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Effects of Mass Selection on Husk and Cob Color in Five Purple Field Corn Populations Segregating for Purple Husks

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Abstract: Improvement of anthocyanin levels in husks and cobs of field corn may add economic value to corn coproducts in commercial production. This study aimed to evaluate the response to four cycles of modified mass selection (MMS) for yield, agronomic traits, total anthocyanin yield (TAY), total anthocyanin content (TAC), total phenolic content (TPC) and antioxidant activity determined by 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity assay (DPPH) and trolox equivalent antioxidant capacity assay (TEAC) in corn husk and cob of five purple field corn populations. The improved populations and check varieties were evaluated at two locations for two seasons in 2017/2018. Selection cycle contributed to a large portion of the total variations for TAC, TPC, DPPH and TEAC in corn husk and cob. All tested populations showed progress for days to anthesis, TAY, TAC, TPC, DPPH and TEAC across four cycles of selection. Lack of significant correlation between agronomic traits and anthocyanin concentrations suggested the independent segregation of these traits. MMS was successfully used to develop field corn populations with improved anthocyanin, antioxidant activities and early flowering without significant yield loss. The populations with the highest selection gains for anthocyanin in husk and cob were identified. Visual selection for dark purple husks and cobs boosted anthocyanin levels and antioxidant activity in selected populations.

Keywords: maize; plant breeding; population improvement; response to selection; phytochemical

1. Introduction

Field corn is an important crop in the world and an excellent source of food and animal feed. The kernels provide abundant nutrients, including carbohydrates, oil and protein [1]. The diverse pigmentation of corn is due to the presence of phytochemicals such as carotenoids [1] and anthocyanins, a class of phlobaphene pigment [2]. Purple corn, in particular, is rich in anthocyanins and related traits such as total phenolic compound and antioxidant activity [3–5], and accumulation of high value compounds such as anthocyanins in specific tissues makes it possible to develop production strategies for high value chemicals using these tissues as feedstock. Anthocyanin is a natural purple pigment and an antioxidant agent [4] with human health benefits. For instance, it reduces the risk of chronic diseases such as cancer [6] and obesity [7]. Safety issues related to purple corn color (PCC), a natural

pigment based on corn anthocyanins, were examined by a clinical study [8], and anthocyanins were suggested as promising natural colorants and supplements [9].

The color in husk and cob of corn is controlled by the pericarp color 1 gene (*p1*). The *p1* gene confers phlobaphene pigmentation in female floral tissue such as corn kernel pericarp and cob glumes [10], husk, silk and tassel glume margins of the male inflorescence [11,12]. The *p1* alleles vary in their tissue specific expression patterns with a two-letter suffix that refers to pericarp and cob glume coloration. For example, the *P1-rr* allele displays red pericarp and red cob glume, the *P1-ww* displays white or colorless pericarp and white cob glumes [13], the *P1-wr* displays white pericarp and red cob glumes [13,14], and the *P1-rw* displays red pericarp and white cob glumes [13,15]. Previous studies assumed that additive genetic effect was important in *P1* gene expression, and the expression was stable across environments [16].

Corn growers leave a large proportion of non-grain tissues in the field after harvest. These parts, including husk and cob, are defined as corn waste. Husk and cob contribute to 20–30% of total corn residue (excluding kernel) and offers a low cost, plentiful and renewable raw material [17]. The waste was used for animal feedstock [18], biofuel or ethanol production [19] and other industrial raw materials [20]. The feeding of the anthocyanin-rich corn silage led to a reduction in aspartate aminotransferase (AST) activity and an increase in superoxide dismutase (SOD) activity in the plasma [21]. In Thailand, the improved population developed by this project is used as roughage and silage for tropical beef cattle [22]. In purple corn, the husk [5,23] and cob [5,23,24] had higher anthocyanin content than the kernel, based on tissue mass [2,25]. Our study used the varieties with purple husk and cob and orange kernels. If conventional breeding can be used to increase levels of pigmentation in the cob and husk, it would improve the feasibility of converting the waste tissues into feedstock for phytochemical production.

Mass selection is a method of population improvement that is based on the selection of desirable individuals in a randomly intermated population [26]. In field corn, this method has been used to enhance ear prolificacy [27] and alter maturity date [28]. Yet, the response to selection in quantitative traits is low [29]. Modified mass selection was developed from classic mass selection in which only selected plants are pollinated. It has been adopted for increasing ear prolificacy of small ear waxy corn [30], ear length [31] and yield [32] of waxy corn. In field corn, the concentrations of some phytochemicals such as oil and protein have been improved through mass selection [33], and the concentrations of methionine [34], oil and fatty acid [35], pro-vitamin A, β -carotene, β -cryptoxanthin and zeaxanthin [36] have also been improved through similar methods. However, population improvement for anthocyanin in purple corn husk and cob has not been reported. Thus, the aim of this study was to evaluate the response to four cycles of modified mass selection in five populations of purple field corn for yield, agronomic traits, total anthocyanin yield (TAY), total anthocyanin content (TAC), total phenolic content (TPC) and antioxidant activity in husk and cob determined by the 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity assay (DPPH) and the trolox equivalent antioxidant capacity assay (TEAC) method.

2. Materials and Methods

2.1. Plant Materials

The corn genotypes used as parents of the base populations consisted of two groups of field corn (Table 1). The genotypes in group 1 including Pacific339, NS3 and NSX were used as maternal parents. Pacific339 is a commercial hybrid from the Pacific company. NS3 and NSX are a commercial hybrid and an elite line, respectively, developed by Nakhon Sawan Field Crops Research Center, Department of Agriculture, Nakhon Sawan, Thailand. All genotypes in this group have orange kernels, green husks and white cobs.

Table 1. Brief information of field corn genotypes used in this study.

Line	Pedigree	Color			Source
		Kernel	Husk	Cob	
Female					
Pacific339		orange	green	white	PS
NSX		orange	green	white	NSFCRC
NS3		orange	green	white	NSFCRC
Male					
PF1	AB/PF	purple	purple	purple	PBRCSA
PF2	TB/KND10//PF	white	purple	purple	PBRCSA
PF3	TL/PF//KND10	yellow	purple	purple	PBRCSA
PF4	DKA/PF//KND	purple	purple	purple	PBRCSA
PF5	WSTS/PF//KND	white	purple	purple	PBRCSA

AB— Khao-Niew-Dum AB; PF— Purple field corn; TB—Tian-Black (Tian-Dum); KND10—Khao-Niew-Dum 10; TL—Tian-Line; DKA—Dok-Koon A; KND—Khao-Niew-Dum; WSTS—White sweeter; PS— Pacific Seeds (Thai) Ltd., Saraburi, Thailand; NSFCRC— Nakhon Sawan Field Crops Research Center, Nakhon Sawan, Thailand; PBRCSA—Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen, Thailand.

Five near inbred lines in group 2 consisting of PF1, PF2, PF3, PF4 and PF5 were used as paternal parents. These lines were improved by the Vegetable Corn Breeding Project, Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen University, Khon Kaen, Thailand. These lines were chosen because of their high anthocyanin concentration and antioxidant activity [5].

Five base populations (C0) of purple field corn including population A (Pacific339 × PF1), population B (Pacific339 × PF2), population C (NSX × PF3), population D (NSX × PF4) and population E (NS3 × PF5) were generated by intercrossing designated orange field corn varieties as maternal parents and purple field corn lines as paternal parents in the dry season of 2014. Each population (A–E) was randomly intermated. The pollen from each population was bulked and used to pollinate all ears in the population in the rainy season 2015 and all pollinated ears in each population were harvested. The F2 generations of the five populations (A–E) were defined as the base populations (C0) for the study.

2.2. Population Improvement

The segregating populations (C0) for cob and husk color contained higher number of pigmented husks and cobs than unpigmented husks and cobs. The five base populations (C0) were further improved through modified mass selection method in the dry season of 2015 to increase husk and cob pigmentation while retaining orange kernels coloration. Modified mass selection was carried out for four consecutive cycles (Figure 1). Phenotypic selection from the C0 generation to the C4 generation was performed at four stages: 1. Selection for good stand and disease-free plants with dark green leaves at pre-reproductive stage; 2. Selection for big purple tassels and early flowering at reproductive stage; 3. Selection for large ears with purple husks at mature stage; 4. Selection for wild-type corn endosperm at dry kernel stage because some populations contained the wx1 allele using potassium iodide (KI) in orange kernels and purple cobs. The selection intensity was 5 to 10 percent in each cycle and the remaining seeds were stored in cool conditions for further evaluation. Modified mass selection for four cycles was completed in the rainy season 2017.

Figure 1. Schematic diagram for population improvement of five purple field corn populations through modified mass selection emphasizing purple husk and cob and orange kernels for four cycles in 2015 to 2017.

2.3. Field Experiment

Five cycles (C0–C4) of five populations (A to E) (25 entries) and three check varieties (KND, KGW and P339) were evaluated in a randomized complete block design (RCBD) with three replications in the dry season (November 2017 to February 2018) and in the rainy season (May to August 2018) at two locations at the Agronomy Field Crop Research Station, Faculty of Agriculture, Khon Kaen University and a farmer’s field in the Uthai Thani Province, Thailand. Irrigation was available at both locations. The climate profile and soil type of these experimental sites were different (Table 2 and Figure A1). The plot size was a 5-m-long four-row plot with a spacing of 0.8 m between rows and 0.25 m between plants within rows. Recommended agricultural practices for commercial corn production were followed.

Table 2. Description of four experimental sites in the study.

Environment	Location	Season	Latitude	Longitude	Altitude (m)	Soil Texture	Average Temperature (°C)
E1	Khon Kaen	Dry 2017/2018	16°28′11.24″ N	102°48′49.46 E	120	Sandy loam	19.1
E2		Rainy 2018					27.7
E3	Uthai Thani	Dry 2017/2018	15°22′57.77″ N	100° 4′42.54″ E	20	Clay loam	26.6
E4		Rainy 2018					29.7

2.4. Sample Preparation and Extraction

Ten randomly selected corn ears from each plot were harvested at physiological maturity (40 days after pollination) and oven-dried at 40 °C for 48 h. The anthocyanins were extracted as described with minor modifications [37,38]. The harvested tissues from each plot were pooled into husk and cob pools and ground into powder and 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 further filtered through Whatman #1 filter paper. After filtration, the retentates were loaded again into 100 mL flasks containing 20 mL of 100% methanol and shaken on a platform shaker for 1 h and filtered through Whatman #1 filter paper. The filtrates

were combined and evaporated in a rotary evaporator to reduce the volume from 40 mL to 10 mL at 40 °C and stored at −20 °C in the dark.

