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Genotypic variability in anthocyanins, total phenolics, and antioxidant activity among diverse waxy corn germplasm

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Abstract Identification of germplasm sources of waxy corn (*Zea mays* L. var. *ceratina*) with high variability for anthocyanins, phenolic compounds, and antioxidant activity is an important phase for waxy corn breeding for improvement of useful phytochemicals. The objectives of this study were to evaluate 49 genotypes of waxy corn for color parameters, monomeric anthocyanin content (MAC) and total phenolic content (TPC), and antioxidant activities. The experiment was conducted under field conditions in a randomized complete block design with three replications for two seasons in the rainy and the dry season 2010. Corn genotypes and seasons were significantly different ($P \leq 0.01$) for most traits under study except for TPC. Variations due to genotype were large for all characters, accounting for 74.43–95.70 % of total variations. The interactions between genotype and season were significant for all characters. Forty-nine corn genotypes were divided into four groups based on antioxidants and their activities. Significant and positive correlations were found among the anthocyanins, phenolics, and antioxidant activities, and correlation coefficients between anthocyanins with 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability and Trolox equivalent antioxidant capacity (TEAC) assays were high (r = 0.94 and 0.88, respectively). All of the Hunterlab color parameters, including chroma and hue angle were highly correlated with anthocyanins, phenolics and their activities and therefore could be used as indirect selection criteria for improving levels of antioxidants and antioxidant activity in waxy corn.

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Introduction

Consumption of fruits, vegetables, and un-polished grains is strongly associated with the reduced risk of developing chronic diseases such as cancer and cardiovascular disease (Isabelle et al. 2010; Liu 2004). Flavonols, flavonones, phenolic compounds, vitamins E and C, carotenoids, and anthocyanins in natural foods might confer these health protective benefits by alleviating oxidative stress such as

preventing free radicals proteins, DNA, and lipids (Duthie et al. 2000; Huang et al. 2005). Anthocyanins are among the most abundant polyphenols in many foods (de Pascual-Teresa and Sanchez-Ballesta 2008). They constitute the largest group of water-soluble pigments in the plant kingdom, being responsible for the vivid blue, purple, and red colors displayed by many flowers, fruits and leaves. These pigments are usually present in red fruits, vegetables, roots, legumes and cereals (Clifford 2000; He and Giusti 2010). Among cereals, corn not only has a sufficient amount of carotenoids, tocopherols, protein, oil, and starch but also has a comparable content of anthocyanin compared with other cereals such as colored rice and wheat (Abdel-Aal et al. 2006; Chander et al. 2008). Purple corn is a special type of corn that is rich in anthocyanin, and can be used as viable a source of antioxidants for functional foods (de Pascual-Teresa et al. 2002). This corn type gained popularity as a functional colorant and functional food ingredient because of its high antioxidant compounds (Montilla et al. 2011).

Waxy corn (*Zea mays* L. var. *ceratina*) is harvested at immature stage and consumed as fresh vegetable in many countries of Asia such as China, Korea, Thailand, Vietnam, Laos and Myanmar, and the demand for waxy corn is increasing (Hu and Xu 2011; Chung et al. 2010). Phytochemical compositions and concentrations vary significantly according to the kernel colors. Especially, waxy corn has various kernel colors, including white, yellow, red, purple, and black. Moreover, the pigmented corn contains anthocyanins, carotenoids, phenolic compounds, and antioxidant activity than non-pigmented corn (Lopez-Martinez et al. 2009; Montilla et al. 2011; Žilić et al. 2012). Pigments and other substances are genetically regulated and can be improved through selection and combined with high-yielding characters (Graham et al. 1999; Rodriguez et al. 2013). Therefore, the opportunity to develop waxy corn varieties with high phytochemicals is great.

Breeding corn may increase antioxidants intake with higher antioxidant properties. The success of breeding depends on identifying genetic variation for the traits, and knowledge of the impacts of environmental parameters on its expression (Conner et al. 2002; Dooner et al. 1991). However, studies on variation among genotypes and the effects of environments are scant. Substantial and significant variations in phytochemical content and antioxidant activity were observed between a set of corn genotypes and even among classes of colors, and the potential health benefits provided by corn depended firstly on corn genotypes (Kuhnen et al. 2011). Kernel color can be easily identified visually and, therefore, can be increased through phenotypic selection (Rodriguez et al. 2013; Shen et al. 2009). Although visual assessment is and an easy method, it lacks consistency due to human is poor in color memory and compromises genotype comparisons (Hasting et al. 2012; Hutchings 1999). The application of colorimetric systems, based on uniform color spaces (CIELUV and CIELAB) and non-uniform color spaces (CIE-XYZ), is of great value for the quantification and characterization of the color properties of diverse pigments and foods (Hernanz et al. 2008; Wrolstad et al. 2005; Yang et al. 2008). Thus, the objectives of this study were to assess variability for color parameters, monomeric anthocyanin content, total phenolic content, and antioxidant activities and to investigate the correlations between these traits and color parameters. The results of this study could provide corn breeders and eventually commercial corn grower with new opportunities to promote the production of corn with enhanced levels of the antioxidant compounds.

