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Pigmentation and Genotype Effects, Phenotypic Stability for Anthocyanins, Phenolic Compounds and Antioxidant Activity in the Corn Tassel

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ABSTRACT

The tassels of corn can be utilized as a co-product for the production of phytochemicals. The objectives were to assess the impact of pigmentation and genotype on the levels of anthocyanins, phenolic compounds and antioxidant activity, and to determine the phenotypic stability of these traits. Sixteen genotypes were evaluated at two locations over two seasons. Corn genotypes in purple or pink pigmentation group had higher anthocyanin concentration and DPPH radical scavenging activity in tassel than those in normal green group. Tassel color can be used as a selection criterion to improve anthocyanin and antioxidant activity, but the trait is not effective to predict the phenolic concentration in the tassel. Genotype is an important source of variation for all parameters. The sensitivity of corn genotypes with high levels of measured compounds to the environment indicates the importance of choosing suitable locations and seasons for the production of high-quality corn tassels as a co-product of grain and vegetable corn. The findings of this study can be valuable for producers who intend to select genotypes for phytochemical production in corn tassels, as well as to corn breeders who aim to develop improved varieties with high yield and high bioactive phytochemicals in tassel.

INTRODUCTION

Corn (*Zea mays* L.) is a staple crop with the ability to grow in both tropical and temperate regions. Corn serves human needs as food, feed and fuel (Aakash et al., 2022). In traditional Chinese and Native American medicine, corn has been used as a medicinal plant to treat several ailments. All parts of the plant, including the kernel, cob, husk and silks contain phytochemicals with health benefit including anthocyanins, flavonoids and phenolic compounds (Simla, Boontang, & Harakotr, 2016). These compounds play a critical role as antioxidants.

The tassel is the male inflorescence. It produces pollen, the male gamete that fertilizes the female gamete to produce corn kernels. Aside from its role in pollination, corn tassel is considered normally not harvested, but it holds the potential to serve as a material for the production of phytochemicals in the related industry, especially the functional food, cosmetics, pharmaceuticals, and healthcare industries (Al-Khayri, Yüksel, A. K., Yüksel, M., Işık, & Dikici, 2022; Elsayed et al., 2022; Yaman, 2022). The reports indicate that the tassel serves as a source of phenolics with significant antioxidant properties (Mohsen & Ammar, 2009). *In vitro* studies

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have reported that the compounds extracted from corn tassels possess an inhibitory effect on the proliferation of gastric cancer cells (Wang et al., 2014). A purified natural compound extracted from sweet corn tassel called Tasselin A has been found to have significant potential in preventing melanin production, and is currently being utilized as an ingredient in skin care products for skin whitening purposes (Wille & Berhow, 2011). Moreover, corn pollen is widely recognized as a nutritious food due to its rich composition of nutrients (Žilić, Vančetović, Janković, & Maksimović, 2014). Bee-pollen in granules collected from the corn tassel has been used in commercially produce therapeutic products that claimed to prevent cell injury and promote health (Khider, Elbanna, Mahmoud, & Owayss, 2013; Pascoal, Rodrigues, Teixeira, Feás, & Estevinho, 2014).

Currently, improvement of grain yield together with bioactive phytochemicals in corn co-products such as in husk, cob and tassel represent the new trend in corn breeding. The genotypes with high grain yield and high bioactive phytochemicals are potentially used for dual purposes. Using corn tassel as a feedstock to extract natural compounds is an excellent approach to enhance the efficiency of corn production, as it helps to minimize agricultural waste while also generating value-added products. These bioactive phytochemicals can be used for creating novel valuable healthcare products. Conversion of agricultural waste into valuable products can generate additional income to corn growers by selling corn tassel as a co-product and also increase profits to related processing industries that use natural compounds as an ingredient in value-added product development. Producing value-added products from agricultural waste aligns with the sustainable development policy of the United Nations (Oleszek, M., Kowalska, Bertuzzi, & Oleszek, W., 2023).

For both breeding and production viewpoints, many factors affect phytochemical quantity, bioactivity and stability. Developmental stage, growing environment and genetic background are the significant factors affecting bioactive phytochemical accumulation (Gu, Wang, Hu, & Hao, 2019). Environment, genotype and genotype by environment interaction are of interest because they play a role in phytochemical accumulation and antioxidant activity such as carotenoids, tocopherols and antioxidant capacity in wheat flour (Lv et al., 2013), phenolics, carotenoids, tocopherols and antioxidant property in wheat bran (Lu et al., 2015). In addition, anthocyanin biosynthesis in grains of Thai black indigenous upland rice (Somsana, Pattanagul, Suriharn, & Sanitchon, 2013) and anthocyanin accumulation with antioxidant activity in purple corn cobs (Jing, Noriega, Schwartz, & Giusti, 2007; Khampas, Lertrat, Lomthaisong, Simla, & Suriharn, 2015) were strongly affected by environment, genotype and their interactions.

Apart from above factors, the pigmentation in corn tassel is also interesting. Pigmentation is related to several measures of phytochemical concentration in the tassel which is related to anthocyanin concentration (Duangpapeng et al., 2018). Anthocyanins are prominent pigments that give many plant tissues their blue, red, and purple coloration (Grotewold, 2006). In purple corn tassels, glume and anther tissues are the main corn floral components that store anthocyanin in the vacuoles (Irani & Grotewold, 2005). Tassel colors vary among corn genotypes, ranging from light yellow to dark purple and genotypes with different tassel pigmentation are also different in bioactive phytochemical content (Duangpapeng, Lertrat, Lomthaisong, Scott, & Suriharn, 2019). Corn genotypes with high levels of phytochemicals and wide adaptability across environments are desired for corn improvement and production. Because genotype by environment interactions strongly influence phytochemical levels, it is necessary to test corn breeding materials across a wide range of environments to identify the superior genotypes.