2.4.1. Determination of Total Anthocyanin Content (TAC)

Total monomeric anthocyanin content in each sample was estimated using the pH differential method [39]. A UV-Vis spectrophotometer (GENESYS 10S, Thermo Scientific, 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 milligrams of cyanidin-3-glucoside equivalents per 100 g dry weight (mg CGE/100 g DW) of samples, anthocyanin pigment (cyanidin-3-glucoside equivalents, mg/L) calculated using the following equation:

$$\text{TAC} = \frac{A \times \text{MW} \times \text{DF} \times 10^3}{\epsilon \times l}, \quad (1)$$

where $A = (A_{510 \text{ nm}} - A_{700 \text{ nm}})_{\text{pH}1.0} - (A_{510 \text{ nm}} - A_{700 \text{ nm}})_{\text{pH}4.5}$; 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 $\text{L} \times \text{mol}^{-1} \times \text{cm}^{-1}$, for cyd-3-glu and 10^3 = factor for conversion from g to mg. Then, TAC was converted into TAY by following this equation:

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

2.4.2. Determination of Total Phenolic Content (TPC)

Total phenolic content in each sample was determined according to Folin–Ciocâlteu's phenol reagent (FC reagent) procedure with minor modification [40]. 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. After 120 min at room temperature, the absorbance of the mixture was read at 765 nm using a UV-Visible spectrophotometer. A series of gallic acid solutions (10–100 mg/L) was used as a reference standard. The total phenolic content (TPC) was expressed as mg gallic acid equivalents/100 g dry weight of samples (mg GAE/100 g DW).

2.4.3. Determination of Antioxidant Assay

The 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity assay (DPPH) was determined by measuring the capacity of bleaching a black colored methanol solution of DPPH radicals as reported by [40]. Briefly, the reaction for each sample was prepared by mixing 4.5 mL of the methanolic solution of DPPH (0.065 mM) and 0.5 mL of a sample extract. The reaction was conducted at room temperature for 30 min before the absorbance was recorded at 517 nm. A series of Trolox solutions (100–1000 μM) was used as a reference standard. Values were expressed as millimoles Trolox equivalents (TE) per 100 g of dry weight (mmol TE/100 g DW).

The trolox equivalent antioxidant capacity assay (TEAC) for each sample was determined according to the method described [40] with minor modifications. Briefly, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS^+) radical cations were generated by a reaction of 7 mmol/L ABTS^+ and 2.45 mmol/L potassium persulfate. The reaction mixture was left in the dark at room temperature for 16–24 h before use and the mixture were used within 2 days. The ABTS^+ solution was diluted with methanol to an absorbance of 0.700 ± 0.050 at 734 nm). Fifty microliters of the diluted extract were mixed with 2.0 mL of diluted ABTS^+ solution for 6 min at room temperature and the absorbance was immediately recorded at 734 nm. A series of Trolox solutions (100–1000 μM) was used as a reference standard. The value was expressed as millimoles Trolox equivalents (TE) per 100 g of dry weight (mmol TE/100 g DW).

2.5. Statistical Analysis

The data of 25 entries (5 populations and 5 cycles) tested at four environments were analyzed for yield, agronomic traits, total anthocyanin yield (TAY), total anthocyanin content (TAC), total phenolic content (TPC) and antioxidant activity determined by the DPPH and TEAC methods. Analysis of variance (ANOVA) was performed separately for each location and error variances were tested for homogeneity. The data with variance homogeneity were further combined in combined ANOVA. The statistical model for the analysis is as follows [41];

$$Y_{ijkl} = \mu + B_i + E_j + P_k + C_l + EP_{jk} + EC_{jl} + PC_{kl} + EPC_{jkl} + e_{ij} + e_{ijk} + e_{ijkl} \quad (3)$$

where Y_{ijkl} is the observed value of each measurement, μ is mean of all observations in the experiment, B_i is the effect of the i th block, E_j is the effect of the j th environment, P_k is the effect of the k th population (A–E), C_l is the effect of the l th cycle (C0–C4) effects, EP_{jk} is interaction between environment and population effects, EC_{jl} is interaction between environment and cycle effects, PC_{kl} is interaction between population and cycle effects, EPC_{jkl} is interaction between environment, population and cycle effects, e_{ij} is error of the E_j , e_{ijk} is error of the P_k , EP_{jk} , and e_{ijkl} is error of the C_l , EC_{jl} , PC_{kl} and EPC_{jkl} . Mean differences were compared by least significant difference (LSD) at 0.05-probability level.

Estimates of broad-sense heritability for the five populations in each environment were calculated by partitioning variance components of cycle mean squares to pooled variance (σ_E^2) and genotypic variance (σ_G^2) and then broad-sense heritability estimates (h_b^2) were calculated as follows [42]:

$$h_b^2 = \frac{\sigma_G^2}{\sigma_P^2} \quad (4)$$

$$\sigma_P^2 = \sigma_G^2 + \frac{\sigma_E^2}{r} \quad (5)$$

where h_b^2 is broad sense heritability, σ_G^2 is genotypic variation, σ_P^2 is phenotypic variation and r is no. of replications.

A simple linear regression analysis was analyzed to determine the response to selection. Simple linear regression analysis was calculated, and the estimates were tested for significance according to Gomez and Gomez (1984). The estimated linear regression was calculated as follows:

$$\hat{Y} = a + bX \quad (6)$$

where $\hat{Y}$ is the value of the dependent variable (Y) that is being predicted or explained; a or alpha, a constant, equals the value of Y when the value of X = 0; b or beta, the coefficient of X, the slope of the regression line, how much Y changes for each one-unit change in X; X is the value of the Independent variable (X), what is predicting or explaining the value of Y;

Test for the significance of b value was calculated as follows:

$$S_{y \cdot x}^2 = \frac{\sum y^2 - \frac{(\sum xy)^2}{\sum x^2}}{n - 2}, \quad (7)$$

$$t_b = \frac{b}{\sqrt{\frac{S_{y \cdot x}^2}{\sum x^2}}} \quad (8)$$

where $S_{y \cdot x}^2$ is the residual mean square; n = pairs of values of (Y) and (X); t_b = compare the computed t_b value to the tabular t values with (n-2) degrees of freedom; b is judged to be significantly different

from zero if absolute value of the computed t_b value is greater than the tabular t value at the prescribed level of significance.

Simple linear correlation analysis was calculated, and the correlation coefficients were tested for significance [41]. The simple linear correlation coefficient (r) was calculated as follows:

$$r = \frac{\sum xy}{\sqrt{(\sum x^2)(\sum y^2)}}, \quad (9)$$

where r is declared significant at the level of significant if the absolute value of the computed r value is greater than the corresponding tubular r value at the level of significance with $(n-2)$ degrees of freedom.

3. Results and Discussion

3.1. Analysis of Variance

Mean squares for all parameters from combined analysis of variance across four environments are presented in Tables 3 and 4. Environment, cycle and environment by cycle interaction were highly significant ($p \leq 0.01$) for all traits (Table 3). Population and environment by population interactions were highly significant for most traits except for cob mass. The interactions between population and cycle were also significant for most traits except for cob mass and days to anthesis. The interactions among environment, population and cycle were highly significant for most observed traits except for cob mass, days to anthesis and ear height. All sources of variation (SOV) were highly significant for TAC, TPC, DPPH and TEAC in corn husks and cobs (Table 4).

Table 3. Mean squares for grain yield, agronomic traits and total anthocyanin yield (TAY) of five populations and five cycles evaluated across four environments.

SOV	df	Grain Yield	Husk Mass	Cob Mass	Anthesis Day	Plant Height	Ear Height	TAY	
								Husk	Cob
Environment (E)	3	4807,326 ** (9.9)	1,304,911 ** (63.8)	767,987 ** (56.7)	417.6 ** (66.7)	17,188 ** (60.6)	10,297 ** (58.4)	261.9 ** (5.0)	235.4 ** (17.0)
Error (a)	8	187,251 (1.0)	5007 (0.7)	6332 (1.2)	0.4 (0.2)	423 (4.0)	124 (1.9)	0.5 (0.0)	0.3 (0.1)
Population (P)	4	4,442,353 ** (12.2)	27,888 ** (1.8)	4032ns (0.4)	4.4 ** (0.9)	633 ** (3.0)	755 ** (5.7)	10.5 ** (0.0)	10.1 ** (1.0)
E × P	12	1,227,452 ** (10.1)	32,021 ** (6.3)	7265ns (2.1)	3.4 ** (2.2)	134* (1.9)	98 ** (2.2)	3.0 ** (0.0)	3.2 ** (1.0)
Error (b)	32	315,902 (6.9)	3573 (1.9)	6297 (5.0)	0.7 (1.2)	54 (2.0)	34 (2.1)	0.6 (0.1)	0.3 (0.2)
Cycle (C)	4	2,407,467 ** (6.6)	34,362 ** (2.2)	23,619 ** (2.3)	94.5 ** (20.1)	761 ** (3.6)	1247 ** (9.4)	3114.9 ** (88.0)	580.4 ** (57.0)
E × C	12	755,443 ** (6.2)	12,801 ** (2.5)	16,269 ** (4.8)	6.1 ** (3.9)	301 ** (4.2)	203 ** (4.6)	46.9 ** (4.0)	58.3 ** (17.0)
P × C	16	486,361 * (5.3)	10,016 ** (2.6)	5131ns (2.0)	0.5ns (0.4)	221 ** (4.2)	90 ** (2.7)	3.6 ** (0.0)	4.7 ** (1.0)
E × P × C	48	402,421 * (13.2)	7887 ** (6.2)	4764ns (5.6)	0.4ns (0.9)	66 ** (3.7)	30ns (2.7)	2.0 ** (0.0)	1.9 ** (2.0)
Pooled error (c)	160	261,380 (28.6)	4623 (12.1)	5028 (19.8)	0.4 (3.4)	69 (12.9)	34 (10.3)	0.6 (0.7)	0.3 (1.0)
CV (a) ¹		6.2	8.3	8.7	1.2	10.9	11.7	8.6	14.3
CV (b) ¹		8.1	7.0	8.7	1.5	3.9	6.1	9.7	12.3
CV (c) ¹		7.3	8.0	7.8	1.2	4.4	6.1	9.4	12.3

ns, *, ** not significant and significant at 0.05 and 0.01 probability levels, respectively; the number within the parentheses is relative percentage of sum squares to total sum of squares; ¹ Coefficient of variation (CV) is a measure of (a) environment, (b) population and population by environment interaction, cycle and cycle by environment interaction, cycle by population interaction and (c) cycle by population by environment interaction.

Environment effects accounted for a large portion (56.7% to 66.7%) of the total variations for husk mass, cob mass, days to anthesis, plant height and ear height (Table 3). Populations responded differently to individual environments for husk mass, cob mass, days to anthesis, plant height and ear height (Tables A1, A1 and A3). However, the dry season was higher than the rainy season at both Khon Kaen location and Uthai Thani location. Growing season was the most important environmental

factors affecting performance of this corn populations. The large effects of the environment have also been reported in quality protein maize for yield [43] and flowering traits [44]. The effect of environment also affected anthocyanin content, antioxidant activity in corn cobs [45], total anthocyanin content and total phenolic content in husk, cob, silk and tassel in purple waxy corn [46]. Days to tasseling and days to silking in purple waxy corn were also greatly affected by environment [32]. In this study, as the crop was irrigated, soil moisture may not cause large differences. However, the large differences would be possible due to the growing season and elevation of the experimental sites. Differences in average daily temperature at the sites for example are likely associated with the differences in accumulations of corn heat units (CHU) [47]. CHUs are known to control the developmental program of corn and affect such traits as flowering date [48]. The results suggested that environmental factors were important for the expression of these traits and different genotypes responded differently to the different environmental factors. In addition, environment effects accounted for small portions of the total variations for TAY in husk and cob, TAC, TPC, DPPH and TEAC (Tables 3 and 4). The populations responded in a similar pattern in individual environments (Tables A4–A8).