Materials and methods

Waxy corn germplasm and crop management

Forty-nine genotypes of waxy corn (Table 1) collected from China, England, Japan, Laos, Myanmar, South Korea, Taiwan, Thailand, USA, and Vietnam were evaluated in this study. The Vegetable Corn Improvement Project, Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen University in Thailand collected these genotypes, which included commercial and landrace cultivars. The entries were assigned in a randomized complete block design with three replications for two seasons in the rainy season (June–August 2010) and the dry season (November 2010–January 2011) at the Research Farm, Khon Kaen University. The plot size was a single-row plot with 5 m long with spacing 0.8 m between row and 0.25 m between plants within rows. Soil preparation, planting and other agronomic practices were carried out uniformly according to the recommendations for

Table 1 Waxy corn genotypes, origin and pigmentation

Entry no.	Genotypes	Origin	Pigmentation
1	KKU-WX211001	Thailand	Light purple
2	KKU-WX211002	Thailand	Light purple
3	KKU-WX211003	Thailand	Light purple
4	KKU-WX211004	Thailand	Purple–yellow
5	KKU-WX212003	Laos	Light purple
6	KKU-WX212004	Laos	Light purple
7	KKU-WX212005	South Korea	Light purple
8	KKU-WX212006	China	Dark pink
9	KKU-WX212007	Laos	Purple–white
10	KKU-WX212008	South Korea	purple–white
11	KKU-WX221001	Thailand	Light purple
12	KKU-WX221058	Thailand	Yellow–white–purple
13	KKU-WX211022	Thailand	Yellow–purple
14	KKU-WX211024	Thailand	Light purple
15	KKU-WX212006	Japan	Dark pink
16	KKU-WX221059	Thailand	Light purple
17	KKU-WX221060	Thailand	Light purple
18	KK composite	Thailand	Yellow–purple–white
19	KKU-WX111031	Thailand	Purplish black
20	KKU-WX111033	Thailand	Purple–white–yellow
21	KKU-WX111034	Thailand	Purple–yellow
22	KKU-WX111035	Thailand	Purple–white
23	KKU-WX112091	Thailand	Light purple
24	KKU-WX112092	Thailand	Purple–white–yellow
25	KKU-WX112096	Thailand	Purple–white
26	KKU-WX112097	Thailand	Purple–white–yellow
27	KKU-WX112098	Thailand	Light purple
28	KKU-WX112099	Thailand	White–yellow–purple
29	KKU-WX121063	Laos	Purple–white–yellow
30	KKU-WX121065	Laos	Purple–white
31	KKU-WX121069	China	Light purple
32	KKU-WX112095	Thailand	Light purple
33	KKU-WX121068	Taiwan	Light purple
34	KKU-WX122026	Taiwan	Light purple
35	KKU-WX122027	Vietnam	White–pink
36	KKU-WX122028	Vietnam	Light purple
37	KKU-WX122031	China	Purple–white
38	KKU-WX122032	Myanmar	Light purple
39	KKU-WX122033	China	Light purple–white
40	KKU-WX122035	Thailand	Purplish black
41	KKU-WX122036	Thailand	Purplish black
42	Local Isan2	Thailand	Purple–white
43	Sweet violet	Thailand	Light purple–white
44	Japan stripe	Japan	Stripe red
45	Europe stripe	England	Stripe red

Table 1 continued

Entry no.	Genotypes	Origin	Pigmentation
46	Thai mountaineer	Thailand	Purple–white
47	KKU KNG	Thailand	Purplish black
48	White	Thailand	Creamy white
49	Yellow	Thailand	Creamy yellow

commercial production of corn. The populations were sib-pollinated with the plants in the same populations to avoid contamination of stray pollens. Five ears for each population were randomly harvested at physiological maturity (35 days after pollination; DAP) and oven-dried at 40 °C (moisture content ≤ 13 %). The kernels were manually separated from the cobs and then color values were measured prior to milling. The samples were milled into fine powder (CM190, Cemotec™), sieved through 60-mesh screen mesh, thoroughly mixed and stored at −20 °C until analysis. Maximum and minimum temperatures during the two seasons were record daily by a meteorological station near the experimental site.

