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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. F_1 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 F_1 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
		Female		
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
		Male		
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 F_1 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 40 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 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 °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 °C to reduce the volume from 40 mL to 10 mL and the concentrated solution was stored at −20 °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;

$$\mathrm{TAC} = \frac{\mathrm{A} \times \mathrm{MW} \times \mathrm{DF} \times 10^3}{\varepsilon \times 1} \tag{1}$$

where A = (A510 nm − A700 nm) pH 1.0 − (A510 nm − A700 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; ε = 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;

$$\mathrm{TAY} = \frac{\mathrm{TAC}\ (\mathrm{mg\ CGE} / 100\ \mathrm{g\ DW})}{\mathrm{Dry\ matter\ yield}\ (\mathrm{kg} / \mathrm{ha})} \tag{2}$$

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_2CO_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;

$$\text{Scavenging rate }(\%) = \left(\frac{1-(\text{A1}-\text{As})}{\text{A0}}\right)\times 100 \quad (3)$$

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;

$$\text{Y}_{ijkd} = \mu + L_d + R_k(L_d) + m_i + f_j + m_i \times f_j + L_d \times m_i + L_d \times f_j + L_d \times m_i \times f_j + e_{ijkd} \quad (4)$$

where Y_{ijkd} is the observed value in location d, replication k, male i, and female j; µ 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_{ijkd} 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;

$$\text{MPH} = \left[\frac{\text{F}_1-\text{MP}}{\text{MP}}\right]\times 100 \quad (5)$$

where F_1 is the mean performance of the cross; MP is the mid-parent value given by (P1 + P2)/2; P1 and P2 are the mean values of parent 1 and parent 2 averaged across locations, respectively. HPH was calculated as;

$$HPH = \left[\frac{F_1 - HP}{HP}\right] \times 100 \quad (6)$$

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
							Husk	Cob	kg ha^{-1}
Location (L)	1	1098.9 **	147,951 **	102,998 **	44,161 **	436,476 **	12.2 **	51.5 **	181,288,043 **
Hybrid	24	9.2 **	597 **	143 **	35,840 **	13,226 **	27.7 **	23.1 **	2,850,862 **
Hybrid × L	24	0.5 ns	329 **	89 **	24,363 **	7686 ns	3.4 **	4.4 **	888,633 **
GCA female	4	40.9 **	1512 **	245 **	91,596 **	18,082 **	92.7 **	28.5 **	5,786,196 **
GCA male	4	5.8 **	1571 **	210 **	34,406 **	5055 ns	5.6 **	51.1 **	751,590 **
SCA	16	2.2 **	125 ns	101 **	22,259 **	14,055 **	17.0 **	14.7 **	2,641,847 **
GCA female × L	4	1.1 ns	1326 **	178 **	5176 ns	20,163 **	2.9 **	0.3 ns	1,034,618 **
GCA male × L	4	0.0 ns	88 ns	176 **	10,143 ns	6298 ns	3.5 **	17.4 **	97,905 **
SCA × L	16	6.9 ns	2246 ns	710 ns	523,438 **	78,611 ns	55.0 **	34.9 **	1,049,818 **
Error	96	0.8	128	37	5486	4882	0.2	0.2	14,090
% 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	14	47	41.4	70.8	40.8	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	Husk				Cob			
		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 **	34.2 **
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 **	292.1 **	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		5.2	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		Grain Yield (kg ha^{-1})
						Husk	Cob	
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
Takfa1	−1.2 **	−2.8	−1.1	−23.4	2.4	−0.5	−0.8 *	228.3 *
Takfa2	0.7 *	5.5 *	1.0	14.6	28.0 **	−1.1 *	0.9 **	−484.9 **
Takfa3	1.7 **	3.1	1.4 *	76.3 **	13.0 *	3.1 **	−1.3 **	555.0 **
KKU–PFC1	−0.6 **	0.0	−1.4	−35.4 **	−4.2	0.1	−0.3	165.5 **
KKU–PFC2	−0.1	−11.8 **	−3.8 **	−36.3 **	−1.2	−0.6 **	−0.2	−203.5 **
KKU–PFC3	0.0	6.8 **	2.0 *	16.4 *	−18.9 **	0.2 *	0.5	68.8
KKU–PFC4	0.1	4.7 *	2.8 **	38.1 **	13.6 **	0.5 **	1.8 **	99.0 *
KKU–PFC5	0.6 **	0.3	0.4	17.1 *	10.6 *	−0.2 *	−1.8 **	−129.9 *
SE Female	0.5	3.2	1.3	24.7	11.0	0.8	0.4	196.4
SE Male	0.2	3.2	1.2	15.1	5.8	0.2	0.6	70.8

