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
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      | purple      | purple          | purple         | PBRCSA         |
|        | PF2      | white       | purple          | purple         | PBRCSA         |
|        | PF3      | yellow      | purple          | purple         | PBRCSA         |
|        | PF4      | purple      | purple          | purple         | PBRCSA         |
|        | PF5      | white       | purple          | purple         | PBRCSA         |

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.
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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:

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

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; \( \varepsilon = 26,900 \) molar extinction coefficient, in \( L \times mol^{-1} \times cm^{-1} \), for cyd-3-glu and \( 10^3 \) = factor for conversion from g to mg. Then, TAC was converted into TAY by following this equation:

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

### 2.4.2. Determination of Total Phenolic Content (TPC)

Total phenolic content in each sample was determined according to Folin–Ciocălăteu’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]:

\[ Y_{ijkl} = \mu + B_i + E_j + P_k + C_l + EP_{jk} + EC_{jl} + PC_{kl} + EPC_{jkl} + e_{ij} + e_{ijk} + e_{ijkl} \]  

where \( Y_{ijkl} \) is the observed value of each measurement, \( \mu \) 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), \( EP_{jk} \) 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 (\( \sigma^2_E \)) and genotypic variance (\( \sigma^2_G \)) and then broad-sense heritability estimates (\( h^2_b \)) were calculated as follows [42]:

\[ h^2_b = \frac{\sigma^2_G}{\sigma^2_P} \]  

\[ \sigma^2_P = \sigma^2_G + \sigma^2_E + \frac{r}{n} \]  

where \( h^2_b \) is broad sense heritability, \( \sigma^2_G \) is genotypic variation, \( \sigma^2_P \) 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:

\[ \hat{Y} = a + bX \]  

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:

\[ S^2_{y|x} = \frac{\sum y^2 - (\sum xy)^2}{n - 2}, \]  

\[ t_b = \frac{b}{\sqrt{S^2_{y|x} \sum x^2}} \]  

where \( S^2_{y|x} \) 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:

\[
r = \frac{\sum xy}{\sqrt{(\sum x^2)(\sum y^2)}},
\]

(9)

