RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
52 (1) 30-44, 2020

QUANTITATIVE INHERITANCE OF TOTAL ANTHOCYANIN CONTENT IN THE TASSEL OF SMALL-EAR WAXY CORN (*Zea mays* var. *ceratina*)

P. DUANGPAPENG[1], K. LERTRAT[2], K. LOMTHAISONG[3], F.S. AGUILAR[4], M.P. SCOTT[5], and B. SURIHARN[1,2*]

[1]Department of Agronomy, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand
[2]Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand
[3]Department of Biochemistry, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand
[4]Department of Agronomy, Iowa State University, Ames, IA 50011, USA
[5]USDA-ARS Corn Insects and Crop Genetics Research Unit, Iowa State University, Ames, IA 50011, USA
Corresponding author email: sphala@kku.ac.th
Email addresses of co-authors: prakasitkku@gmail.com, kamol9@gmail.com, kholom@kku.ac.th, fsilvaag@iastate.edu, paul.scott@usda.gov

SUMMARY

Information on the gene effects governing the inheritance of traits is very important for crop breeding and germplasm management. The objective of this study was to estimate genetic effects for total anthocyanin content in the tassel of small-ear waxy corn. Six generations (P_1, P_2, F_1, F_2, BC_{11}, and BC_{12}) were developed from two crosses of small-ear waxy corn (TB1 × TW1 and TC1 × TB3). TB1 and TB3 had purple tassels, and TW1 and TC1 had green tassels. All generations of each cross were evaluated under field conditions in the rainy season of 2017 and the dry season of 2017/2018. Season, generation, and their (S × G) interaction had significant effects in TB1 × TW1 and TC1 × TB3. For both crosses, generation had the largest effect on the total variance followed by season and S × G interaction. A three-parameter model was adequate for estimating genetic effects. Additive gene effects were significant for controlling the inheritance of total anthocyanin concentration in tassels. The significant additive effect suggested that selection for anthocyanin content in early generations will be effective, and hybrid combinations obtained from superior parental lines with unrelated genetic backgrounds may be an effective option to increase anthocyanin concentration in the tassels of small-ear waxy corn.

Keywords: *Zea mays* L., floral corn, phytochemicals, generation mean analysis, three-parameter model

Key findings: Additive gene effects were significant in controlling anthocyanin concentration in the tassel. Corn tassels with high anthocyanin concentration can be

developed by creating a large population of waxy corn germplasm and improving this germplasm through phenotypic mass selection, recurrent selection, or pedigree selection for tassels with high anthocyanin concentration. Superior lines can be improved from these improved populations to develop hybrid combinations.

Manuscript received: December 20, 2019; Decision on manuscript: January 15, 2020; Accepted: January 28, 2020.

Communicating Editor: Dr. Samrin Gul

INTRODUCTION

Anthocyanins are a class of phenolic phytochemicals that are naturally occurring and synthesized via the flavonoid pathway in plant tissues. These compounds are pigments with a range of red, orange, purple, and blue colors depending on pH values and chemical structure. Anthocyanin is found in many food sources, such as fruits and vegetables, which are well-known as rich sources of phytochemicals and antioxidant agents (Tsuda, 2012; Zhu, 2018).

Purple corn and its coproducts including cobs, silk, and husks, have been reported to be a good source of anthocyanins (Simla *et al*., 2016; Nawaz *et al*., 2018). Anthocyanins extracted from corn coproducts have many health benefits, such as reducing the risk of cancer (Jing *et al*., 2008; Long *et al*., 2013; Thiraphatthanavong *et al*., 2014; Mazewski *et al*., 2017; Intuyod *et al*., 2018), regulating blood pressure (Finkel *et al*., 2013), protecting against neuronal cell death, enhancing memory (Kirisattayakul *et al*., 2017), and controlling obesity (Chaiittianan *et al*., 2017).

The tassel is the male flower of corn, and after pollination it is normally not harvested in corn grain production systems. Anthocyanins accumulate in the vacuoles of tassel cells (Irani and Grotewold, 2005) in some varieties, causing the coloration of tissues. Anthocyanins in waxy and small-ear waxy corn tassels are purple and exhibit high genetic variability (Duangpapeng *et al*., 2019). Purple waxy corn tassels have high levels of anthocyanins and antioxidants and have therefore been proposed to be used as low-cost raw material for agroindustrial applications (Duangpapeng *et al*., 2018). Moreover, the compounds extracted from ground corn tassel have the potential to be used as an ingredient in dietary supplement products (Mohsen and Ammar, 2009; Žilić *et al*., 2014). A purified compound called "Tasselin A" plays a vital role in inhibiting melanin production. This compound is used in skin-whitening products (Wille and Berhow, 2011). Given that the polysaccharide, saponin, and flavonoid compounds extracted from dry corn tassel inhibit gastric cancer cells *in vitro*, they may have potential uses as ingredients for natural medicinal compound development (Wang *et al*., 2014).

The landraces and open-pollinated varieties of small-ear waxy corn have been cultivated in many countries in East and Southeast Asia. A high number of ears per plant, good eating quality, broad adaptability, disease resistance, and high uniformity are important characters for small-ear waxy corn breeding (Lertrat and Thongnarin, 2008;

Kesornkeaw *et al*., 2009). In addition to agronomic traits, pigmented tassels and other coproducts may be useful for adding value to new varieties. The genetic enhancement of tassel pigmentation through conventional phenotypic selection may be an effective method to enhance anthocyanin levels in tassel. A better understanding of the role of gene effects on anthocyanin accumulation in the tassel is important for the success of genetic improvement.

Generation mean analysis has been used to estimate the effects of genes on polygenic traits, such as yield (Rahman and Saad, 2000; Rashwan, 2010; Rao *et al*., 2017), quality traits (Ramli *et al*., 2016; Srinivas and Bhadru, 2015), biotic and abiotic stress (Carson, 2001; Naveed *et al*., 2009; Kere *et al*., 2013), and morphophysiological characters (Sharma *et al*., 2013; Cao *et al*., 2016; Uzokwe *et al*., 2017; Sun *et al*., 2019) in many crops. It can be used to determine the relative contributions of additive, dominant, and epistatic gene effects (Mather and Jinks, 1982) to the expression of a trait.