*, ** 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	Husk				Cob			
	TAC	TPC	DPPH	TEAC	TAC	TPC	DPPH	TEAC
	mg CGE/100 g DW	mg GAE/100 g DW	%	mmol TE/100 g DW	mg CGE/100 g DW	mg GAE/100 g DW	%	mmol TE/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
Takfa1	−38.7	11.7	0.7	−0.3	−100.9 *	−77.7 *	−3.9 **	−0.7 *
Takfa2	−131.5 *	−191.8 **	−5.3 **	−1.7 *	83.8 *	173.6 **	1.9 *	1.9 **
Takfa3	299.7 **	356.7 **	9.0 **	3.7 **	−158.6 **	−179.6 **	−3.7 **	−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})
						Husk	Cob	
NakhonSawan1	53	135	60	624	617	0	0	2634
NakhonSawan2	54	147	66	665	621	0	0	2930
Takfa1	53	135	60	669	655	0	0	2916
Takfa2	55	140	62	648	669	0	0	2205
Takfa3	57	142	65	595	680	0	1	1597
KKU–PFC1	43	174	78	427	515	8	9	2693
KKU–PFC2	43	157	75	394	498	5	5	2544
KKU–PFC3	45	174	80	468	554	5	4	2590
KKU–PFC4	46	174	83	480	547	4	4	2677
KKU–PFC5	46	172	77	610	578	6	2	2630
NakhonSawan1 × KKU–PFC1	53	184	82	799	780	4	5	4347
NakhonSawan1 × KKU–PFC2	54	173	81	712	763	5	4	3899
NakhonSawan1 × KKU–PFC3	53	189	89	758	750	5	5	3802
NakhonSawan1 × KKU–PFC4	53	190	91	819	771	1	5	4667
NakhonSawan1 × KKU–PFC5	54	180	82	750	852	2	4	2996
NakhonSawan2 × KKU–PFC1	53	200	90	815	814	3	2	4352
NakhonSawan2 × KKU–PFC2	53	182	83	852	871	3	6	4441
NakhonSawan2 × KKU–PFC3	53	208	98	805	843	4	6	4665
NakhonSawan2 × KKU–PFC4	53	204	95	930	792	5	8	4627
NakhonSawan2 × KKU–PFC5	54	204	95	844	757	2	2	4192
Takfa1 × KKU–PFC1	52	197	89	856	779	5	2	4975
Takfa1 × KKU–PFC2	52	176	86	764	789	4	1	4684
Takfa1 × KKU–PFC3	54	193	82	861	797	3	3	5292
Takfa1 × KKU–PFC4	53	197	91	805	859	3	7	4001
Takfa1 × KKU–PFC5	53	195	92	809	893	4	1	3930
Takfa2 × KKU–PFC1	54	198	88	766	868	2	6	4285
Takfa2 × KKU–PFC2	55	189	94	870	860	3	5	3262
Takfa2 × KKU–PFC3	54	213	91	945	844	4	6	3976
Takfa2 × KKU–PFC4	54	199	88	839	904	5	4	2921
Takfa2 × KKU–PFC5	56	201	90	864	769	2	1	4871
Takfa3 × KKU–PFC1	55	193	90	799	844	5	2	4608
Takfa3 × KKU–PFC2	56	193	84	831	816	7	2	4437
Takfa3 × KKU–PFC3	55	203	95	924	777	8	2	4349
Takfa3 × KKU–PFC4	56	206	94	1009	847	7	5	6019
Takfa3 × KKU–PFC5	56	193	89	1029	887	11	2	5101
Pacific339	62	213	85	1179	834	0	0	6442
CP301	62	191	82	1097	859	0	0	5869

Table 6. *Cont.*

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})
						Husk	Cob	
Pioneer4546	61	195	82	1117	916	0	0	6054
Syngenta6248	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.