where \( r \) is declared significant at the level of significance if the absolute value of the computed \( r \) value is greater than the corresponding tabular \( r \) 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   | Husk Mass | Cob Mass | Anthesis Day | Plant Height | Ear Height | TAY  |
|--------------|-----|---------|-----------|----------|--------------|--------------|------------|------|
| Environment (E) | 3   | 1,007,326 ** | 1,304,911 ** | 767,967 ** | 417.6 ** | 17,188 ** | 10,297 ** | 2619 ** 235.4 ** |
|              |     | (9.9) | (6.3)     | (56.7)   | (66.7) | (60.6) | (58.4) | (5.0) (17.0) |
| Error (a)    | 8   | 1,007,326 ** | 1,304,911 ** | 767,967 ** | 417.6 ** | 17,188 ** | 10,297 ** | 2619 ** 235.4 ** |
|              |     | (1.0) | (0.7)     | (1.2)    | (0.2) | (4.0) | (1.9) | (0.0) (0.1) |
| Population (P) | 4   | 4,442,353 ** | 27,888 ** | 4032ns   | 4.4 ** | 134 ** | 98 ** | 10.5 ** 10.1 ** |
|              |     | (12.2) | (1.8)     | (0.4)    | (0.9) | (3.0) | (5.7) | (0.0) (1.0) |
| E × P        | 12  | 1,227,452 ** | 32,021 ** | 7266ns   | 3.4 ** | 147 ** | 67 ** | 3.0 ** 3.2 ** |
|              |     | (10.1) | (6.3)     | (2.1)    | (2.2) | (1.9) | (2.2) | (0.0) (1.0) |
| Error (b)    | 32  | 315,902 ** | 3753 **   | 6297     | 0.7   | 54    | 34    | 0.6    0.3 |
|              |     | (6.9)  | (1.9)     | (5.0)     | (1.2) | (2.0) | (2.1) | (1.0) (0.2) |
| Cycle (C)    | 4   | 2,407,467 ** | 34,362 ** | 23,619 ** | 94.5 ** | 761 ** | 1247 ** | 31149 ** 580.4 ** |
|              |     | (6.6)  | (2.2)     | (2.3)    | (20.1) | (3.6) | (9.4) | (88.0) (57.0) |
| E × C        | 12  | 755,443 ** | 12,801 ** | 16,269 ** | 6.1 ** | 301 ** | 203 ** | 46.9 ** 58.3 ** |
|              |     | (6.2)  | (2.5)     | (4.8)    | (3.9) | (4.2) | (4.6) | (4.0) (17.0) |
| P × C        | 16  | 406,361 *  | 10,016 ** | 5311ns   | 0.5ns | 221 ** | 90 ** | 3.6 ** 4.7 ** |
|              |     | (5.3)  | (2.6)     | (2.0)    | (0.4) | (4.2) | (2.7) | (0.0) (1.0) |
| E × P × C    | 48  | 401,421 ** | 7887 **   | 4764ns   | 0.4ns | 66ns  | 36ns  | 2.0 ** 1.9 ** |
|              |     | (13.2) | (6.2)     | (5.6)    | (0.9) | (3.7) | (2.7) | (0.0) (2.0) |
| Pooled error (c) | 160 | 261,380 | 4623 | 9288 | 0.4 | 69 | 34 | 0.6 | 0.3 |
| CV (a)       |     | 6.2    | 8.3       | 8.7      | 1.2   | 10.9 | 11.7 | 8.6 | 14.3 |
| CV (b)       |     | 8.1    | 7.0       | 8.7      | 1.5   | 3.9  | 6.1  | 9.7 | 12.3 |
| CV (c)       |     | 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 ** | 440,334 ** | 174,023 ** | 2,262,768 ** | 1,483,875 ** | 179,389 ** | 1292 ** | 2,517,996 ** |
| Error (a) | 8  | (0.4) | (0.7) | (10.5) | (4.7) | (12.4) | (0.8) | (0.3) | (10.6) |
| Population (P) | 4  | 35,357 ** | 133,723 ** | 1512 ** | 165,909 ** | 103,969 ** | 211,480 ** | 417 ** | 91,273 ** |
| ExP | 12 | (0.1) | (0.3) | (0.1) | (0.5) | (1.2) | (1.3) | (0.1) | (0.5) |
| Error (b) | 32 | (0.2) | (0.2) | (0.1) | (0.2) | (1.2) | (1.1) | (0.6) | (0.4) |
| Cycle (C) | 4  | 39,180,000 ** | 46,300,000 ** | 1,048,430 ** | 32,890,000 ** | 5,970,024 ** | 13,570,000 ** | 274,538 ** | 14,200,000 ** |
| ExC | 12 | (98.1) | (96.6) | (84.7) | (91.6) | (66.3) | (82.2) | (93.5) | (80.0) |
| PoC | 16 | 24,714 ** | 53,517 ** | 728 ** | 30,065 ** | 47,058 ** | 85,366 ** | 238 ** | 26,893 ** |
| ExPoC | 48 | (0.4) | (0.8) | (3.5) | (1.8) | (14.2) | (6.4) | (2.7) | (6.5) |
| Pooled error (c) | 160 | 3.8 | 4.5 | 2.9 | 3.8 | 6.3 | 3.5 | 4.8 | 7.1 |