To the best of our knowledge, no report on gene effects on anthocyanin concentration in corn tassel is available in the literature. Therefore, the objective of the present study was to determine the gene effects controlling anthocyanin concentration in the tassel in two crosses of small-ear waxy corn. The information obtained here will facilitate planning and guide corn breeders seeking to develop small-ear waxy corn with increased levels of anthocyanins in the tassel.

MATERIALS AND METHODS

Plant materials and experimental design

Four inbred lines of small-ear waxy corn were developed through five/six generations of inbreeding. The inbred lines TB1, TB3, TC1, and TW1 were used to generate six populations derived from each of the two crosses (Table 1). TB1 and TB3 are sister lines with high anthocyanin content in their tassels, and TC1 and TW1 are agronomically superior lines with low anthocyanin content. Two F_1-hybrids were generated from the cross between TB1 (P_1, high anthocyanin levels) and TW1 (P_2, low anthocyanin levels) and the cross between TC1 (P_1, low anthocyanin levels) and TB3 (P_2, high anthocyanin levels) in the rainy season of 2016. These F_1 hybrids were then self-pollinated to generate F_2 populations, and individuals in the F_1 populations were back-crossed to both parental lines to generate backcross generations (BC_{11} and BC_{12}) in the dry season of 2016/2017.

Six generations (P_1, P_2, F_1, F_2, BC_{11}, and BC_{12}) from each cross were evaluated in a randomized complete block design with four replications for two seasons in the 2017 rainy season 2017 and 2017/2018 dry season at the Vegetable Research Station, Khon Kaen University, Thailand. The data for solar radiation, temperature, relative humidity, and rainfall of two seasons are presented in Figure 1.

Plot sizes were varied in accordance with the segregation levels of the generations as follows. Single-row plots were used for parental lines

Table 1. Descriptions of the tassels of small-ear waxy corn inbred lines.

Cross	Parental lines	Pedigree	Phenotype
1	TB1 (P_1)	[(TB × KND-S_1) × TLK-F_1]-1-S_6	High anthocyanin content, purple-green glumes, and red-purple anthers
	TW1 (P_2)	[(101L × TLK) × 101LAB]-1-S_6	Low anthocyanin content, green glumes, and green anthers
2	TC1 (P_1)	[(TB × TLK) × KND] × 8Y-1-S_6	Low anthocyanin content, green glumes, and pink anthers
	TB3 (P_2)	[(TB × KND-S_1) × TLK-F_1]-3-S_5	High anthocyanin content, purple-green glumes, and dark-purple anthers

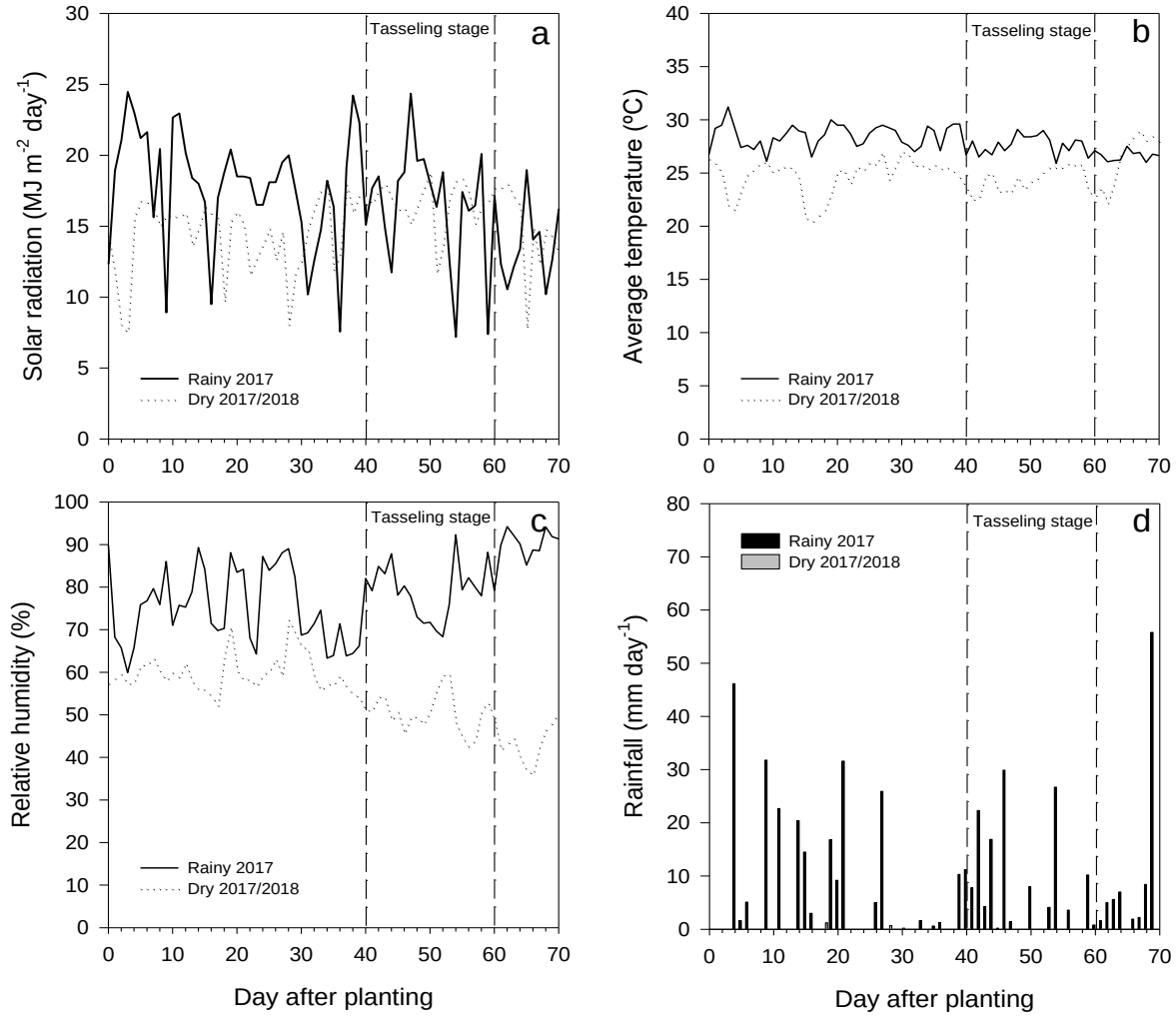

Figure 1. Solar radiation (a), average temperature (b), relative humidity, (c) and daily rainfall (d) during crop growth at the Vegetable Research Station, Khon Kaen University in the rainy season of 2017 and the dry season of 2017/2018.

