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Variability in Anthocyanins, Phenolic Compounds and Antioxidant Capacity in the Tassels of Collected Waxy Corn Germplasm

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Abstract: Corn tassel is a valuable co-product and an excellent source of phytochemicals with bioactive properties. The information on the genetic diversity in the tassel properties of waxy corn germplasm is important for creating new varieties that can have the potential for the commercial production of tassels as a co-product. Therefore, the objective of this study was to evaluate the potential of corn tassels in a set of waxy corn germplasm for the extraction of phenolic compounds with an antioxidant activity. The experiment was carried out under field conditions in the rainy season 2017 and the dry season 2017/2018. Fifty waxy corn genotypes were evaluated. Data were collected for the total anthocyanin content (TAC), total phenolic content (TPC) and the antioxidant activity was determined by the 2,2-diphenyl−1-picrylhydrazyl (DPPH) radical scavenging activity and Trolox equivalent antioxidant capacity (TEAC) assays. The season (S) had small effect on all of the parameters, accounting for 0.2–8.7% of the total variance. The genotype (G) was the largest variance component in the TAC and DPPH radical scavenging activity, accounting for 83.5–97.5% of the total variance. The G and S × G interaction contributed approximately equally to the total variance in the TPC and TEAC. Based on the TAC, TPC and antioxidant capacity variation, the genotypes were classified into seven groups. The tassels of corn genotypes belonging to three of these clusters (clusters E, F and G) had high levels of phytochemicals along with an antioxidant capacity. A significant correlation coefficient was found between the TAC and DPPH (r = 0.70 **). The TPC showed a moderate relationship with the DPPH and TEAC assays (r = 0.60 ** and 0.76 **, respectively). The information obtained from this study can be used for germplasm management and waxy corn breeding for enhancing levels of bioactive properties in waxy corn tassels.

Keywords: *Zea may* L.; floral corn; phytochemicals; genetic diversity; cluster analysis

1. Introduction

Immature ears of small-ear waxy and waxy corn (*Zea mays* L. var. *ceratina*) are consumed as a vegetable in many Asian countries [1]. In Thailand, purple waxy corn is considered a special corn type because it is rich in phenolic compounds and anthocyanin in the kernels, cobs, silks and husks [2], as well as in the tassel [3]. Colored waxy corn is a good source of phytochemicals that are beneficial to

health [4–6]. Natural bioactive compounds in waxy corn with antioxidant activity could reduce the risk of chronic diseases such as cardiovascular diseases, cancer and obesity [7]. In vitro studies indicated that the purple waxy corn kernel extract alone and in combination with ginger has a protection effect against diabetic cataract [8]. Many researchers are interested in the genetic variation in corn kernels because the diverse corn germplasm is a significant source of nutrients, phytochemicals and antioxidants [9–12].

Capturing the value from co-products of grain or ear production is economically beneficial. Low value organs such as husks, cobs and tassels can be an inexpensive feedstock for the extraction of valuable chemicals. In purple waxy corn, these organs often contain anthocyanin pigments that have pro-health effects. For example, the combination of purple waxy corn cob and pandan leaf extract provided neuroprotective and memory-enhancing effects in menopause [13]. Moreover, an anthocyanin complex from purple waxy corn cobs and petals of blue butterfly pea can be anti-inflammatory, inhibit oxidative stress, reduce liver injury and periductal fibrosis, and it can be a chemopreventive agent to prevent cholangiocarcinoma caused by *Opisthorchis viverrini* infection [14,15].

The male corn flower or tassel is a co-product from corn production that can be used as a raw material to develop value-added products. Phenolic compounds extracted from ground corn tassel are bioactive phytochemicals that provide an anti-oxidative activity [16]. Whole corn pollen and bee pollen granules are recognized as a super food because they are excellent sources of nutrition, non-toxic and have a wide range of physiological properties in clinical studies [17]. Corn pollen is useful for developing functional food products [18,19].

Corn tassel has been used as a traditional medicine in China [20]. The authors also reported that flavonoids, saponin and polysaccharide from corn tassel could inhibit the proliferation of MGC80-3 gastric cancer cells *in vitro.* Moreover, a purified 4-hydroxyl−1-oxindole-3-acetic acid named "Tasselin A" extracted from sweet corn tassel played an important role in inhibiting melanin production. This compound was used as an ingredient in skin care products, and the product was registered in a U.S. patent in 2010 [21]. The previous work suggested that chemicals obtained from corn tassels are capable of creating innovative and novel functional food, pharmacy and cosmetic products.

The improvement of the phytochemical levels in tassels along with other traits may enhance profitability for growers, processors and related industries. However, success in crop improvement depends on efficient selection methods and genetic variation in breeding populations [22]. The information on the genotypic variability of bioactive phytochemicals in corn co-products from difference genetic resources is of great significance.