Table 4. Mean squares for total anthocyanin content (TAC), total phenolic content (TPC) and antioxidant activity determined by the DPPH and the TEAC methods of five populations and five cycles evaluated across four environments.

SOV	df	Husk				Cob			
		TAC	TPC	DPPH	TEAC	TAC	TPC	DPPH	TEAC
Environment (E)	3	224,274 ** (0.4)	440,534 ** (0.7)	174,023 ** (10.5)	2,262,768 ** (4.7)	1,483,875 ** (12.4)	179,389 ** (0.8)	1292 ** (0.3)	2,517,996 ** (10.6)
Error (a)	8	1257 (0.0)	2564 (0.0)	26 (0.0)	1094 (0.0)	720 (0.0)	792 (0.0)	28 (0.0)	2577 (0.0)
Population (P)	4	55,357 ** (0.1)	133,723 ** (0.3)	1512 ** (0.1)	165,909 ** (0.5)	103,969 ** (1.2)	211,480 ** (1.3)	417 ** (0.1)	91,273 ** (0.5)
ExP	12	29,413 ** (0.2)	34,349 ** (0.2)	581 ** (0.1)	18,513 ** (0.2)	35,550 ** (1.2)	60,721 ** (1.1)	600 ** (0.6)	23,256 ** (0.4)
Error (b)	32	662 (0.0)	1154 (0.0)	15 (0.0)	944 (0.0)	234 (0.0)	1309 (0.1)	31 (0.1)	1141 (0.1)
Cycle (C)	4	39,180,000 ** (98.1)	46,300,000 ** (96.6)	1,048,430 ** (84.7)	32,890,000 ** (91.6)	5,970,024 ** (66.3)	13,570,000 ** (82.2)	274,538 ** (93.5)	14,200,000 ** (80.0)
ExC	12	52,823 ** (0.4)	122,870 ** (0.8)	14,594 ** (3.5)	2,170,888 ** (1.8)	425,840 ** (14.2)	461,858 ** (8.4)	2662 ** (2.7)	384,372 ** (6.5)
PxC	16	24,714 ** (0.2)	53,517 ** (0.4)	728 ** (0.2)	30,065 ** (0.3)	47,058 ** (2.1)	85,369 ** (2.1)	238 ** (0.3)	26,893 ** (0.6)
ExPxC	48	12,199 ** (0.4)	35,064 ** (0.9)	715 ** (0.7)	21,193 ** (0.7)	19,106 ** (2.5)	53,219 ** (3.9)	487 ** (2.0)	14,603 ** (1.0)
Pooled error (c)	160	810 (0.1)	1205 (0.1)	20 (0.1)	648 (0.1)	345 (0.2)	1004 (0.2)	23 (0.3)	1029 (0.2)
CV (a) ¹		3.8	4.5	2.9	3.8	6.3	3.5	4.8	7.1
CV (b) ¹		2.7	3.0	2.1	3.5	3.6	4.5	5.0	4.7
CV (c) ¹		3.0	3.1	2.5	2.9	4.3	3.9	4.4	4.5

** significant at 0.01 probability levels; the number within the parentheses is relative percentage of sum squares to total sum of squares; TAC—mg CGE/g 100 DW; TPC—mg GAE/100 g DW; TEAC—mmol TE/100 g DW; DPPH—mmol TE/100 g DW; ¹ Coefficient of variation (CV) is a measure of (a) environment, (b) population and population by environment interaction, cycle and cycle by environment interaction, cycle by population interaction and (c) cycle by population by environment interaction.

Cycle contributed to a large portion (66.3% to 98.1%) of the total variations for TAC, TPC, DPPH and TEAC in corn husk and cob (Table 4). The results demonstrated the effectiveness of improving anthocyanin levels and antioxidant activity through modified mass selection. Genotype effects were predominant in anthocyanin concentrations in both kernel [49] and cob [45] of purple waxy corn and other phytochemicals in field corn such as total phenolics [50] and various carotenoids [51]. The significant interaction between population and cycle with a small contribution indicated the differential responses to selection among the populations.

3.2. Response to Selection

A negative linear response to selection was found for (number of) days to anthesis ranging from −0.66 days per cycle to −0.85 days per cycle (Table 5). Modified mass selection effectively improved early flowering among populations by three or four days. The results supported previous findings on

Spanish synthetic maize populations [28] and purple waxy corn populations [32]. The genetic gain for reduction in days to anthesis increased in association with cycles of selection. Responses to selection among populations were not significant for grain yield, husk mass, cob mass, plant height and ear height (Table 5). A strong emphasis was placed on selecting plants with pigmented husks during population improvement. Yield was selected indirectly through selection for large ears. The improved populations showed appreciable gains for agronomic performance without significant yield loss. The response for yield in these populations would be possible due to unintentional selection [52]. Visual selection of desirable individuals at each stage may increase the selection efficiency. Heritability estimates (h_b^2) for GY ranged from 0 to 0.98 (Table 5). Heritability estimates were high in some environments and low in some environments. The results indicated that environmental effect was important for the variation in grain yield. Heritability estimates (h_b^2) for TAY in husk and cob ranged from 0.97 to 0.99 (Table 5). High heritability estimates indicated that the trait was stable across environments and selection in any environment was effective.

Table 5. Means for grain yield, agronomic traits and total anthocyanin yield (TAY) of (A–E) five populations and (C0–C4) five cycles evaluated across four environments.

Population	Cycle	Grain Yield ²	Husk Mass	Cob Mass	Anthesis Day	Plant Height	Ear Height	TAY ³	
		(kg ha ⁻¹)	(kg ha ⁻¹)	(kg ha ⁻¹)	(DAP)	(cm)	(cm)	Husk	Cob
A	C0	7112bc ¹	840d–h	897a–d	56b	186.6c–g	98.0d–f	1.0l	0.9j
	C1	7571a	842d–h	921a–c	55de	182.8e–h	95.7e–i	2.6k	1.8hi
	C2	7588a	859b–f	897a–d	54fg	185.6d–h	93.8g–k	5.6g	3.3f
	C3	7050bc	860b–f	943ab	54gh	184.3e–h	91.8i–k	13.8d	4.8e
	C4	7069bc	868b–e	947ab	53kl	188.1b–e	94.0f–k	17.5b	8.2b
	ΔC	–43	28	50	–3	1.5	–4.0	16.4	7.3
	b	–61ns	7 **	12ns	–1 **	0.5ns	–1.2ns	4.4 **	1.8 **
r		0.35ns	0.96 **	0.80ns	0.99 **	0.35ns	0.81ns	0.97 **	0.97 **
B	C0	7236ab	869b–e	911a–c	56b	192.2a–c	97.0d–h	0.9l	0.9j
	C1	7616a	909ab	931a–c	55cd	186.9c–f	95.1f–i	2.9jk	1.7i
	C2	7230ab	847c–f	882cd	54fg	181.1f–h	84.0l	5.2gh	2.7g
	C3	6815cd	871b–e	897a–d	53hij	179.3h	80.5l	13.6d	4.7e
	C4	6946b–d	901a–c	950ab	53l	191.8a–d	96.0e–j	18.3a	7.9b
	ΔC	290	32	39	–3	–0.3	–1.0	17.4	7.1
	b	–138ns	3ns	4ns	–1 **	–0.8ns	–1.7ns	4.6 **	1.7 **
r		0.71ns	0.16ns	0.25ns	0.99 **	0.22ns	0.34ns	0.97 **	0.96 **
C	C0	6373e	802gh	883cd	56a	197.3a	99.9b–e	0.8l	1.1j
	C1	6945b–d	874b–e	907a–d	55bc	195.3a	103.0a–c	3.9i	2.1h
	C2	6572de	834e–h	923a–c	54ef	180.1gh	89.3k	4.9h	3.4f
	C3	6289e	809f–h	918a–c	54f–h	187.6b–f	90.3jk	12.4e	6.6d
	C4	6787cd	873b–e	913a–c	53j–l	187.4b–f	97.0d–h	17.4b	7.9b
	ΔC	414	72	31	–3	–9.9	–2.9	16.5	6.8
	b	17ns	8ns	7ns	–1 **	–2.8ns	–1.9ns	4.2 **	1.8 **
r		0.10ns	0.36ns	0.72ns	0.99 **	0.63ns	0.49ns	0.97 **	0.98 **
D	C0	7046bc	791h	851d	57a	182.6e–h	101.1a–d	1.1l	0.8j
	C1	6864b–d	842d–h	911abc	55bc	195.1a	98.6c–f	3.4ij	1.5i
	C2	6961b–d	794gh	908a–d	54ef	186.0c–g	93.6g–k	4.8h	3.4f
	C3	6266e	825e–h	901a–d	54f–h	181.3f–h	92.3h–k	13.2d	7.5c
	C4	7008bc	905ab	953a	53i–k	185.5d–h	95.3e–i	17.7b	10.1a
	ΔC	–38	114	102	–4	2.9	–5.8	16.6	9.2
	b	–67ns	21ns	19ns	–1 **	–0.8ns	–1.8ns	4.3 **	2.4 **
r		0.33ns	0.72ns	0.85ns	0.97 **	0.23ns	0.78ns	0.96 **	0.97 **
E	C0	6965bcd	822e–h	923a–c	57a	197.3a	103.5ab	1.0l	0.9j
	C1	6949bcd	847c–g	951a	55bc	197.0a	105.4a	3.4ij	2.1h
	C2	6994bcd	873b–e	902a–d	54ef	186.1c–g	94.2f–j	6.6f	2.7g
	C3	6834bcd	942a	892b–d	54g–i	192.0a–d	97.4d–g	15.1c	6.4d
	C4	7135bc	893a–d	955a	53j–l	193.6ab	101.6a–d	18.6a	8.2b
	ΔC	170	71	33	–4	–3.7	–1.9	17.6	7.3
	b	23ns	24ns	1ns	–1 **	–1.2ns	–1.2ns	4.7 **	1.9 **
r		0.33ns	0.82ns	0.04ns	0.99 **	0.43ns	0.41ns	0.98 **	1.0 **
Check var.	KND	6882	809	976	45	195.8	97.5	1.6	4.9
	KGW	6858	711	890	48	201.0	95.1	1.4	2.0
	P339	10,871	1537	1216	60	188.2	102.9	0.1	0.0

ns and ** not significant and significant at 0.01 probability levels; ¹ means in a column followed by the same letter are not significantly different at 0.05 probability levels; b = response to selection; TAY—kg CGE/DW ha⁻¹; r—correlation coefficient; DAP—days after planting; ²: broad sense heritability (h_b^2) for grain yield, ranged from 0 to 0.98; ³: broad sense heritability (h_b^2) for TAY in husk and cob, ranged from 0.97 to 0.99; ΔC = selection differential mean C0 and C4.

