Combining Ability and Heterosis for Agronomic Traits, Husk and Cob Pigment Concentration of Maize

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Abstract: The objective of this study was to identify the maize inbred lines with good general combining ability (GCA), good specific combining ability (SCA), high heterosis for yield and phytochemicals, and the crosses with high yield of yellow kernels and high anthocyanin content in cobs and husk, which was probably related to the high antioxidant activity. The parental lines including five unpigmented females and five pigmented males were crossed in North Carolina design II. The parents, the resulting 25 hybrids, and 5 controls were evaluated at two locations in the dry season of 2016/2017. Additive and non-additive gene effects controlled the inheritance of grain yield, agronomic traits, and phytochemicals. KKU–PFC2 and KKU–PFC4 had the highest GCA effects for phytochemical traits in husk and cob, whereas Takfa1 and Takfa3 were good combiners for grain yield. F1 hybrids had significantly higher total anthocyanin content (TAC), total phenolic content (TPC), (2,2-diphenyl-1-picrylhydrazyl) (DPPH), and trolox equivalent antioxidant capacity (TEAC) in husk and cob than pigmented control cultivars. The hybrids superior for individual traits were identified, but the experiment was not able to identify superior hybrids for multiple traits. The Takfa3 × KKU–PFC5 and NakhonSuwan2 × KKU–PFC4 had the highest anthocyanin in husk and cobs, respectively. The breeding strategies to develop maize varieties with high anthocyanins and normal yellow kernels and utilization of the hybrids are discussed.

Keywords: maize; plant breeding; hybrids; phytochemicals; anthocyanins; general combining ability (GCA); specific combining ability (SCA)

1. Introduction

Field corn is one of the most important cereal crops in the world, and it is used in human and animal diets [1]. Yellow corn is a source of provitamin A carotenoids required for growth, and it is used as a coloring agent for eggs and skin in poultry to better match the preference of customers [2]. Moreover, purple corn kernel is rich in anthocyanins and phenolic compounds [3–5], and these phytochemicals are also found at high concentrations in Husk [6,7] and cob [7,8]. Anthocyanins and phenolic compounds are known to have beneficial antioxidant properties [9]. The compounds help prevent several non-contagious diseases such as cancer [10,11], cardiovascular disease [12], obesity [13,14], and diabetes [15]. Recently, anthocyanin extracted from purple corn has been used...
as a cosmetic ingredient in lipstick [16], a dietary supplement [17], and a food colorant in the food industries of many countries including Germany, France, Italy, and Japan [14].

Production of field corn generates large amount of corn waste including stem, husk, and cob. However, only a small part of this corn waste is utilized, for example as animal feed [18], bio-ethanol [19], emulsified oil absorption [20], and particleboard panels [21]. Extraction of anthocyanin from husk and cob is an interesting way to effectively utilize corn waste to create value-added product, and development of corn with yellow kernels and high anthocyanins in husk and cob is important to achieve this goal. The compounds produced in this way would provide health benefits as they have potent antioxidant, anti-inflammatory, antimutagenic, anticarcinogenic, and anti-angiogenesis properties [14].

Combining ability identified the best inbred lines and the promising hybrid combinations for production of maize hybrids [22,23]. General combining ability, specific combining ability, and heterosis are evaluated in the course of choosing suitable parental lines for hybrid development [24]. In maize, combining ability study has been used to identify superior parents and specific hybrids for yield and agronomic traits [25], yield and quality traits in baby corn [26], yield and drought-tolerance [27], early maturity in quality protein maize [28], forage and grain [29], resistance to northern leaf blight [30], stem borer resistance [31], traits relevant to the production of cellulosic ethanol [32], total phenols and secondary traits in colored maize [33], and β-carotene content of maize [34].

However, to our knowledge so far, the information on combining ability for anthocyanin concentration in husk and cob of purple field corn has not been reported in the open literature. The objective of this study was to identify the maize inbred lines with good general combining ability (GCA), good specific combining ability (SCA), high heterosis for yield and phytochemicals, and the crosses with high yield of yellow kernels and high anthocyanin content in cobs and husk, which was probably related to the high antioxidant activity. A better understanding on combining ability patterns in this germplasm will allow breeders to make better decisions about which inbreds are to be combined to achieve better hybrid performance. The information obtained will be useful for development of corn hybrids with anthocyanins in cob and husk.

2. Materials and Methods

2.1. Plant Materials

Two groups of maize inbred lines were used in this study (Table 1). The first group had five field corn inbred lines including NakhonSawan1, NakhonSawan2, Takfa1, Takfa2, and Takfa3 and was used as female parents. They had orange kernels, green husk, and white cobs and had been improved for good yield and agronomic traits by the Nakhon Sawan Field Crops Research Center, Department of Agriculture, Nakhon Sawan, Thailand. The second group had five field corn inbred lines consisting of KKU–PFC1, KKU–PFC2, KKU–PFC3, KKU–PFC4, and KKU–PFC5 and was used as male parents. This second group had a mixture of purple, white, and yellow kernels, purple husk, and purple cobs. They were improved for high anthocyanin content in both corn husk and cob through mass selection for five consecutive generations by the Corn Breeding Project, Plant Breeding Research Center for Sustainable Agriculture, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand [7]. These two groups were crossed in a North Carolina design (NCD) II fashion [35] to generate 25 F1 hybrids. This mating design involves making all possible hybrids between a group of inbreds designated as males and a group of different inbreds designated as females. It was chosen because it allows estimation of genetic effects related to combining ability.
Table 1. List of parent materials used in this study.

| No. | Lines            | Kernel Color | Husk Color | Cob Color |
|-----|------------------|--------------|------------|-----------|
| 1   | NakhonSawan1     | Orange       | Green      | White     |
| 2   | NakhonSawan2     | Orange       | Green      | White     |
| 3   | Takfa1           | Orange       | Green      | White     |
| 4   | Takfa2           | Orange       | Green      | White     |
| 5   | Takfa3           | Orange       | Green      | White     |

| No. | Lines        | Kernel Color | Husk Color | Cob Color |
|-----|--------------|--------------|------------|-----------|
| 1   | KKU–PFC1     | Purple       | Purple     | Purple    |
| 2   | KKU–PFC2     | White        | Purple     | Purple    |
| 3   | KKU–PFC3     | Yellow       | Purple     | Purple    |
| 4   | KKU–PFC4     | Purple       | Purple     | Purple    |
| 5   | KKU–PFC5     | White        | Purple     | Purple    |

2.2. Field Experiment

A total number of 40 entries including 10 parents, 25 F₁ hybrids, 4 commercial field corn hybrids (Pacific339, CP301, Pioneer4546, and Syngenta6248), and 1 commercial waxy corn hybrid (Fancy111) were evaluated in this experiment. Pacific339, CP301, Pioneer4546, and Syngenta6248 have orange kernels, white husk, and white cobs. Fancy111 has purple kernels, purple-green husk, and purple cobs.

The entries were arranged in a randomized complete block design with three replications at two locations in the dry season (December 2016–April 2017). The first location was in an upland paddy field (after rice harvest) with irrigation at the Field Crop Research Station in Khon Kaen Province (16°28'11.24" N 102°48'49.46 E and altitude 190 m). The second location was in a lowland farmer’s field with irrigation in the Uthai Thani province (15°22'57.77" N 100°4'42.54" E and altitude 20 m), Thailand. Khon Kaen and Uthai Thani differed in soil type, temperature, rainfall, relative humidity, and solar radiation (Figure A1 and Table A1). Each plot consisted of two rows with 5 m long, inter-row spacing of 0.8 m, and intra-row spacing of 0.25 m. Crop management followed the recommendations for commercial production of corn in Thailand. The location at Khon Kaen University was planted on 22 November 2016, and the location in Uthai Thani was planted on 10 December 2016.

A mixed chemical fertilizer with the formula 15-15-15 of N-P-K was incorporated into the soil at the rate of 125 kg ha⁻¹ at planting. Nitrogen fertilizer in the form of urea (46-0-0) was applied to the crop at the rate of 320 kg ha⁻¹ at two splits at 14 days after planting (DAP) and 30 DAP. At 50 DAP, a mixed chemical fertilizer with the formula 13-13-21 of N-P-K was applied to the crop at the rate of 160 kg ha⁻¹. For all crop cycles, nitrogen was applied at the rate of 334 kg ha⁻¹, phosphorus was applied at the rate of 334 kg ha⁻¹, and potassium was applied at the rate of 52 kg ha⁻¹.

