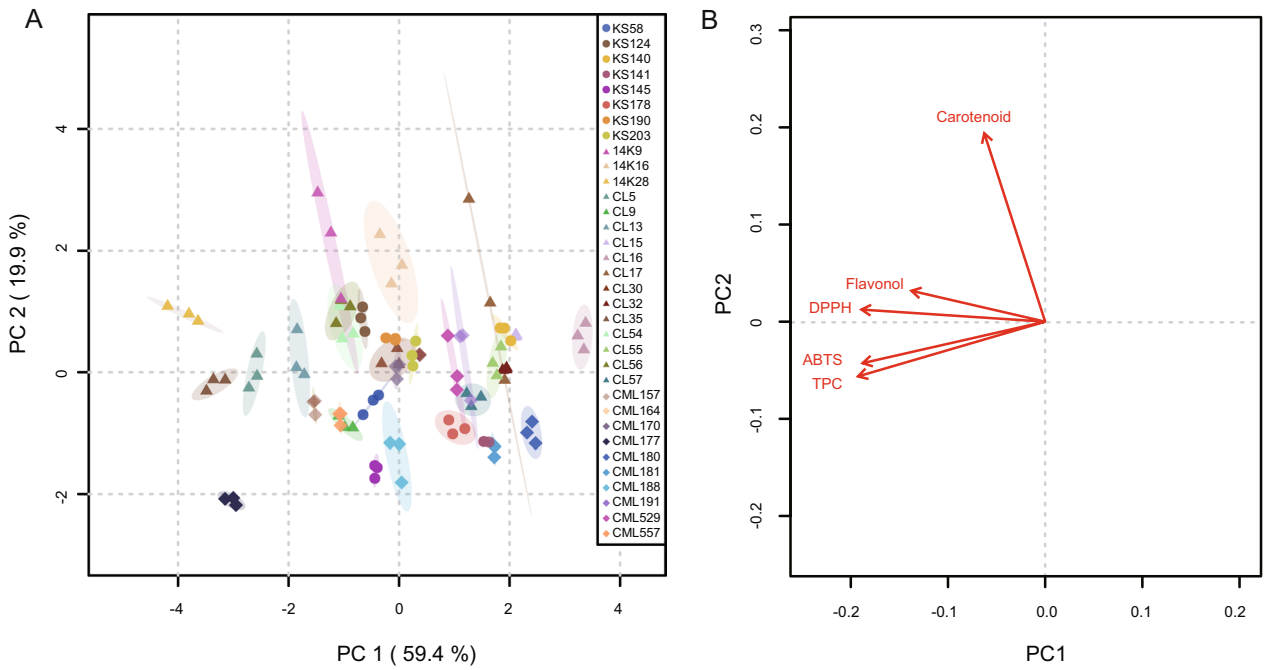


**Fig. 2** PCA of 34 inbred lines based on four antioxidant activities and carotenoid contents

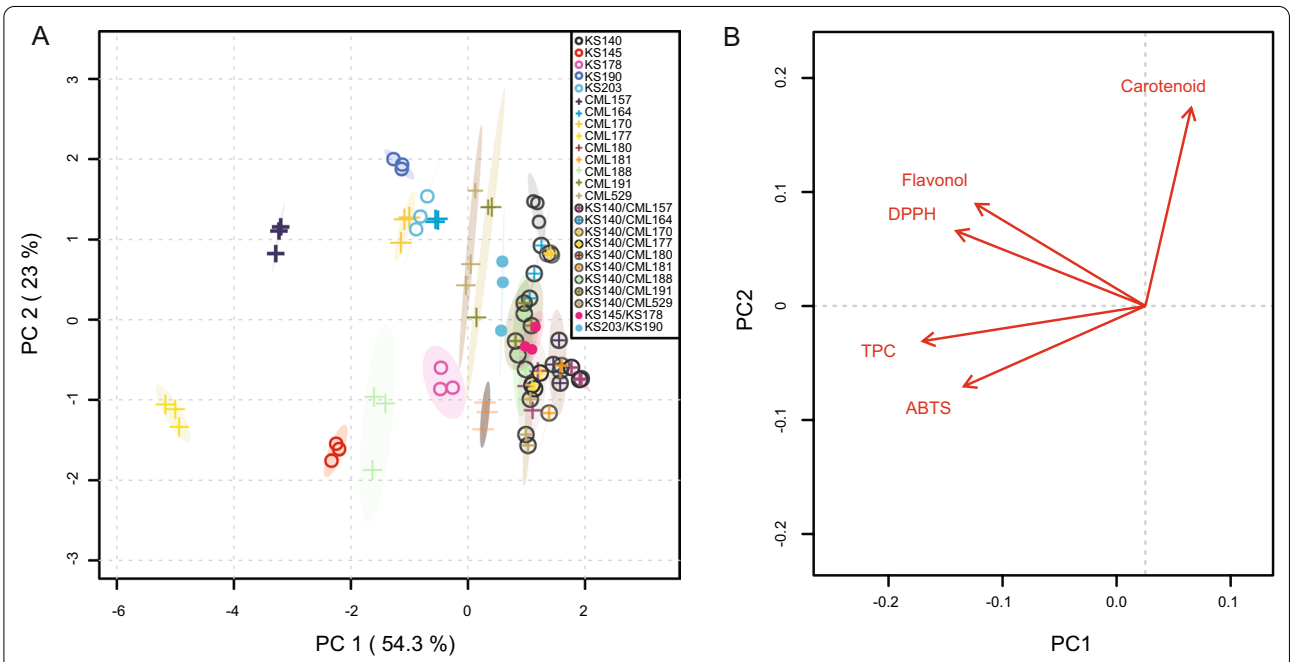


**Fig. 3** PCA of 11 $F_1$ hybrids with their parents based on four antioxidant activities and carotenoid contents

also suggest that antioxidant activities of yellow and white corn can be greatly improved by sophisticated breeding strategies.

### Correlations among antioxidant measurement methods and carotenoid contents

In general, antioxidant activities did not differ among samples from different geographic locations. Only