In corn husk, significant and positive linear responses to selection were observed for TAY (Table 5), TAC, TPC, DPPH and TEAC (Table 6). Among the five populations, population E (NS3 × PF5) showed the most impressive selection progress for phytochemical attributes such as TAY (4.69 kg CGE/DW ha^{−1} cycle^{−1}), TAC (512.88 mg CGE/DW cycle^{−1}), TPC (539.67 mg GAE/100 g DW cycle^{−1}), DPPH (83.10 mmol TE/100 g DW cycle^{−1}) and TEAC (493.37 mmol TE/100 g DW cycle^{−1}).

Table 6. Means for total anthocyanin content (TAC), total phenolic content (TPC) and antioxidant activity determined by the DPPH and the TEAC methods of (A–E) five populations and (C0–C4) five cycles evaluated across four environments.

Population	Cycle	Husk				Cob			
		TAC	TPC	DPPH	TEAC	TAC	TPC	DPPH	TEAC
A	C0	123.6no ¹	179.0p	37.7op	171.0p	96.6n	256.0q	36.6kl	141.5p
	C1	308.4m	484.3m	94.8m	334.4n	196.6k	435.1n	52.7j	357.8k
	C2	643.6i	896.7h	129.6h	554.7k	355.1g	714.4i	91.7g	583.7h
	C3	1601.5e	1812.1d	307.1e	1266.8i	504.2f	1002.5h	157.2d	1009.5e
	C4	1984.8c	2337.3b	344.7c	1893.1c	826.8b	1594.3b	191.1b	1320.2c
	ΔC	1861.2	2158.3	307.0	1722.1	730.2	1338.3	154.6	1178.7
b		501.6 **	564.4 **	82.6 **	437.7 **	176.8 **	324.4 **	41.4 **	300.9 **
	r	0.97 **	0.98 **	0.97 **	0.96 **	1.00 **	0.97 **	0.98 **	0.99 **
B	C0	109.4o	210.1o	40.2o	166.9p	93.1n	262.3q	33.8l	189.1o
	C1	321.9m	471.3m	98.6kl	303.0o	180.6l	504.5m	53.3j	357.8k
	C2	601.4j	647.7j	116.4j	555.8k	312.1h	593.6l	102.0e	502.7i
	C3	1546.4f	1781.0ef	284.4g	1273.2i	515.0f	987.1h	157.3d	976.7f
	C4	2016.5b	2248.2c	325.0d	1826.4e	806.1c	1264.8d	203.3a	1313.5c
	ΔC	1907.1	2038.1	284.8	1659.5	713.0	1002.5	169.5	1124.4
b		503.9 **	538.6 *	75.5 **	428.9 **	176.0 **	248.8 **	44.3 **	286.8 **
	r	0.97 **	0.96 **	0.96 **	0.96 **	0.97 **	0.98 **	0.99 **	0.98 **
C	C0	102.2o	212.5o	35.8p	172.3p	118.9m	315.7p	38.4k	216.8mn
	C1	441.6k	675.6j	99.0k	384.0m	235.1j	596.6l	59.2hi	444.8j
	C2	583.5j	750.3i	123.6i	518.9l	356.5g	727.4i	105.5e	637.0g
	C3	1522.8g	1760.1f	281.2g	1306.3h	694.1e	1127.4f	165.2c	1022.8e
	C4	1972.0c	2342.4b	351.5b	1854.6d	827.1b	1443.6c	189.6b	1327.5c
	ΔC	1869.8	2129.9	315.6	1682.3	708.2	1127.9	151.2	1110.7
b		482.1 **	534.4 **	81.3 **	428.7 *	187.5 **	278.7 **	40.9 **	279.9 **
	r	0.97 **	0.97 **	0.97 **	0.96 **	0.98 **	0.99 **	0.99 **	0.99 **
D	C0	144.8n	260.1n	40.5o	167.1p	97.7n	358.1o	39.8k	238.4m
	C1	394.4l	554.2l	95.4lm	338.0n	169.2l	499.7m	56.0ij	378.0k
	C2	584.4j	736.8i	116.6j	530.8l	363.4g	635.6k	97.5f	589.7h
	C3	1588.4e	1792.9de	296.7f	1387.7g	782.5d	1177.9e	166.1c	1092.0d
	C4	1944.7d	2266.3c	344.5s	1957.5b	1018.2a	1728.8a	201.3a	1531.6a
	ΔC	1799.9	2006.2	304.0	1790.4	920.5	1370.7	161.5	1293.2
b		479.4 **	525.1 **	81.3 **	463.1 *	245.4 **	342.0 *	43.3 **	330.0 **
	r	0.96 **	0.96 **	0.97 **	0.96 **	0.97 **	0.95 *	0.98 **	0.97 **
E	C0	121.0o	277.9n	48.1n	172.3p	101.1n	298.0p	36.7kl	201.8no
	C1	406.6l	590.1k	93.7m	391.6m	224.7j	502.8m	60.7h	328.9l
	C2	752.1h	972.7g	130.6h	648.2j	297.2 i	670.3j	105.8e	582.6h
	C3	1600.0e	1799.4de	296.2f	1489.5f	685.5e	1045.2g	165.1c	1086.2d
	C4	2088.7a	2371.6a	362.3a	2090.2a	831.4b	1425.8c	191.6b	1397.5b
	ΔC	1967.7	2093.7	314.2	1917.9	730.3	1127.8	154.9	1195.7
b		512.9 **	539.7 **	83.1 **	493.4 **	192.1 **	279.8 **	41.4 **	314.9 **
	r	0.98 **	0.98 **	0.97 **	0.97 **	0.97 **	0.98 **	0.99 **	0.98 **
Check var.	KND	192.8	335.9	50.1	229.1	494.4	987.6	130.9	981.0
	KGW	177.8	313.6	46.4	205.7	208.7	440.8	86.8	564.5
	P339	6.0	34.9	14.8	40.9	3.6	87.9	13.0	99.1

ns, * and ** non-significant and significant at 0.05 and 0.01 probability levels, respectively; ¹ means in a column followed by the same letter are not significantly different at 0.05 probability levels; b—response to selection; TAC—mg CGE/g 100 DW; TPC—mg GAE/100 g DW; TEAC—mmol TE/100 g DW; DPPH—mmol TE/100 g DW; r—correlation coefficient; ΔC—selection differential mean C0 and C4.

Similar favorable responses were also found in corn cob. The responses to selection were positive and significant for TAY, TAC, TPC, DPPH and TEAC. Among the five populations, population D (NSX × PF4) had the best responses for TAY (2.44 kg CGE/DW ha^{−1} cycle^{−1}), TAC (245.43 mg CGE/DW cycle^{−1}), TPC (341.96 mg GAE/100 g DW cycle^{−1}), DPPH (43.31 mmol TE/100 g DW cycle^{−1}) and TEAC (330.04 mmol TE/100 g DW cycle^{−1}).

The authors were not able to determine whether the changes observed in the populations are due to the responses to selection or genetic drift. Comparison of the changes in the selected traits and unselected traits may reveal the likelihood of genetic drift [53]. In this study, days to anthesis, purple husk and purple cob were selected intentionally, and the traits changed in the course of the experiment. However, husk mass, cob mass, plant height and ear height were not selected and they were not affected by selection. These observations suggested that the observed changes in the selected traits were most likely due to selection.

The responses to modified mass selection for purple cob and purple husk in this study agreed well with the previous findings on selection of corn for pigmented corn cob [16] and pigmented corn kernel [54]. The authors pointed out that modified mass selection for color cob and color husk could increase anthocyanin content in just one cycle of selection without subsequent cycles. In our study, both genetic gains and mean values of phytochemical traits consistently increased as the populations were advanced in the subsequent selection cycles. Two factors, namely allele fixation by selection and genetic correlation with the targeted traits [52] may be responsible for the responses to selection for these traits.

Anthocyanin concentration in corn is heritable and regulated by multiple dominant genes [55,56], including the P1 dominant allele that controls purple coloration in the corn cob and husk [11,57]. Allele frequency in the base population may be low [21], allele frequency may increase in response to selection for the plants with colored husks. High heritability estimates increased selection efficiency and resulted in the increases in the frequency of pigmented plants in each population and the mean value of the population. It is also possible that the level of pigmentation in individual plants increased as a result of selection. Modified mass selection by visual selection of colored plants is effective in increasing the number of colored plants and color intensity in the improved populations. In addition, the genetic correlation between visually scored color and phytochemical content could be the cause of the increased anthocyanin concentration in the improved populations. Purple color is strongly related to anthocyanin concentration and antioxidant activities in purple waxy corn [49].

Our results demonstrated the efficacy of modified mass selection to increase anthocyanin concentration in the improved populations after four cycles of selection (Figure 2). The C4 populations had higher anthocyanin concentrations than all check varieties. This study suggested that breeders can apply visual selection for dark purple husk and cob to boost the anthocyanin levels and antioxidant activity. Others studies, visual selection was also effective for increasing carotenoid content [1] and anthocyanin content in kernel [54]. The populations will be further improved for uniformity of colored plants and resistance to diseases and pests, and evaluation of stability for yield and phytochemicals will be carried out prior to use of these improved populations.

Figure 2. Comparison of purple husk and cob of (a) purple field corn and (b) white husk and cob of normal field corn.

3.3. Correlation

Most correlation coefficients between total anthocyanin yield (TAY) in husk and cob and agronomic parameters including grain yield, husk mass, cob mass, days to anthesis, plant height and ear height were negative and low or not significant, ranging from -0.03 to -0.44 (Table 7). For husk mass and cob mass, the correlations were low, ranging from -0.03 to -0.12 . For grain yield, days to anthesis, plant height and ear height, the correlations were higher, ranging from -0.18 to -0.44 . The results may indicate that increase in TAY was somewhat detrimental to grain yield and other agronomic parameters, especially for days to anthesis. In corn, early maturity is preferable if it does not cause significant yield reduction [32].

Table 7. Pearson correlation coefficients between grain yield, agronomic traits, total anthocyanin content (TAC), total phenolic content (TPC) and antioxidant activity determined by the DPPH and the TEAC methods and total anthocyanin yield (TAY).

Parameter	Total Anthocyanin Yield (TAY)	
	Husk	Cob
Grain yield	-0.28 ns	-0.35 ns
Husk Mass	-0.03 ns	-0.12 ns
Cob Mass	-0.04 ns	-0.03 ns
Days to anthesis	-0.24 ns	-0.44 *
Plant height	-0.24 ns	-0.18 ns
Ear height	-0.31 ns	-0.27 ns
Husk		
TAC	1.00 **	0.94 **
TPC	1.00 **	0.94 **
DPPH	0.99 **	0.93 **
TEAC	0.99 **	0.95 **
Cob		
TAC	0.94 **	1.00 **
TPC	0.93 **	0.99 **
DPPH	0.94 **	0.97 **
TEAC	0.93 **	0.98 **

ns, * and ** not significant and significant at 0.05 and 0.01 probability levels, respectively.

Days to maturity is positively and significantly correlated with grain yield in many cereal crops such as maize [58], rice [59], sorghum [60] and pearl millet [61]. The low grain yield in early mature genotypes would be because the crops need more time to accumulate biomass and then the accumulated biomass is partitioned into economic yield. However, early mature genotypes can have higher yield than late mature genotypes in some cases. In maize, early mature hybrids with higher seedling strength had higher grain yield than late mature genotypes with poor seed vigor [62], and the genotypes with early maturity and resistance to late season drought had higher grain yield than late maturing genotypes [63].