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		Grain Yield (kg ha^{-1})
						Husk	Cob	
NakhonSawan1 × KKU–PFC1	−3.4 **	−3.4 **	−0.2 **	−34.6 **	58.3 **	−1.2 **	1.6 **	−816.4 **
NakhonSawan1 × KKU–PFC2	4.1 **	2.3 **	0.3 **	−22.2 **	−68.5 **	−1.3 **	−1.3 **	−133.7 *
NakhonSawan1 × KKU–PFC3	3.3 **	3.8 **	−0.5 **	−26.8 **	59.2 **	−0.1	0.2	−516.4 **
NakhonSawan1 × KKU–PFC4	1.1 *	−0.4	0.4 **	−10.1 *	−91.0 **	−1.1 **	−1.5 **	1138.3 **
NakhonSawan1 × KKU–PFC5	−5.0 **	−2.3 **	0.0	93.8 **	42.0 **	3.7 **	1.1 **	328.2 **
NakhonSawan2 × KKU–PFC1	1.0 *	−1.6 **	0.4 **	66.4 **	1.0	0.9 **	0.5 **	239.6 **
NakhonSawan2 × KKU–PFC2	0.0	−1.0 *	0.1 *	1.1	2.4	−0.1	−2.1 **	−269.3 **
NakhonSawan2 × KKU–PFC3	5.4 **	2.1 **	−0.4 **	72.3 **	−40.7 **	1.9 **	−0.5 **	233.0 **
NakhonSawan2 × KKU–PFC4	−2.1 **	−0.4	0.3 **	−55.7 **	23.4 **	−0.6 **	1.8 **	256.8 **
NakhonSawan2 × 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 *	−50.8 **	−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 **	−55.9 **	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 10). 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 NakhonSuwan2 × 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	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 *	−2.5 *	−51.1 *	8.6	−45.6	63.8 *	57.9 *
NakhonSawan1 × KKU–PFC2	12.7 *	2.2	16.3	7.7	24.6	10.5	40.4 *	14.2 *	38.6 *	26.4 *	102.7 *	1.8	33.7 *	−32.9	51.1 *	44.7 *
NakhonSawan1 × KKU–PFC3	8.6 *	0.3	22.2	8.7	28.5	11.3	39.4 *	21.9 *	29.5 *	19.7	97.5 *	−0.8	121.9 *	11.5	46.1 *	39.7 *
NakhonSawan1 × KKU–PFC4	7.1	−0.3	22.3	9.1	30.7	11	50.6 *	32.6 *	33.8 *	22.2 *	−30.5	−65	106.6 *	3.7	77.3 *	70.0 *
NakhonSawan1 × KKU–PFC5	9.1 *	1.6	16.4	3.5	22.6	7.2	21.7 *	17.1 *	47.0 *	41.3 *	−3.4	−51.5	369.2 *	136.3 *	14.0 *	9.2 *
NakhonSawan2 × KKU–PFC1	8.6 *	−2.5	23.8 *	14.4	29.2	18	50.3 *	23.8 *	43.5 *	31.1 *	−27.5	−63.7	−49	−74.4	55.2 *	47.1 *
NakhonSawan2 × KKU–PFC2	8.5 *	−2.5	18.8	14.7	18.4	10.9	61.6 *	29.3 *	56.0 *	40.8 *	25	−37.4	110.6 *	5.6	62.8 *	50.4 *