Atrazine, a pre emergence herbicide, was applied to the crop at the rate 1875 g/375 L water per ha at planting. No other weed control was practiced after application of pre-emergence herbicide. Dimethomorph at the rate of 20 g/20 L water was applied to the crop at 14 and 30 DAP to control downy mildew, and Carbosulfan at the concentration of 20% w/v emulsifiable concentrate and the rate of 60 mL/20 L water was applied to the crop at 14 and 30 DAP to control insects. Inbreds and hybrids were grouped prior to randomization and both groups were planted in the each replication. The placement of the two groups in each replication was randomly determined as well. This method reduced the competition between inbreds and hybrids.

2.3. Data Collection

Data were recorded for number of days to anthesis, plant height, ear height, husk yield, cob yield, and grain yield. Days to anthesis was recorded as number of days between planting and 50% of pollen shed. Plant height was recorded in cm from ground level to the base of the tassel, and ear height was recorded in cm from ground level to the ear-bearing node of the uppermost ear. Plant height and...
ear height were measured on 10 randomly chosen plants in each plot after reproductive stage. Husk yield and cob yield were recorded as dry husk mass and cob mass per plot and converted to kg per hectare. The ears were shelled. Grain moisture was measured by a grain moisture tester (model EE-KU) developed by EE-KU Lab, Bangkok, Thailand according to the manufacturer’s directions. Grain yield was expressed as kg ha\(^{-1}\) at 15% moisture content.

2.4. Sample Preparation and Extraction

Ten ears from each replication of each treatment were randomly harvested at physiological maturity (approximately 40 days after pollination for parental lines and approximately 50 days after pollination for hybrids) and oven-dried at 40 \(^\circ\)C for 48 h. The anthocyanin extraction was performed as described in [36,37]. Husk and cobs were harvested from each replication and were ground into powder separately. The powdered samples of approximately 2 g were loaded into 100 mL flasks containing 20 mL of 100% methanol. The flasks were shaken on a multi-stirrer at 200 rpm for 1 h at room temperature. The samples were filtered through Whatman #1 filter paper. After filtration, the retentates were loaded again into 100-mL flasks containing 20 mL of 100% methanol, shaken on a platform shaker for 1 h, and again filtered through Whatman #1 filter paper. The two filtrates were combined and evaporated in a rotary evaporator at 40 \(^\circ\)C to reduce the volume from 40 mL to 10 mL and the concentrated solution was stored at \(-20\) \(^\circ\)C.

2.4.1. Determination of Total Anthocyanin Content (TAC)

Total monomeric anthocyanin content in each sample was estimated using the pH differential method [37]. A UV–vis spectrophotometer (GENESYS 10S, ThermoScientific, Waltham, MA, USA) was used to measure the absorbance at 510 and 700 nm in a cuvette with a 1 cm path length. Total monomeric anthocyanin concentration (TAC) was expressed as mg of cyanidin-3-glucoside equivalents per 100 g dry weight (mg CGE/100g DW) of samples, anthocyanin pigment (cyanidin-3-glucoside equivalents, mg/L) calculated using the following equation;

\[
TAC = \frac{A \times MW \times DF \times 10^3}{\epsilon \times l}
\]

where \(A = (A_{510 \ nm} - A_{700 \ nm}) \ pH \ 1.0 - (A_{510 \ nm} - A_{700 \ nm}) \ pH \ 4.5\); MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu); DF = dilution factor; \(l = \) pathlength in cm; \(\epsilon = 26,900\) molar extinction coefficient, in L × mol\(^{-1}\) × cm\(^{-1}\), for cyd-3-glu and \(10^3\) = factor for conversion from g to mg. Then, TAC was converted into total anthocyanin yield (TAY) by following this equation;

\[
TAY = \frac{TAC \ (mg \ CGE/100 \ g \ DW)}{Dry \ matter \ yield \ (kg/ha)}
\]

2.4.2. Determination of Total Phenolic Content (TPC)

Total phenolic content in each sample was determined according to Folin–Ciocalteau’s phenol reagent (FC reagent) procedure with minor modification [38]. The reaction was prepared by mixing 0.5 mL methanol extract, 2.5 mL water, and 0.5 mL FC reagent, which was pre-diluted from 2 M to 1 M with distilled water. The mixture was set aside at room temperature for eight minutes and 1.5 mL Na\(_2\)CO\(_3\) solution was added to the mixture. The solution was allowed to stand for 120 min at room temperature. Then, the absorbance was read at 765 nm using a UV–visible spectrophotometer. Gallic acid solutions (10–100 mg/L) were used as reference standards. The total phenolic content (TPC) was expressed as mg gallic acid equivalents/100 g dry weight of samples (mg GAE/100g DW).
2.4.3. Determination of Antioxidant Assay

The assay of DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical-scavenging activity was performed by measuring the capacity for bleaching a black-colored methanol solution of DPPH radicals as reported by [39]. Briefly, the reaction for each sample was prepared by mixing 4.5 mL methanolic solution of DPPH (0.065 mM) and 0.5 mL of solution extract or a standard solution. The reaction was conducted at room temperature for 30 min before the absorbance was recorded at 517 nm. The radical-scavenging activity of the extracts was calculated as follows;

$$\text{Scavenging rate} \% = \left(1 - \frac{A_1 - A_s}{A_0}\right) \times 100 \quad (3)$$

where Ao is the absorbance of the control solution (0.5 mL extraction solvent in 4.5 mL of DPPH solution), A1 is the absorbance of the extracts in DPPH solution, and As, which is a term for correction of errors arising from unequal color of the sample solutions, is the absorbance of the extract solution without DPPH. The value was expressed as percentage (%) of DPPH free radical-scavenging activity assay.

The trolox equivalent antioxidant capacity assay (TEAC) for each sample was executed according to the method described by [39] with minor modifications. Briefly, ABTS$^+$ radical cations were generated by a reaction of 7 mmol/l ABTS and 2.45 mmol/L potassium persulfate. The reaction mixture was allowed to stand in the dark at room temperature for 16–24 h before use and the mixture was used within 2 days. The ABTS$^+$ solution was diluted with methanol to an absorbance of 0.700 ± 0.050 at 734 nm. The diluted extract of 50 microliters was mixed with 2.0 mL of diluted ABTS$^+$ solution for 6 min at room temperature, and the absorbance was immediately recorded at 734 nm. Trolox solution (100–1000 µM) was used as a reference standard. The value was expressed as millimoles of trolox equivalents (TE) per 100 g of dry weight (mmol TE/100 g DW).

2.5. Statistical Analysis

Analysis of variance was performed separately for each location and error variances were tested for homogeneity [40]. Error variances were homogeneous, so the data from the two locations were combined. The following statistical model was used;

$$Y_{ijkl} = \mu + L_d + R_k(L_d) + m_i + f_j + m_i \times f_j + L_d \times m_i + L_d \times f_j + L_d \times m_i \times f_j + e_{ijkl} \quad (4)$$

where $Y_{ijkl}$ is the observed value in location $d$, replication $k$, male $i$, and female $j$; $\mu$ is the grand mean, $L_d$ is the location effect ($d = 1,2$), $R_k(L_d)$ is the effect of replicate $k$ nested in location $d$ ($k = 1,2,3$); $m_i$ is the male effect ($i = 1,2,3,4,5$); $f_j$ is the female effect ($j = 1,2,3,4,5$); $m_i \times f_j$ is the interaction between male and female; $L_d \times m_i$ is the interaction between location $d$ and male $i$; $L_d \times f_j$ is the interaction between location $d$ and female $j$; $L_d \times m_i \times f_j$ is the interaction between location $d$, female $j$ and male $i$; and $e_{ijkl}$ is the pooled error effect. Calculations were performed with AGD-R [41].

Variances of hybrid effect were further partitioned into due to GCA and SCA, and GCA effect of parents and SCA effects of hybrids were calculated based on means of 25 hybrids for agronomic traits, total anthocyanin content, total phenolic content, and antioxidant activity to obtain estimates of SCA of the hybrids and GCA of the parents. Mid-parent heterosis (MPH) and high parent heterosis (HPH) of each hybrid for all traits were calculated and expressed in percentages using trait means of parents and hybrids across two locations. For each trait, the mid-parent value of a cross was calculated as the mean of the parental lines averaged across locations. Hence, MPH was computed as;

$$\text{MPH} = \left[\frac{F_1 - \text{MP}}{\text{MP}}\right] \times 100 \quad (5)$$
where $F_1$ is the mean performance of the cross; MP is the mid-parent value given by $(P_1 + P_2)/2$; $P_1$ and $P_2$ are the mean values of parent 1 and parent 2 averaged across locations, respectively. HPH was calculated as:

$$\text{HPH} = \left[ \frac{F_1 - \text{HP}}{\text{HP}} \right] \times 100$$  \hspace{1cm} (6)

where HP = the better parental mean across locations. The test for significance of MPH and HPH was done by comparing mean values of MP or HP to the hybrid value using Student’s $t$ test at 0.05 probability level.