** 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 ($h^2_T$) for FY 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 ($h^2_T$) 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 $^2$ (kg ha$^{-1}$) | Husk Mass (kg ha$^{-1}$) | Cob Mass (kg ha$^{-1}$) | Anthesis Day (DAP) | Plant Height (cm) | Ear Height (cm) | TAY $^3$ (kg CGE) |
|------------------|-------------------------------|--------------------------|-------------------------|-------------------|-----------------|---------------|-------------------|
|                  |                               |                          |                         |                   |                 |               | Husk Cob         |
| C0               | 6037e 1                       | 802g                     | 883cd                   | 56a               | 197.3a          | 99.9b–e       |                   |
| C1               | 6945b–d                       | 474a                     | 693b                    | 50c               | 193.5a          | 93.6f–j       |                   |
| C2               | 6572e–d                       | 493a                     | 693b                    | 54f               | 186.0c          | 92.9c–f       |                   |
| C3               | 6865b                          | 682d                     | 593a                    | 50g               | 183.1c          | 93.6f–j       |                   |
| C4               | 6946d–d                       | 503a                     | 693b                    | 50f               | 186.1d          | 92.9c–f       |                   |
|                  |                               |                          |                         |                   |                 |               |                   |
| D0               | 7046bc                         | 581e                     | 681d                    | 57a               | 182.6e          | 101.1a–d      |                   |
| D1               | 6845b–d                       | 482d                     | 691c                    | 52a               | 195.1a          | 98.6c–f       |                   |
| D2               | 6916b–d                       | 494a                     | 693b                    | 54f               | 186.0c          | 93.6g–k       |                   |
| D3               | 6946e                          | 503a                     | 693b                    | 50f               | 183.1c          | 93.6f–j       |                   |
| D4               | 7008bc                         | 595b                      | 693d                    | 53g               | 185.4d          | 95.3e–i       |                   |
|                  |                               |                          |                         |                   |                 |               |                   |
| E0               | 6965bd                         | 822e                     | 923a                    | 57a               | 197.3a          | 103.5b        |                   |
| E1               | 6949bc                         | 484g                     | 951a                    | 59c               | 197.0a          | 105.4a        |                   |
| E2               | 6994b–d                       | 473e                     | 902a                    | 54f               | 186.1c          | 94.2e–f       |                   |
| E3               | 6834bd                         | 892d–e                   | 54g–i                   | 192.0d            | 93.6g–j        | 15.1c         |                   |
| E4               | 7153bc                         | 953a–d                   | 593b                    | 193.6ab           | 101.6a–d       | 18.6a         |                   |
|                  |                               |                          |                         |                   |                 |               |                   |
| Check var        |                               |                          |                         |                   |                 |               |                   |
| KND              | 6892                             | 809                       | 976                      | 45                | 195.8           | 97.5          |                   |
| KGW              | 6658                             | 711                       | 880                      | 48                | 201.0           | 95.1          |                   |
| P339             | 10,871                          | 1537                      | 1216                     | 60                | 188.2           | 102.9         |                   |