The objective of this study was to better understand the potential of purple waxy corn tassels as co-products for the extraction of phenolic compounds with an antioxidant activity. Therefore, we evaluated the genotypic variability in anthocyanins, phenolic compounds, and antioxidant activity in a set of tassels of waxy corn genotypes. This information allowed us to classify the waxy breeding germplasm into groups based on the chemical properties of the tassel. The information obtained in this study will guide further research into how best to process tassels in order to maximize the value of a purple waxy corn production system. It will also provide guidance to breeders wishing to optimize tassel composition for the production of anthocyanins, phenolic compounds and/or antioxidants.

2. Materials and Methods

2.1. Plant Materials and Experimental Design

Twenty-six waxy and fifteen small-ear waxy corn genotypes consisting of landraces and open-pollinated varieties were collected from different countries including Thailand, Laos, Myanmar, Vietnam, the Philippines, Taiwan, China, Korea and Japan. Forty-one genotypes in total were selected based on diverse genetic background, which was determined using 13 RAPD markers (Supplementary Figure S1). Six open-pollinated varieties consisting of KKU-KND, KKU-SLE, KKU-Tein composite, KKU-Tein white, KKU-Tein yellow and KKU-Tein bi-color, as well as three improved populations

including KKU-PFC1, KKU-PFC2 and KKU-PFC3 developed by the Vegetable Corn Improvement Project, in the Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen University (KKU) of Thailand, were also included in the study.

The fifty corn genotypes were evaluated in a randomized complete block design (RCBD) with three replications for two seasons in the rainy season (July–August 2017) and the dry season (December 2017–January 2018) at the Vegetable Research Station, KKU. The crop was planted in single-row plots with 5 m in length and a spacing of 80 cm between rows and 25 cm between plants within rows. The crop management was carried out according to the standard method for commercial corn production in Thailand, and irrigation was available for an optimum growth and yield.

2.2. Chemicals and Reagents

All chemicals and reagents were analytical grade. Methanol was purchased from LCI Labscan Co., Ltd., Bangkok, Thailand. Citric acid, gallic acid, potassium chloride and sodium acetate trihydrate were purchased from Ajax Finechem Pty Ltd., Taren Point, New South Wales, Australia. Folin-Ciocalteu reagent was purchased from Loba Chemie Pvt. Ltd., Mumbai, India. 2,2-diphenyl−1-picrylhydrazyl (DPPH), 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich Co., St. Louis, MO, USA.

2.3. Sample Preparation and Extraction

Fifteen uniform whole tassels at the first day of the pollen shed stage were harvested from each plot. This stage was the optimal time for the tassel harvest for the phytochemicals production according to Duangpapeng et al. [3]. The tassels were cut into small pieces, dipped in liquid nitrogen to stop enzymatic activity and freeze-dried using a freeze dryer (Gamma 2−16 LSCplus, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). The samples were ground into powder, sieved through a 40-mesh sieve and stored at −20 °C. The method for extraction was slightly modified from the method of Yang et al. [23]. Briefly, 10 mL of the acidified methanol solution [1% citric acid (CA) in 80% methanol (MeOH); extraction solution] was added to 0.5 g of ground tissue, mixed well and incubated at 4 °C for 24 h. Subsequently, the sample was centrifuged at 5000 rpm for 15 min. The supernatant was filtered through Whatman No.1 filter paper. The final volume was adjusted to 10 mL with extraction solvent and stored at −20 °C until the analysis.

2.4. Total Anthocyanin Content (TAC)

The pH differential method was used to measure the TAC [24]. The dilution was made in the samples in order to measure the TAC. Each appropriately diluted sample was divided and mixed with pH 1.0 or 4.5 buffer, incubated for 15 min under dark conditions, and absorbance was measured using a UV-vis spectrophotometer (GENESYS 10S, ThermoScientific, Waltham, MA, USA) at 510 and 700 nm wavelengths, respectively. The results were calculated with the following equation;

$$TAC = \frac{A \times MW \times DF \times 1000}{\varepsilon \times 1} \quad (1)$$

where A was the absorbance of the diluted sample, calculated from $A = (A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}$, MW was the molecular weight of cyanidin-3-glucoside (449.2 g mol^{-1}), DF was the dilution factor and 1000 was a conversion unit of molar to ppm and the molar absorptivity (ϵ) of 26,900 $M^{-1}cm^{-1}$. Anthocyanin levels were expressed as microgram cyanidin-3-glucoside equivalent per gram of dry weight (μg CGE g^{-1} DW).