Enhanced comprehension of the impact of tassel pigmentation, environment, genotype, and genotype-environment interaction on the phenotypic stability of phytochemical levels and antioxidant activity facilitates breeders and producers in choosing the most suitable genotypes for both general and specific adaptation. Varieties with high levels of phytochemicals and good environmental stability are desirable for corn production with the tassel as a secondary product for extraction of useful phytochemicals. This study aimed to assess the impact of tassel pigmentation, genotype and environment on the concentration of anthocyanin, phenolic compounds and antioxidant capacity, and determine the phenotypic stability of these traits in corn tassel of elite hybrids in Thailand.

MATERIALS AND METHODS

Plant Materials, Study Sites and Experimental Design

The study utilized a total of fourteen commercially available hybrids in Thailand and two pre-commercial elite hybrid varieties from Khon Kaen

University (KKU). The hybrids are classified into three distinct groups based on visual evaluation of tassel pigmentation: green, pink and purple (Table 1). Sixteen genotypes were assessed using a Randomized Complete Block Design (RCBD) with three replications. The trials were performed under field conditions in two locations (Khon Kaen University and Uthai Thani province) over two seasons (the cold season of 2015/2016 and the rainy season of 2016). These two locations were selected because they are the hubs of vegetable corn production in the Northeast and in the Central Plain of Thailand, respectively.

The experimental plot consisted of four-row plots 5 meters long, with row spacing is 80 cm and a spacing of 25 cm plant to plant within a row. Standard commercial corn production practices used in Thailand were followed (Worrajinda, Lertrat, & Suriharn, 2013). An irrigation system was available at both locations in both seasons. A mini-sprinkler system was installed at Khon Kaen and the crop was planted on flat soil, whereas a furrow irrigation system was installed at Uthai Thani and the crop was planted on soil ridges.

Soil and Weather Data

The experimental fields at Khon Kaen location (N 16° 28' E 102° 49') had sandy loam soil and an altitude of 200 masl. The crop in the cold season was planted on December 4, 2015, and the crop in the rainy season was planted on June 22, 2016. The Uthai Thani location (N 15° 22' E 100° 1') had clay loam soil with an altitude of 20 masl. The crop in the cold season was planted on November 21, 2015, and the crop in the rainy season was planted on June 13, 2016.

Data on the weather was collected from the meteorological stations that were closest to each of the locations (Fig. 1). During the tasseling stage, the average daily temperatures in the four environments varied between 24.3 to 29.9ºC. The solar radiation ranged from 14.7 to 18.6 MJ/m^2/day, and relative humidity values ranged from 63.7 to 77.2%. The total rainfall varied from 10.8 to 530.7 mm over the course of the growing season. The Khon Kaen location in the cold season had the lowest average temperature, solar radiation and relative humidity followed by the Uthai Thani location in the cold season, the Khon Kaen location in the rainy season and the Uthai Thani location in the rainy season, respectively. Total rainfall during the tasseling stage at the Khon Kaen location in the cold season was 20.0 mm, whereas total rainfall at the Khon Kaen location during the tasseling stage in the rainy season was 91.9 mm. The Uthai Thani location in the cold season did not have rainfall, and it had rainfall of 116.3 mm during the tasseling stage in the rainy season. Because rainfall was not sufficient for optimal growth of the crop, irrigation was used to provide optimal growing conditions.

Table 1. Tassel pigmentation group, tassel characters and origins of sixteen corn genotypes used in the study

Pigmentation	Entries	Genotypes	Type	Glume color	Anther color	Origin
Green	G1	Sugar75	Sweet	Green	Yellow	Syngenta Seed Co., Ltd.
	G2	Hibrix3	Sweet	Green	Yellow	Pacific Seeds (Thai) Co., Ltd.
	G3	Jumbo sweet	Sweet	Light-green	Light-yellow	East West Seed Co., Ltd.
	G4	Top sweet	Sweet	Light-green	Light-yellow	Chia Tai Co., Ltd.
	G5	Freshy	Sweet	Green	Light-yellow	Advance Seed Co., Ltd.
	G6	White green	Waxy	Green	Yellow-pink	Chia Tai Co., Ltd.
	G7	Sweet violet	Waxy	Green	Light green	East West Seed Co., Ltd.
Pink	G8	PAC339	Field	Red-green	Yellow-pink	Pacific Seeds (Thai) Co., Ltd.
	G9	P4546	Field	Green	Pink	Pioneer Hi-Bred (Thailand) Co., Ltd.
	G10	P4554	Field	Pink-green	Pink	Pioneer Hi-Bred (Thailand) Co., Ltd.
	G11	S6248	Field	Red-green	Pink	Syngenta Seed Co., Ltd.
	G12	CP301	Field	Red-green	Pink	Charoen Pokphand Produce Co., Ltd.
	G13	Muang tam	Waxy	Red-green	Green-pink	Syngenta Seed Co., Ltd.
Purple	G14	Fancy111	Waxy	Purple-green	Purple	Pacific Seeds (Thai) Co., Ltd.
	G15	KGW1	Waxy	Purple-green	Purple	Khon Kaen University
	G16	KGW2	Waxy	Purple-green	Purple	Khon Kaen University