3. Results and Discussion

3.1. Analysis of Variance

Locations were significantly different for most traits except for cob DPPH (Tables 2 and 3), indicating that the location was an important source of variations in agronomic traits and phytochemical content. Soil heterogeneity, temperature, and nutrient availability are the factors affecting anthocyanin pigment accumulation [42,43]. The effects of hybrids were also significant for all traits, suggesting that selection on the tested hybrids would be possible. Hybrid × location interactions were significant for most traits excluding cob weight and days to anthesis, demonstrating that hybrids responded differentially to environments although the magnitudes of interaction effects were small. These interaction effects, although small, could confound the selection of superior hybrids, and multi-location testing of the hybrids is still required.

The significance of GCA and SCA effects revealed the presence of both additive and non-additive gene effects for most traits. Additive gene effects were predominant for husk weight, anthesis day, plant height, ear height, husk TAY, husk TAC, husk TPC, husk DPPH, husk TEAC, cob TAY, cob TAC, cob TPC, and cob DPPH, whereas overwhelming non-additive gene effects were noticed for grain yield, cob weight, and cob TEAC.

Based on the results, three breeding strategies should be devised for the most effective selection programs. Because the interactions between genotype and environment were significant for yield, agronomic traits, and anthocyanin content, evaluation of breeding lines and hybrids in multi-location trials is required. As the purple color was expressed in the $F_1$ generation and gene expression for anthocyanins was additive, visual selection of colored plants using simple or modified mass selection would be effective for improving anthocyanins in husk and cob in early cycles of selection. Breeders could also perform visual selection for early flowering and lodging tolerance to fix the favorable alleles. In the latter selection cycles, when the colored plants are more uniform, chemical analysis of anthocyanins should be performed and selection for grain yield and cob weight should also be carried out to ensure cultivars with the greatest overall value are selected.

Female GCA effects were larger than male GCA effects for most traits shown in Table 4 except plant height and TAY in cob. Female GCA effects were larger than male GCA effects for all phytochemical traits in husk traits, but female GCA effects were smaller than male GCA effects for all phytochemical traits in cob (Table 5). This may reflect the difference in the genetic control of phytochemical accumulation in cob and husk tissues.
Table 2. Mean squares for agronomic traits, anthocyanin yield, and grain yield of hybrids evaluated across two locations.

| Source of Variation | df | Anthesis Day (day) | Plant Height (cm) | Ear Height (cm) | Husk Mass (kg ha\(^{-1}\)) | Cob Mass (kg ha\(^{-1}\)) | TAY | Grain Yield |
|---------------------|----|---------------------|-------------------|-----------------|-----------------------------|-----------------------------|-----|-------------|
|                     |    |                     |                   |                 |                             |                             |     |             |
| Location (L)        | 1  | 1098.9 **           | 147.951 **        | 102.998 **      | 44.161 **                   | 436.476 **                  | 12.2 | 51.5 **     |
| Hybrid              | 24 | 9.2 **              | 597 **            | 143 **          | 35.840 **                   | 13.226 **                   | 27.7 | 23.1 **     |
| Hybrid × L          | 24 | 0.5 ns              | 329 **            | 89 **           | 24.363 **                   | 7686 ns                     | 3.4  | 4.4 **      |
| GCA female          | 4  | 40.9 **             | 1512 **           | 245 **          | 91.596 **                   | 18.082 **                   | 92.7 | 28.5 **     |
| GCA male            | 4  | 3.8 **              | 1571 **           | 210 **          | 34.406 **                   | 5055 ns                     | 5.6  | 51.1 **     |
| SCA                 | 16 | 2.2 **              | 125 ns            | 101 **          | 22.259 **                   | 14.055 **                   | 17.0 | 14.7 **     |
| GCA female × L      | 4  | 1.1 ns              | 1326 **           | 178 **          | 5176 ns                     | 20.163 **                   | 2.9  | 0.3 ns      |
| GCA male × L        | 4  | 0.0 ns              | 88 ns             | 176 **          | 10,143 ns                   | 6298 ns                     | 3.5  | 17.4 **     |
| SCA × L             | 16 | 6.9 ns              | 2246 ns           | 710 ns          | 523,438 **                  | 78,611 ns                   | 55.0 | 34.9 **     |
| Error               | 96 | 0.8                 | 128               | 37              | 5486                        | 4882                        | 0.2  | 0.2         |

% SS GCA female 73.9 42.2 28.6 42.6 22.8 55.8 20.6 33.8
% SS GCA male 10.5 43.8 24.4 16 6.4 3.4 37 4.4
% SS SCA 15.6 47 470 42.4 61.8

ns and ** nonsignificant and significant at the 0.01 probability level. % SS, proportional contribution of the sum of squares; TAY, total anthocyanin yield (kg CGE/DW ha\(^{-1}\)).

Table 3. Sums of squares for total anthocyanin content (TAC), total phenolic content (TPC), and antioxidant activity determined by (2,2-diphenyl-1-picrylhydrazyl) (DPPH) and trolox equivalent antioxidant capacity (TEAC) method of parents and their hybrids evaluated across two locations.

| Source of Variation | df | TAC (mg CGE/100 g DW) | TPC (mg GAE/100 g DW) | DPPH (%) | TEAC (mmol TE/100 g DW) | TAC (mg CGE/100 g DW) | TPC (mg GAE/100 g DW) | DPPH (%) | TEAC (mmol TE/100 g DW) |
|---------------------|----|-----------------------|-----------------------|----------|-------------------------|-----------------------|-----------------------|----------|-------------------------|
|                     |    |                       |                       |          |                         |                       |                       |          |                         |
| Location (L)        | 1  | 272,450 **            | 1,077,998 **         | 205.5 ** | 108.5 **                | 290,542 **            | 857,825 **            | 2.5 ns   | 2.1 **                  |
| Hybrid              | 24 | 288,280 **            | 549,025 **           | 415.2 ** | 43.9 **                 | 315,603 **            | 547,021 **            | 368.2 ** | 42.2 **                 |
| Hybrid × L          | 24 | 51,424 **             | 142,574 **           | 53.4 **  | 12.3 **                 | 49,328 **             | 217,542 **            | 83.7 **  | 10.5 **                 |
| GCA female          | 4  | 884,742 **            | 1,349,445 **         | 927.6 ** | 134.5 **                | 433,796 **            | 773,513 **            | 360.3 ** | 46.4 **                 |
| GCA male            | 4  | 90,326 **             | 483,590 **           | 395.2 ** | 43.3 **                 | 719,558 **            | 1,024,086 **          | 766.9 ** | 49.3 **                 |
| SCA                 | 16 | 188,653 **            | 365,279 **           | 392.3 ** | 21.4 **                 | 185,065 **            | 371,132 **            | 270.5 ** | 27.3 **                 |
| GCA female × L      | 4  | 41,053 **             | 144,920 **           | 37.5 **  | 22.7 **                 | 3388 **               | 154,720 **            | 48.7 **  | 8.0 **                  |
| GCA male × L        | 4  | 75,216 **             | 146,508 **           | 77.0 **  | 17.9 **                 | 192,258 **            | 695,223 **            | 271.9 ** | 26.3 **                 |
| SCA × L             | 16 | 769,089 **            | 2,256,069 **         | 823.8 ** | 133.9 **                | 401,291 **            | 1,821,247 **          | 726.4 ** | 115.9 **                |
| Error               | 96 | 680                   | 525                  | 1.0      | 0.1                     | 606                   | 1629                  | 1.1      | 0.0                     |

% SS GCA female 51.2 40.9 37.2 51.1 22.9 23.6 16.3 22.6
% SS GCA male 14.7 15.9 16.4 38.0 31.2 34.7 24.1
% SS SCA 43.6 44.4 46.9 32.5 39.1 45.2 49.0 53.3

ns and ** nonsignificant and significant at the 0.01 probability level. % SS, proportional contribution of the sum of squares; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical scavenging ability; TEAC, trolox equivalent antioxidant activity.
Table 4. General combining ability effects (GCA) for agronomic traits, anthocyanin yield, and grain yield of parents across two locations.