2.5. Total Phenolic Content (TPC)

The TPC was measured using Folin-Ciocalteu (F-C) reagent with a minor modification of the method of Hu and Xu [25]. Briefly, 0.5 mL of 10× of the diluted sample with the extraction solvent, 2.5 mL of deionized water and 0.5 mL of 1 M F-C reagent were mixed. Then, 1.5 mL of a 7.5% Na_2CO_3 solution was added, mixed well and stored at room temperature for 2 h. The optical absorbance was measured at 765 nm with a UV-vis spectrophotometer (GENESYS 10S, ThermoScientific, Waltham, MA, USA). Gallic acid (GA) solutions (10–100 μg mL^{-1}) were used to make a standard curve for calibration. The TPC was expressed as milligram GA equivalent per gram dry weight (mg GAE g^{-1} DW).

2.6. Antioxidant Capacity by DPPH and TEAC Assay

The DPPH radical scavenging activity was measured using the method described by Hu and Xu [25] with a minor modification. The portion of 0.5 mL of the sample extract 10× diluted in the extraction solvent was mixed with 4.5 mL of the 60 μM DPPH radical solution in methanol. The reaction was stored in dark conditions for 30 min, and the absorbance was measured at 517 nm using a UV-vis spectrophotometer (GENESYS 10S, ThermoScientific, Waltham, MA, USA).

The Trolox equivalent antioxidant capacity (TEAC) was determined according to the method described by Re et al. [26]. The stock solution of ABTS radical cation was obtained by mixing 7 mM of ABTS and 2.45 mM $K_2S_2O_8$. The reaction was stored in the dark at room temperature for 16–24 h before use; the solution was used within 2 days. The fresh working solution was prepared by a diluted ABTS stock solution with methanol for an absorbance of 0.700 ± 0.05 and was measured at 734 nm. All the samples were diluted with methanol for 10× dilutions. Fifty microliters of the diluted sample and 1.9 mL of fresh ABTS radical cation solution were mixed. The reaction was set aside for 6 min at room temperature and the optical absorbance was measured at 734 nm with a UV-vis spectrophotometer (GENESYS 10S, ThermoScientific, Waltham, MA, USA).

Trolox solution (10–100 μM) was used as the reference compound in both the DPPH and TEAC methods. The results were expressed as micromole Trolox equivalent per gram of dry weight (μmol TE g^{-1} DW).

2.7. Statistical Analysis

An analysis of variance (ANOVA) was carried out on each trait in each season, and error variances were used for a homogeneity test [27]. The combined analysis of variances of two seasons was performed for all traits. The least significant difference (LSD) was used to compare the means at $p \leq 0.05$. The variance was partitioned into components taking the percentage of the sum of squares, which were calculated through the weighted of the variance in each component compared to the total variances. A two-way Ward's clustering analysis was performed using a matrix of data of all traits across fifty genotypes to construct the dendrogram. The calculations were done using JMP Pro software (version 13.0, SAS institute Inc., Chicago, IL, USA). A Pearson correlation analysis was to evaluate the relationships between the traits measured, using the Statistix10 software program (version 10.0, Analytical Software, Tallahassee, FL, USA).

3. Results and Discussion

3.1. Phytochemicals and Antioxidant Variation in Waxy Corn Germplasm

The effects of the season (S), genotype (G) and season by genotype (S × G) interaction were significant for all traits measured (Table 1). The S effect accounted for a rather small proportion (0.2–8.7%) of the total variance for all traits. Interestingly, the G effect explained nearly all of the variation in the TAC and DPPH radical scavenging activity (97.5% and 83.5%, respectively). The results showed that a high G variability, along with low S × G interactions of total variations, suggested that the anthocyanin accumulation and DPPH radical scavenging activity in waxy corn tassels were stable. Our results were in agreement with the results of other studies and in other crop species such

as the antioxidant activity in potatoes [28], the anthocyanin content and antiradical capacity in colored bran rice [29], and the anthocyanin content and antioxidant capacity in waxy corn kernel [11], corn cob and husk [30]. These data indicated that the selection of the best genotypes for high anthocyanin with antioxidant activity should be possible among germplasm populations. This information is very important for the special waxy corn breeding programs aiming to improve the anthocyanin and antioxidant capacity.

Table 1. Mean squares for the total anthocyanin content, total phenolic content and antioxidant capacity in the tassel of fifty waxy corn genotypes evaluated across two seasons.

Source of Variation	df	Phytochemicals	Antioxidant Capacity		
		TAC	TPC	DPPH	TEAC
Season (S)	1	404,390 ** (0.2) [a]	638.6 ** (8.7)	112.1 * (1.6)	2484.9 * (3.2)
Genotype (G)	49	3,864,741 ** (97.5)	63.4 ** (42.3)	121.5 ** (83.5)	946.9 ** (59.6)
S × G	49	82,648 ** (2.1)	65.8 ** (43.8)	20.2 ** (13.9)	568.7 ** (35.8)
Error	196	1519 (0.2)	1.8 (4.7)	0.1 (0.3)	2.7 (0.7)
C.V. (%)		10.98	3.98	1.97	1.66

df: degree of freedom, *C.V.*: coefficient of variation across all treatments, *TAC*: total anthocyanin content, *TPC*: total phenolic content, *DPPH*: 2,2-diphenyl−1-picrylhydrazyl radical scavenging activity, *TEAC*: Trolox equivalent antioxidant capacity. [a] The number in the parentheses is the percentage of the sum of the squares. *, ** significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