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; $^2$ response to selection; $^3$ broad sense heritability ($h^2_T$) for grain yield, ranged from 0 to 0.98; $^{13}$ broad sense heritability ($h^2_T$) for TAY in husk and cob, ranged from 0.97 to 0.99. $\Delta C$ = 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 x 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 |
|------------|-------|------|-----|
| A          |       |      |     |
| C0         | 123.60 ** | 179.0p | 171.0p |
| C1         | 308.4m | 484.3m | 94.8m |
| C2         | 364.8i | 896.7h | 129.6h |
| C3         | 1601.3e | 1812.1d | 307.1e |
| C4         | 1994.8c | 2327.3b | 344.7c |
| C          |       |       |     |
| A          |       |       |     |
| C1         | 321.9m | 471.3m | 98.6k |
| C2         | 601.4i | 647.7j | 116.4j |
| C3         | 1546.9f | 1781.0ef | 284.4g |
| C4         | 2016.5b | 2248.2c | 325.0d |
| C          |       |       |     |
| C1         | 109.4o | 210.1o | 40.2o |
| C2         | 321.9m | 471.3m | 98.6k |
| C3         | 1546.9f | 1781.0ef | 284.4g |
| C4         | 2016.5b | 2248.2c | 325.0d |
| C          |       |       |     |
| C1         | 109.4o | 210.1o | 40.2o |
| C2         | 321.9m | 471.3m | 98.6k |
| C3         | 1546.9f | 1781.0ef | 284.4g |
| C4         | 2016.5b | 2248.2c | 325.0d |
| C          |       |       |     |
| C1         | 109.4o | 210.1o | 40.2o |
| C2         | 321.9m | 471.3m | 98.6k |
| C3         | 1546.9f | 1781.0ef | 284.4g |
| C4         | 2016.5b | 2248.2c | 325.0d |
| C          |       |       |     |
| C1         | 109.4o | 210.1o | 40.2o |
| C2         | 321.9m | 471.3m | 98.6k |
| C3         | 1546.9f | 1781.0ef | 284.4g |
| C4         | 2016.5b | 2248.2c | 325.0d |
| C          |       |       |     |
| C1         | 109.4o | 210.1o | 40.2o |
| C2         | 321.9m | 471.3m | 98.6k |
| C3         | 1546.9f | 1781.0ef | 284.4g |
| C4         | 2016.5b | 2248.2c | 325.0d |
| C          |       |       |     |
| C1         | 109.4o | 210.1o | 40.2o |
| C2         | 321.9m | 471.3m | 98.6k |
| C3         | 1546.9f | 1781.0ef | 284.4g |
| C4         | 2016.5b | 2248.2c | 325.0d |
| C          |       |       |     |
| C1         | 109.4o | 210.1o | 40.2o |
| C2         | 321.9m | 471.3m | 98.6k |
| C3         | 1546.9f | 1781.0ef | 284.4g |
| C4         | 2016.5b | 2248.2c | 325.0d |
| C          |       |       |     |
| C1         | 109.4o | 210.1o | 40.2o |
| C2         | 321.9m | 471.3m | 98.6k |
| C3         | 1546.9f | 1781.0ef | 284.4g |
| C4         | 2016.5b | 2248.2c | 325.0d |
| C          |       |       |     |
| C1         | 109.4o | 210.1o | 40.2o |
| C2         | 321.9m | 471.3m | 98.6k |
| C3         | 1546.9f | 1781.0ef | 284.4g |
| C4         | 2016.5b | 2248.2c | 325.0d |
| C          |       |       |     |
| C1         | 109.4o | 210.1o | 40.2o |
| C2         | 321.9m | 471.3m | 98.6k |
| C3         | 1546.9f | 1781.0ef | 284.4g |
| C4         | 2016.5b | 2248.2c | 325.0d |
| C          |       |       |     |
| C1         | 109.4o | 210.1o | 40.2o |
| C2         | 321.9m | 471.3m | 98.6k |
| C3         | 1546.9f | 1781.0ef | 284.4g |
| C4         | 2016.5b | 2248.2c | 325.0d |
| C          |       |       |     |
| C1         | 109.4o | 210.1o | 40.2o |
| C2         | 321.9m | 471.3m | 98.6k |
| C3         | 1546.9f | 1781.0ef | 284.4g |
| C4         | 2016.5b | 2248.2c | 325.0d |
| C          |       |       |     |
| C1         | 109.4o | 210.1o | 40.2o |
| C2         | 321.9m | 471.3m | 98.6k |
| C3         | 1546.9f | 1781.0ef | 284.4g |
| C4         | 2016.5b | 2248.2c | 325.0d |
| C          |       |       |     |
| C1         | 109.4o | 210.1o | 40.2o |
| C2         | 321.9m | 471.3m | 98.6k |
| C3         | 1546.9f | 1781.0ef | 284.4g |
| C4         | 2016.5b | 2248.2c | 325.0d |

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 (NS3 x 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 be 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.](image-url)
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                      |
|                      | **ns                           | **ns                          |
|                      | Husk                           | Cob                           |
| 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 **                       |

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.

Author Contributions: Conceptualization, P.K., K.L. (Khomlorn Lomthaisong), K.L. (Kamol Lertrat), B.H. and B.S.; formal analysis, P.K., K.L. (Khomlorn Lomthaisong), M.P.S. and B.S.; methodology, P.K., K.L. (Khomlorn Lomthaisong), B.H. and B.S.; writing—original draft, P.K. and B.S.; writing—review & editing, K.L. (Khomlorn Lomthaisong), K.L. (Kamol Lertrat) B.H. and M.P.S. All authors have read and agreed to the published version of the manuscript.

Funding: The Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program (Grant No PHD/0014/2557).