Color measurements

Color attributes was measured by HunterLab miniscan XE PLUS colorimeter (Mod. PL50, Hunter Associates Laboratory Inc., VA, USA), and the colorimeter was calibrated prior to data collection with a HunterLab calibration standard white reflector plate. The color value was determined from random samples of 100 kernels from five corn ears of purple-colored genotypes which the terminal tip ends of 3 cm were discarded. The color was expressed in C* and h°. The chroma (C*) represents color intensity which is the distance of a color from the origin (a* + b* = 0) in a* and b* plane. Hue angle (h°) expresses in degree ranged from 0° to 360°, where 0° (red) locates on the +a axis and then rotates anticlockwise to 90° (yellow) for the +b* axis, 180° (green) for −a* and 270° (blue) for −b* (McGuire 1992).

Sample extraction

The anthocyanins in ground waxy corn kernels were extracted according to the method described by Rodriguez-Saona and Wrolstad (2001), Jing et al. (2007) with slight modifications. Approximately 2 g of each sample were added to a flask containing 25 mL of 70 % aqueous acetone acidified by the addition of HCl to 0.01 % and mixed well. The flasks were shaken on a platform shaker (LabScientific Inc., Livingston, NJ, USA) at 200 rpm and room temperature for 2 h. Each sample was filtered through Whatman # 1 filter paper under vacuum using a Büchner funnel, and the slurry was washed with 10 mL of acidified 70 % acetone. The filtrate was transferred to a separatory funnel, and 15 mL of chloroform were added. The mixture was gently mixed by turning the funnel upside down a few times. The samples were stored overnight at 4 °C or until a clear partition between the two phases was obtained. The solution was transferred to a centrifuge tube and centrifuged at 11,538 × g and 4 °C for 10 min. The upper aqueous layer containing the acetone/water mixture was collected, and the chloroform/acetone layer was carefully discarded. The residual acetone and chloroform were removed from the anthocyanin extract using a rotary evaporator at 40 °C under vacuum. The volume of the extracts was increased to 25 mL in a volumetric flask by the addition of 0.01 % HCl-acidified methanol.

Monomeric anthocyanin content (MAC)

The total monomeric anthocyanin content was measured using the pH differential method, as described by Giusti and Wrolstad (2001). A UV–vis spectrophotometer (GENESYS 10S, ThermoScientific, Waltham, MA, USA) was used to measure the absorbance at 510 and 700 nm. Anthocyanin levels were expressed as μg of cyanidin-3-glucoside equivalents per g of dry weight (μg CGE/g DW), using the reported molar extinction coefficient of 26,900 M^{-1} cm^{-1} and a molecular weight of 449.2 g/mol.

Total phenolic content (TPC)

The phenolic content was determined using the Folin-Ciocalteu (F–C) assay as described by Hu and Xu (2011). Briefly, the appropriate dilutions of the

extracts were oxidized with F–C reagent for 90 min, and the reaction was neutralized with sodium carbonate. The absorbance of the resulting blue color was measured at 765 nm, and the phenolic content was expressed as mg of gallic acid equivalents per g of dry weight (mg GAE/g DW).

Measurements of antioxidant activity

The activity for scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was assessed by measuring the bleaching of a black-colored methanol solution containing DPPH radicals, as described by Yao et al. (2010). Trolox was used as the reference compound. The results are expressed in μmol of Trolox equivalents per g of dry weight (μmol TE/g DW).

The Trolox equivalent antioxidant capacity (TEAC) assay, which measures the reduction of radical cations of ABTS by antioxidants, was conducted as described by Lopez-Martinez et al. (2009). Trolox was used as the reference compound. The results are expressed in μmol of Trolox equivalents per g of dry weight (μmol TE/g DW).

Statistical analysis

Analysis of variance was performed separately for each season, and error variances were tested for homogeneity (Gomez and Gomez 1984). Where error variances were homogeneous, the data of two seasons for monomeric anthocyanin, phenolic content, antioxidant activity and color parameter were combined. Duncan's multiple range test (DMRT) was used to compare mean difference between groups at 0.05 probability level. The Pearson correlation coefficients were calculated to determine the relationships among variables. Hierarchical agglomerative clustering was then performed for antioxidants and their activity, using the Ward criterion. All calculations were performed using JMP Pro software (version 10.0, SAS institute Inc., Chicago, IL, USA).