The negative correlations between TAY and agronomic parameters would be possibly caused by the effect of environments. The stress environments favor the accumulation of anthocyanins of purple corn cob [37]. In maize, low temperature increases anthocyanins in kernel [64]. Similarly, abiotic stresses and nutrient deficiency also increase anthocyanins in corn kernel [65]. In contrast, optimum environmental factors such as plant population density [66], nutrients [67] and soil moisture [68] promote growth and yield of the crop. Independent segregation of anthocyanins and agronomic traits is preferable for selection of corn genotypes with high anthocyanins and high grain yield. However, there may be genetic relationships between these traits.

The TAC, TPC, DPPH, and TEAC in husk and cob were closely correlated with TAY in husk and cob ($r = 0.93^{**}$ to 1.00^{**}). The correlation coefficients between TAY and antioxidant activity (DPPH and

TEAC) in husk (0.99** and 0.99**) were not different from those in cob (0.97** and 0.98**). The results indicated that TAC and TPC in husk and cob contributed to antioxidant and both husk and cob are promising as raw materials for anthocyanin extraction. Previous studies reported a strong association between kernel color and phytochemicals in corn. Purple color of corn was positively and significantly related to anthocyanins and antioxidant activities [25,49], and visually scored orange kernel color was associated with carotenoids [69,70].

Anthocyanins are a naturally occurring type of flavonoid with antioxidant effects in many foods [71], and phenolic compounds are also found in plant tissues including fruits and vegetables [72]. Diversification of food consumption in our diet can therefore reduce the risk of noncommunicable diseases [73]. Corn can be consumed as both vegetable and cereal, and its byproducts can be used for phytochemical extraction in food industry.

Thus, visual screening for dark purple coloration in corn husk and cob could be applied as one of indirect selection criteria in modified mass selection to gain corn genotypes with high anthocyanins and antioxidant activities. The method can be applied in the early cycles of population improvement when the variation of colored plants is still high.

4. Conclusions

Cycle of selection explained a large portion of the total variance for TAC, TPC, DPPH and TEAC in corn husk and cob. All tested populations showed good progress for days to anthesis, TAY, TAC, TPC, DPPH and TEAC over four cycles of selection. Agronomic traits and anthocyanins could be independently used as complementary criteria of selection because these traits were poorly correlated. Modified mass selection was a successful method for development of the improved field corn populations with increased anthocyanin concentration, antioxidant activity and early flowering without losing significant yield. Two improved populations, D (NSX x PF4) and E (NS3 x PF5), had the highest selection gains per cycle for anthocyanin concentration in the corn cob and husk, respectively. This study provided a new insight into the strategy to enhance anthocyanin concentration in the cob and husk tissue. Visual selection for dark purple husk and cob populations segregating for purple plants was effective at boosting anthocyanin levels and antioxidant activity.

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Appendix A

Figure A1. Rainfall, relative humidity, temperature and solar radiation during the crops growth at four environments. (a) Khon Kaen in the dry season 2017/2018, (b) Khon Kaen in the rainy season 2018, (c) Uthai Thani in the dry season 2017/2018 and (d) Uthai Thani in the rainy season 2018.

Table A1. Means for grain yield and husk mass of (A to E) five populations and (C0 to C4) five cycles evaluated in four environments.

Population	Cycle	Grain Yield (kg ha ⁻¹)				Husk Mass (kg ha ⁻¹)			
		E1	E2	E3	E4	E1	E2	E3	E4
A	C0	7031cd ¹	6562c-f	7213b-f	7643a	1000c-e	825ab	752e-g	782b-g
	C1	7660a	7049a-d	7906a-c	7668a	1033b-d	733b-e	880b-d	723e-g
	C2	7667a	7233a	7882a-c	7568ab	1017b-e	719c-e	954ab	745c-g
	C3	7660a	6827a-e	6618c-g	7093a-e	1024b-d	777a-e	904bc	735d-g
	C4	7479ab	6027f	7633a-d	7137a-e	1108ab	789a-d	800c-f	775b-g
b		90ns	-129ns	-45ns	-159 *	21ns	-3ns	12ns	-0.2ns
r		0.27ns	0.19ns	0.02ns	0.79 *	0.61ns	0.01ns	0.06ns	0.00ns
B	C0	6856de	6581b-f	8131ab	7376a	1087bc	809a-b	838b-e	741c-g
	C1	6943d	7196a-c	8622a	7702a	1118ab	764a-e	842b-e	912a
	C2	7265a-d	6665a-f	7358a-e	7630a	1071bc	796a-d	760d-g	761b-g
	C3	6904d	6507d-f	6696c-g	7155a-e	1115ab	703de	900bc	765b-g
	C4	6979cd	6893a-e	6590c-g	7323a-d	1100 a-c	797a-d	890bc	817a-e
b		21ns	-7ns	-501 *	65ns	2ns	-9ns	16ns	1ns
r		0.04ns	0.00ns	0.80 *	0.21ns	0.03ns	0.10ns	0.21ns	0.00ns
C	C0	5634hi	6476d-f	6732c-g	6652e	840f	736a-e	800c-f	831a-c
	C1	6171fg	6907a-e	7198b-f	7502ab	950de	804a-d	897bc	844ab
	C2	6095fg	7026a-e	6231e-g	6935b-e	914ef	840a	817c-e	766b-g
	C3	5507i	6040f	6895b-g	6713de	952de	680e	833b-e	769b-g
	C4	6938d	6534e-f	6959b-g	6717de	1120ab	781a-e	850b-e	742c-g
b		194ns	-75ns	15ns	-66ns	56ns	-3ns	4ns	-25 *
r		0.30ns	0.09ns	0.00ns	0.09ns	0.75ns	0.01ns	0.02ns	0.81 *
D	C0	7044cd	6655a-f	6973b-g	7510ab	1025b-d	807a-d	615h	716fg
	C1	6268fg	6798a-e	7177b-f	7212a-e	1077bc	789a-d	780c-f	721e-g
	C2	7206b-d	6650a-f	6735c-g	7255a-e	1029b-d	749a-e	687f-h	710g
	C3	6001gh	6377ef	5889fg	6798c-e	1080bc	812a-c	643gh	763b-g
	C4	6450ef	7215ab	7246b-f	7122 a-e	1195a	782a-e	848b-e	794b-g
b		-146ns	70ns	-74ns	-119ns	34ns	0ns	33ns	20ns
r		0.20ns	0.13ns	0.05ns	0.54ns	0.63ns	0.03ns	0.29ns	0.74ns
E	C0	6441ef	6504d-f	7548a-e	7365a-d	993c-e	743a-e	785c-f	766b-g
	C1	6998cd	6531d-f	6568c-g	7700a	1055b-d	729b-e	830b-e	776b-g
	C2	6897d	7214ab	6449d-g	7417a-c	1122ab	763a-e	883b-d	726e-g
	C3	7380a-c	6606a-f	5678g	7671a	1113ab	792a-d	1038a	823a-d
	C4	7589ab	6821a-e	6486d-g	7646a	1100a-c	792a-d	868b-e	810b-f
b		268 *	71ns	-301ns	53ns	27ns	16 *	37ns	13ns
r		0.90 *	0.14ns	0.51ns	0.29ns	0.65ns	0.80 *	0.38ns	0.31ns
Check var.	KND	6676	6761	7490	6600	1102	667	697	772
	KGW	7330	6628	6164	7309	1097	512	643	591
	P339	9297	10,773	12,419	10,995	1687	1545	1257	1660

ns and * not significant and significant at 0.05 probability levels; ¹ means in a column followed by the same letter are not significantly different at 0.05 probability levels; b—response to selection; r—correlation coefficient; E1—Khon Kaen, dry season; E2—Khon Kaen, rainy season; E3—Uthai Thani, dry season; E4—Uthai Thani, rainy season.

Table A2. Means for cob mass and anthesis day of (A–E) five populations and (C0–C4) five cycles evaluated in four environments.

Population	Cycle	Cob Mass (kg ha ⁻¹)				Anthesis Day (DAP)			
		E1	E2	E3	E4	E1	E2	E3	E4
A	C0	1017b-e ¹	828a-c	853cd	892a-c	60ab	54b-d	55cd	52a-d
	C1	1067a-c	833a-c	946a-d	838a-d	59cd	53d-g	54d-f	52c-e
	C2	1094ab	777bc	899b-d	819b-d	58d-f	53e-g	54e-g	51de
	C3	1115ab	835a-c	955a-d	866a-d	58e-g	52g	54e-g	51ef
	C4	1117ab	870ab	986a-c	815b-d	56i-k	53fg	52i	50f
b		25 *	9ns	28ns	-13ns	-1 **	-0.4 *	-1 *	-1 **
r		0.89 *	0.17ns	0.69ns	0.37ns	0.97 **	0.80 *	0.89 *	0.96 **

Table A2. Cont.

Population	Cycle	Cob Mass (kg ha ⁻¹)				Anthesis Day (DAP)			
		E1	E2	E3	E4	E1	E2	E3	E4
B	C0	1028a-e	890ab	918a-d	809b-d	59bc	54b-d	56bc	53a-c
	C1	1095ab	828a-c	913a-d	888a-c	59c-e	54b-d	55c-e	52b-e
	C2	1088ab	750c	842d	846a-d	57g-i	54b-e	53f-h	52c-e
	C3	1108ab	777bc	897b-d	805b-d	56h-j	53e-g	53g-i	51de
	C4	1128a	791a-c	976a-d	905ab	55j-L	53fg	52i	50f
b		21 *	-25ns	10ns	11ns	-1 **	-1 *	-1 **	-1 **
r		0.81 *	0.53ns	0.11ns	0.15ns	0.95 **	0.89 *	0.98 **	0.92 **
C	C0	935e	860a-c	905a-d	831b-d	60ab	55b	56ab	53ab
	C1	946de	822a-c	917a-d	942a	59cd	55bc	55cd	52a-d
	C2	1042a-d	797a-c	1030a-b	825b-d	57f-h	54c-f	54e-g	52a-b
	C3	1033a-e	819a-c	948a-d	872a-d	56 h-j	53d-g	53f-h	52a-d
	C4	1100ab	869ab	914a-d	770d	55l	53e-g	52i	52b-e
b		42 *	2ns	5ns	-19ns	-1 **	-1 **	-1 **	-0.2ns
r		0.90 *	0.01ns	0.02ns	0.27ns	1.00 **	0.96 **	0.97 **	0.75ns
D	C0	950de	825a-c	837d	793cd	60ab	56a	57ab	53a
	C1	980c-e	902a	919a-d	843a-d	59c-e	54b-d	55c-e	53ab
	C2	1017b-e	825a-c	921a-d	870a-d	57f-i	54b-e	53f-h	53a-c
	C3	1083ab	777bc	925a-d	819b-d	56i-k	54c-f	53g-i	53a-c
	C4	1119a	837a-c	959a-d	897a-c	55l	54c-f	52hi	52c-e
b		44 **	-10ns	25ns	19ns	-1 **	-1 **	-1 *	-0.3 *
r		0.98 **	0.13ns	0.77 *	0.51ns	0.99 **	0.72 **	0.91 *	0.86 *
E	C0	973c-e	883ab	937a-d	897a-c	61a	55b	57a	53ab
	C1	1038a-d	820a-c	1043a	901a-c	59c-e	54b-e	56ab	52b-e
	C2	1068a-c	794a-c	848cd	898a-c	57f-h	53d-g	55c-e	52b-e
	C3	1028a-e	779bc	938a-d	821b-d	56h-j	53fg	54e-g	52c-e
	C4	1122a	862a-c	988a-c	850a-d	55kl	53e-g	53g-i	51ef
b		29ns	-8ns	-0.2ns	-17ns	-1 **	-1 *	-1 **	-0.4 *
r		0.69ns	0.09ns	0.00ns	0.58ns	0.97 **	0.83 *	0.98 **	0.90 *
Check var.	KND	1155	877	1073	798	50	42	46	42
	KGW	1127	744	868	822	51	46	49	45
	P339	1273	1150	1210	1229	64	57	62	56

ns, * and ** not significant and significant at 0.05 and 0.01 probability levels, respectively; ¹ means in a column followed by the same letter are not significantly different at 0.05 probability levels; b—response to selection; r—correlation coefficient; DAP—days after planting; E1—Khon Kaen, dry season; E2—Khon Kaen, rainy season; E3—Uthai Thani, dry season; E4—Uthai Thani, rainy season.