and F_1 hybrids (non segregating). Two-row plots were used for backcross generations (segregating), and six-row plots were used for the F_2 generation (highly segregating). All plots were 5 m in length, and the crop was planted with a spacing of 80 cm between rows and 25 cm between plants within rows. The plots were managed to avoid environmental drought, water logging, weeds, diseases, and insects in accordance with the Thailand agricultural recommendations (Worrajinda *et al*., 2013).

Conventional tillage was practiced for soil preparation, and raised beds were constructed through rotary strip tillage technique to provide an optimum environment for germination. The seeds were planted at the rate of three seeds per hill. Corn seedlings were thinned to a single plant per hill at 14 days after planting. Fertilizer (15 N–15 P–15 K) was applied during soil preparation at the rate of 171 kg h^{-1}. In addition, the 15–15–15 formula fertilizer was applied at the rate of 93.75 kg h^{-1} plus urea (46–0–0) at the rate of 93.75 kg h^{-1} at 14 days after planting, and 125 kg h^{-1} 15–15–15 fertilizer plus 62.50 kg h^{-1} urea was applied at 30 days after planting. The crop was irrigated using a minisprinkler system to avoid drought stress. Pesticides were applied to obtain optimum growth and yield in both seasons.

Sample preparation and anthocyanin measurement

Whole tassels were harvested on the first day of pollen shed (Duangpapeng *et al*., 2018). Five tassels were harvested in each plot for nonsegregating generations, including parental lines and F_1 hybrids. Ten tassels per plot were collected for backcross generations, and 30 tassels per plot were collected for the F_2 generation. The individual tassels in each generation were cut into small pieces, dipped in liquid nitrogen, and freeze-dried. Each tassel was ground into powder and sieved through a 40-mesh screen.

Anthocyanin extraction was performed on individual tassels following the method of Yang *et al*. (2008). Briefly, 10 mL of acidified methanol solution (1% citric acid in 80% methanol) was added to 0.5 g of ground sample, mixed, and incubated at 4 °C for 24 h. Then, the sample was centrifuged at 5,000 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 analysis.

Total anthocyanin content (TAC) was measured using the pH differential method (Giusti and Wrolstad, 2001). Each appropriately diluted extract was divided and mixed with pH 1.0 or 4.5 buffers and incubated for 15 min in the dark. A UV–vis spectrophotometer was used to measure absorbance at 510 and 700 nm. TAC was calculated using the following equation:

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

where A is the absorbance difference of the diluted sample and was calculated from $A = (A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}$, MW is molecular weight of cyanidin-3-glucoside (449.2 g mol^{-1}), DF is the dilution factor, 1,000 is the conversion factor from gram to milligram, and ε is the molar absorptivity of 26,900 $M^{-1}cm^{-1}$. Anthocyanin levels were calculated as

microgram cyanidin-3-glucoside equivalent per gram of dry weight (µg CGE g^{-1} DW).

Statistical analysis

The data for the anthocyanin content of all samples in each treatment, including three repeated measurements on corn tassels, were used in the statistical analysis. The combined analysis of variance (ANOVA) across two seasons was performed (Gomez and Gomez, 1984) using Statistix10 software program (Analytical Software, Tallahassee, FL, USA). The statistical model was as follows:

$$Y_{ijk} = m + B_i + S_j + G_k + SG_{jk} + e_{ij} + e_{ijk}$$

where Y_{ijk} is mean of the parameter that responds to season j in generation k and block i, m is an overall mean, B_i is the block effect, S_j is the season effect, G_k is the generation effect, SG_{jk} is the interaction effect between season and generation, e_{ij} is the season error effect, and e_{ijk} is the pooled error effect.

Variance was partitioned into components by taking the percentages of the sum of squares calculated through the weighted variance of each component compared with the total variance. Least significant difference (LSD) was used to compare means at the $P \leq 0.05$ level. The results are presented as mean ± standard error (SE).

Generation mean analysis

Data were separately analyzed for each cross by fitting the fixed effects linear model

$$Y_{ijkl} = S_i + B_{j(i)} + G_k + p_{ik} + \mu_{ik(l)} + \varepsilon_{ijk}$$

where Y_{ijkl} is the response variable for plant l in generation k in replication j and season i; S_i is the effect of season i; $B_{j(i)}$ is the effect of replication j nested in season i; G_k is the effect of generation k; p_{ik} is the interaction between season i and generation k; $\mu_{ik(l)}$ is the effect of a plant nested in generation and season; and ε_{ijl} is the random residual term. All effects were fitted as fixed effects except for the environmental interaction p_{ik} and $\mu_{ik(l)}$.

The full linear mixed model with homogeneous variances was fitted to identify outliers. The presence of outliers was tested by applying Bonferroni correction at the 2% level of significance to the Studentized residuals. No data points met this criterion. Thus, no outliers were removed. Eight models containing all fixed effects but with different combinations of the random effects and in heterogeneity/homogeneity in the residual variance ($V(\varepsilon_{ijkl}) = \sigma^2_e I$ vs. $V(\varepsilon_{ijkl}) = \sigma^2_{e(i)} I$) were evaluated. The best fitting model was selected in accordance with the lowest value for the Bayesian information criterion (BIC) (Schwarz, 1978). The selected model was then fitted in SAS software to test for fixed effects and to obtain the best linear unbiased estimators for each generation. All analyses were done using the MIXED procedure in SAS 9.4 software (SAS Institute, Cary, NC, USA).