The variation in the TPC and TEAC was split more evenly between the genotype and S × G interaction. The G and S × G interaction were sources of variation effects in the TPC, accounting for 42.3 and 43.8% of the total variances, respectively. At the same time, the G and S × G interaction accounted for 59.6 and 35.8% of the variance in the TEAC, respectively (Table 1). These results indicated that the performance of corn genotypes in different environments was not stable. In a previous investigation, Žilić et al. [19] reported that corn pollen which is contained inside the tassel was sensitive to dehydration. Pollen grains had about 55–60% of moisture content, and the pollen was highly viable at a low temperature and high relative humidity [31]. Environmental factors greatly affected the physical structure and chemical composition of corn pollen [32]. Environmental change also affected the enzyme activities, leading to a change in the quantity of phenolic compounds. Therefore, the evaluation of corn genotypes in multi-location trials is essential for identifying and selecting the best genotypes for the TPC and TEAC.

Different values between the rainy and dry season were observed for all parameters. Although the season effect explained less than 10% of the total variation in any trait (Table 1), there was a consistent trend in which the samples produced in the dry season had higher levels of all the traits that were measured (Figure 1). Phenolic compounds including anthocyanins are natural metabolites in plants. They play an important role as a reducing agent and anti-radical activity. The plant phenolic concentration is often influenced by environmental stress such as the high or low temperature, high solar radiation and water stress [33].