Results

Variations in color parameters, antioxidants and antioxidant activity

Differences in seasons were significant ($P \leq 0.01$) for chroma (C*), and hue angle (h°) (Table 2). However,

Table 2 Mean squares for color parameters, monomeric anthocyanin content, total phenolic content and antioxidant activity in kernels of 49 waxy corn genotypes evaluated in the rainy and dry seasons 2010

SOV	df	Color parameters		Antioxidants		Antioxidant capacity	
		Chroma (C*)	Hue angle (h°)	MAC	TPC	DPPH	TEAC
Season (S)	1	211.5* (3.64)[a]	6830.9** (9.71)	167,905** (1.05)	0.003NS (0)	150.3** (0.83)	5211.4** (1.33)
Genotype (G)	48	223.9** (88.67)	2276.2** (74.43)	314,420** (94.85)	13.9** (92.30)	360.8** (95.70)	7596.8** (93.62)
S × G	48	17.5** (6.94)	355.2** (11.62)	13,174** (3.97)	1.04** (6.90)	11.7** (3.11)	383.4** (4.73)
Error	192	0.5 (0.73)	30.5 (3.99)	105.0 (0.13)	0.03 (0.71)	0.3 (0.36)	6.2 (0.3)
C.V. (%)		4.25	5.29	4.41	7.25	5.28	6.58

SOV source of variance, *df* degree of freedom, *MAC* monomeric anthocyanin content, *TPC* total phenolic content, *DPPH* 2,2-diphenyl-1-picrylhydrazyl radical scavenging ability, *TEAC* Trolox equivalent antioxidant capacity, *NS* non-significant

*, ** Significant at $P \leq 0.05$ and 0.01, respectively

[a] Number within the parentheses is percentage of sum squares to total sum of squares

Table 3 Means for colors, antioxidant content, and antioxidant activity in kernels of 49 waxy corn genotypes evaluated in the rainy and dry seasons 2010

Parameters	Seasons		Minimum	Maximum	Average
	Rainy	Dry			
Colors					
Chroma (C*)	15.07 ± 5.88	16.84 ± 6.76	2.9	29.4	15.95 ± 6.37
Hue angle (h°)	292.73 ± 21.67	314.03 ± 40.42	274.5	355.0	306.88 ± 22.18
Antioxidant content					
MAC (µg CGE/g DW)	225.23 ± 1.34	273.03 ± 1.35	2.5	1,063.0	249.13 ± 3.57
TPC (mg GAE/g DW)	6.74 ± 0.01	6.70 ± 0.01	4.3	11.8	6.73 ± 0.01
Antioxidant activity					
DPPH (µmol TE/g DW)	10.25 ± 0.02	11.68 ± 0.02	0.1	39.4	10.96 ± 0.02
TEAC (µmol TE/g DW)	34.62 ± 0.22	41.22 ± 0.22	0.1	184.6	37.91 ± 0.22

Data are expressed as the mean ± SD of triplicate samples

MAC monomeric anthocyanin content, *TPC* total phenolic content, *DPPH* 2,2-diphenyl-1-picrylhydrazyl radical scavenging ability, *TEAC* Trolox equivalent antioxidant capacity

variations in seasons had small contributions to total variations for these traits (3.64 and 9.71 %, respectively). The results indicated that the traits were consistent across seasons. Corn genotypes were significantly different for all traits, and there were large contributions of corn genotype to total variations for C* and h° (88.67 and 74.43 %, respectively). The interactions between genotype by season were significant ($P \leq 0.01$) for all traits. However, most interactions had small contributions (6.94 % for C* and 11.62 % for h°) to total variations for these traits. Although the differences between seasons were low for these parameters, the maximum and minimum values for these parameters were rather wide, ranging from 2.9 to 29.4 for C*, and 274.5° to 355.0° for h° (Table 3). The averaged means of two-season data were 15.95 and 306.88° for C* and h°, respectively.

Genotype was the most important source of variations in monomeric anthocyanin content (MAC), accounting for 94.85 % of total variation, whereas season accounted for only 1.05 % although the difference between seasons was significant (Table 2). Genotype by season interaction was significant ($P \leq 0.01$) and contributed to 3.97 % of total variation. The monomeric anthocyanin contents of corn grown in the rainy season and the dry season ranged from 2.5 to 1,063.0 µg CGE/g DW (Table 3). The averaged means of two-season data was 249.13 µg CGE/g DW. Genotype was still the main source of variation in total phenolic content (TPC), accounting

for 92.3 % of total variation, whereas difference in seasons was not significant (Table 2). Genotype by season interaction was significant ($P \leq 0.01$) and contributed to 6.9 % of total variation. TPC values ranged from 4.3 to 11.8 mg GAE/g DW (Table 3). The averaged means of two-season data was 6.73 mg GAE/g DW.

Genotype contributed to the largest portions of total variations in 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability (95.70 %) and Trolox equivalent antioxidant capacity (TEAC) (93.62 %), whereas season contributed to the smallest portions for DPPH (0.83 %) and TEAC (1.33 %) (Table 2). The differences among genotypes were significant ($P \leq 0.01$) for these traits, and the interactions between genotype and season were also significant ($P \leq 0.01$). However, the contributions of the interactions between genotype and season were small, accounting for only 3.11 % for DPPH and 4.73 % for TEAC. DPPH values ranging from 0.1 to 39.4 µM TE/g DW were observed among waxy corn genotypes, and the averaged means of two-season data was 10.96 µM TE/g DW. Similarly, TEAC values ranging from 0.1 to 184.6 µM TE/g DW were found, and the averaged means of two-season data was 37.91 µM TE/g DW.