| Parental Lines          | Anthesis Day (day) | Plant Height (cm) | Ear Height (cm) | Husk Mass (kg ha\(^{-1}\)) | Cob Mass (kg ha\(^{-1}\)) | TAY (kg ha\(^{-1}\)) | Grain Yield (kg ha\(^{-1}\)) |
|-------------------------|--------------------|-------------------|----------------|----------------------------|---------------------------|---------------------|-----------------------------|
|                         |                    |                   |                |                           |                           |                     |                             |
| NakhonSawan1            | −0.5 *             | −11.2 **          | −4.4 **        | −74.7 **                  | −37.7 **                  | −0.8 *              | 0.5 *                       | −405.8 *                    |
| NakhonSawan2            | −0.7 *             | 5.4 *             | 3.1 *          | 7.1                       | −5.7                      | −0.7                | 0.7 *                       | 107.4                       |
| Takia1                  | −1.2 **            | −2.8              | −1.1           | −23.4                     | 2.4                       | −0.5                | −0.8 *                      | 228.3 *                     |
| Takia2                  | 0.7 *              | 5.5 *             | 1.0            | 14.6                      | 28.0 **                   | −1.1 *              | 0.9 **                      | −484.9 **                   |
| Takia3                  | 1.7 **             | 3.1               | 1.4 *          | 76.3 **                   | 13.0 *                    | 3.1 **              | −1.3 **                     | 555.0 **                    |
|                         |                    |                   |                |                           |                           |                     |                             |
|                         | −0.6 **            | 0.0               | −1.4           | −35.4 **                  | −4.2                      | 0.1                 | −0.3                        | 165.5 **                    |
|                         | −11.8 **           | 2.0 *             | −3.8 **        | −36.3 **                  | −1.2                      | −0.6 **             | −0.2                        | −203.5 **                   |
|                         | 6.8 **             | 2.8 **            | 11.7           | 16.4 *                    | −18.9 **                  | 0.2 *               | 0.5                         | 68.8                        |
|                         | 4.7 *              | 0.3               | 0.4            | 17.1 *                    | 10.6 *                    | 0.7 *               | −1.8 **                     | −129.9 *                    |
|                         | 0.6 **             |                   |                |                           |                           |                     |                             |
|                         | −0.7             | 11.7              | 0.7            | −109.0 *                  | −3.9 **                   | 0.2                 | −0.2                        | −77.7 *                     |
|                         | −191.8 **          | −5.3 **           | −1.7 *         | 83.8 *                    | 3.7 **                    | −179.6 **           | 1.9 *                       | 1.9 **                      |
|                         | 356.7 **           | 9.0 **            | −198.6 **      | −179.6 **                 | −37.7 **                  | −1.4 **             |                             |                             |
|                         | 76.8              | 94.8              | 2.5            | 0.9                       | 53.8                      | 71.8                | 1.5                         | 0.6                         |
|                         | 24.5              | 56.8              | 1.6            | 0.5                       | 69.3                      | 82.6                | 2.3                         | 0.6                         |

*, ** indicate that the estimates were significantly different from zero at ≥SE and ≥2SE, respectively. SE, standard error of the general combining ability effects; TAY, total anthocyanin yield (kg CGE/DW ha\(^{-1}\)).

Table 5. General combining ability effects (GCA) for total anthocyanin content (TAC), total phenolic content (TPC), and antioxidant activity determined by DPPH and TEAC method of parents across two locations.

| Parental Lines    | TAC (mg CGE/100 g DW) | TPC (mg GAE/100 g DW) | DPPH (% mmol TE/100 g DW) | TEAC (mg CGE/100 g DW) | TAC (mg CGE/100 g DW) | TPC (mg GAE/100 g DW) | DPPH (% mmol TE/100 g DW) | TEAC (mg CGE/100 g DW) |
|-------------------|-----------------------|-----------------------|---------------------------|------------------------|-----------------------|-----------------------|---------------------------|------------------------|
| NakhonSawan1      | −43.1                 | −78                   | −1                        | −1.1 *                 | 84.7 *                | 166.7 **              | 2.6 *                     | 0                     |
| NakhonSawan2      | −86.5 *               | −98.5 *               | −3.5 *                    | −0.6                   | 91.0 *                | −83.0 *               | 3.1 **                    | 0.2                   |
| Takia1            | −38.7                 | 11.7                  | 0.7                       | −0.3                   | −100.9 *              | −77.7 *               | −3.9 **                   | −0.7 *                 |
| Takia2            | −131.5 *              | −191.8 **             | −5.3 **                   | −1.7 *                 | 83.8 *                | 173.6 **              | 1.9 *                     | 1.9 **                 |
| Takia3            | 299.7 **              | 356.7 **              | 9.0 **                    | 3.7 **                 | −198.6 **             | −179.6 **             | −37.7 **                  | −1.4 **                |
|KKU–PFC1           | −51.1 **              | −36.5                 | −0.8                      | −0.2                   | −33.7                 | 21.7                  | −1.3                      | 0.4                   |
|KKU–PFC2           | 60.3 **               | 119.5 **              | 3.1 *                     | 1.3 **                 | −25.3                 | −1.7                  | −0.8                      | 0.5                   |
|KKU–PFC3           | 57.5 **               | 143.9 **              | 4.2 **                    | 1.2 **                 | 62.4                  | −10.7                 | 2                         | 0.6 *                  |
|KKU–PFC4           | −44.7 *               | −148.3 **             | −4.6 **                   | −1.4 **                | 210.7 **              | 256.0 **              | 7.0 **                    | 0.8                   |
|KKU–PFC5           | −22                   | −78.6 *               | −1.9 *                    | −0.8 *                 | −214.1 **             | −265.4 **             | −6.9 **                   | −2.3 **                |
|SE Female          | 76.8                  | 94.8                  | 2.5                       | 0.9                    | 53.8                  | 71.8                  | 1.5                       | 0.6                   |
|SE Male            | 24.5                  | 56.8                  | 1.6                       | 0.5                    | 69.3                  | 82.6                  | 2.3                       | 0.6                   |

*, ** indicate that the estimates were significantly different from zero at ≥SE and ≥2SE, respectively. SE, standard error of the general combining ability effects; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical scavenging ability; TEAC, trolox equivalent antioxidant activity.
3.2. General Combining Ability Effects

The effects of general combining ability (GCA) are useful for identification of superior parents for direct use in breeding programs [33,44]. The selected inbred lines should have high GCA that is significantly different from zero and a high mean value to predict the best progeny based on GCA. The GCA effects of 10 parental lines for grain yield, agronomic traits, TAC, TPC, and antioxidant activity determined by the DPPH and TEAC methods across two locations are shown in Tables 4 and 5. The female lines had greater ranges of effects than the male lines for all agronomic traits (Table 4). This could be a property of the germplasm or it could be due to the direction of the cross. The cross of all possible combinations and reciprocal cross in diallel mating scheme might differentiate these possibilities.

3.3. Specific Combining Ability Effects and Heterosis

Specific combining ability (SCA) describes the performance of the crosses relative to the averaged performance of hybrids in the experiment. SCA is related to non-additive gene effects such as dominance and epistasis. The hybrids combinations that showed high and significant SCA effects may be valuable in a breeding programs [45–47].

The detailed characterization of the hybrids based on grain yield, husk weigh, cob weight, days to anthesis, TAY in husk and cob, SCA effects for these traits, and heterosis are shown in Tables 6–8. Grain yield is the first priority for most maize breeding programs. Many maize breeders value early maturity to reduce crop loss from late season drought, and early cultivars are easily integrated into cropping systems. However, early maturity should not cause significant yield reduction.

Table 6. Mean performance for agronomic traits, anthocyanin yield, and grain yield of parents, their hybrids, and control cultivars across two locations.

| Lines/Hybrids          | Anthesis Day (day) | Plant Height (cm) | Ear Height (cm) | Husk Mass (kg ha⁻¹) | Cob Mass (kg ha⁻¹) | TAY | Grain Yield (kg ha⁻¹) |
|------------------------|--------------------|-------------------|-----------------|---------------------|--------------------|-----|----------------------|
| NakhonSawan1 × KKU–PFC1| 53                 | 135               | 60              | 624                 | 617                | 0   | 0                    | 2634  |
| NakhonSawan1 × KKU–PFC2| 53                 | 135               | 60              | 624                 | 617                | 0   | 0                    | 2930  |
| Taka1 × KKU–PFC1       | 43                 | 157               | 75              | 394                 | 498                | 5   | 5                    | 2544  |
| Taka1 × KKU–PFC2       | 43                 | 174               | 80              | 468                 | 554                | 5   | 4                    | 2590  |
| Taka3 × KKU–PFC1       | 46                 | 174               | 83              | 480                 | 547                | 4   | 4                    | 2677  |
| Pacific339             | 62                 | 213               | 85              | 1179                | 834                | 0   | 0                    | 6442  |
| CP301                  | 62                 | 191               | 82              | 1097                | 859                | 0   | 0                    | 5869  |
was not able to identify the superior hybrids for multiple traits such as grain yield, early maturity, and anthocyanin concentration in husk and cob. Two hybrids were selected based on high mean values for these traits for further evaluation and study.

| Lines/Hybrids | Anthesis Day (day) | Plant Height (cm) | Ear Height (cm) | Husk Mass (kg ha\(^{-1}\)) | Cob Mass (kg ha\(^{-1}\)) | TAY Husk | TAY Cob | Grain Yield (kg ha\(^{-1}\)) |
|---------------|--------------------|-------------------|----------------|--------------------------|--------------------------|---------|---------|-------------------------|
| Pioneer4546   | 61                 | 195               | 82             | 1117                     | 916                      | 0       | 0       | 6054                    |
| Syngenta248   | 64                 | 193               | 95             | 956                      | 988                      | 0       | 0       | 6221                    |
| Fancy111      | 48                 | 174               | 73             | 577                      | 944                      | 1       | 1       | 3794                    |
| Mean          | 53                 | 184               | 84             | 789                      | 775                      | 3       | 3       | 4062                    |
| LSD (0.05)    | 1                  | 13                | 7              | 78                       | 74                       | 1       | 1       | 134                     |
| SE            | 1                  | 3                 | 2              | 29                       | 19                       | 0       | 0       | 188.7                   |

TAY, total anthocyanin yield (kg CGE/DW ha\(^{-1}\)); LSD, least significant difference value at 0.05 probability; SE, standard error.