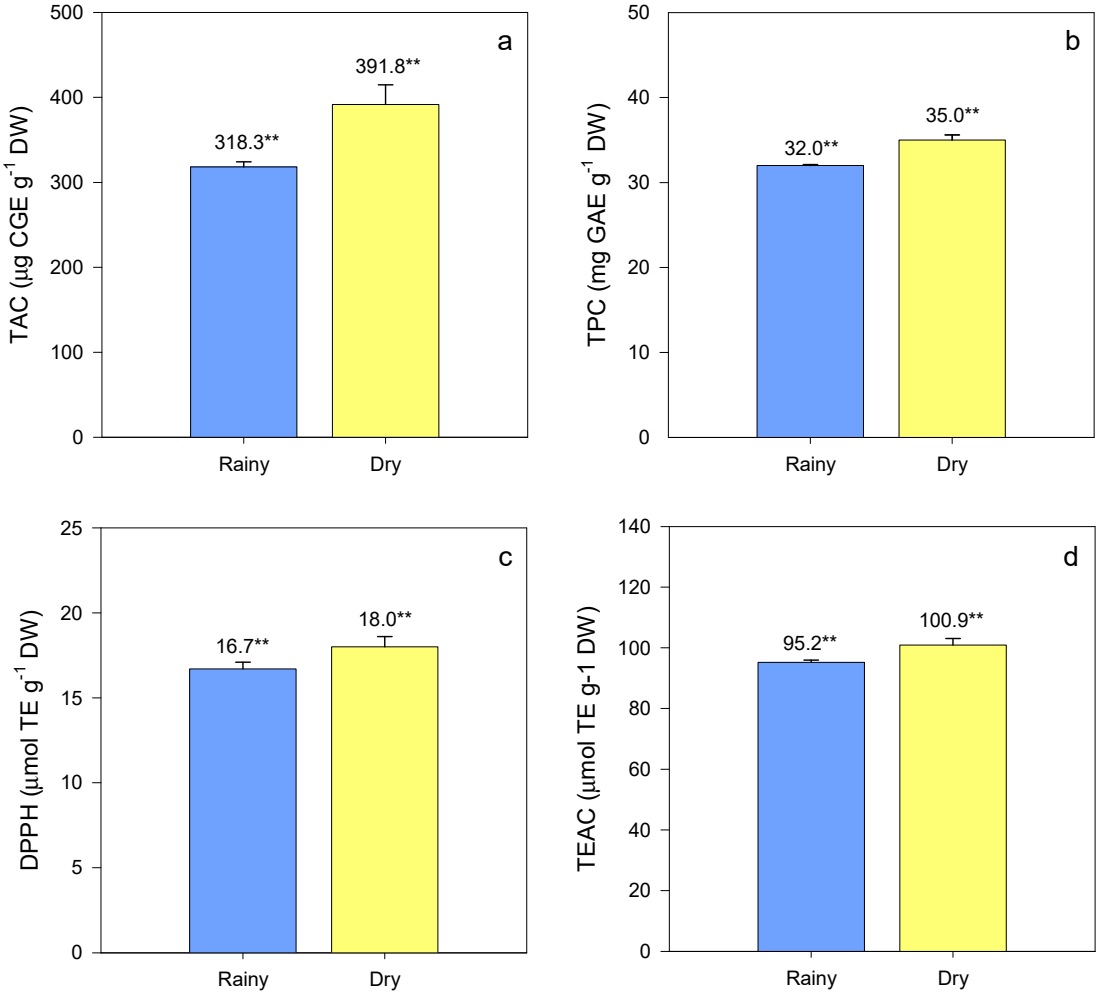

Figure 1. Means for (**a**) the total anthocyanin content (TAC), (**b**) total phenolic content (TPC), (**c**) DPPH radical scavenging activity (DPPH) and (**d**) Trolox equivalent antioxidant capacity (TEAC) in the tassel of waxy corn genotypes evaluated in the rainy season 2017 and dry season 2017/2018. ** significant difference at $p \leq 0.01$ by the least significant difference (LSD). Data are expressed as the mean ± SD.

In this study, the differential environment between seasons may be an important factor which affected the phenol accumulation in the tassels. The average temperature during the dry season was rather lower than for the rainy season. This factor might be the cause of the high TPC, TAC and antioxidant capacity. Khampas et al. [34] reported that a low temperature is a significant factor that affected anthocyanin accumulation in corn cobs. High solar radiation and low temperature induce anthocyanin synthesis and phenolic compounds accumulation in purple waxy corn ear and its components [35]. Therefore, it will be interesting to evaluate additional environments or years to determine what aspects of the production influence the levels of the compounds of interest.

3.2. Cluster Analysis

The average values of the individual genotypes across two seasons ranged from 27.6 to 4050.5 µg CGE g^{-1} DW, 26.1 to 39.6 µg GAE g^{-1} DW, 9.9 to 33.5 µmol TE g^{-1} DW and 66.4 to 124.8 µmol TE g^{-1} DW for TAC, TPC and the antioxidant capacity determined by the DPPH and TEAC methods, respectively (Supplementary Table S1). Based on the TAC, TPC and antioxidant capacity (DPPH and TEAC) in the tassels of waxy corn germplasm, genotypes were classified into seven distinct clusters (Figure 2).