Acknowledgments: The study was funded by the Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program (Grant No PHD/0014/2557) and the Senior Research Scholar Project of Sanum Jogloy (Project no. RTA6180002). The authors would like to thank the National Science and Technology Development Agency through the National Center for Genetic Engineering and Biotechnology, Bangkok, Thailand (Grant No P-17-51695) and the Plant Breeding Research Center for Sustainable Agriculture, Faculty of Agriculture, Khon Kaen University, Thailand. The materials were supported by the Nakhon Sawan Field Crops Research Center, Department of Agriculture, Thailand. The authors wish to thank Abil Dermail for helpful discussions. This research was supported in part by the U.S. Department of Agriculture, Agricultural Research Service. USDA is an equal opportunity employer. Mention of trade names or commercial products in this report is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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. 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 | 906a–c | 7668a | 1033b–d | 733b–e | 880b–d | 723e–g |
| C2         | 7676a | 7233a  | 7882a–c | 7568ab | 1017b–e | 719c–e | 954ab  | 745c–g |
| C3         | 7660b | 6827a–e | 6618c–g | 7093a–e | 1024b–e | 773a–g | 904bc  | 735c–g |
| C4         | 7492ab| 6027f  | 7633a–d | 7173a–e | 1108b–e | 789d–g | 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 850bc 817a–e

b 194ns –75ns –75ns –66ns 15ns –3ns 16 * 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 789d–g 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 7122a–e 1195a 782a–e 848b–e 749b–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 6973b–g 7510ab 1025b–d 807a–d 615h 716fg
C1 6998cd 6531d–f 6568a–g 7700a 1055b–d 729b–e 830a–c 776b–g
C2 6897d 7214ab 6449g–d 7417c–e 1122b–d 763a–c 883b–f 726g–c
C3 7380a–c 6606a–f 5678g 7671a 1113b–d 792a–c 1038a–c 823a–d
C4 7589ab 6821a–e 6436d–g 746a 1100a–c 792a–c 868b–e 810b–f

b 268 * 71ns –301ns 53ns 25ns 16 * 37ns 13ns
r 0.90 * 0.14ns 0.51ns 0.29ns 0.65ns 0.80 * 0.96 ** 0.31ns

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–d | 946a–d | 838a–d | 59cd | 53g–g | 54d–f | 52c–e |
| C2         | 1094ab | 777bc | 899b–d | 819b–d | 58d–f | 53g–g | 54e–g | 51d–e |
| C3         | 1115ab | 835a–c | 955a–d | 866a–d | 58e–g | 52g | 54e–g | 51e–f |
| C4         | 1117ab | 870a | 986a–c | 815b–d | 56i–k | 53g | 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 | 54d-b | 56bc | 53a-c |
|            | C1    | 1095ab | 828a-c | 913a-d | 888a-c | 59c-e | 54d-b | 55c-e | 52b-e |
|            | C2    | 1088ab | 750c | 842d | 846a-d | 57g-i | 54b-e | 53f-h | 52e-c |
|            | C3    | 1106ab | 777bc | 897b-d | 805b-d | 56h-j | 53g-e | 53g-i | 51de |
|            | C4    | 1128a | 791a-e | 976a-d | 905ab | 59j-L | 53g-h | 52i | 50f |
| b         |       | 21 * | -25ns | 10ns | 11ns | -1 ** | -1 | -1 ** | -1 ** |
| r         |       | 0.81 * | 0.53ns | 0.11ns | 0.95 ** | 0.89 * | 0.98 ** | 0.92 ** |
| C          | C0    | 935e | 860a-c | 905a-d | 831b-d | 60ab | 59b | 56ab | 53ab |
|            | C1    | 946de | 822a-c | 917a-d | 942a | 59cd | 55b | 55cd | 52a-d |
|            | C2    | 1042a-d | 797a-c | 1030a-b | 825b-d | 57f-h | 54c-f | 53g-e | 52a-b |
|            | C3    | 1033a-e | 819a-c | 948a-d | 872a-d | 56h-j | 53g-d | 53f-h | 52d-e |
|            | C4    | 1100ab | 869ab | 914a-d | 770d | 55f-g | 52i | 52j | 50f |
| b         |       | 42 * | -10ns | 25ns | 19ns | -1 ** | -1 ** | -1 ** | -0.2ns |
| r         |       | 0.90 * | 0.01ns | 0.02ns | 0.27ns | 1.00 ** | 0.96 ** | 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 | 52c-a |
|            | C3    | 1083ab | 777bc | 925a-d | 819b-d | 56k-i | 54c-f | 53g-i | 52c-a |
|            | C4    | 1119a | 837a-c | 959a-d | 897a-c | 55f-g | 52i | 52h-e | 52c-e |
| b         |       | 44 * | -10ns | 25ns | 19ns | -1 ** | -1 ** | -1 ** | -0.3 * |
| r         |       | 0.98 ** | 0.13ns | 0.77 * | 0.51ns | 1.00 ** | 0.96 ** | 0.97 ** | 0.75ns |
| 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    | 1086a-c | 794a-c | 848cd | 899a-c | 57f-h | 53d-g | 53g-i | 52c-e |
|            | C3    | 1028a-e | 779bc | 938a-d | 821b-d | 56j-i | 53g-h | 54e-g | 52c-e |
|            | C4    | 1122a | 862a-c | 988a-c | 897a-c | 55l-i | 52h-e | 52b-e | 52c-e |
| b         |       | 29ns | -8ns | 0.2ns | -2.8 ** | 0.8ns | -2.8 * | 0.0ns | -2.7ns |
| 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