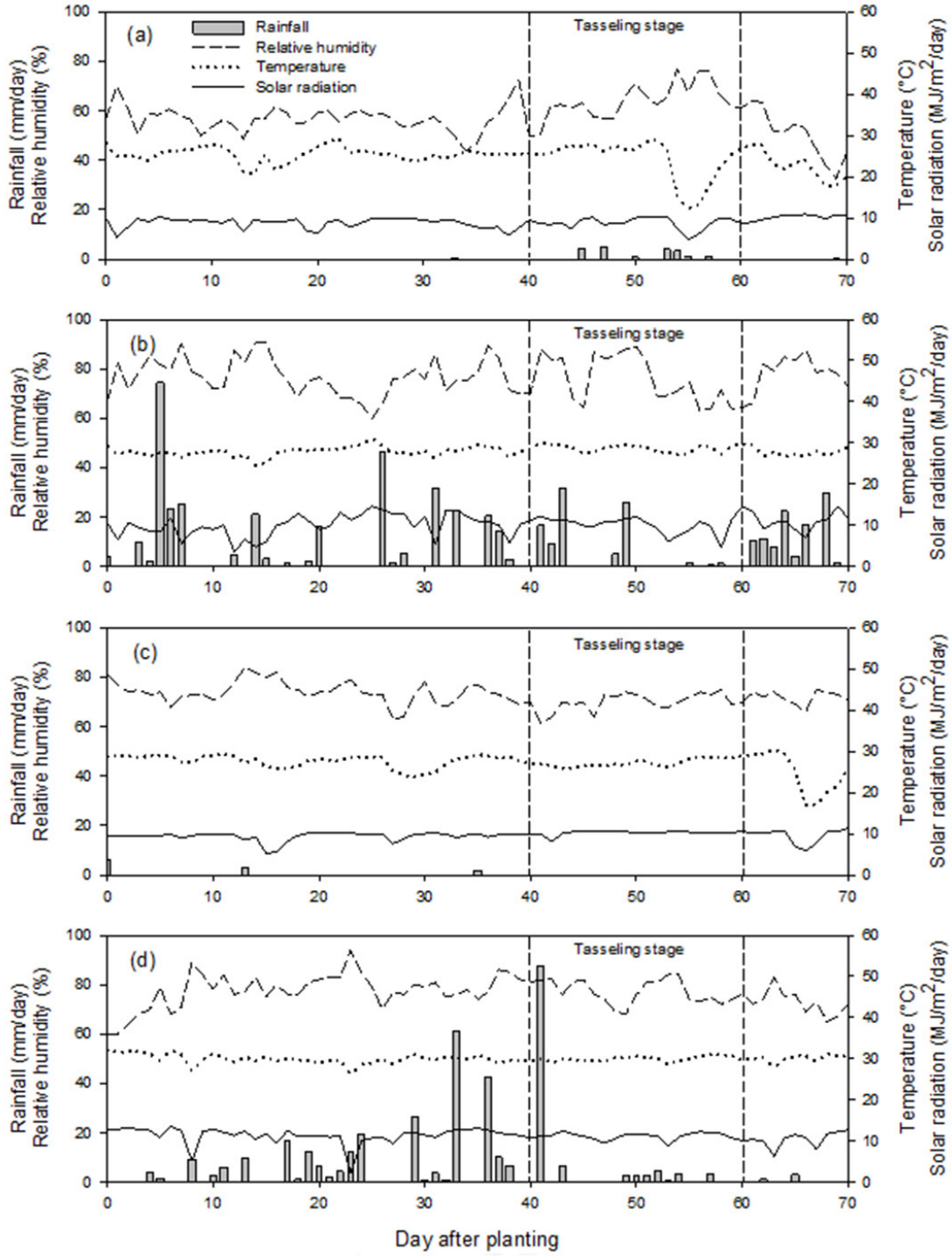


Fig. 1. Total rainfall, relative humidity, average temperature and solar radiation throughout the growth period of the crops across four different environments: Khon Kaen location in the cold season 2015/2016 (a), Khon Kaen location in the rainy season 2016 (b), Uthai Thani location in the cold season 2015/2016 (c), and Uthai Thani location in the rainy season 2016 (d)

Sample Preparation and Extraction

Sample preparation was carried out as previously reported (Duangpapeng et al., 2018). The tassel samples were fragmented into small pieces, rapidly immersed in liquid nitrogen, and subsequently freeze-dried. The samples were powdered, sieved and stored at a temperature of -20 ºC until they were ready for analysis. In this research, all reagents and chemicals used were of analytical grade. The sample extraction method was described previously (Yang, Fan, Gu, Han, & Chen, 2008), with slight modification. Briefly, 10 ml extraction solvent, a mixture of 80% (v/v) methanol and 1% (v/v) citric acid, was added to 0.5 g of the sample. The samples were thoroughly mixed and incubated for 24 hours at a temperature of 4ºC. Subsequently, they were centrifuged at 5,000 rounds per minute for 15 minutes. The supernatant was filtered, and the final volume was made up to 10 ml and stored at -20ºC for further analysis.

Measurement of Anthocyanin Concentration

The pH differential method was used to measure the total anthocyanin content (Giusti & Wrolstad, 2001). The samples were diluted as necessary, then separately mixed with pH 1.0 or 4.5 buffer and stored for 15 minutes in darkness. The absorbance at 510 and 700 nm was read using a UV-vis spectrophotometer. TAC was calculated from the equation (1):

$$TAC\ (mg/l) = (A \times MW \times DF \times 1{,}000) / (\varepsilon \times 1) \quad ....(1)$$

Where: *A* = the absorbance of the diluted sample calculated from $A = (A_{510}-A_{700})_{pH1.0} - (A_{510}-A_{700})_{pH4.5}$; *MW* = the molecular weight of cyanidin-3-glucoside (*MW* = 449.2); *DF* = the dilution factor; *1,000* = the conversion factor from gram to milligram; and ε = the molar absorptivity (ε = 26,900). The result was expressed as micrograms of c-3-g equivalents per gram of sample dried weight (μg CGE/g DW).