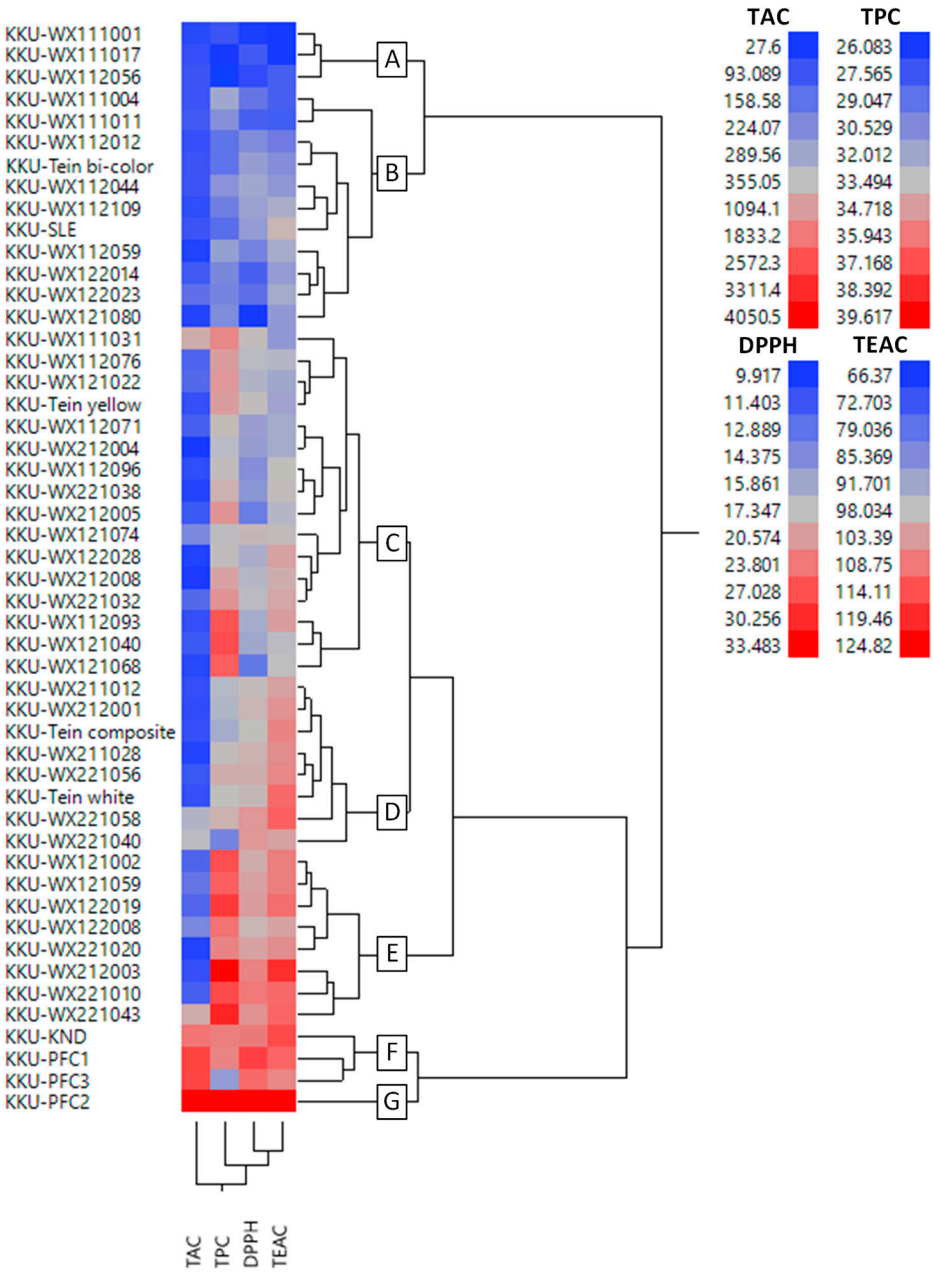

Figure 2. Seven clusters of the relationships based on the total anthocyanin content (TAC), total phenolic content (TPC) and antioxidant capacity determined by DPPH and TEAC assays in the tassel among 50 waxy corn genotypes. A two-way Ward's clustering analysis was used.

Cluster A consisted of 3 corn genotypes including KKU-WX111001, KKU-WX111017 and KKU-WX112056. This group had the lowest values of all of the parameters. Cluster B had 11 corn genotypes including KKU-WX111004, KKU-WX111011, KKU-WX112012, KKU-Tein bi-color, KKU-WX112044, KKU-WX112109, KKU-SLE, KKU-WX112059, KKU-WX122014, KKU-WX112023 and KKU-WX121080. This group showed low TAC values, but the values of the TPC and antioxidant capacity in this cluster were higher than those in cluster A.

Cluster C was the largest group and consisted of 16 corn genotypes with low TAC values, high TPC values and rather high levels for the antioxidant capacity. The genotypes in this cluster were KKU-WX111031, KKU-WX112076, KKU-WX121022, KKU-Tein yellow, KKU-WX112071, KKU-WX212004, KKU-WX112096, KKU-WX221038, KKU-WX212005, KKU-WX121074, KKU-WX122028, KKU-WX212008, KKU-WX221032, KKU-WX112093, KKU-WX121040, and

KKU-WX121068. The analysis within the group found that KKU-WX112093, KKU-WX121040 and KKU-WX121068 had high TPC values. KKU-WX111031 showed high TPC and rather high TAC values, whereas KKU-WX121068 and KKU-WX212005 had low DPPH values.

Cluster D consisted of 8 corn genotypes including KKU-WX211012, KKU-WX212001, KKU-Tein composite, KKU-WX211028, KKU-WX221056, KKU-Tein white, KKU-WX221058 and KKU-WX221040. Most of them showed low TAC values, and high TPC values with an antioxidant capacity.

Cluster E consisted of 8 corn genotypes including KKU-WX121002, KKU-WX121059, KKU-WX122019, KKU-WX122008, KKU-WX221020, KKU-WX212003, KKU-WX221010 and KKU-WX221043. This group showed low TAC values, high TPC values and levels of antioxidant capacity. KKU-WX212003 from the Lao People's Democratic Republic had the highest TPC values and TEAC.

Cluster F consisted of 3 corn genotypes including KKU-KND, KKU-PFC1 and KKU-PFC3. This group showed high TAC values, TPC values and high levels of antioxidant capacity. Cluster G had the highest values of all the parameters, and consisted of the KKU-PFC2 genotype only.

The descriptions of seven clusters based on TAC, TPC, DPPH and TEAC (Figure 2) are presented in Table 2. Cluster A was characterized by a low TAC, TPC, DPPH and TEAC, and corn genotypes in this group were not suitable for co-product production. Cluster B had relatively high antioxidant capacity although it had low anthocyanins and phenolic compounds. In contrast to cluster B, cluster C had a low TAC, but it had rather high TPC, DPPH and TEAC. The corn varieties in cluster C are interesting extractions of the phenolic compounds with an antioxidant capacity. The varieties in cluster D are also interesting because they were low in TAC, with relatively high TPC values, but the TEAC was high. The varieties in this group may contain novel antioxidant compounds that are not abundant in other varieties. The chemical compositions of these varieties should be further analyzed to determine the compounds that contributed to a high TEAC.

Table 2. Means across two seasons for the total anthocyanin content, total phenolic content and antioxidant capacity in the tassel of seven clusters of waxy corn genotypes.