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### Table 7. Specific combining ability effects (SCA) for agronomic traits, anthocyanin yield, and grain yield of hybrids across two locations.

| Hybrids | Anthesis Day (day) | Plant Height (cm) | Ear Height (cm) | Husk Mass (kg ha\(^{-1}\)) | Cob Mass (kg ha\(^{-1}\)) | TAY Husk | TAY Cob | Grain Yield (kg ha\(^{-1}\)) |
|---------|--------------------|-------------------|----------------|--------------------------|--------------------------|---------|---------|-------------------------|
| NakhonSawan 1 × KKU-PFC1 | −3.4 ** | −3.4 ** | −0.2 ** | −34.6 ** | 58.3 ** | −1.2 ** | 1.6 ** | −816.4 ** |
| NakhonSawan 1 × KKU-PFC2 | 4.1 ** | 2.3 ** | 0.3 ** | −22.2 ** | −68.5 ** | −1.3 ** | −1.3 ** | −133.7 ** |
| NakhonSawan 1 × KKU-PFC3 | 3.3 ** | 3.8 ** | −0.5 ** | −26.8 ** | 59.2 ** | −0.1 ** | 0.2 | −516.4 ** |
| NakhonSawan 1 × KKU-PFC4 | 1.1 | −0.4 | 0.4 ** | −10.1 | −91.0 ** | −1.1 ** | −1.5 ** | 1138.3 ** |
| NakhonSawan 1 × KKU-PFC5 | −5.0 ** | −2.3 ** | 0.0 | 93.8 ** | 42.0 ** | 3.7 ** | 1.1 ** | 328.2 ** |
| NakhonSawan 2 × KKU-PFC1 | 1.0 | −1.6 ** | 0.4 ** | 66.4 ** | 1.0 | 0.9 ** | 0.5 ** | 239.6 ** |
| NakhonSawan 2 × KKU-PFC2 | −1.0 | −2.1 ** | 0.1 | 13.0 | −40.7 ** | 1.9 ** | −0.5 ** | 233.0 ** |
| NakhonSawan 2 × KKU-PFC3 | 5.4 ** | 2.1 ** | −0.4 | −72.3 ** | 23.4 ** | −0.6 ** | 1.8 ** | 256.8 ** |
| NakhonSawan 2 × KKU-PFC4 | −2.1 ** | −0.4 | 0.3 ** | −55.7 ** | 23.4 ** | −0.6 ** | 1.8 ** | 256.8 ** |
| NakhonSawan 2 × KKU-PFC5 | −4.2 ** | 0.9 | −0.3 | −84.1 ** | 13.8 ** | −2.2 ** | 0.2 | −460.1 ** |
| Takfa1 × KKU-PFC1 | 1.2 | −0.5 | 0.8 ** | −19.3 | −19.0 ** | 1.7 ** | −0.5 ** | 160.7 ** |
| Takfa1 × KKU-PFC2 | −5.5 ** | −5.8 ** | −0.5 | 39.0 ** | 56.5 ** | −0.5 ** | 1.2 | 189.1 ** |
| Takfa1 × KKU-PFC3 | −3.6 ** | 1.5 ** | −0.5 | −18.8 | −33.1 ** | −0.1 | −1.3 | 311.0 ** |
| Takfa1 × KKU-PFC4 | 1.1 | 7.2 ** | 0.1 | 50.0 ** | 12.2 | −0.2 | 0.9 ** | −398.1 ** |
| Takfa1 × KKU-PFC5 | 6.8 ** | −2.4 ** | 0.1 | −50.6 | −16.5 ** | −1.0 ** | −0.2 | −262.8 ** |
| Takfa2 × KKU-PFC1 | −0.7 | 1.9 | −0.3 | −25.6 | −14.4 | 0.7 ** | −0.1 | −209.3 ** |
| Takfa2 × KKU-PFC2 | 1.6 ** | 4.1 ** | −0.1 | −60.7 ** | 46.6 | 0.0 | 1.1 | 141.0 ** |
| Takfa2 × KKU-PFC3 | −5.6 ** | −7.7 ** | 1.1 | 25.6 | −7.8 | −1.1 ** | −0.7 ** | 646.9 ** |
| Takfa2 × KKU-PFC4 | 5.9 ** | −1.2 | −0.1 | 71.8 ** | 13.8 | 0.3 | 1.1 | 44.2 |
| Takfa2 × KKU-PFC5 | −1.2 | 3.0 | −0.6 | −11.1 | −38.2 | 0.1 | −1.3 | −622.7 ** |
| Takfa3 × KKU-PFC1 | 2.0 ** | 3.6 ** | −0.7 | 13.1 | −25.8 ** | −2.3 ** | −1.5 | 625.4 ** |
| Takfa3 × KKU-PFC2 | −0.2 | 0.4 | 0.2 ** | 42.8 ** | −37.0 ** | 1.9 | 1.1 | 73.0 |
| Takfa3 × KKU-PFC3 | 0.5 | 0.3 | 0.4 ** | −52.4 | 22.4 | −0.6 ** | 2.4 ** | −674.6 ** |
| Takfa3 × KKU-PFC4 | −6.0 ** | −5.1 | −0.7 | −55.8 | 41.5 ** | 1.5 ** | −2.2 | −1041.2 ** |
| Takfa3 × KKU-PFC5 | 3.6 ** | 0.8 | 0.8 ** | 52.3 ** | −1.1 | −0.6 ** | 0.2 | 1017.4 ** |
| SE | 0.7 | 0.7 | 0.1 | 9.9 | 7.9 | 0.3 | 0.3 | 108.4 |

*, ** indicate that the estimates were significantly different from zero at ≥SE and ≥2SE, respectively. SE, standard error of the general combining ability effects; TAY, total anthocyanin yield (kg CGE/DW ha\(^{-1}\)).

An important objective of this research project is to find hybrids with high anthocyanin yield. This would be the combination of high anthocyanin concentration in husk and cob and high weights of husk and cob. In this study, superior hybrids for individual traits were identified. However, the study was not able to identify the superior hybrids for multiple traits such as grain yield, early maturity, and high anthocyanins. It may be helpful to implement a selection index in order to develop cultivars with optimal value considering both grain and phytochemical yield.