FGW 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    | 198.3-c | 162.0a-e | 197.0b-f | 195.3a-f | 101.3e-h | 85.3a-d | 101.3b-e | 104.0a-c |
|            | C1    | 187.7i | 169.0a-c | 183.7e-f | 190.7c-i | 99.3f-j | 80.0h-g | 101.3b-e | 102.0a-e |
|            | C2    | 198.7c | 170.0a-c | 189.3b-f | 184.3f-j | 104.0b-g | 78.3e-f | 101.3a-c | 89.3b-c |
|            | C3    | 204.0a-f | 159.7c | 183.7e-f | 183.7f-j | 102.3b-c | 74.0b-f | 100.0b-e | 91.0b-c |
|            | C4    | 207.7a-c | 163.7a-c | 197.0b-f | 183.7e-f | 103.7b-c | 74.3f-j | 102.0b-e | 96.0b-c |
| 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 | 1073 | 798 | 50 | 42 | 46 | 42 |
|            | 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 | 69.3k | 96.3d-e | 78.7j |
|            | C3    | 189.0f-h | 158.0c-e | 181.0b-f | 183.7j | 90.7i | 64.0k | 95.3d-e | 78.7k |
|            | C4    | 202.0a-g | 172.3a-c | 197.7a-d | 103.7b-c | 79.3c-i | 100.3b-e | 101.0a-c |
| b         |       | -0.9ns | -1.1ns | -0.6ns | -0.7ns | -0.3ns | -3.4ns | -1.1ns | -2.1ns |
| r         |       | 0.06ns | 0.08ns | 0.03ns | 0.01ns | 0.00ns | 0.32ns | 0.20ns | 0.07ns |
null
### 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| 1.2k| 0.8l| 1.0m| 1.0m| 1.0l| 1.0k| 0.9m|
|            | C1    | 3.7hi| 3.1h–j| 3.0g| 3.8g| 2.3k–i| 1.9j–l| 2.2h–k| 2.5j–l|
|            | C2    | 5.9p**| 4.0**| 4.5**| 4.6**| 3.0*| 1.1*| 2.6*| 0.9**|
| b          | r     | 0.97**| 0.96**| 0.92**| 0.93**| 0.91*| 0.91*| 0.88*| 0.98**|

Check var.