Cluster analysis

A dendrogram, which was constructed based on MAC, TPC, DPPH, and TEAC, could classify the corn

Fig. 1 Dendogram of genetic relationships among 48 waxy corn genotypes. Four main clusters (*A* to *D*) were formed. Two ways clustering method was Ward's cluster analysis based on antioxidants and antioxidant activities traits (scale: Distance scale) [a]
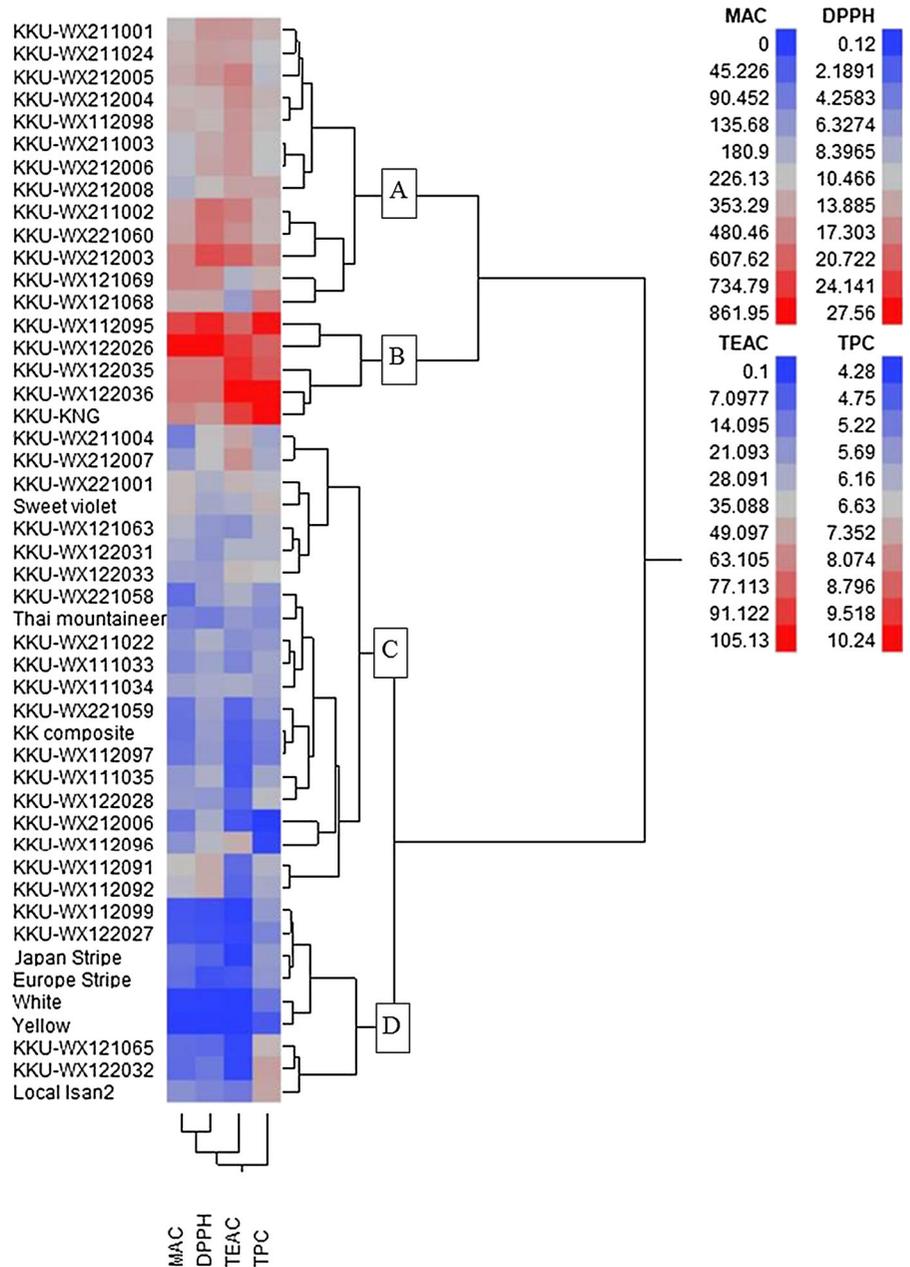
[a] Cluster together *columns* as well as *rows*; the *columns* must be measured on the same scale

genotypes into four distinct groups (Fig. 1). KKU-WX111031 was considered as the out-group and it was not included in the dendrogram because it had the extremely high values of antioxidants and their activities. Therefore, forty-eight genotypes were included in the analysis.