Although all F\(_1\) hybrids had purple husk and cob depicted by higher mean TAY, TAC, TPC, DPPH, and TEAC than all controls, including purple Fancy111 (Table 9), significant SCA effects (Table 10) and heterosis (Table 11) were observed for some parameters in some hybrids. The hybrids were classified into four groups based on total anthocyanin content (Table 11). Group I had a positive SCA effect for anthocyanins in husk and cob. Group II had positive SCA effect for anthocyanins in husk only. Group III had positive SCA anthocyanins in cob only, and group IV had negative SCA effect for anthocyanins in husk and cob. However, significant and positive or negative SCA effects showed that the hybrids performed better or poorer than what would be expected from the GCA effects of their respective parents. As the major breeding objective was to select the hybrids with high anthocyanins in husk and cobs, two hybrids were selected based on high mean values for these traits for further evaluation and possible release. Takfa3 × KKU-PFC5 had the highest means for TAC, TPC, DPPH, and TEAC in husk, and NakhonSwan2 × KKU-PFC4 had the highest means for TAC, TPC, DPPH, and TEAC in cob.
Table 8. Mid-parents heterosis (MP) and high-parents heterosis (HP) estimates for agronomic traits, anthocyanin yield, and grain yield of hybrids across two locations.

| Hybrids                  | Anthesis Day | Plant Height | Ear Height | Husk Mass | Cob Mass | TAY | Grain Yield |
|--------------------------|--------------|--------------|------------|-----------|----------|-----|-------------|
|                          | MP           | HP           | MP         | HP        | MP       | HP  | MP          | HP  | MP          | HP  |
| NakhonSawan1 × KKU–PFC1  | 10.8*        | 0.3          | 18.2       | 5.1       | 23.2     | 7.4  | 53.9*       | 29.3* | 38.6*       | 24.1* |
| NakhonSawan1 × KKU–PFC2  | 12.7*        | 2.2          | 16.3       | 7.7       | 24.6     | 10.5 | 40.4*       | 14.2* | 38.6*       | 26.4* |
| NakhonSawan1 × KKU–PFC3  | 8.6*         | 0.3          | 22.2       | 8.7       | 28.5     | 11.3 | 39.4*       | 21.9* | 29.5*       | 19.7  |
| NakhonSawan1 × KKU–PFC4  | 7.1          | −0.3         | 22.3       | 9.1       | 30.7     | 11   | 50.6*       | 32.6* | 33.8*       | 22.2* |
| NakhonSawan1 × KKU–PFC5  | 9.1*         | 1.6          | 16.4       | 3.5       | 22.6     | 7.2  | 21.7*       | 17.1* | 47.0*       | 41.3* |
| NakhonSawan2 × KKU–PFC1  | 8.6*         | −2.5         | 23.8*      | 14.4      | 29.2     | 18   | 50.3*       | 23.8* | 43.5*       | 31.1* |
| NakhonSawan2 × KKU–PFC2  | 8.5*         | −2.5         | 18.8       | 14.7      | 18.4     | 10.9 | 61.6*       | 29.3* | 56.0*       | 40.8* |
| NakhonSawan2 × KKU–PFC3  | 7.5*         | −1.6         | 29.2*      | 19.5      | 38.9     | 26.1 | 42.8*       | 23.7  | 43.3*       | 35.8* |
| NakhonSawan2 × KKU–PFC4  | 7.4*         | −0.9         | 26.3*      | 16.4      | 29.3     | 15   | 62.8*       | 39.9* | 35.9*       | 27.7* |
| NakhonSawan2 × KKU–PFC5  | 8.8*         | 0.3          | 27.6*      | 18        | 36.5     | 25.5 | 32.8*       | 21.7* | 26.1*       | 19.0* |
| Takfa × KKU–PFC1         | 7.9*         | −2.2         | 28.6*      | 14.4      | 28.6     | 14   | 57.9*       | 28.5* | 32.7*       | 18.6* |
| Takfa × KKU–PFC2         | 8.8*         | −1.2         | 21.1*      | 11.8      | 29.8     | 17.5 | 45.1*       | 16.0* | 37.3*       | 21.4* |
| Takfa × KKU–PFC3         | 10.1*        | 1.9          | 24.8*      | 10.9      | 14.5     | 0.4  | 51.7*       | 29.9* | 31.2*       | 21.2* |
| Takfa × KKU–PFC4         | 8.0*         | 0.7          | 28.0*      | 14.2      | 26.8     | 9.6  | 41.0*       | 21.6* | 43.2*       | 32.0* |
| Takfa × KKU–PFC5         | 7.4*         | 0            | 27.5*      | 13.2      | 37.2     | 22.4 | 27.1*       | 18.5* | 45.5*       | 37.0* |
| Takfa × KKU–PFC1         | 10.8*        | −1.5         | 25.6*      | 13.3      | 26.2     | 13   | 44.1*       | 20.2* | 47.1*       | 30.9* |
| Takfa × KKU–PFC2         | 11.3*        | −0.9         | 27.2*      | 20.5      | 40.6     | 29.7 | 68.1*       | 39.9* | 48.1*       | 30.0* |
| Takfa × KKU–PFC3         | 8.9*         | −1.2         | 35.5*      | 22.5      | 28.5     | 14.2 | 72.6*       | 49.5* | 38.6*       | 27.3* |
| Takfa × KKU–PFC4         | 7.2*         | −2.1         | 27.1*      | 15.3      | 21.4     | 6.1  | 49.0*       | 29.1* | 48.9*       | 34.9* |
| Takfa × KKU–PFC5         | 10.5*        | 0.9          | 30.0*      | 19        | 28.4     | 16.8 | 38.9*       | 27.7* | 23.8*       | 15.5  |
| Takfa × KKU–PFC1         | 9.6*         | −3.8         | 21.4       | 10.7      | 25.1     | 15.2 | 56.9*       | 35.4* | 42.9*       | 25.6* |
| Takfa × KKU–PFC2         | 11.5*        | −2           | 27.6*      | 21.1      | 20.6     | 12.2 | 70.4*       | 41.9* | 38.6*       | 20.2* |
| Takfa × KKU–PFC3         | 8.2*         | −3.2         | 28.3*      | 16.7      | 31.3     | 19.8 | 77.3*       | 59.5* | 27.0*       | 15.9  |
| Takfa × KKU–PFC4         | 10.3*        | −0.6         | 29.5*      | 17        | 24.2     | 11.2 | 92.9*       | 73.5* | 38.5*       | 25.4* |
| Takfa × KKU–PFC5         | 9.7*         | −1.2         | 21.3       | 10.1      | 22.7     | 13.2 | 73.4*       | 65.9* | 40.9*       | 30.1* |

TAY, total anthocyanin yield; * significant differences based on Student’s t-test at 0.05 probability for MP and HP.
Table 9. Mean performance for total anthocyanin content (TAC), total phenolic content (TPC), and antioxidant activity determined by DPPH and TEAC method of parents and their hybrids evaluated across two locations of parents, their hybrids, and control cultivars across two locations.