**Table 1** Antioxidant contents of corn samples analyzed in this study

| Line | Type | Source | Polyphenol (μg $g^{-1}$)[a] | Flavonoid (μg $g^{-1}$)[b] | DPPH (μmol $g^{-1}$)[c] | ABTS (μmol $g^{-1}$)[c] | Carotenoid (mg $g^{-1}$) |
|---|---|---|---|---|---|---|---|
| KS85 | Dent | NICS | 1846.9 ± 8.2 | 454.0 ± 33.5 | 223.0 ± 11.2 | 364.1 ± 8.2 | 1.20 ± 0.33 |
| KS124 | Dent | NICS | 1873.5 ± 14.2 | 360.5 ± 13.9 | 239.2 ± 0.5 | 361.5 ± 1.9 | 4.00 ± 0.33 |
| KS140 | Flint | NICS | 1335.4 ± 4.0 | 184.2 ± 3.1 | 171.8 ± 4.4 | 299.4 ± 2.1 | 3.07 ± 0.19 |
| KS141 | Flint | NICS | 1511.6 ± 7.1 | 200.1 ± 5.5 | 176.4 ± 2.7 | 332.0 ± 1.3 | 0.00 ± 0.00 |
| KS145 | Dent | NICS | 2384.7 ± 8.1 | 260.9 ± 22.0 | 124.3 ± 3.5 | 454.8 ± 0.4 | 1.07 ± 0.19 |
| KS178 | Dent | NICS | 1863.5 ± 90.3 | 194.5 ± 8.4 | 138.3 ± 1.4 | 375.0 ± 1.0 | 1.33 ± 0.19 |
| KS190 | Dent | NICS | 1876.3 ± 16.1 | 395.9 ± 11.3 | 206.3 ± 5.3 | 336.1 ± 2.4 | 3.20 ± 0.00 |
| KS203 | Dent | NICS | 1690.7 ± 4.0 | 313.4 ± 16.5 | 206.5 ± 4.5 | 351.3 ± 3.0 | 2.80 ± 0.33 |
| 14K9 | Flint | VLM | 2115.1 ± 10.7 | 290.4 ± 19.6 | 242.4 ± 1.5 | 381.1 ± 3.3 | 6.93 ± 1.32 |
| 14K16 | Flint | VLM | 1713.8 ± 89.4 | 412.8 ± 8.4 | 188.1 ± 3.0 | 349.9 ± 4.8 | 5.47 ± 0.68 |
| 14K28 | Flint | VLM | 2706.3 ± 31.1 | 418.9 ± 89.3 | 316.4 ± 2.1 | 474.1 ± 1.5 | 5.60 ± 0.00 |
| CL5 | Flint | VLM | 2590.4 ± 17.8 | 330.5 ± 30.3 | 278.7 ± 3.6 | 445.1 ± 2.0 | 3.73 ± 0.50 |
| CL9 | Flint | VLM | 2079.0 ± 26.8 | 217.4 ± 5.5 | 252.0 ± 4.6 | 425.2 ± 5.9 | 1.73 ± 0.19 |
| CL13 | Flint | VLM | 2296.4 ± 35.1 | 266.3 ± 24.4 | 258.6 ± 4.6 | 437.8 ± 3.6 | 4.00 ± 0.57 |
| CL15 | Flint | VLM | 1420.8 ± 18.6 | 130.1 ± 6.3 | 155.9 ± 2.4 | 300.7 ± 1.9 | 3.20 ± 0.00 |
| CL16 | Flint | VLM | 1184.1 ± 6.9 | 75.7 ± 6.2 | 126.4 ± 5.1 | 257.7 ± 3.8 | 2.80 ± 0.33 |
| CL17 | Flint | VLM | 1537.0 ± 4.0 | 142.7 ± 5.4 | 162.1 ± 3.8 | 312.5 ± 3.4 | 4.67 ± 2.29 |
| CL30 | Flint | VLM | 1853.7 ± 73.3 | 206.6 ± 25.6 | 214.2 ± 3.0 | 391.5 ± 1.0 | 3.33 ± 0.19 |
| CL32 | Flint | VLM | 1451.0 ± 33.0 | 100.5 ± 0.0 | 168.1 ± 2.0 | 322.7 ± 1.0 | 2.40 ± 0.00 |
| CL35 | Flint | VLM | 2647.3 ± 39.1 | 346.4 ± 32.7 | 322.7 ± 3.0 | 464.0 ± 1.1 | 3.47 ± 0.19 |
| CL54 | Flint | VLM | 1962.7 ± 84.3 | 141.2 ± 9.6 | 270.4 ± 5.1 | 415.9 ± 1.8 | 4.67 ± 0.38 |
| CL55 | Flint | VLM | 1431.1 ± 22.9 | 154.1 ± 6.4 | 177.3 ± 0.7 | 313.7 ± 6.1 | 2.40 ± 0.33 |