| KND | KGW | P339 |
|-----|-----|------|
| 3.0| 0.9 | 0.2  |

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    | 102.7h*| 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| 822.6h| 1937.7d|
|            | C3    | 1875.5e| 1271.5d| 1586.7e| 1489.2f| 1927.2e| 1890.1g| 1779.7h| 1612.8i|
|            | C4    | 2251.8a| 2029.5ab| 1953.6a| 1704.1d| 2685.4a| 2253.4c| 2472.6a| 1937.7d|
| b          | r     | 0.95**| 0.94**| 0.93**| 0.92**| 0.95**| 0.97**| 0.94**| 0.97**|
| C          | 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| 478.7l| 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| 2472.6a| 1937.7d|
| b          | r     | 0.95**| 0.94**| 0.93**| 0.92**| 0.95**| 0.97**| 0.94**| 0.97**|
| D          | C0    | 118.6n| 127.1mn| 64.1q| 99.1p| 297.6m| 250.4m| 155.9q| 146.2a|
|            | C1    | 523.9j| 425.0k| 395.8l| 421.6l| 654.1i| 784.0h| 537.2m| 727.1j|
|            | C2    | 762.8h| 511.8k| 534.6ij| 462.9kl| 988.1h| 523.2j–l| 632.9i| 446.5n|
|            | C3    | 1699.1f| 1397.5f| 1359.7g| 1220.9h| 2046.5f| 1704.7h| 2004.7c| 1220.3g|
|            | C4    | 2156.7b| 1993.2bc| 1879.6c| 1704.1d| 2510.7b| 2204.7c| 2177.3d| 1220.3g|
| b          | r     | 0.96**| 0.95**| 0.94**| 0.93**| 0.96**| 0.86*| 0.91*| 0.87*|
| E          | C0    | 104.6n| 159.7m| 91.1p| 120.4no| 396.2l| 291.2m| 169.7q| 284.7p|
|            | C1    | 357.3l| 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| 822.6h| 1937.7d|
|            | C3    | 1875.5e| 1271.5d| 1586.7e| 1489.2f| 1927.2e| 1890.1g| 1779.7h| 1612.8i|
|            | C4    | 2251.8a| 2029.5ab| 1953.6a| 1704.1d| 2685.4a| 2253.4c| 2472.6a| 1937.7d|
| b          | r     | 0.95**| 0.94**| 0.93**| 0.92**| 0.95**| 0.97**| 0.94**| 0.97**|

* 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 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) |
|------------|-------|-------------------------|-------------------------|
| A          | C0    | 27.3*                   | 34.1p                   |
|            | C1    | 61.4p                   | 46.5i                   |
|            | C2    | 96.2*                   | 99.2f                   |
|            | C3    | 250.9e                  | 234.5c                  |
|            | C4    | 298.0a                  | 265.2b                  |
| b          |       | 73.1**                  | 105.1**                 |
| r          |       | 0.92 **                 | 0.91 **                 |
| B          | C0    | 22.1t                   | 51.8o                   |
|            | C1    | 67.5o                   | 130.4h                  |
|            | C2    | 228.3h                  | 224.7d                  |
|            | C3    | 304.9e                  | 327.6f                  |
|            | C4    | 325.8c                  | 453.7d                  |
| b          |       | 70.5 *                  | 98.6 *                  |
| r          |       | 0.93 **                 | 0.94 **                 |
| C          | C0    | 33.7r                   | 46.3o                   |
|            | C1    | 74.6n                   | 149.8i                  |
|            | C2    | 112.7i                  | 143.2ij                 |
|            | C3    | 235.4g                  | 350.0e                  |
|            | C4    | 589.6c                  | 427.2b                  |
| b          |       | 65.9 **                 | 99.4 **                 |
| r          |       | 0.97 **                 | 0.95 **                 |
| D          | C0    | 25.6st                  | 48.5o                   |
|            | C1    | 81.2m                   | 100.4m                  |
|            | C2    | 110.1j                  | 143.2ij                 |
|            | C3    | 235.4g                  | 350.0e                  |
|            | C4    | 589.6c                  | 427.2b                  |
| b          |       | 68.2 **                 | 100.7 **                |
| r          |       | 0.96 **                 | 0.95 **                 |
| E          | C0    | 38.6q                   | 62.9n                   |
|            | C1    | 62.3p                   | 135.5k                  |
|            | C2    | 120.9i                  | 171.9g                  |
|            | C3    | 240.8f                  | 367.6d                  |
|            | C4    | 279.9i                  | 451.4a                  |
| b          |       | 65.9 **                 | 100.9 **                |
| r          |       | 0.95 **                 | 0.97 **                 |
| Check var. |      | KND 60.5                | 44.8h                   |
|            |      | GKW 49.3               | 46.8h                   |
|            |      | P339 12.8             | 15.6h                   |

* 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/g 100 DW) |
|------------|-------|-----------------------|-----------------------|
| A          | C0    | 143.8op                | 59.2l                  |
|            | C1    | 197.4m                 | 184.8i                 |
|            | C2    | 432.9j                 | 276.5h                 |
|            | C3    | 577.8g                 | 416.4e                 |
|            | C4    | 1137.5e               | 487.1c                |
| b          |       | 236.8*                 | 108.7*                 |
| r          |       | 0.88 *                 | 0.91 **                |
| B          | C0    | 130.6op               | 62.1l                  |
|            | C1    | 169.3n                | 176.1j                 |
|            | C2    | 278.0k                 | 308.4g                 |
|            | C3    | 643.2f                 | 370.0f                 |
|            | C4    | 1005.4d               | 576.3e                 |
| b          |       | 234.4 *               | 122.2 *                |
| r          |       | 0.88 *               | 0.97 **                |