Measurement of Phenolic Compounds

The Folin-Ciocalteu phenol reagent method with slightly modified was used to measure the total phenolic content (Hu & Xu, 2011). In brief, 0.5 ml of extracted sample, 2.5 ml of deionized water, and 0.5 of 1 M Folin-Ciocalteu reagent were combined and stored for 8 minutes. Then, 1.5 ml of 7.5% Na_2CO_3 solution was added, thoroughly mixed, and the mixture was stored for 2 hours. A UV-visible spectrophotometer was used to read the absorbance at 765 nm. Gallic acid solutions (10-100 μg/ml) were utilized to create a reference curve for calibration. The result was expressed as milligrams of gallic acid equivalents per gram of sample dried weight (mg GAE/g DW).

Measurement of Antioxidant Activity

The analysis of DPPH radical scavenging activity was conducted based on the original method with minor modifications (Hu & Xu, 2011). In brief, 0.5 ml of the extracted sample was mixed with 4.5 ml of 60 μM DPPH radical solution in methanol. The sample was thoroughly mixed and stored for 30 minutes in dark conditions at room temperature. The absorbance was read at 517 nm using a UV-vis spectrophotometer.

The Trolox equivalent antioxidant capacity (TEAC) was measured according to a previous method with slight modifications (Hu & Xu, 2011). Five milliliters of 7 mM ABTS and 5 ml of 2.45 mM $K_2O_2S_8$ were mixed to prepare ABTS radical cation stock solution. The resulting mixture was incubated in darkness for 16-24 hours before use. The stock solution was diluted with methanol until the absorbance reached 0.70±0.05 at 734 nm to prepare the ABTS radical cation working solution. All extracted samples were diluted with an extraction solvent to 20-80% inhibition of blank absorbance. Fifty microliters of diluted sample were thoroughly mixed with 1.9 ml of fresh ABTS working solution. The reaction was left to incubate for 6 minutes, and absorbance was read at 734 nm using a UV-vis spectrophotometer.

A reference curve was established using varying concentrations (10-100 μM) of Trolox solution for both methods. The results are presented as micromoles of Trolox equivalents per gram of sample dried weight (μmol TE/g DW).

Statistical Analysis

One hundred and ninety-two datapoints for each trait were tested for normal distribution. The full linear mixed model with homogeneous variances was fitted in order to detect outliers. The outliers for each trait (three-, six-, seven- and eight-datapoints for TAC, TPC, DPPH and TEAC, respectively) were removed. The datasets with the outliers removed were subjected to a combined analysis of variance (ANOVA) across four environments using JMP Pro version 14 software (SAS Institute Inc., USA). For pigmentation group analysis, combined ANOVA was performed with the following model (2):

$$Y_{ij} = m + E_i + P_j + EP_{ij} + e_{ij} \quad \text{.....(2)}$$

Where: Y_{ij} = the observation data of pigmentation group j at environment i; m = grand mean value across environments; E_i = environment effect; P_j = pigmentation group effect; EP_{ij} = environment by pigmentation group interaction effects; and e_{ij} = pooled error.

Combined ANOVA for genotypic analysis was performed using the model (3):

$$Y_{ij} = m + E_i + G_j + EG_{ij} + e_{ij} \quad \text{.....(3)}$$

Where: Y_{ij} = the observed value of genotype j at environment i; m = grand mean value across environments; E_i = environment effect; G_j = genotype effect; EG_{ij} = environment by genotype interaction effects; and e_{ij} = pooled error.

Means for pigmentation groups and genotypes were compared by Student's T-test at $\alpha < 0.05$ level. Data are expressed in mean ± standard deviation (SD). SAS version 9.4 software (SAS Institute, USA) was used to perform principal component analysis and generate graphical images of Additive main effects and multiplicative interaction (AMMI) for exploring phenotypic stability.

RESULTS AND DISCUSSION

Effect of Pigmentation on Phytochemicals and Antioxidant Activity

Pigmentation groups showed significant variation ($P \leq 0.001$) for TAC, TPC and antioxidant activity (Table 2). The results suggested that the observed variation in tassel color was unlikely to be due to chance. The models used indicated had R^2 values that varied from 0.36 to 0.99. High R^2 values suggest that the model explained a large proportion of the observed variation, whereas low R^2 suggested that the model was explained less variation and more variation can be attributed to error.

Table 2. Average values for anthocyanin concentration, phenolic compounds, and antioxidant activity in tassels of different tassel pigmentation groups evaluated across four distinct environments

Pigmentation	Phytochemical			Antioxidant activity	
	TAC (μg CGE/g DW)	TPC (mg GAE/g DW)	TAC:TPC ratio (%)	DPPH (μmol TE/g DW)	TEAC (μmol TE/g DW)
Green	28.1±11.7 c	24.6±4.1 b	0.11	10.2±5.0 b	38.6±13.6 b
Pink	131.1±53.9 b	26.9±4.0 a	0.49	14.3±5.4 a	46.9±15.5 a
Purple	1,153.6±175.0 a	23.5±2.9 b	4.91	13.3±5.4 a	39.8±12.1 b
P-value					
Environment (E)	0.0032	<0.001		<0.001	<0.001
Pigmented (P)	<0.001	<0.001		<0.001	<0.001
E×P	0.3874	0.0112		0.0004	0.3136
Model R^2	0.99	0.36		0.78	0.68

Remarks: TAC = total anthocyanin content, TPC = total phenolic content, DPPH = 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, TEAC = Trolox equivalent antioxidant capacity. Data are presented as the mean ± standard deviation across environments. Means with the same letter(s) within the same column are not significantly different at α < 0.05 level