| CL56 | Flint | VLM | 2174.0 ± 70.7 | 232.8 ± 22.6 | 227.9 ± 1.6 | 401.9 ± 3.0 | 5.20 ± 0.33 |
| CL57 | Flint | VLM | 1738.7 ± 112.9 | 74.7 ± 11.1 | 167.7 ± 1.9 | 357.7 ± 5.3 | 2.13 ± 0.19 |
| CML157 | Flint | CIMMYT | 2103.3 ± 12.3 | 591.5 ± 3.2 | 250.4 ± 1.0 | 366.3 ± 3.8 | 1.07 ± 0.19 |
| CML164 | Flint | CIMMYT | 1896.1 ± 17.7 | 199.3 ± 0.0 | 212.2 ± 1.6 | 339.4 ± 1.0 | 3.20 ± 0.00 |
| CML170 | Flint | CIMMYT | 1990.4 ± 18.0 | 228.6 ± 3.2 | 242.8 ± 1.0 | 333.2 ± 2.7 | 2.67 ± 0.19 |
| CML177 | Flint | CIMMYT | 2992.4 ± 41.0 | 277.3 ± 37.6 | 313.4 ± 1.3 | 451.1 ± 1.7 | 0.40 ± 0.00 |
| CML180 | Flint | CIMMYT | 1593.4 ± 30.1 | 113.9 ± 16.4 | 149.7 ± 0.5 | 288.5 ± 2.5 | 0.40 ± 0.33 |
| CML181 | Flint | CIMMYT | 1741.5 ± 4.1 | 108.2 ± 9.6 | 170.4 ± 1.8 | 322.7 ± 3.3 | 0.27 ± 0.19 |
| CML188 | Flint | CIMMYT | 2189.1 ± 10.7 | 95.8 ± 16.4 | 199.2 ± 0.5 | 413.3 ± 2.6 | 1.20 ± 0.57 |
| CML191 | Flint | CIMMYT | 1684.1 ± 22.6 | 152.5 ± 3.2 | 187.8 ± 2.9 | 325.7 ± 2.8 | 2.93 ± 0.94 |
| CML529 | Flint | CIMMYT | 1833.9 ± 21.5 | 163.0 ± 8.3 | 199.9 ± 2.6 | 312.3 ± 1.5 | 2.67 ± 0.68 |
| CML557 | Flint | CIMMYT | 2498.2 ± 4.1 | 198.5 ± 3.2 | 242.4 ± 2.4 | 389.7 ± 1.3 | 2.27 ± 0.19 |
| KS140/CML157 | Flint | F1 | 1281.3 ± 10.8 | 74.3 ± 19.1 | 116.3 ± 2.3 | 309.5 ± 0.7 | 1.20 ± 0.00 |
| KS140/CML164 | Flint | F1 | 1501.2 ± 25.4 | 97.6 ± 6.3 | 133.9 ± 2.9 | 340.3 ± 7.4 | 3.20 ± 0.33 |
| KS140/CML170 | Flint | F1 | 1370.3 ± 18.0 | 117.5 ± 6.4 | 135.9 ± 4.0 | 327.4 ± 1.8 | 3.20 ± 0.00 |
| KS140/CML177 | Flint | F1 | 1436.2 ± 8.2 | 113.0 ± 19.1 | 135.8 ± 3.8 | 320.4 ± 0.7 | 0.93 ± 0.19 |
| KS140/CML180 | Flint | F1 | 1370.0 ± 29.4 | 83.3 ± 6.3 | 118.1 ± 4.1 | 326.9 ± 3.2 | 1.60 ± 0.33 |
| KS140/CML181 | Flint | F1 | 1427.8 ± 31.1 | 91.4 ± 5.5 | 104.1 ± 5.4 | 326.1 ± 4.0 | 1.33 ± 0.38 |
| KS140/CML188 | Flint | F1 | 1565.4 ± 4.1 | 113.4 ± 14.6 | 129.1 ± 4.5 | 336.7 ± 2.1 | 1.87 ± 0.38 |
| KS140/CML191 | Flint | F1 | 1466.9 ± 28.2 | 138.5 ± 19.6 | 137.2 ± 4.4 | 334.4 ± 5.8 | 2.00 ± 0.33 |
| KS140/CML529 | Flint | F1 | 1499.8 ± 8.2 | 102.7 ± 14.7 | 131.4 ± 4.4 | 324.3 ± 2.9 | 0.27 ± 0.38 |
| Sinhwangok (KS178/145) | Dent | F1 | 1549.3 ± 10.8 | 118.3 ± 5.5 | 110.6 ± 3.5 | 342.4 ± 1.3 | 2.13 ± 0.19 |
| Hwangdaok (KS203/190) | Dent | F1 | 1775.5 ± 54.4 | 128.4 ± 12.8 | 101.3 ± 5.3 | 371.8 ± 1.9 | 3.47 ± 0.50 |