The estimation of the genetic parameters was performed by using a weighted least squares procedure, where the weight was the inverse of the square standard error (se^2) for each generation (Mather and Jinks, 1982). The test for model selection (two-, three-, and four-parameter model) was conducted with

Table 2. Mean squares for TAC in the tassel of six generations of two small-ear waxy corn crosses evaluated across two seasons.

Source of variance	d.f.	TB1 × TW1	TC1 × TB3
Season (S)	1	4,467,579** (22.1)	26,090,000** (38.6)
Rep. within S (a)	6	3,936 (0.1)	2,970 (0.0)
Generation (G)	5	2,599,302** (64.4)	6,235,361** (46.1)
S × G	5	508,645** (12.6)	1,986,519** (14.7)
Pooled error (b)	30	5,292 (0.8)	12,033 (0.5)
C.V. (a) (%)		9.4	3.9
C.V. (b) (%)		10.9	7.8

d.f. = degree of freedom, C.V. = coefficient of variation across all treatments, ** = Significant at $P \leq 0.01$ level. The number in the parentheses is the percentage of the sum of the squares.

a joint scaling test by adding the genetic parameters one by one to the model and testing for adequacy by examining the reduction in the residual sum of squares using the χ^2 test (Table 4). The model that resulted in no reduction in the residual sum of squares was deemed adequate (Cavalli, 1952; Lynch and Walsh, 1998). The goodness of fit test was performed by assuming that the product of the square difference between the observed and expected means and its corresponding weight followed a χ^2 distribution with the degrees of freedom equal to the number of generations (*n*) minus the number of parameters (*p*) to be estimated (Mather and Jinks, 1982). In this study, Gamble's (1962) notation was used to define gene effects, i.e., *m* is the mean, *a* is additive effects, *d* is dominance effects, *aa* is additive-by-additive epistatic effects, *ad* is additive-by-dominance epistatic effects, and *dd* is dominance-by-dominance epistatic effects.

RESULTS AND DISCUSSION

The information obtained in this study is important for the breeding of small-ear waxy corn for high anthocyanin content in tassels. In hybrid seed production, two inbred parents are grown side-by-side, and the tassels of the female parent are normally removed to prevent pollination by the female parent. The removed tassels are normally discarded as waste. The seed production system might be more effective if the tassel is used as a coproduct to produce anthocyanins. In this study, the high parent in each cross had the highest anthocyanin content across seasons. Therefore, the high parent can be used as the female parent in seed production. Under optimal conditions, harvesting tassels from commercially produced grain is also possible because corn tassels normally produce pollen in excess of what is required for the complete pollination of a field. Therefore, hybrid seed production would be a good option for the utilization of corn tassels as coproducts for anthocyanin production.

Anthocyanin variation analysis

The effects of season, generation, and season by generation (S × G) interaction on TAC were statistically significant ($P \leq 0.01$) for both crosses (Table 2). Generation had the largest

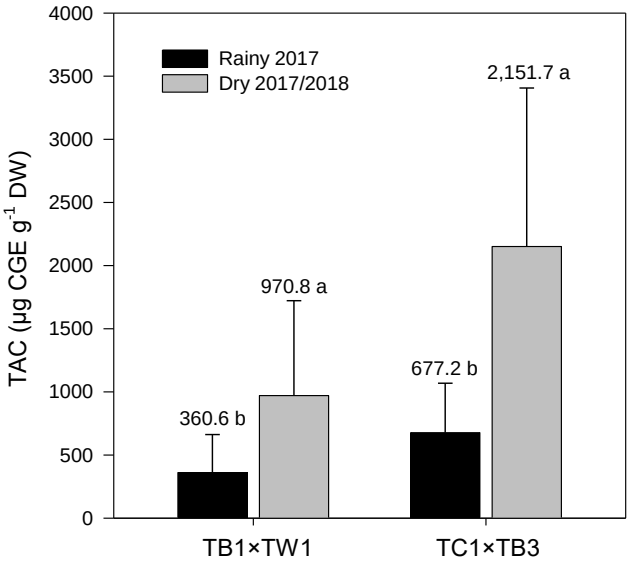

Figure 2. Means of each season for TAC in the tassel of small-ear waxy corn evaluated in the rainy season 2017 and the dry season 2017/2018. Different letters within a cross indicate significant differences at $P \leq 0.05$ level as determined by the least significant difference (LSD). Data are expressed as the mean ± standard error.

effect on TAC in TB1 × TW1 followed by season and S × G interaction with the percentages of 64.4%, 22.1%, and 12.6%, respectively. Similarly, the proportions of total variance in TC1 × TB3 were mainly explained by generation followed by season with the percentages of 46.1% and 38.6%, respectively, whereas S × G interaction had the smallest contribution, accounting for 14.7% of the total variance. The results for two crosses suggested that generation was the main cause of variation in TAC. High variation in TAC due to generation is expected because the parents in each cross had contrasting TAC values.

The moderate proportions of the total variance and significant difference between seasons in both crosses indicated that growing seasons are also important to determining anthocyanin content in the tassels of small-ear waxy corn. Growing small-ear waxy corn in the dry season resulted in the highest anthocyanin content with 970.8 and 2,151.7 µg CGE g^{-1} DW for TB1 × TW1 and TC1 × TB3, respectively. Moreover, TAC for TC1 × TB3 in the dry season was three times higher than that in the rainy season (Figure 2).

Environmental factors, including solar radiation, temperature, and nitrogen level, are important for regulating anthocyanin accumulation in plants (Gu *et al*., 2019). In this study, high temperature, high relative humidity, and high daily rainfall in the rainy season may result in lower anthocyanin level (Figure 1). In general, high anthocyanin level is promoted by low temperature. Higher anthocyanin level in the cold environment during the dry season agreed with this theory. Moreover, the

results were consistent with observations on anthocyanin accumulation in corn cobs (Khampas *et al*., 2015) and anthocyanin biosynthesis in apples (Ban *et al*., 2007; Xie *et al*., 2012). In addition to temperature, nitrogen level plays an important role in anthocyanin accumulation. Low nitrogen levels increase anthocyanin accumulation, whereas high nitrogen supply can reduce it (Wang *et al*., 2018; Gu *et al*., 2019). In this study, nitrogen fertilizer was applied to the crop at the most optimum level, and the difference in nitrogen application between two seasons was expected to be not significant. Therefore, different environmental conditions might be the main cause of lower anthocyanin content in the rainy season.