Clusters	No. Genotype	Phytochemicals		Antioxidant Capacity	
		TAC (μg CGE g^{-1} DW)	TPC (mg GAE g^{-1} DW)	DPPH (μmol TE g^{-1} DW)	TEAC (μmol TE g^{-1} DW)
A	3	61.9 ± 19.0	28.4 ± 7.5	11.9 ± 3.0	75.3 ± 24.7
B	11	92.0 ± 36.2	29.8 ± 4.3	13.4 ± 2.7	85.9 ± 14.9
C	16	135.6 ± 179.4	34.8 ± 3.5	16.0 ± 2.2	96.8 ± 9.5
D	8	139.7 ± 161.5	33.0 ± 3.4	19.0 ± 2.8	106.3 ± 5.2
E	8	210.3 ± 238.8	37.4 ± 3.8	20.8 ± 2.2	109.3 ± 10.6
F	3	2,511.1 ± 180.7	34.2 ± 4.3	25.5 ± 3.6	110.9 ± 10.7
G	1	4,050.5 ± 180.7	39.6 ± 2.1	33.5 ± 3.2	124.8 ± 3.0

TAC: total anthocyanin content, *TPC*: total phenolic content, *DPPH*: 2,2-diphenyl−1-picrylhydrazyl radical scavenging activity, *TEAC*: Trolox equivalent antioxidant capacity. Data are expressed as the mean ± SD of three replicates across two seasons.

The waxy corn genotypes in clusters E, F and G are ideally suitable for the production of phytochemicals and antioxidants (Figure 3). The varieties in cluster E are excellent for the production of phenolic compounds and antioxidants because of a high TPC, DPPH and TEAC. The varieties in clusters F and G are suitable for the production of phytochemicals with an antioxidant capacity because of the high TAC, TPC, DPPH and TEAC.