Table A3. Means for plant height and ear height of (A–E) five populations and (C0–C4) five cycles evaluated in four environments.

Population	Cycle	Plant Height (cm)				Ear Height (cm)			
		E1	E2	E3	E4	E1	E2	E3	E4
A	C0	198.3c-h ¹	162.0a-e	190.7b-f	195.3a-f	101.3e-h	85.3a-d	101.3b-e	104.0a-c
	C1	187.7i	169.0a-e	183.7ef	190.7c-i	99.3f-h	80.0b-h	101.3b-e	102.0a-e
	C2	198.7c-h	170.0a-e	189.3b-f	184.3f-j	104.0b-h	78.3c-j	103.7a-e	89.3h-i
	C3	204.0a-f	159.7c-e	190.0b-f	183.7f-j	102.3c-h	74.0f-j	100.0b-e	91.0f-h
	C4	207.7a-c	163.7a-e	196.3a-f	184.7e-j	103.7b-h	74.3f-j	102.0b-e	96.0b-g
b		3.5ns	-6.0ns	1.8ns	-2.8 *	0.8ns	-2.8 *	0.0ns	-2.7ns
r		0.54ns	0.04ns	0.38ns	0.77 *	0.41ns	0.91 *	0.00ns	0.43ns
B	C0	203.0a-g	172.0a-c	197.7a-e	196.0a-f	98.0g-i	87.0a-c	102.7a-e	100.3a-f
	C1	196.0d-i	169.3a-e	191.0b-f	191.3b-h	101.7d-h	82.3a-f	99.0c-e	97.3b-g
	C2	196.3d-i	169.3a-e	182.0f	176.7j	91.7i	69.3jk	96.3de	78.7jk
	C3	189.0hi	158.0c-e	189.3b-f	181.0h-j	90.7i	64.0k	92.3e	75.0k
	C4	202.0a-g	172.3a-c	195.3a-f	197.7a-d	103.3b-h	79.3c-i	100.3b-e	101.0a-e
b		-0.9ns	-1.1ns	-0.6ns	-0.7ns	-0.03ns	-3.4ns	-1.1ns	-2.1ns
r		0.06ns	0.08ns	0.03ns	0.01ns	0.00ns	0.32ns	0.20ns	0.07ns

Table A3. Cont.

Population	Cycle	Plant Height (cm)				Ear Height (cm)			
		E1	E2	E3	E4	E1	E2	E3	E4
C	C0	204.7a–e	172.0a–c	207.7a	205.0a	104.0b–h	81.7a–g	109.3a–c	104.7ab
	C1	205.3a–d	171.0a–d	203.7ab	201.0a–c	110.0ab	87.0a–c	111.3ab	103.7a–d
	C2	206.7a–c	154.3e	185.0d–f	174.3j	107.7b–e	70.3i–k	100.7b–e	78.7j
	C3	207.3a–c	160.0b–e	198.7a–d	184.3f–j	105.3b–g	72.0h–k	103.3a–e	80.3i–k
	C4	205.7a–d	158.7c–e	200.0a–c	185.3d–j	105.7b–f	75.7e–j	114.0a	92.7e–h
b		0.4ns	–3.8ns	–2.0ns	–5.6ns	–0.1ns	–2.7ns	0.1ns	–4.7ns
	r	0.35ns	0.57ns	0.14ns	0.49ns	0.01ns	0.38ns	0.00ns	0.37ns
D	C0	194.0g–i	159.3c–e	198.7a–d	178.3ij	107.3b–e	84.7a–e	114.3a	98.0b–g
	C1	196.7d–i	175.7ab	207.3a	200.7a–c	97.7hi	84.0a–e	111.7ab	101.0a–e
	C2	194.3f–i	177.7a	196.3a–f	175.7j	105.3b–g	76.7d–j	106.7a–d	85.7h–j
	C3	199.0c–g	154.7e	190.3b–f	181.3g–j	98.0g–i	72.7g–k	104.0a–e	94.7c–h
	C4	195.3e–i	156.0de	197.3a–e	193.3a–h	97.3hi	79.7c–i	107.3a–d	97.0b–g
b		0.5ns	–2.8ns	–2.0ns	1.1ns	–2.0ns	–2.1ns	–2.2ns	–0.8ns
	r	0.15ns	0.16ns	0.26ns	0.02ns	0.42ns	0.45ns	0.69ns	0.05ns
E	C0	202.7a–g	175.7ab	207.0a	203.7ab	107.0b–e	91.0a	107.7a–d	108.3a
	C1	211.7a	172.7a–c	206.3a	197.3a–e	116.0a	87.0a–c	110.7a–c	108.0a
	C2	210.3ab	171.7a–d	186.3c–f	176.0j	109.3a–c	76.0d–j	105.0a–d	86.3h–j
	C3	207.3a–c	164.3a–e	202.3ab	194.0a–g	107.3b–e	79.0c–i	109.0a–c	94.3d–h
	C4	201.0b–g	175.7ab	199.7a–c	198.0a–d	109.0a–d	89.3ab	104.7a–d	103.3a–d
b		–1.0ns	–0.8ns	–1.9ns	–1.5ns	–0.5ns	–1.1ns	–0.8ns	–2.4ns
	r	0.07ns	0.08ns	0.12ns	0.05ns	0.04ns	0.07ns	0.22ns	0.15ns
Check var.	KND	196	178.7	204.7	203.7	105	85	102	98
	KGW	215.3	175.7	214.7	198.3	109	82	99.3	90
	P339	207	173.3	198.3	174	112	87	116	96.7

ns and * not significant and significant at 0.05 probability levels; ¹ means in a column followed by the same letter are not significantly different at 0.05 probability levels; b—response to selection; r—correlation coefficient; DAP—days after planting; E1—Khon Kaen, dry season; E2—Khon Kaen, rainy season; E3—Uthai Thani, dry season; E4—Uthai Thani, rainy season.

Table A4. Means for total anthocyanin yield (TAY) in husk and cob of (A–E) five populations and (C0–C4) five cycles evaluated in four environments.

Population	Cycle	TAY in Husk (kg CGE/DW ha ^{–1})				TAY in Cob (kg CGE/DW ha ^{–1})			
		E1	E2	E3	E4	E1	E2	E3	E4
A	C0	1.0j ¹	0.8l	1.2kl	1.1j	1.5lm	0.5m	1.1j–l	0.5m
	C1	2.9i	2.1jk	3.0ij	2.4i	2.1j–l	1.5l	1.8h–l	1.8l
	C2	7.4f	4.9ef	5.8g	4.1fg	4.7g	2.1i–k	3.9fg	2.3i–l
	C3	17.1d	12.9b	14.3cd	10.9de	6.5f	3.5de	6.1e	3.3e–g
	C4	25.0a	16.0a	15.6bc	13.2c	12.7bc	4.2bc	11.5b	4.2cd
b		6.2 **	4.1 **	4.0 **	3.3 **	2.7 *	1.0 **	2.5 *	0.9 **
	r	0.94 **	0.93 **	0.93 **	0.92 **	0.89 *	0.99 **	0.90 *	0.98 **
B	C0	0.6j	1.1kl	1.0l	0.9j	1.3lm	0.6m	1.1j–l	0.4m
	C1	4.0g–i	2.2i–k	2.9ij	2.7hi	1.9j–l	1.5l	1.4i–l	2.0kl
	C2	8.0f	4.9ef	3.9h–j	4.1fg	3.0hi	2.3h–j	2.9gh	2.7hi
	C3	19.0c	10.9c	13.8d	10.7e	7.1f	2.9fg	5.6e	3.3e–g
	C4	24.7a	15.9a	17.1a	15.5b	12.0c	4.6b	9.6cd	5.4b
b		6.3 **	3.8 **	4.3 **	3.7 **	2.7 *	0.9 **	2.1 *	1.1 **
	r	0.96 **	0.94 **	0.89 **	0.92 **	0.88 *	0.97 **	0.89 *	0.96 **
C	C0	1.0j	1.0kl	0.5l	0.8j	1.5k–m	0.8m	1.3j–l	0.7m
	C1	5.0g	3.4g–i	3.5h–j	3.6gh	2.4ij	1.9j–l	2.3h–j	2.0kl
	C2	7.0f	4.5fg	4.7gh	3.5gh	4.4g	2.5g–i	4.2f	2.4h–k
	C3	16.2d	9.5d	12.1e	11.9d	10.9d	3.1ef	9.6cd	2.9gh
	C4	24.2ab	15.6a	15.9ab	13.8c	13.3b	3.9cd	10.7bc	3.8de
b		5.8 **	3.5 **	3.9 **	3.4 *	3.2 *	0.7 **	2.6 **	0.7 **
	r	0.94 **	0.92 **	0.94 **	0.89 *	0.92 *	0.99 **	0.93 **	0.96 **
D	C0	0.7j	1.5kl	0.9l	1.2j	1.0m	0.8m	0.8l	0.8m
	C1	4.9gh	3.0h–j	2.5jk	3.1g–i	1.4lm	1.7kl	1.1j–l	1.9kl
	C2	7.8f	3.8f–h	4.2hi	3.3g–i	5.1g	2.2i–k	3.0f–h	3.2fg
	C3	18.6c	12.2b	10.1f	11.9d	12.8bc	2.8f–h	10.9b	3.3e–g
	C4	24.3a	15.3a	15.6bc	15.5b	16.0a	4.6b	13.6a	6.0a
b		6.1 **	3.7 *	3.7 **	3.7 *	4.4 **	0.8 **	3.5 *	1.2 **
	r	0.96 **	0.89 *	0.92 **	0.88 *	0.92 **	0.93 **	0.88 *	0.92 **

Table A4. Cont.