Means for the anthocyanin content of six generations of TB1 × TW1 indicated that TB1 (recurrent parent) had the highest TAC of 1,646.4 μg CGE g^{-1} DW. The F_1 generation of TB1 × TW1 had a mean TAC of 718.8 μg CGE g^{-1} DW, which was 14.7% lower than that of the mid-parent. The F_2 generation was a highly segregating population, and the mean value of this generation was significantly lower than that of its corresponding F_1 generations. The reductions in anthocyanin content in the F_2 generation were 24.0% and 35.2% lower than those in the F_1 generation and midparent, respectively. The mean value for TAC in both backcross generations was intermediate between F_1 and the recurrent parent. However, the mean TAC values in the backcross generation to the high parent was 15.2% higher than that in the F_1 generation (Table 3).

In TC1 × TB3, significant differences in anthocyanin content were observed among generations, and TB3 had the highest anthocyanin content of 2,601.2 μg CGE g^{-1} DW. The mean TAC of the F_1 generation was not different from that of the midparent. The mean TAC of the F_2 generation was also not different from that of the F_1 generation. The mean TAC of the backcross generations of TC1 × TB3 were in a similar pattern of those of TB1 and TW1. Both backcrosses were intermediate between F_1 and the recurrent parent (Table 3).

Estimation of genetic components

Models with increasing numbers of gene effects were evaluated starting with additive only (*m*, *a*), dominance only (*m*, *d*), additive + dominance (*m*, *a*, *d*), additive + dominance + additive by additive (*m*, *a*, *d*, *aa*), additive + dominance + additive by dominance (*m*, *a*, *d*, *ad*), and additive + dominance + dominance by dominance (*m*, *a*, *d*, *dd*) as summarized in Table 4. All models, except for the dominance-only model, were effective for explaining genetic effects for both crosses. The additive model was significant, whereas the dominance model was nonsignificant. The results for both simple models indicated that additive gene effects were important for anthocyanin inheritance in the tassel for both TB1 × TW1 and TC1 × TB3.

The additive + dominance model was selected as the model that can best explain gene effects because it was the simplest model that was sufficient to explain the data and described additive and dominance effects. The χ^2 test (df = 3) was not significant for both TB1 × TW1 and TC1 × TB3, indicating that the three-parameter (*m*, *a*, *d*) model adequately

Table 3. Observed means and expected means for TAC in the tassel for the six generations of two small-ear waxy corn crosses.

Generations	TB1×TW1		TC1×TB3	
	Observed	Expected	Observed	Expected
P_1	1,646.4 ± 865.8 a	1,624.1 ± 252.1	90.5 ± 59.4 e	126.1 ± 494.4
P_2	38.2 ± 24.5 f	53.2 ± 252.4	2,601.2 ± 1,623.3 a	2,561.2 ± 494.4
F_1	718.8 ± 359.2 c	717.5 ± 252.1	1,299.7 ± 927.9 c	1,294.2 ± 494.4
F_2	546.0 ± 569.9 d	546.0 ± 244.6	1,263.5 ± 1,143.1 c	1,264.7 ± 480.2
BC_{11}	847.2 ± 661.6 b	842.7 ± 247.6	1,059.7 ± 920.2 d	1,067.1 ± 486.0
BC_{12}	197.7 ± 218.4 e	205.7 ± 247.6	2,172.2 ± 1,184.6 b	2,166.3 ± 486.0
MP	842.3	838.7	1,345.9	1,343.7

P_1; Parental line 1, P_2; Parental line 2, F_1; First filial generation of crosses, F_2; Second filial generation of crosses, BC_{11}; First backcross generation with parental line 1, BC_{12}; First backcross generation with parental line 2 and MP; mid-parent value. Data are expressed as the mean ± standard error (µg CGE g^{-1} DW) of four replicates. Means with different letters in the same column indicate significant differences at $P \leq 0.05$ level, as determined by the least significant difference (LSD).

Table 4. Estimates of gene effects with their standard errors and goodness of fit test for TAC in the tassel of two small-ear waxy corn crosses.

Crosses	Model	Mean (*m*)	Additive (*a*)	Dominance (*d*)	Additive × additive (*aa*)	Additive × dominance (*ad*)	Dominance × dominance (*dd*)	Goodness-of-fit test	
								df	χ^2
TB1×TW1	1	662.0 ± 72.3*	755.1 ± 112.9*	-	-	-	-	4	2.02 ns
	2	750.6 ± 396.3	-	−211.0 ± 734.8	-	-	-	4	24.09**
	3	749.9 ± 115.1*	755.0 ± 113.3*	−209.9 ± 213.5	-	-	-	3	1.53 ns
	4	257.1 ± 260.4	754.9 ± 80.3*	417.9 ± 349.6	560.3 ± 281.3	-	-	2	0.51 ns
	5	749.8 ± 134.2*	785.6 ± 148.3*	−209.9 ± 248.9	-	−297.2 ± 653.5	-	2	1.38 ns
	6	838.7 ± 48.5*	754.9 ± 43.2*	−1,107.2 ± 223.3*	-	-	985.9 ± 228.5*	2	0.15 ns
TC1×TB3	1	1,414.1 ± 75.2*	−1,193.3 ± 117.4*		-	-	-	4	0.57 ns
	2	1,407.9 ± 617.8	-	14.8 ± 1145.6	-	-	-	4	15.23**
	3	1,407.9 ± 137.6*	−1,193.3 ± 135.5*	14.8 ± 255.2	-	-	-	3	0.57 ns
	4	1,512.4 ± 532.7	−1,193.3 ± 164.2*	−118.3 ± 714.8	−118.8 ± 575.2	-	-	2	0.56 ns
	5	1,407.9 ± 165.0*	−1,217.6 ± 182.2*	14.8 ± 306.0	-	236.7 ± 803.8	-	2	0.54 ns
	6	1,343.7 ± 152.2*	−1,193.3 ± 135.6*	664.9 ± 701.1	-	-	-714.4 ± 717.4	2	0.38 ns

ns, non-significant, *,** significant at $P \leq 0.05$ and 0.01 level, respectively.