NakhonSawan2 × KKU–PFC3	7.5 *	−1.6	29.2 *	19.5	38.9	26.1	42.8 *	23.7	43.3 *	35.8 *	66.5 *	−16.5	201.6 *	51.3 *	69.8 *	58.0 *
NakhonSawan2 × KKU–PFC4	7.4 *	−0.9	26.3 *	16.4	29.3	15	62.8 *	39.9 *	35.9 *	27.7 *	208.2 *	54.8 *	234.4 *	67.7 *	65.8 *	56.2 *
NakhonSawan2 × KKU–PFC5	8.8 *	0.3	27.6 *	18	36.5	25.5	32.8 *	21.7 *	26.1 *	19.0 *	0.1	−49.8	51.9	−23.5	51.5 *	41.8 *
Takfa1 × KKU–PFC1	7.9 *	−2.2	28.6 *	14.4	28.6	14	57.9 *	28.5 *	32.7 *	18.6 *	35.7	−32	−47.5	−73.7	78.6 *	69.4 *
Takfa1 × KKU–PFC2	8.8 *	−1.2	21.1 *	11.8	29.8	17.5	45.1 *	16.0 *	37.3 *	21.4 *	49.6 *	−25	−44	−71.9	72.3 *	60.3 *
Takfa1 × KKU–PFC3	10.1 *	1.9	24.8 *	10.9	14.5	0.4	51.7 *	29.9 *	31.2 *	21.2 *	44.4	−27.6	32	−33.6	93.2 *	81.1 *
Takfa1 × KKU–PFC4	8.0 *	0.7	28.0 *	14.2	26.8	9.6	41.0 *	21.6 *	43.2 *	32.0 *	67.3 *	−16	219.6 *	60.7 *	44.4 *	36.2
Takfa1 × KKU–PFC5	7.4	0	27.5 *	13.2	37.2	22.4	27.1 *	18.5 *	45.5 *	37.0 *	41	−29.3	39.2 *	−29.6	42.8 *	34.1
Takfa2 × KKU–PFC1	10.8 *	−1.5	25.6 *	13.3	26.2	13	44.1 *	20.2 *	47.1 *	30.9 *	−47.8	−73.9	45.7 *	−26.9	76.0 *	62.3 *
Takfa2 × KKU–PFC2	11.3 *	−0.9 *	27.2 *	20.5	40.6	29.7	68.1 *	35.9 *	48.1 *	30.0 *	23.3	−38.2	103.0 *	1.8	38.1 *	30.8
Takfa2 × KKU–PFC3	8.9 *	−1.2 *	35.5 *	22.5	28.5	14.2	72.6 *	49.5 *	38.6 *	27.3 *	70.6 *	−14.5	200.4 *	50.9 *	66.8 *	56.8 *
Takfa2 × KKU–PFC4	7.2 *	−2.1	27.1 *	15.3	21.4	6.1	49.0 *	29.1 *	48.9 *	34.9 *	154.8 *	27.8	93.5 *	−2.9	20.1 *	10.7 *
Takfa2 × KKU–PFC5	10.5 *	0.9	30.0 *	19	28.4	16.8	38.9 *	27.7 *	23.8 *	15.5	−16.1	−58	45.7	−26.7	102.6 *	89.0 *
Takfa3 × KKU–PFC1	9.6 *	−3.8	21.4	10.7	25.1	15.2	56.9 *	35.4 *	42.9 *	25.6 *	18.1	−40.8	−44.9	−70.5	113.4 *	73.9 *
Takfa3 × KKU–PFC2	11.5 *	−2	27.6 *	21.1	20.6	12.2	70.4 *	41.9 *	38.6 *	20.2 *	147.8 *	24.3	−25.9	−59	111.8 *	78.5 *
Takfa3 × KKU–PFC3	8.2 *	−3.2	28.3 *	16.7	31.3	19.8	77.3 *	59.5 *	27.0 *	15.9	235.2 *	68.3 *	−26.8	−58.4	106.4 *	71.1 *
Takfa3 × KKU–PFC4	10.3 *	−0.6	29.5 *	17	24.2	11.2	92.9 *	73.5 *	38.5 *	25.4 *	290.8 *	96.6 *	78.8 *	0.8	180.8 *	128.0 *
Takfa3 × KKU–PFC5	9.7 *	−1.2	21.3	10.1	22.7	13.2	73.4 *	65.9 *	40.9 *	30.1 *	318.9 *	110.3 *	48.7 *	−2.8	139.4 *	98.5 *