| Lines/Hybrids          | TAC (mg CGE/100 g DW) | TPC (mg GAE/100 g DW) | DPPH (%) | TEAC (mmol TE/100 g DW) | TAC (mg CGE/100 g DW) | TPC (mg GAE/100 g DW) | DPPH (%) | TEAC (mmol TE/100 g DW) |
|------------------------|-----------------------|-----------------------|----------|-------------------------|-----------------------|-----------------------|----------|-------------------------|
| NakhonSawan1           | 4                     | 49                    | 1        | 5                       | 3                     | 58                    | 7        | 5                       |
| NakhonSawan2           | 2                     | 41                    | 1        | 6                       | 2                     | 68                    | 3        | 4                       |
| Takfa1                 | 2                     | 25                    | 2        | 6                       | 4                     | 54                    | 2        | 3                       |
| Takfa2                 | 2                     | 20                    | 2        | 5                       | 2                     | 82                    | 2        | 4                       |
| Takfa3                 | 3                     | 75                    | 3        | 6                       | 82                    | 235                   | 8        | 5                       |
| KUU–PFC1               | 1869                  | 2799                  | 64       | 22                      | 1728                  | 2428                  | 61       | 19                      |
| KUU–PFC2               | 1384                  | 1762                  | 45       | 18                      | 1067                  | 1764                  | 44       | 16                      |
| KUU–PFC3               | 1036                  | 1710                  | 42       | 17                      | 735                   | 1531                  | 37       | 17                      |
| KKU–PFC4               | 737                   | 1013                  | 31       | 15                      | 813                   | 1567                  | 37       | 15                      |
| KKU–PFC5               | 894                   | 1284                  | 31       | 15                      | 381                   | 718                   | 16       | 7                       |
| NakhonSawan1 × KUU–PFC1 | 487                   | 843                   | 28       | 12                      | 577                   | 1112                  | 26       | 10                      |
| NakhonSawan1 × KUU–PFC2 | 765                   | 1193                  | 36       | 13                      | 466                   | 1056                  | 24       | 9                       |
| NakhonSawan1 × KUU–PFC3 | 628                   | 1113                  | 31       | 12                      | 611                   | 1095                  | 26       | 10                      |
| NakhonSawan1 × KUU–PFC4 | 131                   | 262                   | 9        | 6                       | 605                   | 1186                  | 25       | 8                       |
| NakhonSawan1 × KUU–PFC5 | 330                   | 554                   | 17       | 8                       | 479                   | 1130                  | 27       | 10                      |
| NakhonSawan2 × KUU–PFC1 | 353                   | 742                   | 22       | 10                      | 270                   | 759                   | 16       | 7                       |
| NakhonSawan2 × KUU–PFC2 | 390                   | 691                   | 20       | 11                      | 640                   | 1165                  | 30       | 11                      |
| NakhonSawan2 × KUU–PFC3 | 504                   | 946                   | 24       | 12                      | 724                   | 648                   | 34       | 12                      |
| NakhonSawan2 × KUU–PFC4 | 599                   | 882                   | 26       | 12                      | 935                   | 1124                  | 37       | 11                      |
| NakhonSawan2 × KUU–PFC5 | 279                   | 601                   | 17       | 8                       | 200                   | 636                   | 14       | 7                       |
| Takfa1 × KUU–PFC1      | 609                   | 1160                  | 31       | 13                      | 293                   | 733                   | 17       | 9                       |
| Takfa1 × KUU–PFC2      | 525                   | 1027                  | 30       | 12                      | 188                   | 632                   | 13       | 7                       |
| Takfa1 × KUU–PFC3      | 379                   | 720                   | 31       | 11                      | 339                   | 897                   | 20       | 8                       |
| Takfa1 × KUU–PFC4      | 381                   | 716                   | 14       | 10                      | 829                   | 1577                  | 34       | 14                      |
| Takfa1 × KUU–PFC5      | 470                   | 790                   | 24       | 10                      | 160                   | 518                   | 13       | 6                       |
| Takfa2 × KUU–PFC1      | 270                   | 360                   | 13       | 8                       | 720                   | 1361                  | 33       | 13                      |
| Takfa2 × KUU–PFC2      | 373                   | 738                   | 22       | 11                      | 625                   | 1129                  | 26       | 12                      |
| Takfa2 × KUU–PFC3      | 440                   | 875                   | 23       | 10                      | 730                   | 1485                  | 32       | 13                      |
| Takfa2 × KUU–PFC4      | 546                   | 878                   | 28       | 10                      | 473                   | 1121                  | 25       | 11                      |
| Takfa2 × KUU–PFC5      | 271                   | 545                   | 16       | 9                       | 184                   | 516                   | 10       | 8                       |
| Takfa3 × KUU–PFC1      | 583                   | 1067                  | 28       | 13                      | 286                   | 889                   | 18       | 10                      |
| Takfa3 × KUU–PFC2      | 805                   | 1303                  | 35       | 15                      | 268                   | 755                   | 18       | 11                      |
| Takfa3 × KUU–PFC3      | 893                   | 1419                  | 38       | 17                      | 222                   | 567                   | 14       | 6                       |
| Takfa3 × KUU–PFC4      | 677                   | 876                   | 27       | 12                      | 525                   | 1018                  | 30       | 8                       |
| Takfa3 × KUU–PFC5      | 1097                  | 1472                  | 43       | 17                      | 219                   | 619                   | 18       | 4                       |
| Pacific339             | 2                     | 165                   | 1        | 9                       | 8                     | 319                   | 4        | 1                       |
| Pioneer4546            | 3                     | 76                    | 2        | 6                       | 2                     | 35                    | 2        | 1                       |
| Syngenta2248           | 3                     | 71                    | 1        | 6                       | 5                     | 54                    | 3        | 1                       |
| Fancy111               | 157                   | 247                   | 6        | 8                       | 125                   | 508                   | 13       | 3                       |
| Mean                   | 472                   | 779                   | 22       | 11                      | 413                   | 830                   | 21       | 8                       |
| LSD (0.05)             | 55                    | 52                    | 2        | 1                       | 50                    | 80                    | 2        | 1                       |
| SE                     | 65                    | 93                    | 2        | 1                       | 58                    | 87                    | 2        | 1                       |

DPPH, 2,2-diphenyl-1-picrylhydrazyl radical scavenging ability; TEAC, trolox equivalent antioxidant activity; LSD, least significant difference value at 0.05 probability; SE, standard Error.
Table 10. Specific combining ability effects (SCA) for total anthocyanin content (TAC), total phenolic content (TPC), and antioxidant activity determined by DPPH and TEAC method of hybrids across two locations.

| Hybrids                     | TAC (mg CGE/100 g DW) | TPC (mg GAE/100 g DW) | DPPH (%) | TEAC (mmol TE/100 g DW) | TAC (mg CGE/100 g DW) | TPC (mg GAE/100 g DW) | DPPH (%) | TEAC (mmol TE/100 g DW) |
|-----------------------------|-----------------------|-----------------------|----------|-------------------------|-----------------------|-----------------------|----------|-------------------------|
|                             |                       |                       |          |                         |                       |                       |          |                         |
| NakhonSawan1 × KKU–PFC1     | -116.1 **             | -160.2 **             | -1.8 **  | 145.8 **                | 279.6 **              | 8.4 **                | 2.6 **   |
| NakhonSawan1 × KKU–PFC2     | -124.2 **             | -93.0 **              | -1.4 **  | -139.6 **               | 34.8                  | -5.1 **               | -0.2 **  |
| NakhonSawan1 × KKU–PFC3     | 19.4                  | -14.2                 | -0.4**   | 12.3                    | -88.0 **              | 0.1                  | -0.3 **  |
| NakhonSawan1 × KKU–PFC4     | -87.2 **              | -55.7 *               | 0.4      | -148.0 **               | -341.0 **             | -8.5 **              | -0.8 **  |
| NakhonSawan1 × KKU–PFC5     | 308.2 **              | 323.1 **              | 3.2      | 129.5 **                | 114.6 **              | 5.1 **               | -1.3 **  |
| NakhonSawan2 × KKU–PFC1     | 69.6 **               | 86.6 **               | 4.9      | 62.8 **                 | -25.7                 | 1.5                  | 0.6      |
| NakhonSawan2 × KKU–PFC2     | -21.1                 | 6.0                   | -0.4     | -250.3 **               | -129.1 **             | -8.8 **              | -2.7 **  |
| NakhonSawan2 × KKU–PFC3     | 187.3 **              | 313.6 **              | 1.9      | -35.6                   | -159.9 **             | -1.4 **              | -0.4     |
| NakhonSawan2 × KKU–PFC4     | -59.0 **              | -282.5 **             | -1.7 **  | 207.5 **                | 217.1 **              | 9.0                  | 1.2 **   |
| NakhonSawan2 × KKU–PFC5     | -176.9 **             | -123.7 **             | -1.4 **  | 15.6                    | 97.6 **               | -0.3                 | 1.3 **   |
| Takfa1 × KKU–PFC1           | 236.7 **              | 280.3 **              | 8.2      | 19.2                    | -56.4                 | -0.6                 | -0.9 **  |
| Takfa1 × KKU–PFC2           | -94.7 **              | -200.6 **             | -4.8     | -0.9                    | 111.6 **              | 300.3 **             | 4.7 **   |
| Takfa1 × KKU–PFC3           | -8.1                  | 25.0                  | -0.1     | -148.3 **               | -238.1 **             | -5.3 **              | -2.2 **  |
| Takfa1 × KKU–PFC4           | -67.5 **              | -61.0 *               | -1.5 **  | -103.6 **               | 8.5                   | 2.2 **               | -0.1     |
| Takfa1 × KKU–PFC5           | -66.4 **              | -43.7 **              | -0.9     | -10.5                   | -12.6                 | -1.1 **              | 2.5 **   |
| Takfa2 × KKU–PFC1           | 236.7 **              | 176.2 **              | 8.2      | 19.2                    | -56.4                 | -0.6                 | -0.9 **  |
| Takfa2 × KKU–PFC2           | 102.0 **              | 176.2 **              | 8.2 **   | 1.0                     | -10.2                 | -2.0 **              | 0.4      |
| Takfa2 × KKU–PFC3           | 21.4                  | 30.1                  | -1.7     | 107.6                   | -207.6 **             | 5.5 **               | 2.2 **   |
| Takfa2 × KKU–PFC4           | -151.2 **             | -306.4 **             | -1.5 **  | -85.1 **                | 36.7                  | -1.2 **              | -1.4 **  |
| Takfa2 × KKU–PFC5           | 3.2                   | 52.2 *                | -0.6     | 121.5 **                | 373.0 **              | 4.8 **               | 0.9 **   |
| Takfa3 × KKU–PFC1           | 24.7                  | 47.9 **               | 0.1      | -145.0 **               | -191.8 **             | -7.0 **              | -2.1 **  |
| Takfa3 × KKU–PFC2           | -292.2 **             | -383.0 **             | -10.3 ** | -2.4                    | -153.1 **             | -7.4 **              | -2.8 **  |
| Takfa3 × KKU–PFC3           | 218.6 **              | 257.5 **              | 8.3      | 2.5 **                  | 170.6 **              | 1.6                  | 3.8 **   |
| Takfa3 × KKU–PFC4           | -47.3 *               | -17.9                 | -7.4     | 256.8 **                | 449.2 **              | 7.9 **               | 4.3 **   |
| Takfa3 × KKU–PFC5           | 210.5 **              | 346.9 **              | 12.1     | 2.0 **                  | -284.6 **             | -7.5 **              | -1.6 **  |
| Takfa3 × KKU–PFC6           | -89.5 **              | -203.6 **             | -2.7     | -21.1                   | 10.3                  | -7.7                 | 3.3 **   |
| SE                          | 29.0                  | 40.3                  | 1.1      | 0.3                     | 28.7                  | 40.6                 | 1.1      |