There were 13 corn genotypes in group A consisting of KKU-WX211001, KKU-WX211024, KKU-WX211005, KKU-WX211004, KKU-WX112098,

KKU-WX211003, KKU-WX212006, KKU-WX212008, KKU-WX211002, KKU-WX221060, KKU-WX212003, KKU-WX121069, and KKU-WX121068. Most corn genotypes this group had high values of MAC, TPC, DPPH, and TEAC except for KKU-WX121068, which showed the low value of TEAC.

Group B comprised 5 corn genotypes with the highest values of antioxidants and their activities (Table 4). This

Table 4 List of top high and low performing genotypes for monomeric anthocyanin content, total phenolic content and antioxidant activities in kernel of waxy corn genotypes averaged over two seasons

Genotypes/Groups	MAC (μg CGE/g DW)	TPC (mg GAE/g DW)	Antioxidant activities (μmol TE/g DW)	
			DPPH	TEAC
High group				
KKU-WX111031	1,063.0 ± 108.4a[a]	11.8 ± 0.4a	36.8 ± 2.8a	178.4 ± 8.1a
KKU-WX112095	699.3 ± 47.2c	10.1 ± 0.3b	26.1 ± 2.2c	78.0 ± 10.3f
KKU-WX122026	870.0 ± 59.4b	8.8 ± 0.3 cd	27.6 ± 1.5b	91.4 ± 8.3e
KKU-WX122035	544.9 ± 135.7f	9.0 ± 0.2c	19.0 ± 2.7f	95.8 ± 13.5d
KKU-WX122036	636.4 ± 202.7e	10.2 ± 0.8b	21.6 ± 4.4e	105.1 ± 15.9b
KKU KNG	664.9 ± 256.5d	10.2 ± 0.3b	21.4 ± 5.5e	99.1 ± 20.5c
Low group				
KKU-WX112099	29.6 ± 5.1ć	5.8 ± 0.6o-t	1.4 ± 0.4é	1.2 ± 0.5w
KKU-WX122027	35.9 ± 15.2b́ć	5.4 ± 0.6stu	1.4 ± 0.5é	7.1 ± 6.2stu
Japan stripe	75.1 ± 7.8zá	5.8 ± 0.6o-t	2.6 ± 0.4x	0.6 ± 0.6w
Euro stripe	74.8 ± 27.7zá	5.7 ± 0.6q-t	1.4 ± 0.5y	2.5 ± 1.6vw
KKU-WX121065	70.6 ± 14.6á	7.0 ± 0.7g-l	3.0 ± 1.0ć	1.5 ± 0.8w
KKU-WX122032	70.5 ± 20.8á	7.5 ± 1.5fg	3.7 ± 0.6b́	1.2 ± 0.8w
Local Isan	126.4 ± 12.9uv	7.4 ± 0.1fgh	4.8 ± 1.2zá	14.1 ± 4.8r
White	2.5 ± 1.2d́	5.2 ± 0.9uv	0.3 ± 0.2f́	0.5 ± 0.3w
Yellow	ND	4.7 ± 1.1vw	0.1 ± 0.1f́	0.1 ± 0.5w

Data are expressed as the mean ± SD of six determinations

MAC monomeric anthocyanin content, *TPC* total phenolic content, *DPPH* 2,2-diphenyl-1-picrylhydrazyl radical scavenging ability, *TEAC* Trolox equivalent antioxidant capacity, *ND* not detected

[a] Means in the same columns with different letters are significant ($P \leq 0.05$) determined by DMRT

group consisted of KKU-WX112095, KKU-WX122026, KKU-WX122035, KKU-WX122036, and KKU KNG. Most genotypes were collected in Thailand except for KKU-WX122026 from Taiwan.

Group C was the largest group consisting of KKU-WX211004, KKU-WX212007, KKU-WX221001, Sweet violet, KKU-WX121063, KKU-WX122031, KKU-WX122033, KKU-WX221058, Thai mountaineer, KKU-211022, KKU-WX111033, KKU-WX111034, KKU-WX221059, KK composite, KKU-112097, KKU-111035, KKU-WX122028, KKU-WX212006, KKU-WX122096, KKU-WX122091, and KKU-WX122092. This group showed medium values of antioxidants and antioxidant activities. However, the genotypes KKU-WX211004 and KKU-WX212007 showed high TEAC values, and KKU-WX122091 and KKU-WX122092 demonstrated high DPPH values.