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	Husk				Cob			
	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
KKU–PFC1	1869	2799	64	22	1728	2428	61	19
KKU–PFC2	1384	1762	45	18	1067	1764	44	16
KKU–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 × KKU–PFC1	487	843	28	12	577	1112	26	10
NakhonSawan1 × KKU–PFC2	765	1193	36	13	466	1056	24	9
NakhonSawan1 × KKU–PFC3	628	1113	31	12	611	1095	26	10
NakhonSawan1 × KKU–PFC4	131	262	9	6	605	1186	25	8
NakhonSawan1 × KKU–PFC5	330	554	17	8	479	1130	27	10
NakhonSawan2 × KKU–PFC1	353	742	22	10	270	759	16	7
NakhonSawan2 × KKU–PFC2	390	691	20	11	640	1165	30	11
NakhonSawan2 × KKU–PFC3	504	946	24	12	724	648	34	12
NakhonSawan2 × KKU–PFC4	599	882	26	12	935	1124	37	11
NakhonSawan2 × KKU–PFC5	279	601	17	8	200	636	14	7
Takfa1 × KKU–PFC1	609	1160	31	13	293	733	17	9
Takfa1 × KKU–PFC2	525	1027	30	12	188	632	13	7
Takfa1 × KKU–PFC3	379	720	31	11	339	897	20	8
Takfa1 × KKU–PFC4	381	716	14	10	829	1577	34	14
Takfa1 × KKU–PFC5	470	790	24	10	160	518	13	6
Takfa2 × KKU–PFC1	270	360	13	8	720	1361	33	13
Takfa2 × KKU–PFC2	373	738	22	11	625	1129	26	12
Takfa2 × KKU–PFC3	440	875	23	10	730	1485	32	13
Takfa2 × KKU–PFC4	546	878	28	10	473	1121	25	11
Takfa2 × KKU–PFC5	271	545	16	9	184	516	10	8
Takfa3 × KKU–PFC1	583	1067	28	13	286	889	18	10
Takfa3 × KKU–PFC2	805	1303	35	15	268	755	18	11
Takfa3 × KKU–PFC3	893	1419	38	17	222	567	14	6
Takfa3 × KKU–PFC4	677	876	27	12	525	1018	30	8
Takfa3 × KKU–PFC5	1097	1472	43	17	219	619	18	4
Pacific339	2	165	1	9	8	319	4	1
CP301	2	69	1	5	5	70	3	1
Pioneer4546	3	76	2	6	2	35	2	1
Syngenta6248	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	Husk				Cob			
	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 **	−5.3 **	−1.8 **	145.8 **	279.6 **	8.4 **	2.6 **
NakhonSawan1 × KKU–PFC2	−124.2 **	−93.0 **	−2.8 **	−1.4 **	−139.6 **	34.8	−5.1 **	−0.2