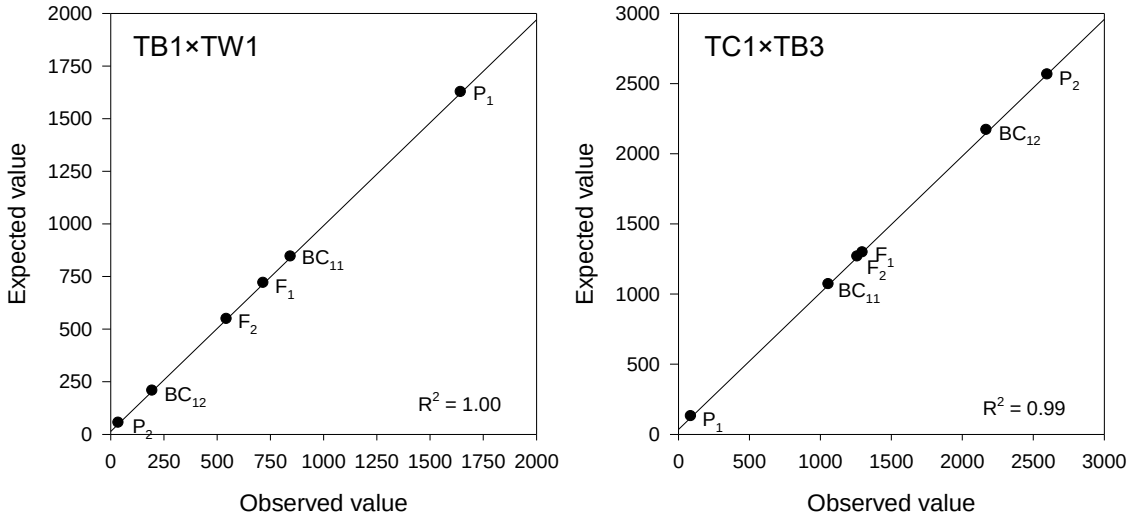

Figure 3. Relationships between the observed means and expected means predicted by the additive + dominance model for TAC in the tassels of six generations.

explained the gene effects for TAC. The results were in agreement with the inheritance of anthocyanin in purple eggplant (Sabolu *et al*., 2014). Moreover, the expected means for TAC using this model were highly related to the observed mean values. The regression coefficients were significant for TB1 × TW1 (r^2 = 1.00) and TC1 × TB3 (r^2 = 0.99) (Figure 3).

The estimates of genetic effects were significantly ($P \leq 0.05$) different from zero for the mean and additive effects, whereas the estimates were not significant for dominance effects in both crosses. Given that dominance effects were not significant and epistatic effects were not considered in the best-fit model, we concluded that both of these genetic effects had very little impact on the inheritance of TAC in tassels. The significance of the mean component "(*m*)" indicates that anthocyanin content in tassels is quantitatively inherited (Kere *et al*., 2013). Additive gene effects were the major component of the inheritance for TAC in corn tassel. The significant additive gene effect for anthocyanin content in this study was in agreement with a previous report on red table grape (Liang *et al*., 2011), black and violet pepper (Stommel *et al*., 2014), purple red-leafed rapeseed (Dai *et al*., 2016), and purple corn cob (Harakotr *et al*., 2016).

In this case, recessive sun-red alleles or the *pl* gene is the major gene controlling the anthocyanin biosynthesis pathway in corn plants. Light plays an important role in the activation of the *pl* gene to induce anthocyanin pigmentation in vegetative and floral organs (Cone *et al*., 1993). Anthocyanins accumulate in the vacuoles of tassel tissues, and their level is closely related to purple color expression in the tassel (Irani and Grotewold, 2005). Therefore, phenotypic selection through simple methods, such as mass selection, simple recurrent selection, and pedigree selection, may be effective for the improvement of small-ear waxy corn for high anthocyanins in tassel.

In addition, additive gene effect had a positive sign in TB1 × TW1 and a negative sign in TC1 × TB3. The difference in signs did not have genetic consequences because it merely reflected which parent (donor or recipient) had high anthocyanin content. The results indicated that parental lines with high TAC influenced the expression of anthocyanins in tassels. Hybrids with high anthocyanin content may be obtained from the crosses of inbred lines with high anthocyanin content and different genetic backgrounds.

CONCLUSION

Season, generation, and S × E interaction provided significant contributions to the variance in anthocyanin content in corn tassels. The sister lines TB1 and TB3 were parents with high anthocyanin content. Additive gene effects were significant in controlling anthocyanin content in corn tassel, whereas nonadditive gene effects were not significant. The use of simple selection and pedigree selection in early generations for population improvement for anthocyanin content in corn tassels could be effective to develop genotypes with high TAC. Inbred lines with high anthocyanin and good combining ability for agronomic traits may be effective hybrid combinations. The information obtained from this study may help corn breeders design effective approaches for improving anthocyanin concentration in waxy corn tassels.

ACKNOWLEDGEMENTS

The study was funded by the Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program (Grant No. PHD/0039/2558). It was supported in part by the U.S. Department of Agriculture, Agricultural Research Service. The authors also acknowledge the National Science and Technology Development Agency, Thailand (Grant No. P-17-51695) and the Plant Breeding Research Center for Sustainable Agriculture, Faculty of Agriculture, Khon Kaen University, Thailand for the support plant materials and providing research facilities. The authors wish to thank Dr. Kendall Lamkey and Dr. Jode Edwards for their helpful discussions.

REFERENCES

Ban Y, Honda C, Hatsuyama Y, Igarashi M, Bessho H, Moriguchi T (2007). Isolation and functional analysis of a MYB transcription factor gene that is a key regulator for the development of red coloration in apple skin. *Plant Cell Physiol.* 48: 958–970.