[a] μg gallic acid equivalents $g^{-1}$ DW

[b] μg catechin equivalents $g^{-1}$ DW

[c] μmol Trolox equivalents $g^{-1}$ DW

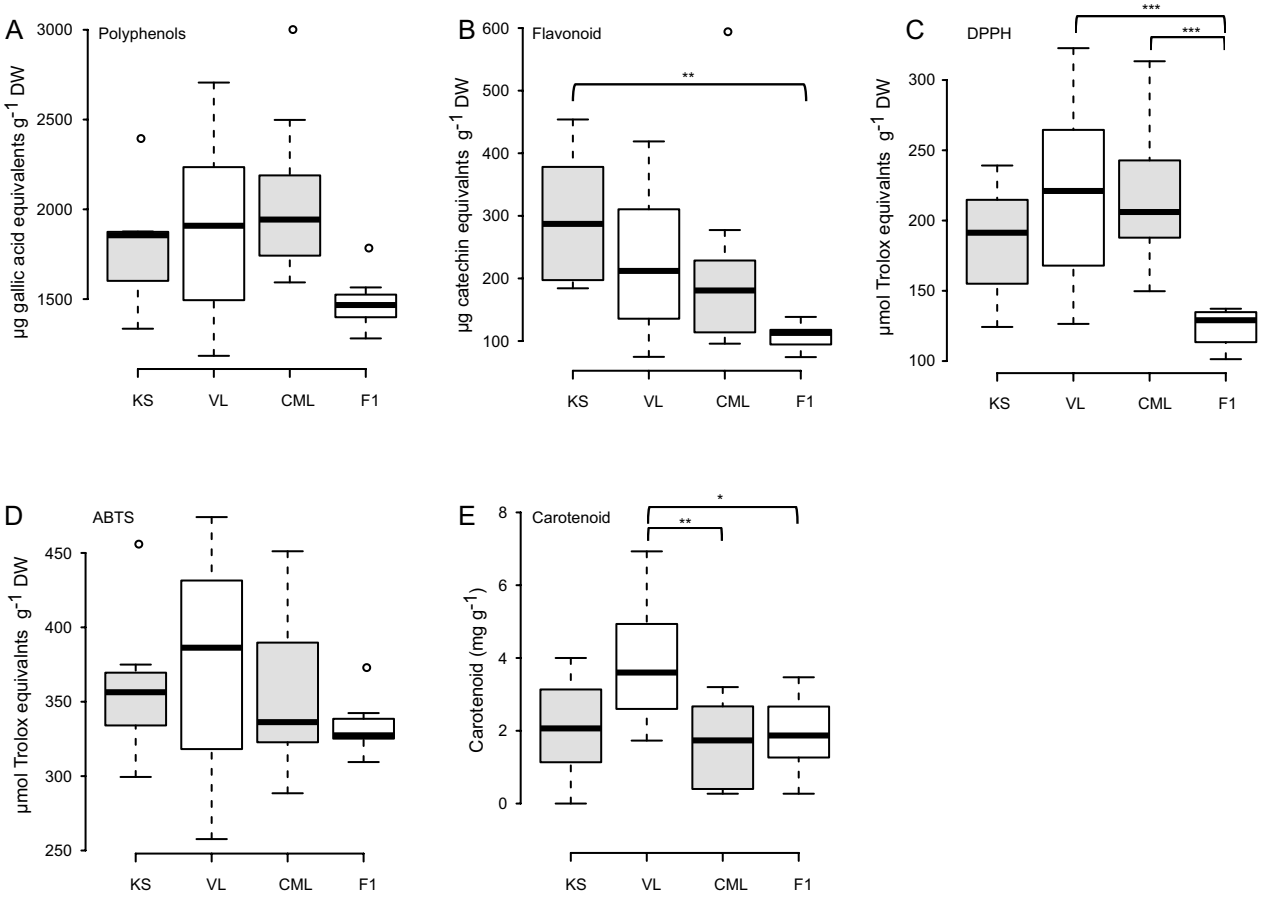


**Fig. 4** Boxplots of antioxidant activities and carotenoid contents of corn samples based on geographic location, including polyphenols (**A**), flavonoid (**B**), DPPH (**C**), ABTS (**D**), and carotenoid contents (**E**). Significant differences among samples are indicated by $*p < 0.01$, $**p < 0.005$, and $***p < 0.001$

carotenoid contents significantly differed between samples from Vietnamese lines and CMLs ($p < 0.005$; Fig. 4). By contrast, $F_1$ hybrids showed significant differences in flavonoid and carotenoid contents, and DPPH activity for at least one of the samples from different geographic regions (Fig. 4).

Correlation analysis of the results obtained using different methods to measure antioxidant activity revealed high correlation coefficients, confirming the reliability of the different methods. DPPH and ABTS activities showed higher correlation coefficients with polyphenol contents (0.667 and 0.806, respectively) than flavonoid contents (0.444 and 0.243, respectively). A relatively weak correlation was observed for carotenoid contents (Table 2).

We then analyzed the correlation coefficients between antioxidant activities and color values. Polyphenol contents showed significant correlations with *b* values and carotenoid contents with *a* values (Table 3). Therefore, the *a* (green/red) and *b* (blue/yellow) color values of the kernels could be used as indicators of carotenoid contents and polyphenol contents, respectively. In purple waxy corn, kernel color is thought to reflect anthocyanin levels [16]. Lower correlation coefficients could be caused by the greater genetic diversity of the corn samples examined in this study.

Furthermore, carotenoid color can be divided into the redness and yellowness of the kernel. Kernel color values