NakhonSawan1 × KKU–PFC3	19.4	−14.2	−0.2	−0.4*	12.3	−88.0 **	0.1	−0.3 *
NakhonSawan1 × KKU–PFC4	−87.2 **	−55.7 *	−2.2 *	0.4 *	−148.0 **	−341.0 **	−8.5 **	−0.8 **
NakhonSawan1 × KKU–PFC5	308.2 **	323.1 **	10.5 **	3.2 **	129.5 **	114.6 **	5.1 **	−1.3 **
NakhonSawan2 × KKU–PFC1	69.6 **	86.6 **	4.9 *	1.6 **	62.8 **	−25.7	1.5 *	0.6 *
NakhonSawan2 × KKU–PFC2	−21.1	6.0	0.9	−0.4 *	−250.3 **	−129.1 **	−8.8 **	−2.7 **
NakhonSawan2 × KKU–PFC3	187.3 **	313.6 **	6.1 **	1.9 **	−35.6 *	−159.9 **	−1.4 *	−0.4
NakhonSawan2 × KKU–PFC4	−59.0 **	−282.5 **	−6.6 **	−1.7 **	207.5 **	217.1 **	9.0	1.2 **
NakhonSawan2 × KKU–PFC5	−176.9 **	−123.7 **	−5.2 **	−1.4 **	15.6	97.6 **	−0.3	1.3 **
Takfa1 × KKU–PFC1	236.7 **	280.3 **	8.2 **	1.9 **	−56.4 *	−58.1 *	−0.6	−0.9 **
Takfa1 × KKU–PFC2	−94.7 **	−200.6 **	−4.8 **	−0.9 **	111.6 **	300.3 **	4.7 **	0.7 **
Takfa1 × KKU–PFC3	−8.1	25.0	0.6	−0.1	−148.3 **	−238.1 **	−5.3 **	−2.2 **
Takfa1 × KKU–PFC4	−67.5 **	−61.0 *	−1.5 *	−0.1	103.6 **	8.5	2.2 **	−0.1
Takfa1 × KKU–PFC5	−66.4 **	−43.7 *	−2.5 **	−0.9 **	−10.5	−12.6	−1.1 *	2.5 **
Takfa2 × KKU–PFC1	102.0 **	176.2 **	2.6 **	0.8 **	1.0	−10.2	−2.0 **	0.4 *
Takfa2 × KKU–PFC2	21.4	30.1	−1.7 *	0.1	107.6 *	−207.6 **	5.5 **	2.2 **
Takfa2 × KKU–PFC3	−151.2 **	−306.4 **	1.0	−1.5 **	−85.1 **	36.7	−1.2 *	−1.4 *
Takfa2 × KKU–PFC4	3.2	52.2 *	−1.7*	−0.6 **	121.5 **	373.0 **	4.8 **	0.9 **
Takfa2 × KKU–PFC5	24.7	47.9 *	−0.1	1.2 **	−145.0 **	−191.8 **	−7.0 **	−2.1 **
Takfa3 × KKU–PFC1	−292.2 **	−383.0 **	−10.3 **	−2.4 **	−153.1 **	−185.6 **	−7.4 **	−2.8 **
Takfa3 × KKU–PFC2	218.6 **	257.5 **	8.3 **	2.5 **	170.6 **	1.6	3.8 **	0.1
Takfa3 × KKU–PFC3	−47.3 *	−17.9	−7.4 **	0.0	256.8 **	449.2 **	7.9 **	4.3 **
Takfa3 × KKU–PFC4	210.5 **	346.9 **	12.1 **	2.0 **	−284.6 **	−257.5 **	−7.5 **	−1.2 **
Takfa3 × KKU–PFC5	−89.5 **	−203.6 **	−2.7 **	−2.1 **	10.3	−7.7	3.3 **	−0.4 *
SE	29.0	40.3	1.1	0.3	28.7	40.6	1.1	0.3