Group D comprised 9 corn genotypes including KKU-WX122099, KKU-WX122027, Japan stripe,

Table 5 Pearson correlation coefficients between color parameters, monomeric anthocyanin content, total phenolic content, and antioxidant activities of purple colored waxy corn genotypes

	Color parameters		MAC	TPC	DPPH
	C*	h°			
C*					
h°	0.76**				
MAC	0.68**	0.84**			
TPC	0.75**	0.80**	0.84**		
DPPH	0.57**	0.77**	0.94**	0.78**	
TEAC	0.60**	0.78**	0.88**	0.79**	0.89**

Values are from six determinations

C* chroma, *h°* hue angle, *MAC* monomeric anthocyanin content, *TPC* total phenolic content, *DPPH* 2,2-diphenyl-1-picrylhydrazyl radical scavenging ability, *TEAC* Trolox equivalent antioxidant capacity

** The correlation is significant at $P \leq 0.01$

Euro stripe, KKU-WX121065, KKU-WX122032, Local Isan, White, and Yellow (Table 4). This group had the lowest values of antioxidants except for KKU-WX122032 and Local Isan, which showed high TPC.

Relationship among monomeric anthocyanin, phenolic content, antioxidant activity, and color traits

The chromatic parameter (C*) was significantly correlated with hue angle (h°) (0.76; $P \leq 0.01$) (Table 5). C* was positively correlated with monomeric anthocyanin content (MAC), total phenolic content (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability and Trolox equivalent antioxidant capacity (TEAC), and the correlation coefficients ranged from 0.57 to 0.75; $P \leq 0.01$. Similarly, color parameter h° was also significantly correlated with antioxidants and their properties, and the correlation coefficients ranged from 0.77 to 0.84; $P \leq 0.01$. The correlation coefficients among MAC, TPC, DPPH and TEAC were positive and significant, ranging from 0.78 to 0.94; $P \leq 0.01$.

Discussion

Variations in color parameters, antioxidants and antioxidant activity

Phytochemicals in food are beneficial to health due to their high antioxidant properties, which is related to prevention of cardiovascular disease, diabetes or obesity and mutagenesis or cancer (He and Giusti 2010; Tsuda et al. 2003). In corn, colored corn has different pigments and other compounds with antioxidant activity that can be highly beneficial for human health (Rodriguez et al. 2013). The hypothesis underlying this investigation is that color parameters are related to antioxidant compounds and their activity, and, therefore, color parameters, monomeric anthocyanin content (MAC), total phenolic content (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability and Trolox equivalent antioxidant capacity (TEAC) were investigated in 49 corn genotypes with different levels of colors from white to purplish black. This investigation also explored the possibility to use color parameters as an alternative

tool to select corn genotypes with high antioxidant activity.

In this study, corn genotypes were significantly different for color traits, MAC, TPC, DPPH, and TEAC, and the contributions of genotype to total variations in these characters were highest, ranging from 74.43 to 95.70 %. Direct comparison of studies in waxy corn is not available. However, genotype was more important source of variations in anthocyanin and other phytochemicals in corn (Chander et al. 2008; Mahan et al. 2013) than environment and genotype by environment interaction. The results indicated that selection for individual characters would be possible as sample variations are available for these characters.

Season had very small effects on total variations in color parameters, MAC, TPC, DPPH, and TEAC accounting for 0 % for TPC to 9.71 % for hue angle. The results indicated that these characters were rather stable. In previous investigation in cereals, environmental factor had small effects on antioxidant characters and this should allow for easy selection (Santra et al. 2005). Therefore, in corn breeding programs, selection of corn genotypes with high antioxidant activity in a few seasons should result in improved anthocyanin concentration combined with other desirable antioxidant compounds. However, environmental factors such as UV radiation, cold temperature, drought and water stress could induce high accumulation of anthocyanin in corn (Jing et al. 2007) and other plants (Chalker-Scott 1999; Liang et al. 2009). The contrasting results among different studies would be possible due to the differences in materials used and experimental conditions.

The interaction effects were generally small compared to main effects except for TPC (6.9 %), color parameter C* (6.94 %) and h° (11.62 %). When low interaction effects were considered, MAC, DPPH, and TEAC were rather stable and might be useful as selection criteria for improvement of corn genotypes with high antioxidant capacity. The low interactions of genotype × environment are preferable, indicating that the relative performance in one season is reliable in predicting relative performance in another season (Knievel et al. 2009; Hasting et al. 2012; Mahan et al. 2013). With low G × E interaction and little error variance, the relative performance across seasons or environment should be similar.

Relationships among color parameters, antioxidants and antioxidant activity

Pearson correlations coefficients among Hunterlab color parameters, chroma (saturation or vividness of color) and hue angle (tint of color, an angular measure) with anthocyanins, phenolic content, and antioxidant activity were explored in this study. The color parameters C* and h° correlated strongly with the levels of anthocyanins, phenolic and their activities (r = 0.60–0.84), except correlation between C* and DPPH (r = 0.57). A positive correlation between antioxidants and antioxidant activity would be expected because any increase in antioxidants would increase color intensity and hue angle. These results were in agreement with those reported by Yang et al. (2008) who evaluated the correlations among the yield of anthocyanins with C* and h° in extractible anthocyanin of purple corncob. Interestingly, the colored waxy corn genotypes (high C* and h° values) may better develop in phytochemicals and/or antioxidant activity. C* and h° may be a suitable for use as indirect selection criteria in screening of corn genotypes with high antioxidants.