**Table 2** Correlation analysis of values from antioxidant assays and carotenoid contents

| $R^2$ | Flavonoid | DPPH | ABTS | Carotenoid |
|---|---|---|---|---|
| Polyphenol | 0.335* | 0.667*** | 0.806*** | 0.041 |
| Flavonoid | | 0.444** | 0.243* | 0.079 |
| DPPH | | | 0.484** | 0.138 |
| ABTS | | | | 0.058 |

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

**Table 3** Correlations between kernel color and the levels of antioxidant compounds

| $R^2$ | Polyphenol | Flavonoid | DPPH | ABTS | Carotenoid |
|---|---|---|---|---|---|
| *L* | 0.000 | 0.026 | 0.002 | 0.045 | 0.216 |
| *a* | 0.002 | 0.048 | 0.003 | 0.013 | 0.352* |
| *b* | 0.238* | 0.056 | 0.163 | 0.097 | 0.027 |

*L* lightness; *a* redness; *b* yellowness

* $p < 0.05$

*a* and *b* increased with increasing carotenoid accumulation in orange corn during seed maturation [20]. In addition, the changes in kernel color values *a* and *b* showed significant consistency, as carotenoid are degraded during postharvest storage [21]. In the current study, the significant partial correlation coefficients between kernel redness and carotenoid contents indicate that *a* value rather than *b* value could be used as an indicator of carotenoid contents, especially for genetically diverse kernels.

#### Authors' contributions

HHB, SL, and GY designed the study. HHB, GY, YSG, JYH carried out the experimental works. HHB, SL, JYH, and GY analyzed and interpreted data. GY and HHB wrote the manuscript. YSG, JYH, YC, JHS and SS prepared plant materials and established analytical methods. SS, TWJ supervised all the steps of this study. All authors read and approved the final manuscript.

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## Declarations

#### Competing interests

The authors declare that they have no competing interests.

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