The group with purple tassels had the highest TAC (1,153.6 μg CGE/g DW) and it also had high DPPH radical scavenging activity (13.3 μmol TE/g DW). The group with pink tassels had the highest TPC (26.9 mg GAE/g DW) and antioxidant activity determined by both DPPH (14.3 μmol TE/g DW) and TEAC (46.9 μmol TE/g DW), whereas the group with green tassels had the low values of all parameters. The ratios of TAC:TPC were 0.11 in the green group, 0.49 in pink the group and 4.91 in the purple group (Table 2). However, the group with green tassel was not significant different from the group with purple tassel for TPC and TEAC. The results indicated that the group with purple tassel and the group with pink tassel had higher anthocyanin concentration and DPPH radical scavenging activity than the group with green tassel. Therefore, tassel color can be used as a selection criterion to improve anthocyanin with DPPH radical scavenging activity. In contrast to TPC and TEAC, TAC and DPPH were not effective for predicting the values of phenolic compounds and TEAC because TAC represents a small portion of the total phenolic concentration, and TPC and TEAC are not correlated with TAC.

In addition, the interaction between environment and pigmentation was significant for TPC and DPPH assay. Corn genotypes in the groups with pigmented tassel responded differently to environments for TPC and DPPH. The results indicated that pigment is not the primary origin of bioactive phytochemicals. It might be viewed as a secondary source of phenolics that accumulate in the tassel. Phenolics are the natural compounds accumulated in plant tissues, and they have a significant role in bioactivities. TPC from the whole corn tassel was significantly correlated with DPPH and TEAC, indicating its important role in antioxidant activity (Duangpapeng, Lertrat, Lomthaisong, Scott, & Suriharn, 2019). The phytochemicals other than phenolics may present in the pollen and play a role in antioxidant activity. The yellow pollen is also a source of volatile oil lipids and quercetin flavonoid pigments, which contribute to antioxidant activities (Žilić, Vančetović, Janković, & Maksimović, 2014). For better understanding on the roles of phytochemicals in antioxidant activity, further investigations on compound purification and evaluation of the role of phytochemicals in antioxidant activity in all types of pigmented tassel are required.

Effect of Genotype on Phytochemicals and Antioxidant Activity

Corn varieties showed significant variation ($P \leq 0.001$) for all traits (Table 3). Variation in environments and genotype by environment interactions also varied significantly ($P \leq 0.001$) for all parameters. The model explained a high percentage of the observed variation in all traits ($R^2 \geq 0.95$).

The TAC ranging from 21.0 to 1,195.2 μg CGE/g DW was observed among corn genotypes. KGW1, KGW2 and Fancy111 in purple group had the highest TAC. The TPC among corn varieties ranged from 17.9 to 30.5 mg GAE/g DW. P4546 exhibited the highest TPC value followed by Hibrix3, PAC339 and CP301, respectively. The ratios of TAC:TPC ranged from 0.09 to 5.38% (Table 3). The results suggest that anthocyanins were not the main phenolic compounds found in the tassel. Identification of non-anthocyanin phenolic compounds and assessment of their function is still required.

The values of antioxidant activity among genotypes varied from 9.0 to 18.2 μmol TE/g DW for DPPH assay and 30.6 to 54.9 μmol TE/g DW for TEAC assay. CP301 had the highest antioxidant activity determined by DPPH assay. PAC339 ranked in the second place followed by P4546, Fancy111, S6248 and KGW2, respectively. Similarly, the TEAC assay showed that P4546, PAC339, and CP301 exhibited the highest antioxidant activity (Table 3). The methods DPPH and TEAC rely on the concept of free radical scavenging, and are commonly employed to screen the antioxidant activity of plant extracts. Polyphenols are well-known as the excellent scavengers of radicals in plant tissues (Šamec, Karalija, Šola, Vujčić Bok, & Salopek-Sondi, 2021). These compounds are crucial for plant defense mechanisms as they can improve tolerance to external biotic and abiotic stresses (Shah & Smith, 2020). In this study, the antioxidant capacities of the extracted compounds from the tassel varied depending on corn genotypes. The corn genotypes with high levels of antioxidant activity were from field corn and waxy corn with pigmented tassel. High antioxidant activity in field corn might be responsible for tolerance to environmental stress. High grain yield and tolerance to environmental stress such as drought and high temperature have been the main objectives of field

corn breeding, while the main objective of vegetable corn breeding has been to improve fresh ear yield and ear quality. Therefore, the observed variations in all traits studied in this research enable corn breeders to choose the most suitable genotypes for incorporation into corn improvement programs and for the production of bioactive phytochemicals.