*, ** indicate that the estimates were significantly different from zero at ≥SE and ≥2SE, respectively; SE, standard error of the general combining ability effects; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical scavenging ability; TEAC, trolox equivalent antioxidant activity.
Table 11. Mid-parents heterosis (MP) and high-parents heterosis (HP) estimates for total anthocyanin content (TAC), total phenolic content (TPC), and antioxidant activity determined by DPPH and TEAC method across two locations.

| Hybrids                  | TAC MP | TPC MP | DPPH MP | TEAC MP | TAC HP | TPC HP | DPPH HP | TEAC HP |
|--------------------------|--------|--------|---------|---------|--------|--------|---------|---------|
| NakhonSawan1 × KKU–PFC1  | −48.5  | −74.2  | −40.8   | −69.9   | −12.7  | −55.5  | −14.6   | −47.9   |
| NakhonSawan1 × KKU–PFC2  | 10.3   | −44.7  | 31.9    | −32.2   | 56.7   | −19.5  | 16.4    | −26.3   |
| NakhonSawan1 × KKU–PFC3  | 21.1   | −39.2  | 26.6    | −34.9   | 44.3   | −25.8  | 13.8    | −26.4   |
| NakhonSawan1 × KKU–PFC4  | −63.2  | −81.5  | −45.8   | −71.4   | −40.0  | −68.8  | −34.5   | −56.4   |
| NakhonSawan1 × KKU–PFC5  | −22.5  | −61.1  | −12.2   | −54.3   | 9.0    | −43.4  | −23.2   | −48.9   |
| NakhonSawan2 × KKU–PFC1  | −62.7  | −81.3  | −47.0   | −73.2   | −32.5  | −65.5  | −27.6   | −54.1   |
| NakhonSawan2 × KKU–PFC2  | −43.1  | −71.5  | −22.9   | −60.5   | −12.1  | −54.7  | −8.5    | −39.7   |
| NakhonSawan2 × KKU–PFC3  | −4.1   | −52.0  | −7.7    | −44.8   | 12.7   | −41.8  | 5.2     | −29.1   |
| NakhonSawan2 × KKU–PFC4  | 59.7   | −20.0  | 64.6    | −14.4   | 59.0   | −17.0  | 14.2    | −20.2   |
| NakhonSawan2 × KKU–PFC5  | −33.9  | −66.9  | −4.3    | −50.7   | 10.6   | −42.3  | −19.3   | −43.7   |
| Takfa1 × KKU–PFC1         | −34.8  | −67.4  | −17.6   | −58.4   | −1.9   | −49.6  | −10.6   | −42.4   |
| Takfa1 × KKU–PFC2         | −23.8  | −61.9  | 15.4    | −41.5   | 28.9   | −33.2  | −0.3    | −33.2   |
| Takfa1 × KKU–PFC3         | −24.9  | −62.4  | −16.6   | −56.7   | 43.8   | −25.2  | −5.9    | −34.9   |
| Takfa1 × KKU–PFC4         | −0.4   | −50.1  | 27.5    | −34.9   | −13.2  | −53.4  | −8.0    | −35.7   |
| Takfa1 × KKU–PFC5         | 4.8    | −47.5  | 21.1    | −38.2   | 49.9   | −21.0  | −7.9    | −34.6   |
| Takfa2 × KKU–PFC1         | −71.2  | −85.6  | −73.7   | −86.7   | −59.6  | −79.1  | −43.6   | −64.5   |
| Takfa2 × KKU–PFC2         | −45.1  | −72.5  | −16.1   | −57.6   | −4.4   | −50.5  | −9.2    | −41.2   |
| Takfa2 × KKU–PFC3         | −15.6  | −57.8  | 0.9     | −49.0   | 3.0    | −46.5  | −7.5    | −38.6   |
| Takfa2 × KKU–PFC4         | 44.7   | −27.5  | 64.1    | −16.4   | 69.5   | −10.3  | 1.2     | −30.9   |
| Takfa2 × KKU–PFC5         | −40.7  | −70.3  | −18.9   | −58.8   | −1.1   | −47.6  | −9.3    | −37.2   |
| Takfa3 × KKU–PFC1         | −37.4  | −68.6  | −25.2   | −61.7   | −13.7  | −55.1  | −2.8    | −38.8   |
| Takfa3 × KKU–PFC2         | 15.1   | −42.3  | 41.9    | −25.9   | 48.6   | −21.0  | 29.7    | −15.2   |
| Takfa3 × KKU–PFC3         | 71.0   | −14.2  | 58.1    | −17.2   | 70.0   | −8.4   | 4.7     | −47.2   |
| Takfa3 × KKU–PFC4         | 81.3   | −8.9   | 63.9    | −12.7   | 61.8   | −12.0  | 12.9    | −22.9   |
| Takfa3 × KKU–PFC5         | 144.7  | 22.8   | 119.7   | 15.6    | 159.6  | 41.1   | 70.3    | 17.8    |

DPPH, 2,2-diphenyl-1-picrylhydrazyl radical scavenging ability; TEAC, trolox equivalent antioxidant activity; * significant differences based on Student’s t-test at 0.05 probability for MP and HP.
High and positive values of heterosis were recorded for all hybrids for grain yield, husk mass, and cob mass. This may be an indicator of genetic divergence between these female lines and male lines used. Similarly, high values of heterosis were reported for all hybrids of elite drought tolerant maize inbred lines possessing genes that are complimentary [27]. The values of heterosis for TAY in husk in some hybrids were higher than for other traits (up to 318.9%). In addition, some hybrids had negative heterosis values for TAY, TAC, TPC, DPPH, and TEAC in both husk and cob. Accumulation of pigments in husk and cob tissue depends on gene combination which may explain the observed heterosis.

The F1 hybrids in this study were crossed between female lines with unpigmented husk and cob and yellow kernels and male lines with purple husk and cob and purple, white, or yellow kernels, resulting in F1 hybrids with purple husk and cob and yellow kernels (Figure 1). It has been observed that the P1 gene [48] affected the expression of color in husk, cob, and kernel in F1 hybrids. The P1 gene has allelic diversity and is involved in the anthocyanin and phlobaphene biosynthetic pathways in plant leaf tissue, pericarp of kernel, and cob glumes [49,50]. The hybrids produced in this study have desirable coloration that meets the needs of the field corn yellow grain market and allows the cobs and husk to be used as feedstock for anthocyanin and phytochemical production.

![Figure 1. F1 hybrid (Takfa3 × KKU–PFC5) and control cultivars showing color of ground husk (A), cross section of ear (B) and kernel.](image)

4. Conclusions

The cross between female parents with normal yellow kernels and cob and male parents with pigmented purple husk and cob generated F1 hybrids with normal yellow kernels and purple husk and cob, and the resulting hybrids can be used for phytochemical production. Takfa3 × KKU–PFC5 and NakhonSuwan2 × KKU–PFC4 were identified as superior hybrids with high anthocyanins and antioxidant activity in husk and cob, respectively. These hybrids will be further evaluated for possible release. Based on GCA, SCA, and heterosis in this study, both additive genes and non-additive genes controlled the inheritance of agronomic traits and phytochemicals, and simultaneous improvement of traits agronomic traits and phytochemicals would be difficult. It may be possible to simultaneously select for agronomic traits and phytochemicals by using a selection index. However, a clear understanding on the value of the phytochemical traits is necessary for development of meaningful weights in the index.

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Appendix A

Figure A1. (a) Temperature, (b) rainfall, (c) relative humidity, and (d) solar radiation at Khon Kaen and Uthai Thani.
Table A1. Soil physical and chemical properties.

| Locations | Soil Type        | pH  (1:1 H₂O) | EC  (1:5 H₂O) (dS/m) | Organic Matter (%) | Total Nitrogen (%) | Available Phosphorus (mg/kg) | Available Potassium (mg/kg) | Exchangeable Calcium (mg/kg) |
|-----------|------------------|---------------|----------------------|-------------------|-------------------|-----------------------------|----------------------------|-----------------------------|
| Khon Kaen | Sandy loam       | 6.34          | 0.06                 | 0.99              | 0.06              | 730.8                       | 730.8                      | 478                         |
| Uthai Thani | Clay loam       | 5.56          | 0.07                 | 1.6               | 0.12              | 85.8                        | 85.8                       | 1860                        |

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