In this study, the relationships among monomeric anthocyanin content (MAC), total phenolic content (TPC) and antioxidant activity (2,2-diphenyl-1-picryl-hydrazyl radical scavenging ability and Trolox equivalent antioxidant capacity) were strong and positive. In previous study, MAC was significantly positive correlated with TPC because anthocyanin is the phenolic subgroup with same biosynthetic pathway and localization (pericarp and/or aleurone layer of kernel) (Hu and Xu 2011; Žilić et al. 2012). As anthocyanin is related to antioxidant in colored corn, breeding for high anthocyanin would result in high antioxidant.

Although MAC and TPC had significant positive correlation with both DPPH and TEAC, antioxidant activities were more closely correlated with anthocyanin than with phenolic compounds. The results suggested that anthocyanins had higher contribution to antioxidant properties than did phenolic compounds due to relatively high concentrations of anthocyanins compared with the overall phenolic compounds. In contrast to this study, phenolic components had higher contribution to antioxidant activity than did anthocyanin pigments (Pitija et al. 2013). The contrasting results of different studies would be possibly due to materials used, evaluation methods for antioxidation,

and complicated extracts containing two or more antioxidants (del Pozo-Insfran et al. 2006). A better understanding on the relationship among phytochemicals and antioxidant activities in corn is important for improvement of corn varieties with high nutritional value.

Cluster analysis

Dendrogram could classified corn genotypes into four distinct clusters and one genotype (KKU-WX111031) was an out-group because of its high values of TAC, TPC, DPPH, and TEAC. Cluster B had the highest values of antioxidants and their activity. The differences between high group (group B) and low group (group D) were significant for TAC, TPC, DPPH, and TEAC, and the genotype with low and high antioxidants and antioxidant activity were readily identified (Table 4). The use of phytochemicals and antioxidant activity in clustering corn genotypes was successful in this study. The use of phytochemicals and antioxidant activity for germplasm in identifying superior genotypes with high antioxidant activity is useful. The corn genotypes in cluster B (KKU-WX112095, KKU-WX122026, KKU-WX122035, KKU-WX122036, and KKU KNG) and the out-group (KKU-WX111031) can be used as parents for improvement of nutraceutical properties in corn.

Implications for waxy corn breeding

The prime goal of corn breeders is always yield enhancement, but views of improving the livings through increased levels of antioxidants and their activity in grains is highly warranted. Thus, high anthocyanins, phenolic compounds and antioxidant activity as percent mean along with wide variation estimates observed for these important traits indicated the considerable scope for improving the phytochemical status of corn. A few breeders have studied the prospects for development of corn hybrids with high functional and antioxidant materials. As a primary step to achieve this goal, it is necessary to investigate the genotypic diversity in the phytochemicals among diverse corn germplasm, to find a way to enrich these compositions by breeding.

In this study, contributions of genotype to total variations in these traits were highest. Elite genotypes were identified as a genetic stock for enhanced levels of antioxidants and their capacity. The pigment was

found in the aleurone layer and pericarp of the kernel. Although the naked-eye color of the kernel and location of the pigment in corn genotypes were the same, the content of antioxidants varied (Moreno et al. 2005). Modern color instrumentation has made a measurement of chroma and hue angle indices and easy (Wrolstad et al. 2005). It is interesting to note here that strong correlations between color parameters C* and h° with antioxidants and antioxidant activity, suggests that color traits could be used as indirect indexes to select corn breeding germplasm with high antioxidant components. Moreover, pre-screening using a colorimeter could significantly reduce the number of samples, expensive extraction and long time for analysis.

Conclusion

The wide range of antioxidants and their activity levels found in this study indicates that adequate genetic variability is present potentially to develop corn varieties with enhanced health benefits. Large contributions of genotypes and low interactions between genotype and environment to total variations in these characters indicated that evaluation for these traits could be carried out in few locations or seasons in order to identify superior genotypes. The relationships of these traits with kernel color traits, including of chroma (C*) and hue angle (h°) could serve as indexes to indirectly select waxy corn genotypes with high anthocyanin, phenolics, and their activities. KKU-WX111031, KKU-WX112095, KKU-WX122026, KKU-WX122035, KKU-WX122036, and KKU-KNG were identified as genotypes with high anthocyanin, high phenolic content and high antioxidant activities. The information is useful for corn breeders to develop superior lines and hybrids with high level of antioxidant compounds.

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