Phenotypic Stability Analysis for Anthocyanins and Phenolic compounds

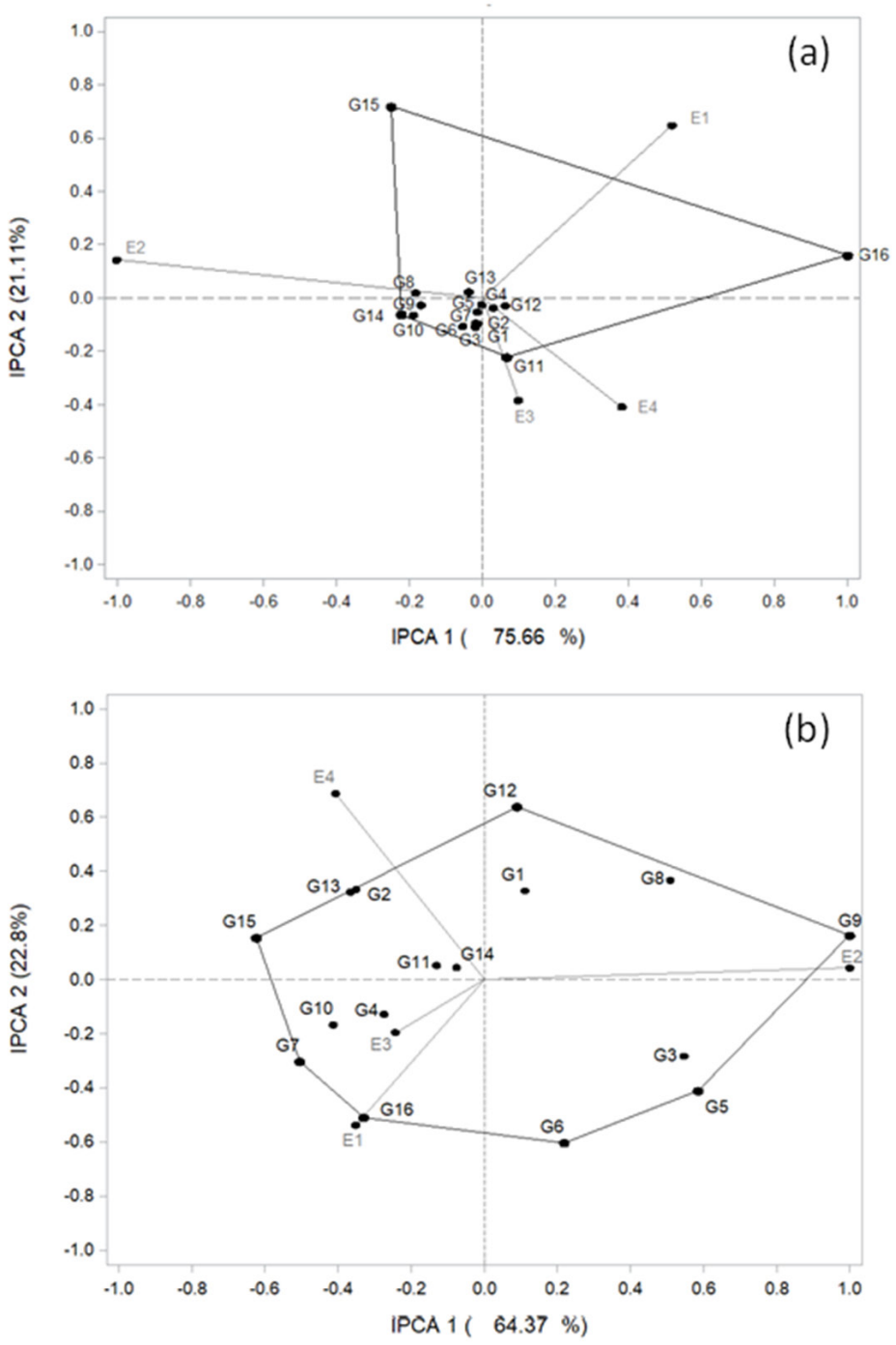
The assessment of corn genotypes across multiple environments facilitates the identification of genotypes with superior characteristics that are adaptable to a broad range of environments, as well as genotypes that are well-suited to specific environments. Phenotypic stability across environments is a valuable characteristic that corn breeders consider when selecting the best varieties. However, the breeding goal should include the selection of corn genotypes that possess broad adaptability, high phytochemical concentration, and substantial antioxidant activity in the tassel.

The polygon view of AMMI biplot analysis to assess the phenotypic stability for TAC presents in Fig. 2a. The genotypes located near the x-axis (IPCA2) and close to the point of origin are considered to be highly stable. On the other hand, the genotypes that are a long distance from the point of origin are considered to be highly sensitive to environmental variation. Among the genotypes with high anthocyanin concentration, Fancy111 (G14) was more stable than KGW1 and KGW2 (G15 and G16). KGW1 was well adapted to E1 and E2 (Khon Kaen location in the cold season and rainy season, respectively), whereas KGW2 was well adapted to E1 and E4 (Khon Kaen location in the cold season and Uthai Thani location in the rainy season).

Table 3. Average values for anthocyanin concentration, phenolic compounds, and antioxidant activity in tassels of sixteen genotypes evaluated across four distinct environments

Genotype	Phytochemical			Antioxidant activity	
	TAC (μg CGE/g DW)	TPC (mg GAE/g DW)	TAC:TPC ratio (%)	DPPH (μmol TE/g DW)	TEAC (μmol TE/g DW)
Sugar75	21.0±5.1 f	24.0±2.6 gh	0.09	11.1±4.2 fg	41.3±15.4 de
Hibrix3	25.4±6.3 f	28.7±3.2 b	0.09	11.6±7.3 ef	43.1±12.6 cd
Jumbo sweet	29.4±9.2 f	27.5±1.5 cd	0.11	9.4±5.5 i	42.5±13.7 d
Top sweet	31.9±19.0 f	24.5±2.4 fg	0.13	9.3±5.7 i	34.9±13.3 f
Freshy	29.5±16.8 f	26.1±1.7 e	0.11	10.4±5.0 h	43.2±17.9 cd
White green	29.3±8.5 f	21.0±1.2 k	0.14	9.1±5.9 i	34.7±13.0 f
Sweet violet	30.1±12.8 f	17.9±2.8 l	0.17	10.6±5.2 gh	30.6±13.2 g
PAC339	145.1±49.1 d	28.4±2.9 bc	0.51	16.5±4.9 b	54.2±13.5 a
P4546	145.9±44.2 d	30.5±3.7 a	0.48	14.1±5.0 c	54.9±16.2 a
P4554	138.5±47.6 d	25.2±2.6 ef	0.55	12.2±2.9 de	46.5±10.1 b
S6248	86.5±28.9 e	27.0±2.1 d	0.32	13.9±6.7 c	42.7±17.4 d
CP301	192.4±44.2 c	28.0±3.3 bc	0.69	18.2±4.3 a	52.0±15.2 a
Muang tam	78.5±32.5 e	21.7±3.2 jk	0.36	9.0±6.1 i	31.3±13.4 g
Fancy111	1,192.2±50.5 a	24.5±2.0 fg	4.87	14.0±7.5 c	35.1±11.8 f
KGW1	1,032.9±157.3 b	23.1±3.9 hi	4.47	12.3±5.3 d	45.6±12.9 bc
KGW2	1,195.2±252.3 a	22.2±2.4 ij	5.38	13.7±5.1 c	38.7±12.2 e
P-value					
Environment (E)	<0.001	<0.001		<0.001	<0.001
Genotype (G)	<0.001	<0.001		<0.001	<0.001
E×G	<0.001	<0.001		<0.001	<0.001
Model R^2	0.99	0.95		0.98	0.97