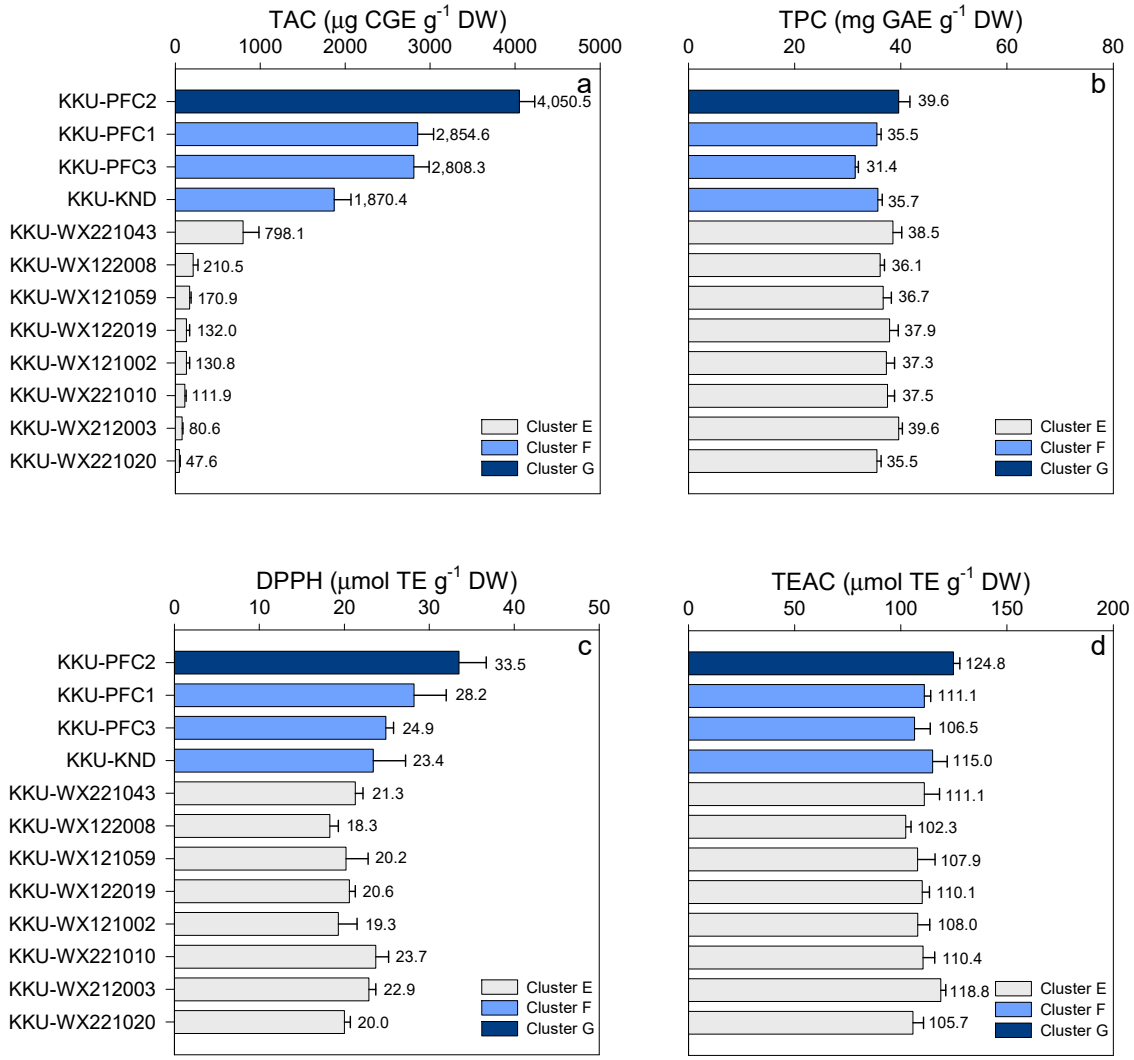

Figure 3. Means for (**a**) the total anthocyanin content, (**b**) total phenolic content, (**c**) DPPH radical scavenging activity and (**d**) Trolox equivalent antioxidant capacity in the tassel of the highest waxy corn genotypes (cluster E, F and G) evaluated across two seasons. Data are expressed as the mean ± SD.

Moreover, the improvement of phytochemical levels in tassels along with agronomic traits is an important way to enhance profitability to corn growers, the food processing industry and related industries. A germplasm evaluation can help with the conservation of crop genetic resources [36], classification of the accessions into heterotic groups [37] and crop improvement [38]. Therefore, the information from the analysis of the genetic diversity is of great value to breeders to generate base populations and to select parental lines for the development of hybrid varieties.

3.3. Pearson Correlation Analysis

The correlation coefficients among the four parameters are presented in Table 3. The TAC was moderately correlated with the DPPH radical scavenging activity (r = 0.70 **) but it had low correlations with the TPC and TEAC (r = 0.19 ** and 0.33 **, respectively). The TPC was significantly correlated with the DPPH and TEAC (r = 0.60 ** and 0.76 **, respectively). The relationship between the DPPH and TEAC was positive and significant (r = 0.73 **) in a set of waxy corn germplasm. The results showed moderate relationships between the phytochemicals and antioxidant capacity.

Table 3. Correlation coefficients (r) among the total anthocyanin content, total phenolic content and antioxidant capacity in the tassel of fifty waxy corn genotypes.

Parameters	TAC	TPC	DPPH
TPC	0.19 **		
DPPH	0.70 **	0.60 **	
TEAC	0.33 **	0.76 **	0.73 **

TAC: total anthocyanin content, *TPC*: total phenolic content, *DPPH*: 2,2-diphenyl−1-picrylhydrazyl radical scavenging activity, *TEAC*: Trolox equivalent antioxidant capacity. ** significant difference at $p \leq 0.01$.

The correlation coefficients between the phytochemicals and antioxidant capacity in this study were low to moderate. In previous studies, higher correlations between these parameters have been reported. The correlation coefficients among the total phenolic compounds and anthocyanin contents, and the antioxidant capacity were high and positive in the corn kernel [5,11,25], corn cob and husk [30]. The correlations among the parameters in this study indicated that an indirect selection for parameters in this set of waxy corn germplasm will be effective to some extent. Once the breeding objectives are identified, corn breeders can use the relationships among these parameters to design breeding strategies for improving the antioxidant activity in the tassels of waxy corn.

4. Conclusions

Wide variations in the total phenolic content, total anthocyanin content and antioxidant capacity were found in a set of waxy corn tassels. Based on the variations in phytochemicals and the antioxidant capacity, the genotypes in this study were classified into seven groups. Clusters E, F and G were most interesting for their high phytochemicals and antioxidant capacity. The total anthocyanin content was moderately correlated with the DPPH radical scavenging capacity, whereas the total phenolic content was moderately correlated with the antioxidant capacity. The information on genetic diversity from this study will help corn breeders to select the best genotypes for phytochemical production and collect the appropriate genotypes for the breeding programs focusing on high levels of phytochemicals and antioxidant capacity in the tassel.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/9/3/158/s1, Figure S1: Dendogram of genetic relationship among 41 corn genotypes were screened with 13 RAPD markers, Table S1: List of waxy corn genotypes, their origin and tassel pigmentation, and average total anthocyanin content, total phenolic content, and antioxidant capacity in the tassel were evaluated across two seasons.

Author Contributions: Conceptualization, P.D., K.L. (Kamol Lertrat) and B.S.; Formal analysis, P.D., M.P.S. and B.S.; Methodology, P.D., K.L. (Khomsorn Lomthaisong) and B.S.; Writing—Original draft, P.D. and B.S.; Writing—Review & editing, K.L. (Kamol Lertrat), K.L. (Khomsorn Lomthaisong) and M.P.S.

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