Population	Cycle	TAY in Husk (kg CGE/DW ha ⁻¹)				TAY in Cob (kg CGE/DW ha ⁻¹)			
		E1	E2	E3	E4	E1	E2	E3	E4
E	C0	1.1j	1.2kl	0.8l	1.0j	1.0m	0.8m	1.0kl	0.9m
	C1	3.7hi	3.1h-j	3.0ij	3.8g	2.3i-k	1.9j-l	2.2h-k	2.2j-l
	C2	9.5e	6.1e	6.0g	5.0f	3.5h	2.2i-k	2.6hi	2.5h-j
	C3	18.5c	12.1bc	15.8ab	13.8c	9.9e	3.2ef	8.7d	3.6ef
	C4	22.9b	16.5a	16.9ab	18.2a	12.1c	5.5a	10.5bc	4.7c
b		5.9 **	4.0 **	4.5 **	4.6 **	3.0 *	1.1 *	2.6 *	0.9 **
r		0.97 **	0.96 **	0.92 **	0.93 **	0.91 *	0.91 *	0.88 *	0.98 **
Check var.	KND	3.0	0.9	1.6	1.1	6.2	3.6	6.2	3.5
	KGW	2.6	0.7	1.5	0.6	3.4	0.6	2.5	1.4
	P339	0.2	0.0	0.1	0.0	0.0	0.1	0.0	0.0

* and ** significant at 0.05 and 0.01 probability levels; ¹ means in a column followed by the same letter are not significantly different at 0.05 probability levels; b—response to selection; r—correlation coefficient; DAP—days after planting; E1—Khon Kaen, dry season; E2—Khon Kaen, rainy season; E3—Uthai Thani, dry season; E4—Uthai Thani, rainy season.

Table A5. Means for total anthocyanin content (TAC), total phenolic content (TPC) of (A–E) five populations and (C0–C4) five cycles in husk evaluated in four environments.

Population	Cycle	TAC (mg CGE/g 100 DW)				TPC (mg GAE/100 g DW)			
		E1	E2	E3	E4	E1	E2	E3	E4
A	C0	102.7n ¹	93.8n	163.6o	134.3no	188.3n	147.2n	197.2q	183.1rs
	C1	275.5m	288.5l	342.8mn	326.9m	362.2l	562.7jk	381.5o	631.0kl
	C2	730.6i	685.1h	609.0i	549.8i	975.1h	949.2g	822.6j	839.9i
	C3	1672.5f	1657.4d	1586.7e	1489.2f	1927.2ef	1890.1d	1779.7gh	1651.2gh
	C4	2251.8a	2029.5ab	1953.6a	1704.1d	2685.4a	2253.4c	2472.6a	1937.7d
b		569.5 **	524.0 **	482.4 **	430.2 **	655.9 **	554.0 **	594.9 **	452.9 **
r		0.95 **	0.94 **	0.93 **	0.92 **	0.95 **	0.97 **	0.94 **	0.97 **
B	C0	56.4o	137.6mn	117.6p	126.1no	129.9o	308.1m	177.2q	225.1qr
	C1	357.3l	289.9l	348.6m	291.8m	564.1j	477.4n	477.4n	365.2o
	C2	744.6hi	614.8i	511.8k	534.6ij	988.1h	523.2j-l	632.9l	446.5n
	C3	1708.5e	1547.8e	1531.9f	1397.6g	1949.8e	1684.6e	1887.6f	1601.8h
	C4	2244.3a	2001.9bc	1924.7b	1895.0c	2510.7b	2204.7c	2177.3d	2100.1c
b		572.7 **	498.7 **	479.8 **	464.7 **	614.7 **	499.9 *	541.0 *	498.7 *
r		0.96 **	0.93 **	0.92 **	0.93 **	0.98 **	0.86 *	0.91 *	0.87 *
C	C0	118.6n	127.1mn	64.1q	99.1o	297.6m	250.4m	155.9q	146.2s
	C1	523.9j	425.0k	395.8l	421.6l	654.1i	784.0h	537.2m	727.1j
	C2	762.8h	532.9j	575.6j	462.9kl	998.4gh	676.9i	746.2k	579.7lm
	C3	1699.1e	1397.5f	1450.9g	1543.8e	2039.4d	1553.3f	1741.7h	1706.1f
	C4	2156.7b	1993.2bc	1879.6c	1858.7c	2426.0c	2413.8a	2292.3c	2237.7b
b		525.1 **	470.5 **	468.6 **	464.1 *	564.2 **	509.6 *	547.7 **	516.2 *
r		0.96 **	0.92 **	0.95 **	0.90 *	0.96 **	0.89 *	0.95 **	0.89 *
D	C0	71.3o	185.8m	151.5o	170.5n	158.0no	316.1m	267.7p	298.4p
	C1	452.1k	377.2k	322.1n	426.0l	694.1i	492.5kl	389.4o	640.7k
	C2	753.1hi	509.1j	613.6i	461.7kl	994.3h	579.2j	834.1j	539.7m
	C3	1716.6e	1504.9e	1572.3e	1559.7e	2046.3d	1655.1e	1809.0g	1661.3fg
	C4	2031.4d	1957.1c	1841.0d	1949.4b	2418.7c	2337.0ab	2116.1e	2193.4b
b		518.5 **	467.0 *	462.9 **	469.2 *	587.7 **	520.4 *	511.6 **	481.1 *
r		0.96 **	0.90 *	0.93 **	0.89 *	0.97 **	0.88 *	0.93 **	0.87 *
E	C0	104.6n	159.7mn	99.1p	120.4no	396.2l	291.2m	169.7q	254.6pq
	C1	351.5l	428.6k	360.5m	485.7jk	467.3k	668.7i	478.8n	745.8j
	C2	850.5g	798.9g	675.7h	683.4h	1043.8g	953.5g	1003.7i	889.9i
	C3	1660.3f	1535.9e	1523.5f	1680.4d	1899.3f	1656.3e	1760.1h	1882.0e
	C4	2085.2c	2077.4a	1942.8ab	2249.3a	2415.3c	2263.9bc	2399.6b	2407.6a
b		527.0 **	494.3 **	485.0 **	545.3 **	547.0 **	493.3 **	574.1 **	544.2 **
r		0.97 **	0.97 **	0.96 **	0.94 **	0.95 **	0.97 **	0.98 **	0.95 **
Check var.	KND	270.3	132.7	229.6	138.5	534.2	185.1	439.2	185.1
	KGW	238.7	128.9	234.0	109.6	446.2	172.3	462.4	173.7
	P339	11.0	1.2	10.8	1.2	44.7	22.5	44.4	28.1

* and ** significant at 0.05 and 0.01 probability levels; ¹ means in a column followed by the same letter are not significantly different at 0.05 probability levels; b—response to selection; r—correlation coefficient; E1—Khon Kaen, dry season; E2—Khon Kaen, rainy season; E3—Uthai Thani, dry season; E4—Uthai Thani, rainy season.

Table A6. Means for antioxidant activity determined by the DPPH and the TEAC methods of (A–E) five populations and (C0–C4) five cycles in husk evaluated in four environments.

Population	Cycle	DPPH (mmol TE/100 g DW)				TEAC (mmol TE/100 g DW)			
		E1	E2	E3	E4	E1	E2	E3	E4
A	C0	27.3s ¹	34.1p	33.8lm	55.7mn	169.0p	117.8p	294.9o	102.1m
	C1	61.4p	110.0l	46.5j	161.4h	526.6l	168.9no	449.0l	193.2k
	C2	96.2l	169.4g	99.2f	153.7i	743.2j	353.6k	789.3h	332.6i
	C3	250.9e	383.1c	243.5c	351.0g	1651.1e	972.5i	1468.6g	974.8g
	C4	298.0a	423.1b	265.2b	392.4d	2218.4a	1789.1d	1980.6b	1584.4d
b		73.1 **	105.1 **	66.0 *	86.3 *	522.3 **	414.6 *	439.1 **	374.6 *
r		0.92 **	0.94 **	0.91 *	0.91 *	0.95 **	0.86 *	0.95 **	0.88 *
B	C0	22.1t	51.8o	37.4kl	49.6no	136.1p	106.9p	269.0op	155.7kl
	C1	67.5o	153.4h	42.6jk	130.9k	489.4m	223.7m	338.5n	160.4kl
	C2	105.1k	130.3k	84.3h	145.6j	885.5h	319.8kl	748.4i	269.3j
	C3	228.3h	327.6f	224.7d	357.0fg	1573.4f	1044.9h	1461.5g	1013.0fg
	C4	294.1ab	363.7d	239.3c	403.0c	2081.4b	1732.5e	1777.7cd	1713.9c
b		70.5 **	79.8 *	58.6 *	93.3 *	497.5 **	407.2 *	414.0 **	396.9 *
r		0.96 **	0.89 *	0.88 *	0.92 *	0.98 **	0.87 *	0.94 **	0.83 *
C	C0	33.7r	46.3o	18.2n	45.1o	265.9o	151.1op	134.2q	138.0lm
	C1	74.6n	149.8hi	49.8ij	122.0l	612.3k	228.5m	446.6l	248.5j
	C2	117.7i	143.8ij	88.0gh	145.0j	834.1i	361.7k	601.3j	278.4j
	C3	229.8h	329.1f	213.5e	352.3g	1640.6e	954.9i	1619.6ef	1010.1fg
	C4	285.8c	453.7a	263.1b	403.2c	1967.2c	1890.1c	1807.2c	1753.9c
b		65.9 **	99.4 **	65.6 **	94.7 **	443.1 **	420.4 *	451.9 **	399.3 *
r		0.97 **	0.92 **	0.94 **	0.93 **	0.96 **	0.83 *	0.92 *	0.84 *
D	C0	25.6st	48.5o	30.9lm	56.9m	160.7p	115.9p	240.2p	151.6kl
	C1	81.2m	100.4m	46.9ij	153.2i	506.1lm	202.8mn	376.6m	266.6j
	C2	110.1j	143.2ij	92.7fg	120.4l	816.0i	288.8l	728.4i	289.8ij
	C3	235.4g	350.0e	219.0de	382.5e	1720.9d	1141.5g	1641.1e	1047.3f
	C4	289.6bc	427.2b	237.8c	423.3b	1997.7c	2127.2a	1758.7d	1946.3b
b		68.2 **	100.7 **	58.6 *	96.2 *	488.9 **	496.1 *	430.2 *	437.0 *
r		0.96 **	0.93 **	0.91 *	0.85 *	0.96 **	0.83 *	0.92 *	0.82 *
E	C0	38.6q	62.9n	29.2m	61.8m	350.2n	130.2op	79.0r	129.7lm
	C1	62.3p	135.5jk	53.9i	122.9l	581.8k	202.3mn	486.6k	295.6ij
	C2	120.9i	171.1g	99.2f	131.3k	980.8g	422.3j	807.4h	382.2h
	C3	240.8f	367.6d	216.2e	360.4f	1673.9e	1370.1f	1592.9f	1321.1e
	C4	278.9d	451.4a	274.1a	444.9a	2218.2a	2060.6b	2037.7a	2044.4a
b		65.9 **	100.9 **	65.2 **	100.4 *	482.8 **	502.9 *	502.4 **	485.5 *
r		0.95 **	0.94 **	0.95 **	0.90*	0.97 **	0.89 *	0.98 **	0.88 *
Check var.	KND	60.5	44.8	46.8	48.3	353.9	120.0	323.8	118.8
	KGW	49.3	46.8	46.2	43.2	298.6	118.0	302.0	104.3
	P339	12.8	15.6	12.8	17.9	33.5	47.7	36.2	46.2

* and ** significant at 0.05 and 0.01 probability levels; ¹ means in a column followed by the same letter are not significantly different at 0.05 probability levels; b—response to selection; r—correlation coefficient; E1—Khon Kaen, dry season; E2—Khon Kaen, rainy season; E3—Uthai Thani, dry season; E4—Uthai Thani, rainy season.