Remarks: TAC = total anthocyanin content, TPC = total phenolic content, DPPH = 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, TEAC = Trolox equivalent antioxidant capacity. Data are presented as the mean ± standard deviation across environments. Means with the same letter(s) within the same column are not significantly different at α<0.05 level



Remarks: E1 = Khon Kaen location in the cold season 2015/2016, E2 = Khon Kaen location in the rainy season 2016, E3 = Uthai Thani location in the cold season 2015/2016, E4 = Uthai Thani location in the rainy season 2016

Fig. 2. AMMI graphical of genotypic main effect plus environment by genotype interactions effect: anthocyanin concentration (a) and phenolic compounds in the tassel of sixteen genotypes (G1 to G16) evaluated across four environments (b)

Environment represents a significant factor affecting biosynthesis of anthocyanins in the tassel. Solar radiation and temperature are known to be major environmental factors affecting anthocyanin accumulation in many crops (Gu, Wang, Hu, & Hao, 2019). Solar radiation induces biosynthesis of anthocyanins in corn tassel by activating the *pl* gene when the floral tissue is exposed to light. The *pl* alleles are the key alleles involved in synthesis of anthocyanins in corn floral tissues. They are classified into two types including dominant (*Pl*) and recessive (*pl*) alleles. The dominant *Pl* allele leads to light-independent pigmentation, while the recessive *pl* allele, also called sun-red allele, leads to light-dependent pigmentation. Therefore, the floral tissue coloration caused by sun-red allele requires a direct light-induced procedure (Cone, Cocciolone, Burr, F. A., & Burr, B., 1993; Cone et al., 1993).

In this study, the Khon Kaen location in the cold season (E1) represented a favorable environment for anthocyanin production in the varieties KGW1 and KGW2 (Fig. 2a). The low temperature observed during tassel emergence at this site (Fig. 1) may have stimulated the accumulation of anthocyanin in the tassel. These findings are consistent with the mechanism of anthocyanin biosynthesis observed in apple fruit peels (Xie et al., 2012) and red-skinned grapes (Gao-Takai et al., 2019). The authors suggest that anthocyanin storage in plant cells is sensitive to temperature. Low temperature induced anthocyanin pigment biosynthesis in the vacuoles of plant cells, whereas high temperature resulted in degradation of anthocyanin pigments (Gu, Wang, Hu, & Hao, 2019; Kim et al., 2017). However, combinations of the factors, particularly solar radiation and temperature can promote anthocyanin pigment and polyphenol accumulation. Jaakola & Hohtola (2010) suggested that the intensity of solar radiation, photoperiod and temperature are the key environmental factors that interact with genotype to influence anthocyanin biosynthesis in plants.