* 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.
### Table A7. Cont.

| Population | Cycle |
|------------|-------|
| C          | C0    | 162.6n | 86.1k | 142.8nm | 84.3n | 274.5no | 391.2n | 208.2pq | 382.8n |
|            | C1    | 256.7kl | 228.6i | 247.6j | 207.4m | 579.5j | 673.1j | 580.3j | 557.1k |
|            | C2    | 420.4kl | 308.2g | 405.3g | 292.1k | 720.3i | 845.8g | 684.4i | 563.6j |
|            | C3    | 1058.1d | 376.3f | 1518.7de | 485.6e | 1718.7h | 985.8g | 1651.5b | 1418.2b |
|            | C4    | 1208.3b | 86.4** | 282.2* | 92.3** | 383.1** | 126.0** | 380.0** | 225.6* |
| b          | r 0.91 | 0.97** | 0.92* | 0.96* | 0.94** | 0.80* | 0.94** | 0.95* |
| D          | C0    | 104.6p | 93.7k | 96.4ap | 86.4p | 372.6p | 91.7p | 282.9p | 384.7p |
|            | C1    | 256.7kl | 228.6i | 247.6j | 207.4m | 579.5j | 673.1j | 580.3j | 557.1k |
|            | C2    | 420.4kl | 308.2g | 405.3g | 292.1k | 720.3i | 845.8g | 684.4i | 563.6j |
|            | C3    | 1058.1d | 376.3f | 1518.7de | 485.6e | 1718.7h | 985.8g | 1651.5b | 1418.2b |
|            | C4    | 1208.3b | 86.4** | 282.2* | 92.3** | 383.1** | 126.0** | 380.0** | 225.6* |
| b          | r 0.93 | 0.96** | 0.88* | 0.95** | 0.90** | 0.89* | 0.86* |
| E          | C0    | 104.6p | 93.7k | 96.4ap | 86.4p | 372.6p | 91.7p | 282.9p | 384.7p |
|            | C1    | 256.7kl | 228.6i | 247.6j | 207.4m | 579.5j | 673.1j | 580.3j | 557.1k |
|            | C2    | 420.4kl | 308.2g | 405.3g | 292.1k | 720.3i | 845.8g | 684.4i | 563.6j |
|            | C3    | 1058.1d | 376.3f | 1518.7de | 485.6e | 1718.7h | 985.8g | 1651.5b | 1418.2b |
|            | C4    | 1208.3b | 86.4** | 282.2* | 92.3** | 383.1** | 126.0** | 380.0** | 225.6* |
| b          | r 0.90 | 0.95** | 0.88* | 0.95** | 0.90** | 0.89* | 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 |
|------------|-------|
| D          | C0    | 162.6no | 86.1k | 142.8nm | 84.3n | 274.5no | 391.2n | 208.2pq | 382.8n |
|            | C1    | 256.7kl | 228.6i | 247.6j | 207.4m | 579.5j | 673.1j | 580.3j | 557.1k |
|            | C2    | 420.4kl | 308.2g | 405.3g | 292.1k | 720.3i | 845.8g | 684.4i | 563.6j |
|            | C3    | 1058.1d | 376.3f | 1518.7de | 485.6e | 1718.7h | 985.8g | 1651.5b | 1418.2b |
|            | C4    | 1208.3b | 86.4** | 282.2* | 92.3** | 383.1** | 126.0** | 380.0** | 225.6* |
| b          | r 0.91 | 0.97** | 0.92* | 0.96* | 0.94** | 0.80* | 0.94** | 0.95* |

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. Cont.

| Population | Cycle | DPPH (mmol TE/100 g DW) | TEAC (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          |       | 49.1    | 34.8    | 48.0    | 33.8    | 384.0   | 266.5   | 351.8   | 257.3   |
| r          |       | 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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