*, ** 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	Husk								Cob							
	TAC		TPC		DPPH		TEAC		TAC		TPC		DPPH		TEAC	
	MP	HP	MP	HP	MP	HP	MP	HP	MP	HP	MP	HP	MP	HP	MP	HP
NakhonSawan1 × KKU–PFC1	−48.5	−74.2	−40.8	−69.9	−12.7	−55.5	−14.6	−47.9	−31.2	−65.5	−8.7	−53.2	−22.7	−56.9	−11.9	−45.9
NakhonSawan1 × KKU–PFC2	10.3	−44.7	31.9 *	−32.2	56.7 *	−19.5	16.4 *	−26.3	−13.0	−56.4	15.8 *	−40.2	−3.9	−44.3	−12.1	−44.4
NakhonSawan1 × KKU–PFC3	21.1 *	−39.2	26.6 *	−34.9	44.3 *	−25.8	13.8 *	−26.4	62.4 *	−18.5	36.1 *	−29.4	18.5	−29.7	−1.1	−38.8
NakhonSawan1 × KKU–PFC4	−63.2	−81.5	−45.8	−71.4	−40.0	−68.8	−34.5	−56.4	46.0 *	−26.8	43.8 *	−25.4	16.2 *	−30.8	−19.4	−49.3
NakhonSawan1 × KKU–PFC5	−22.5	−61.1	−12.2	−54.3	9.0	−43.4	−23.2	−48.9	193.2 *	47.7 *	221.4 *	75.7 *	130.7 *	69.5 *	59.4 *	29.4
NakhonSawan2 × KKU–PFC1	−62.7	−81.3	−47.0	−73.2	−32.5	−65.5	−27.6	−54.1	−68.2	−84.1	−39.0	−68.7	−49.7	−73.7	−33.4	−61.3
NakhonSawan2 × KKU–PFC2	−43.1	−71.5	−22.9	−60.5	−12.1	−54.7	−8.5	−39.7	19.8 *	−40.0	26.9 *	−34.1	30.0 *	−31.1	11.8	−32.4
NakhonSawan2 × KKU–PFC3	−4.1	−52.0	7.7	−44.8	12.7	−41.8	5.2	−29.1	98.9 *	−0.3	−18.9	−57.6	72.9 *	−7.7	21.5	−27.4
NakhonSawan2 × KKU–PFC4	59.7 *	−20.0	64.6 *	−14.4	59.0 *	−17.0	14.2	−20.2	128.1 *	14.3 *	37.7 *	−28.1	88.4 *	1.2	13.6	−30.1
NakhonSawan2 × KKU–PFC5	−33.9	−66.9	−4.3	−50.7	10.6	−42.3	−19.3	−43.7	14.9	−42.2	64.2 *	−9.7	49.5 *	−12.2	32.6	−0.8
Takfa1 × KKU–PFC1	−34.8	−67.4	−17.6	−58.4	−1.9	−49.6	−10.6	−42.4	−65.9	−82.9	−39.9	−69.2	−47.1	−72.6	−22.7	−55.4
Takfa1 × KKU–PFC2	−23.8	−61.9	15.4 *	−41.5	28.9 *	−33.2	−0.3	−33.2	−64.8	−82.3	−30.2	−64.0	−42.7	−69.9	−27.3	−57.2
Takfa1 × KKU–PFC3	−24.9	−62.4	−16.6	−57.6	43.8 *	−25.2	−5.9	−34.9	−7.9	−53.7	12.6	−41.7	3.2	−45.4	−20.9	−54.4
Takfa1 × KKU–PFC4	−0.4 *	−50.1	27.5 *	−34.9	−13.2	−54.3	−8.0	−35.7	101.9 *	1.4 *	92.1 *	−0.6 *	76.4 *	−6.3	54.7 *	−8.5
Takfa1 × KKU–PFC5	4.8	−47.5	21.1	−38.2	49.9 *	−21.0	−7.9	−34.6	−11.4	−55.2	35.6	−26.8	35.0 *	−22.9	32.3	−12.0
Takfa2 × KKU–PFC1	−71.2	−85.6	−73.7	−86.7	−59.6	−79.1	−43.6	−64.5	−15.1	−57.5	11.0	−42.6	4.8	−45.8	15.6	−32.4
Takfa2 × KKU–PFC2	−45.1	−72.5	−16.1	−57.6	−4.4	−50.5	−9.2	−41.2	16.8 *	−41.5	23.0 *	−35.6	15.8 *	−39.3	19.0	−27.9
Takfa2 × KKU–PFC3	−15.6	−57.8	0.9	−49.0	3.0	−46.5	−7.5	−38.6	95.6 *	−1.9	82.1 *	−4.1	65.2 *	−13.0	25.1	−25.8
Takfa2 × KKU–PFC4	44.7 *	−27.5	64.1 *	−16.4	69.5 *	−10.3	1.2	−30.9	16.1	−41.8	33.8	−29.6	26.1 *	−33.2	18.5	−27.8
Takfa2 × KKU–PFC5	−40.7	−70.3	−18.9	−58.8	−1.1	−47.6	−9.3	−37.2	8.5	−45.5	35.6	−23.7	3.2	−40.0	56.4 *	13.9
Takfa3 × KKU–PFC1	−37.4	−68.6	−25.2	−61.7	−13.7	−55.1	−2.8	−38.8	−67.4	−82.8	−30.3	−62.1	−47.1	−70.0	−17.5	−49.4
Takfa3 × KKU–PFC2	15.1	−42.3	41.9 *	−25.9	48.6 *	−21.0	29.7 *	−15.2	−52.7	−74.5	−25.2	−57.4	−32.5	−60.0	6.0	−32.6
Takfa3 × KKU–PFC3	71.0 *	−14.2	58.1 *	−17.2	72.0 *	−8.4	56.5 *	4.7	−47.2	−70.7	−35.8	−63.2	−34.0	−59.7	−41.2	−63.1
Takfa3 × KKU–PFC4	81.3 *	−8.9	63.9 *	−12.7	61.8 *	−12.0	12.9	−22.0	14.1 *	−37.2	11.9 *	−35.3	33.5 *	−18.4	−13.1	−44.1
Takfa3 × KKU–PFC5	144.7 *	22.8 *	119.7 *	15.6 *	159.6 *	41.1 *	70.3 *	17.8 *	3.0	−35.7	31.3	−8.9	44.9 *	8.7 *	−30.7	−43.3