Cao XW, Cu HM, Li J, Xiong AS, Hou XL,Li Y (2016). Heritability and gene effects for tiller number and leaf number in non-heading Chinese cabbage using joint segregation analysis. *Sci. Hort. 2*03: 199–206.

Carson ML (2001). Inheritance of resistance to phaeosphaeria leaf spot of maize. *Plant Dis.* 85: 798–800.

Cavalli LL (1952). An analysis of linkage in quantitative genetics. In: C.R. Rieve, C.H. Waddington, eds., *Quantitative inheritance*. HSOM, London, pp. 135–144.

Chaiittianan R, Sutthanut K, Rattanathongkom A (2017). Purple corn silk: A potential anti-obesity agent with inhibition on

adipogenesis and induction on lipolysis and apoptosis in adipocytes. *J. Ethnopharmacol.* 201: 9–16.

Cone KC, Cocciolone SM, Moehlenkamp CA, Weber T, Drummond BJ, Tagliani LA, Bowen BA, Perrot GH (1993). Role of the regulatory gene *pl* in the photocontrol of maize anthocyanin pigmentation. *Plant Cell*. 5: 1807–1816.

Dai W, Girdthai T, Huang Z, Ketudat-Cairns M, Tang R, Wang S (2016). Genetic analysis for anthocyanin and chlorophyll contents in rapeseed. *Cienc. Rural*. 46: 790–795.

Duangpapeng P, Ketthaisong D, Lomthaisong K, Lertrat K, Scott MP, Suriharn B (2018). Corn tassel: A new source of phytochemicals and antioxidant potential for value-added product development in the agro-industry. *Agron.* 8: 242. doi:10.3390/agronomy8110242.

Duangpapeng P, Lertrat K, Lomthaisong K, Scott MP, Suriharn B (2019). Variability in anthocyanins, phenolic compounds and antioxidant capacity in the tassels of collected waxy corn germplasm. *Agron.* 9: 158. doi:10.3390/agronomy9030158.

Finkel ML, Sanchez S, Mak T, Granstein J, Lefkowitz A (2013). Anthocyanin-rich purple corn extract and its effects on the blood pressure of adults. *Evid.-Based Complementary Altern. Med.* 18: 237–242.

Gamble EE (1962). Gene effects in corn (*Zea mays* L.) I. Separation and relative importance of gene effects for yield. *Can. J. Plant Sci.* 42: 339–48.

Giusti MM, Wrolstad RE (2001). Characterization and measurement of anthocyanins by UV-Visible Spectroscopy. In: R.E. Wrolstad, T.E. Acree, H. An, E.A. Decker, M.H. Penner, D.S. Reid, S.J. Schwartz, C.F. Shoemaker, P. Sporns, eds., *Current protocols in food analytical chemistry.* John Wiley & Sons, New York, pp. F1.2.1–F1.2.13.

Gomez KA, Gomez AA (1984). Statistical procedure for agricultural research. John Wiley & Sons, New York.

Gu, KD, Wang CK, Hu DG, Hao YJ (2019). How do anthocyanins paint our horticultural products?. *Sci. Hort.* 249: 257–262.

Harakort B, Suriharn B, Lertrat K, Scott MP (2016). Genetic analysis of anthocyanin content in purple waxy corn (*Zea mays* L. var. *ceratina* Kulesh) kernel and cob. *SABRAO J. Breed. Genet*. 48: 230–239.

Intuyod K, Priprem A, Pairojkul C, Hahnvajanawong C, Vaeteewoottacharn K, Pinlaor P, Pinlaor S (2018). Anthocyanin complex exerts anti-cholangiocarcinoma activities and improves the efficacy of drug treatment in a gemcitabine-resistant cell line. *Int. J. Oncol.* 52: 1715–1726.

Irani NG, Grotewold EG (2005). Light-induced morphological alteration in anthocyanin-accumulating vacuoles of maize cells. *BMC Plant Biol.* 5: 7, doi:10.1186/1471-2229-5-7.

Jing P, Bomser JA, Schwartz SJ, He J, Magnuson BA, Giusti MM (2008). Structure-function relationships of anthocyanins from various anthocyanin-rich extracts on the inhibition of colon cancer cell growth. *J. Agric. Food Chem.* 56: 9391–9398.

Kere GM, Guo Q, Shen J, Xu J, Chen J (2013). Heritability and gene effects for salinity tolerance in cucumber (*Cucumis sativus* L.) estimated by generation mean analysis. *Sci. Hort.*159: 122–127.

Kesornkeaw P, Lertrat K, Suriharn B (2009). Response to four cycles of mass selection for prolificacy at low and high population density in small ear waxy corn. *Asian J. Plant Sci.*8: 425–432.

Khampas S, Lertrat K, Lomthaisong K, Simla S, Suriharn B (2015). Effect

of location, genotype and their interactions for anthocyanins and antioxidant activities of purple waxy corn cobs. *Turk. J. Field Crops.* 20: 15–23.

Kirisattayakul W, Wattanathorn J, Iamsaard S, Jittiwat J, Suriharn B, Lertrat K (2017). Neuro-protective and memory-enhancing effect of the combined extract of purple waxy corn cob and pandan in ovariectomized rats. *Oxid. Med. Cell. Longev.* doi:10.1155/2017/5187102.

Lertrat K, Thongnarin N (2008). Novel approach to eating quality improvement in local waxy corn: Improvement of sweet taste in local waxy corn variety with mixed kernels from super sweet corn. *Acta Hortic.* 769: 145–150.

Liang Z, Sang M, Wu B, Ma A, Zhao S, Zhong GY, Li S (2011). Inheritance of anthocyanin content in the ripe berries of a tetraploid × diploid grape cross population. *Euphytica* 186: 343–356.

Long N, Suzuki S, Sato S, Naiki-Ito A, Sakatani K, Shirai T, Takahashi S (2012). Purple corn color inhibition of prostate carcinogenesis by targeting cell growth pathways. *Cancer Sci.* 104: 298–303.