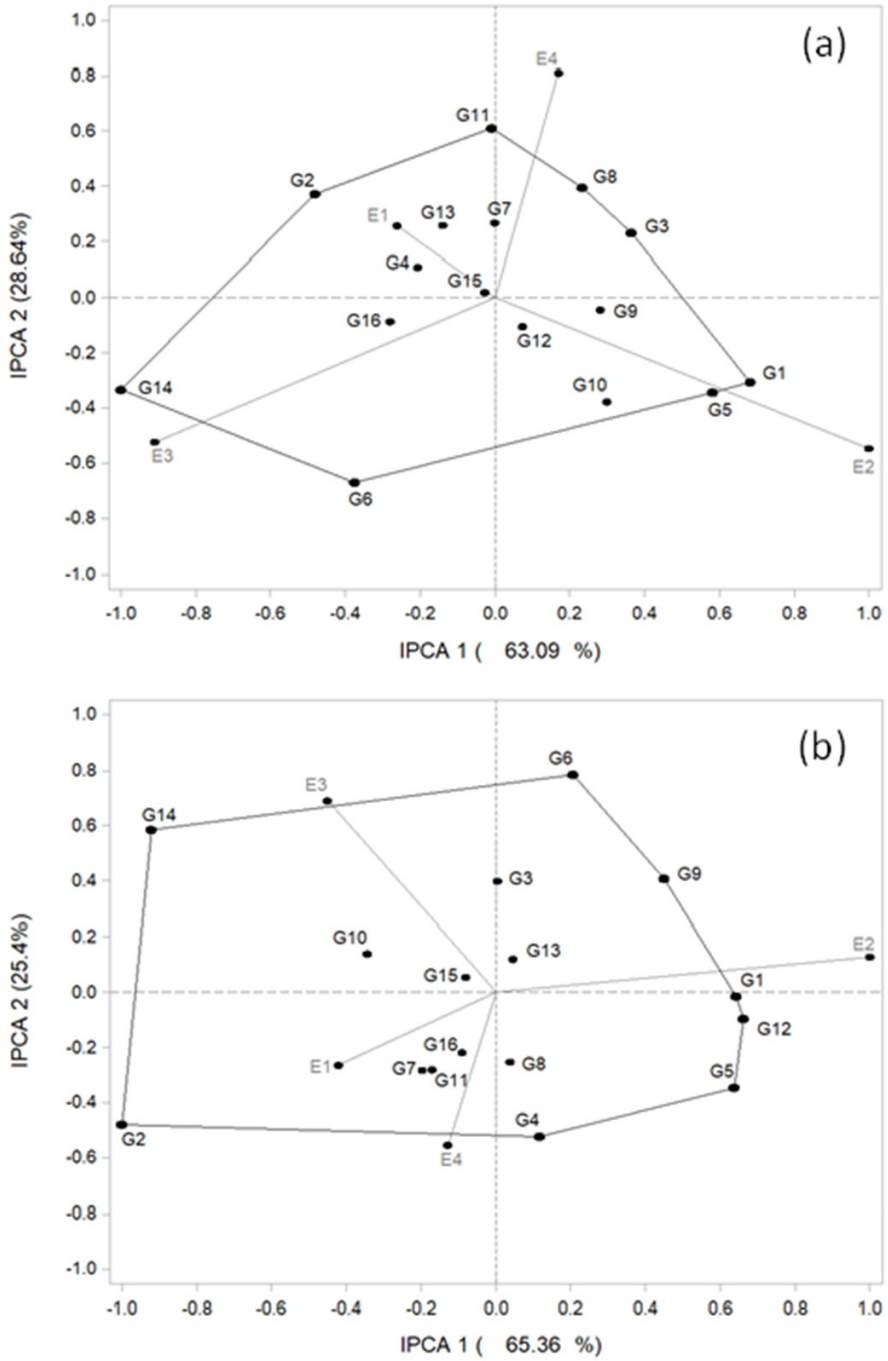
The stability and adaptability of corn genotypes for TPC are shown in Fig. 2b. S6248 and Fancy111 (G11 and G14) had moderate TPC and were located near IPCA2 axis and close to the point of origin, indicating they had highest stability. P4546 (G9) had the highest TPC but had unstable performance, it specific adapted to E2. However, Hibrix3 and CP301 (G2 and G12), which had TPC lower than P4546, showed good adaptation to E4.

Phenotypic Stability Analysis for Antioxidant Activity

Corn genotypes displayed a comparable performance trend for antioxidant activity, as evaluated by both the DPPH and TEAC assays. Hibrix3, White green and Fancy111 (G2, G6 and G14, respectively) are located far from the point of origin, suggesting that their antioxidant activity was not stable across environments. In contrast to these genotypes, KGW1 (G15) with moderate antioxidant activity was more stable for antioxidant activity. In the high group, P4546 and CP301 were more stable than PAC339 (G8) for DPPH radical scavenging activity (Fig. 3a), whereas a high and stable variety for antioxidant activity determined by TEAC could not be identified (Fig. 3b).

In previous investigations, purple corn genotypes with high anthocyanins and antioxidant activity in cobs were sensitive to environments (Khampas, Lertrat, Lomthaisong, Simla, & Suriharn, 2015). Similarly, wheat genotypes had high phenolic compounds with antioxidant properties in bran were also sensitive to environments. The genotypes with the highest levels of these compounds did not perform well in all environments. These genotypes had specific adaptation to favorable environments (Lu et al., 2015).

In this study, corn genotypes that had high levels of phenolics and antioxidant capacity were found to be more susceptible to environmental variations compared to the genotypes that showed intermediate performance for these traits. The presence of phenolic compounds in pollen grains is the primary factor contributing to their antioxidant activity in the tassel (Žilić, Vančetović, Janković, & Maksimović, 2014). Weather parameters especially relative humidity and average temperature during the tasseling period are external environmental factors affecting biosynthesis of these phytochemicals. Changes in relative humidity and temperature are directly related to corn pollen viability because it contains about 60% water. Pollen longevity decreased at high air temperature and low air humidity (Aylor, 2003; Fonseca, & Westgate, 2005). Moreover, rapid environmental change affected physical and chemical properties of pollen grains. Change in environmental conditions can lead to the increase in cellular leakage, decrease enzymatic activity and change in physiological properties (van Bilsen, Hoekstra, Crowe, L., & Crowe, J., 1994).



Remarks: E1 = Khon Kaen location in the cold season 2015/2016, E2 = Khon Kaen location in the rainy season 2016, E3 = Uthai Thani location in the cold season 2015/2016, E4 = Uthai Thani location in the rainy season 2016

Fig. 3. AMMI graphical of genotypic main effect plus environment by genotype interactions effect on antioxidant activity: DPPH radical scavenging activity (a) and Trolox equivalent antioxidant capacity in the tassel of sixteen genotypes (G1 to G16) evaluated across four environments (b)

To optimize the production of these phytochemicals, it will be important to understand what aspects of the environment have the greatest impact on their production and choose production locations most likely to have favorable conditions. In the absence of a better understanding of environmental control of these traits, the value of environmental stability will need to be weighted versus the potential for greater productivity that accompanies low environmental stability. Therefore, identification of corn genotypes with specific adaptation and broad adaptation is a valuable activity.

CONCLUSION

Visually identified pigmentation class (purple, pink or green) and genotype were the main sources of variations for bioactive phytochemicals in the corn tassel. Visual selection for purple tassel color can be used for improving anthocyanin concentration and antioxidant activity. Genotypes with higher bioactive phytochemical compounds were more sensitive to environments than the genotypes with moderate values. Selection of superior genotypes for specific environments is important for production of anthocyanin and phenolic compounds with antioxidant properties in corn tassel. This study provides valuable information that can assist corn breeders in designing experiments or breeding programs for the selection of the best varieties.

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