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 F_1 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 F_1 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 F_1 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.

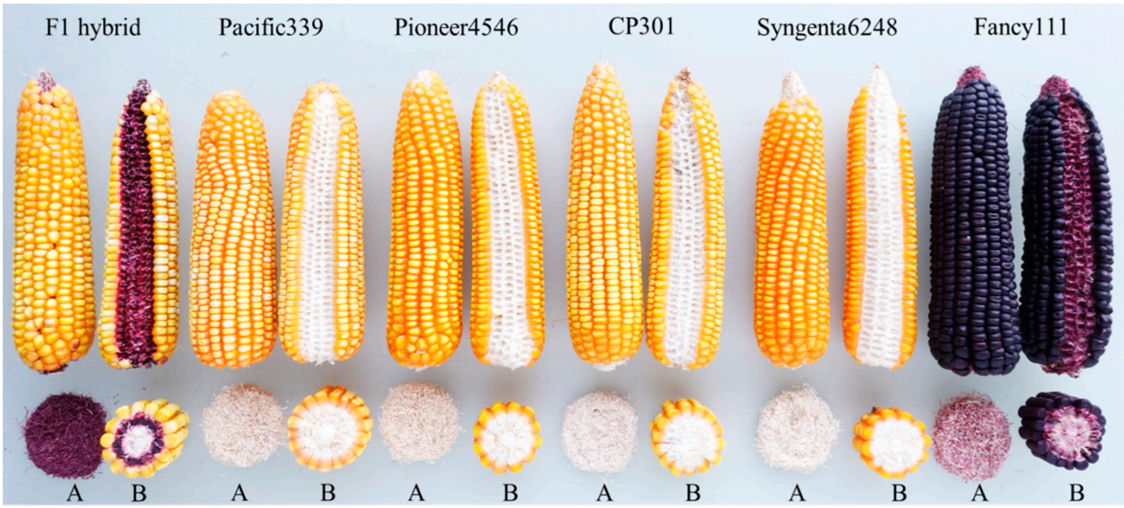

Figure 1. F1 hybrid (Takfa3 × KKU–PFC5) and control cultivars showing color of ground husk (A), cross section of ear (B) and kernel.

4. Conclusions

The cross between female parents with normal yellow kernels and cob and male parents with pigmented purple husk and cob generated F_1 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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Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

Appendix A

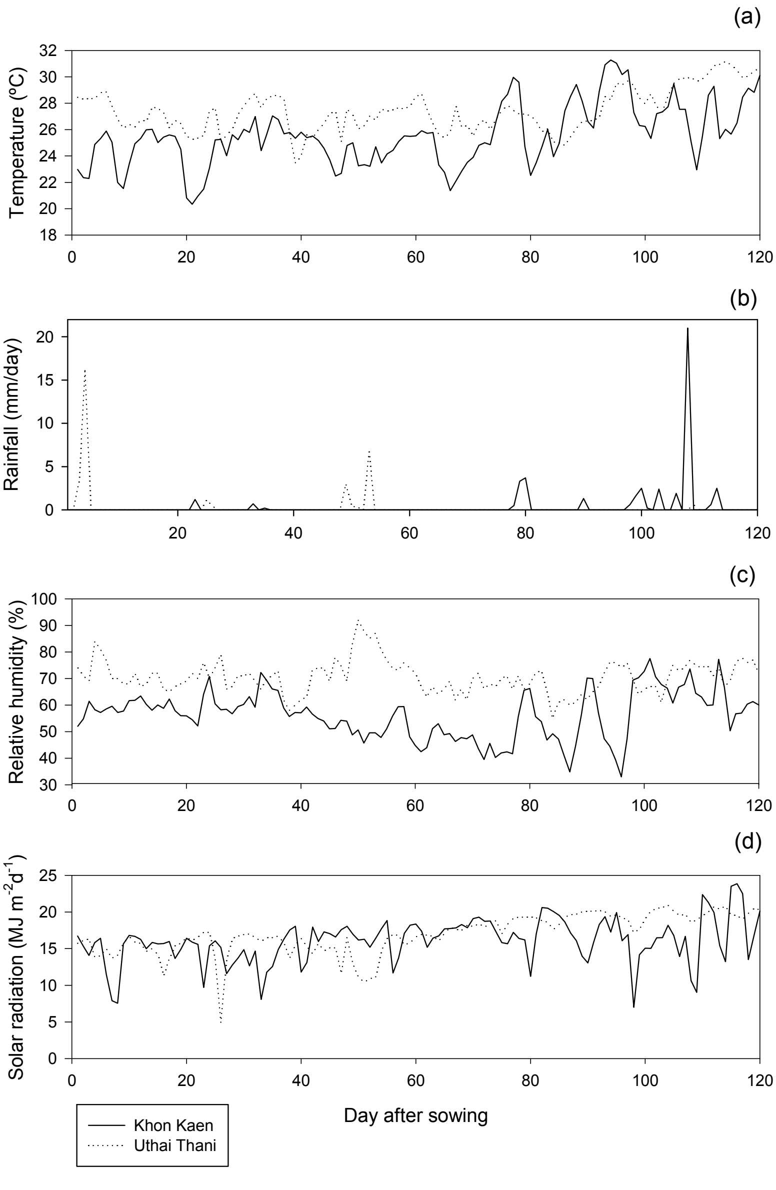

Figure A1. (**a**) Temperature, (**b**) rainfall, (**c**) relative humidity, and (**d**) solar radiation at Khon Kaen and Uthai Thani.

Table A1. Soil physical and chemical properties.

Locations	Soil Type	pH (1:1 H_2O)	EC 1:5 H_2O (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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