Lynch M,Walsh B (1998). Genetics and Analysis of Quantitative Traits. Sinauer Associates, Massachusetts.

Mather K, Jinks JL (1982). Biometrical genetics. Chapman and Hall, London.

Mazewski C, Liang K, Gonzalez de Mejia E (2017). Inhibitory potential of anthocyanin-rich purple and red corn extracts on human colorectal cancer cell proliferation in *vitro*. *J. Funct. Foods.* 34: 254–265.

Mohsen SM, Ammar ASM (2009). Total phenolic contents and antioxidant activity of corn tassel extracts. *Food Chem.* 112: 595–598.

Naveed A, Khan A, Khan IA (2009). Generation mean analysis of water stress tolerance in okra (*Abelmoschous esculentus* L.). *Pak. J. Bot.* 41: 195–205.

Nawaz H, Muzaffar S, Aslam M, Ahmad S (2018). Phytochemical composition: Antioxidant potential and biological activities of corn. In: Amanullah, S. Fahad, eds., *Corn-production and human health in changing climate*. Intech Open, London, pp. 49-68.

Rahman MA, Saad MS (2000). Estimation of additive, dominance and digenic epistatic interaction effects for certain yield characters in *Vigna sesquipedalis* Fruw. *Euphytica* 114: 61–66.

Ramli AB, Rafii MY, Latif MA, Saleh GB, Omar OB, Puteh AB (2016). Generation mean analysis of grain quality traits in selected rice populations derived from different amylose characteristics. *J. Sci. Food Agric.*96: 1593–1600.

Rao M, Grithlahre S, Bisen P, Loitongbam B, Dar MH, Zaidi NW, Singh US, Singh PK (2017). Generation mean analysis for grain yield and its component traits in submergence rice. *SABRAO J. Breed. Genet*. 49: 327–335.

Rashwan AMA (2010). Estimation of some genetic parameters using six populations of two cowpea hybrids. *Asian J. Crop Sci.* 2: 216–267.

Sabolu S, Kathiria KB, Mistry CR, Kumar S (2014). Generation mean analysis of fruit quality traits in eggplant (*Solanum melongena* L.). *Aust. J. Crop Sci.* 8: 242–250.

Schwarz G (1978). Estimating the dimension of a model. *Ann. Stat.* 6: 461–464.

Sharma S, Upadhyaya HD, Gowda CLL, Kumar S, Singh S (2013). Genetic analysis for seed size in three crosses of chickpea (*Cicer arietinum* L.). *Can. J. Plant Sci.* 93: 387–395.

Simla S, Boontang S, Harakotr B (2016). Anthocyanin content, total phenolic content, and antiradical capacity in different ear components of purple waxy corn at two maturation

stages. *Aust. J. Crop Sci.* 10: 675–682.

Srinivas B, Bhadru D (2015). Estimation of genetic parameters through generation mean analysis for fiber quality traits in upland cotton. *SABRAO J. Breed. Genet*. 47: 238–247.

Stommel JR, Pushko M, Haynes KG, Whitaker BD (2014). Differential inheritance of pepper (*Capsicum annuum*) fruit pigments results in black to violet fruit color. *Plant Breed.* 133: 788–793.

Sun XR, Liu L, Zhi XN, Bai JR, Cui YN, Shu JS,Li JM (2019). Genetic analysis of tomato internode length via mixed major gene plus polygene inheritance model. *Sci. Hort.* 246: 759–764.

Thiraphatthanavong P, Wattanathorn J, Muchimapura S, Thukham-mee W, Wannanon P, Tong-un T, Suriharn B, Lertrat K (2014). Preventive effect of *Zea mays* L. (purple waxy corn) on experimental diabetic cataract. *Biomed. Res. Int.* 3: 1–8.

Tsuda T (2012). Dietary anthocyanin-rich plants: Biochemical basis and recent progress in health benefits studies. *Mol. Nutr. Food Res.* 56: 159–170.

Uzokwe VNE, Asafo-Adjei B, Fawole I, Abaidoo R, Odeh IOA, Ojo DK, Dashiell K, Sanginga N (2017). Generation mean analysis of phosphorus-use efficiency in freely nodulating soybean crosses grown in low-phosphorus soil. *Plant Breed.* 136: 139–146.

Wang L, Yu Y, Fang M, Zhan C, Pan H, Wu Y, Gong Z (2014). Antioxidant and antigenotoxic activity of bioactive extracts from corn tassel. *J. Huazhong Univ. Sci. Technol. Med. Sci.* 34: 131–136.

Wang, XF, An JP, Liu X, Su L, You CX, Hao YJ (2018). The nitrate-responsive protein MdBT2 regulates anthocyanin biosynthesis by interacting with the MdMYB1 transcription factor. *Plant Physiol.* 178: 890–906.

Wille JJ, Berhow MA (2011). Bio-actives derived from ripe corn tassels: A possible new natural skin whitener, 4-hydroxy-1-oxindole-3-acetic acid. *Curr. Bioact. Compd.* 7: 126–134.

Worrajinda J, Lertrat K, Suriharn B (2013). Combining ability of super sweet corn inbred lines with different ear sizes for ear number and whole ear weight. *SABRAO J. Breed. Genet*. 45: 468–477.

Xie XB, Li S, Zhang RF, Zhao J, Chen YC, Zhao Q, Hao YJ (2012). The bHLH transcription factor MdbHLH3 promotes anthocyanin accumulation and fruit colouration in response to low temperature in apples. *Plant Cell Environ.* 35: 1884–1897.

Yang Z, Fan G, Gu Z, Han Y, Chen Z (2008). Optimization extraction of anthocyanins from purple corn (*Zea mays* L.) cob using tristimulus colorimetry. *Eur. Food Res. Technol.* 227: 409–415.

Zhu F (2018). Anthocyanins in cereals: Composition and health effects. *Food Res. Int.* 109: 232–249.

Žilić S, Vančetović J, Janković M, Maksimović V (2014). Chemical composition, bioactive compounds, antioxidant capacity and stability of floral maize (*Zea mays* L.) pollen. *J. Funct. Foods.* 10: 65–74.