Table A7. Means for total anthocyanin content (TAC), total phenolic content (TPC) of (A–E) five populations and (C0–C4) five cycles in cob evaluated in four environments.

Population	Cycle	TAC (mg CGE/g 100 DW)				TPC (mg GAE/100 g DW)			
		E1	E2	E3	E4	E1	E2	E3	E4
A	C0	143.8op ¹	59.2l	130.9m–o	52.6o	322.7mn	184.7p	307.9mn	208.7n
	C1	197.4mn	184.8j	187.0kl	217.4m	351.0lm	579.4l	286.3no	523.9k
	C2	432.9i	276.5h	431.5g	279.5k	696.1hi	785.5h	752.4h	623.5ij
	C3	577.8g	416.4e	635.2f	387.5hi	1188.8f	858.9g	1184.2g	777.9gh
	C4	1137.5c	487.1c	1167.4b	515.1d	1504.9e	1603.1a	1492.3d	1776.7a
b		236.8 *	108.7 *	252.1 **	109.5 **	320.2 **	311.6 *	326.7 **	339.0 **
r		0.88 *	0.99 *	0.91 **	0.98 **	0.94 **	0.90 *	0.94 **	0.82 **
B	C0	130.6op	62.1l	122.5n–p	57.1o	260.8op	272.2o	248.1op	268.2m
	C1	169.3no	176.1j	156.9lm	220.1m	384.3l	627.4k	355.3lm	651.2i
	C2	278.0k	308.4g	345.4h	316.6j	568.6j	533.9m	550.2j	721.8h
	C3	643.2f	370.0f	629.0f	418.0fg	1234.1f	660.1jk	1193.8g	860.2f
	C4	1065.4d	576.1b	987.9d	595.0b	1508.4de	1168.5d	1337.2e	1045.1d
b		234.4 *	122.2 **	220.3 **	127.4 **	334.5 **	182.5 *	301.7 **	176.3 **
r		0.88 *	0.97 **	0.92 **	0.99 **	0.93 **	0.78 *	0.93 **	0.93 **

Table A7. Cont.

Population	Cycle	TAC (mg CGE/g 100 DW)				TPC (mg GAE/100 g DW)			
		E1	E2	E3	E4	E1	E2	E3	E4
C	C0	162.6no	86.1k	142.8mn	84.3n	274.5no	391.2n	208.2pq	388.9l
	C1	256.7kl	228.6i	247.6j	207.4m	575.9j	673.1j	580.3j	557.1k
	C2	420.4i	308.2g	405.3g	292.1k	720.3hi	848.5g	684.4i	656.3i
	C3	1058.1d	376.3f	1014.6d	327.6j	1518.7de	743.6hi	1493.0d	754.2gh
	C4	1208.3b	444.5d	1170.2b	485.6e	1718.7b	985.8e	1651.5b	1418.2b
b r		289.3 *	86.4 **	282.2 *	92.3 **	383.1 **	126.0 *	380.0 **	225.6 *
		0.91 *	0.97 **	0.92 *	0.96 **	0.94 **	0.80 *	0.94 **	0.95 *
D	C0	104.6p	93.7k	96.4p	96.2n	211.7pq	511.0m	196.1q	513.5k
	C1	140.1op	191.9j	119.5n–p	225.4lm	459.9k	544.5lm	424.8k	569.6jk
	C2	495.6h	262.6h	326.2hi	369.2i	742.1h	641.6jk	395.5kl	763.2gh
	C3	1180.7b	362.3f	1181.8b	405.4gh	1629.3c	738.5i	1556.0c	787.9g
	C4	1432.8a	552.7b	1418.2a	669.0a	1949.5a	1549.0b	1953.4a	1463.1b
b r		369.7 **	108.8 **	370.6 *	132.6 **	464.5 **	227.0ns	464.6 *	211.8 *
		0.93 **	0.96 **	0.88 *	0.95 **	0.95 **	0.70ns	0.89 *	0.78 *
E	C0	104.6p	96.9k	105.6op	97.1n	179.9q	401.5n	228.9pq	381.7l
	C1	222.9lm	229.4i	207.6k	238.9l	398.4l	624.5k	329.4mn	658.9i
	C2	328.1j	283.4h	301.3i	276.0k	661.1i	921.8f	547.9j	550.3k
	C3	963.9e	412.3e	930.8e	434.8f	1085.4g	865.9g	1282.8f	946.5e
	C4	1080.1d	636.4a	1059.8c	549.1c	1567.0d	1483.5c	1344.2e	1308.5c
b r		269.2 *	126.2 **	263.2 *	110.0 **	346.1 **	240.5 *	318.4 *	214.1 *
		0.90 *	0.95 **	0.89 *	0.98 **	0.97 **	0.88 *	0.90 *	0.86 *
Check var.	KND	542.5	416.9	580.7	437.5	1053.5	874.4	1081.4	941.3
	KGW	307.3	81.0	284.9	161.6	563.4	269.6	467.2	462.9
	P339	3.1	6.5	1.9	2.8	83.5	85.3	76.2	106.8

ns, * and ** not significant and significant at 0.05 and 0.01 probability levels, respectively; ¹ means in a column followed by the same letter are not significantly different at 0.05 probability levels; b—response to selection; r—correlation coefficient; E1—Khon Kaen, dry season; E2—Khon Kaen, rainy season; E3—Uthai Thani, dry season; E4—Uthai Thani, rainy season.

Table A8. Means for antioxidant activity determined by the DPPH and the TEAC methods of (A–E) five populations and (C0–C4) five cycles in cob evaluated in four environments.

Population	Cycle	DPPH (mmol TE/100 g DW)				TEAC (mmol TE/100 g DW)			
		E1	E2	E3	E4	E1	E2	E3	E4
A	C0	38.2n ¹	34.3q	37.9lm	35.8q	176.8o	122.1n	170.0o	97.1o
	C1	47.8m	50.7no	44.3kl	68.0m	404.7l	344.7i–k	372.3m	309.4jk
	C2	97.3i	90.6k	92.2i	86.6k	772.2gh	489.8gh	699.6h	373.1i
	C3	173.6g	144.6f	166.8de	143.9g	1218.7f	795.8e	1263.5f	760.1f
	C4	205.2d	178.4b	197.5c	183.4b	1718.7c	944.4c	1613.0c	1004.9b
b r		46.0 **	38.2 **	44.2 **	37.1 **	389.8 **	209.6 **	377.7 **	266.6 **
		0.95 **	0.98 **	0.94 **	0.97 **	0.98 **	0.99 **	0.98 **	0.96 **
B	C0	30.0o	41.9p	27.8mn	35.7q	232.6n	154.5mn	212.4o	157.1n
	C1	51.2lm	52.1no	42.4l	67.7m	416.6l	281.8kl	391.8m	340.8ij
	C2	84.2j	99.0j	107.9g	117.0i	644.6i	378.9i	556.1jk	431.2h
	C3	182.5f	125.3h	150.8f	170.7cd	1229.3f	597.7f	1186.8g	892.9cd
	C4	208.3cd	207.0a	191.5c	206.4a	1766.8b	1022.6b	1543.0d	921.5c
b r		48.8 **	40.4 **	43.6 **	44.5 **	388.1 **	205.2 *	345.6 **	208.1 **
		0.94 **	0.92 **	0.98 **	0.99 **	0.94 **	0.91 *	0.94 **	0.93 **
C	C0	36.5n	48.1op	24.1n	44.9p	285.0m	196.9m	199.5o	185.9mn
	C1	59.3k	65.7m	54.6k	57.0n	534.7j	374.2ij	531.3k	339.0ij
	C2	98.2i	132.7g	94.1hi	97.2j	755.0h	573.1f	726.7h	493.1g
	C3	193.1e	149.1ef	169.3d	149.3g	1319.6e	747.2e	1280.2f	744.4f
	C4	224.7b	156.8d	211.4b	165.6d–f	1793.9b	884.4cd	1704.4b	927.2c
b r		51.0 *	30.0 *	48.9 **	33.4 **	380.3 **	174.8 **	375.9 **	188.8 **
		0.95 *	0.90 *	0.98 *	0.96 **	0.96 **	1.00 **	0.98 **	0.99 **
D	C0	25.6op	57.1n	23.0n	53.4no	218.5no	280.4kl	178.8o	275.9kl
	C1	57.4kl	52.6no	46.8kl	67.3m	487.0k	307.1j–l	486.2l	231.8lm
	C2	102.0i	76.7l	75.4j	135.9h	808.4g	464.6h	612.7i	473.2gh
	C3	191.1e	154.6de	158.0ef	160.8f	1493.7d	782.5e	1255.8f	835.8e
	C4	239.3a	168.4c	229.4a	168.0c–e	1968.7a	1035.3b	1945.8a	1176.8a
b r		56.1 **	32.5 *	52.4 **	32.3 *	450.7 **	198.5 **	430.4 **	240.6 *
		0.97 **	0.86 *	0.94 **	0.91 *	0.97 **	0.93 **	0.93 **	0.90 *

Table A8. Cont.

Population	Cycle	DPPH				TEAC			
		(mmol TE/100 g DW)				(mmol TE/100 g DW)			
		E1	E2	E3	E4	E1	E2	E3	E4
E	C0	23.4p	51.1no	24.6n	47.7op	208.5no	201.4m	207.3o	189.7mn
	C1	50.2m	69.5m	46.2kl	76.8l	440.1kl	270.9l	324.7n	279.9k
	C2	99.2i	115.7i	104.3gh	104.0j	755.5h	551.7fg	590.1ij	433.1h
	C3	164.1h	160.3d	174.0d	162.1ef	1322.3e	869.2d	1288.2f	865.3de
	C4	212.0c	179.8b	200.6bc	174.2c	1687.2c	1234.9a	1484.5e	1183.4a
b r		49.1 **	34.8 **	48.0 **	33.8 **	384.0 **	266.5 **	351.8 **	257.3 **
		0.98 **	0.98 **	0.97 **	0.97 **	0.98 **	0.96 **	0.93 **	0.93 **
Check var.	KND	139.4	116.1	137.5	130.7	1055.9	848.6	1066.4	953.3
	KGW	98.5	70.5	88.2	90.2	791.4	374.3	627.1	465.3
	P339	14.3	14.2	14.3	9.2	104.2	92.4	108.0	91.7

* and ** significant at 0.05 and 0.01 probability levels; ¹ means in a column followed by the same letter are not significantly different at 0.05 probability levels; b—response to selection; r—correlation coefficient; E1—Khon Kaen, dry season; E2—Khon Kaen, rainy season; E3—Uthai Thani, dry season; E4—Uthai Thani, rainy season.

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