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GENETIC ANALYSIS OF ANTHOCYANIN CONTENT IN PURPLE WAXY CORN (*Zea mays* L. var. *ceratina* Kulesh) KERNEL AND COB

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SUMMARY

Anthocyanins of pigmented corn become a topic of increasing interest recently due to consumer awareness of their various health benefits. A better understanding on the gene effects to anthocyanin is an important for high antioxidants breeding programs. The objective of the present study was to determine gene effect controlling of anthocyanin content in purple waxy corn kernel and cob by generation mean analysis. For the cross of KND $_{10\text{-}4P}$ (P_1) × BW (P_2), F_2, and backcross generations were developed. All generations were planted in the randomized complete block design (RCB) with 3 replications at the Khon Kaen University, Thailand. Genetic analysis showed that the additive gene effects appeared to be a major contribution to the inheritance for pelargonidin-3-glucosid and peonidin-3-glucoside in the purple corn cob, indicate that selection for these anthocyanins is made at early breeding generations may be possible. Moreover, dominance and dominance × dominance epistatic effects were important for all studied traits in the corn kernel and cyanidin-3-glucoside in purple corn cob so initial selection can be carried out in large populations of later generations with increased homozygosity.

Key words: Additive and dominance gene effects, epistasis, anthocyanin, pigmented maize

Key findings: The results confirmed gene effects governing the inheritance of major anthocyanins in waxy corn kernel and cob were dominance and its epistatic effects and that selection for high these antioxidants level would be effective even in later generations. This information would help corn breeders to select for high anthocyanin level.

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INTRODUCTION

Epidemiological studies have confirmed that a high consumption of fruits, vegetables, and whole grains is strongly reduce risk against non-communicable diseases (NCD_s), also known as chronic disease such as; cardiovascular disease (CVD), which is today the largest single contributor to global mortality and will continue to dominate mortality trends in the future (Fuster and Kelly, 2010; Isabelle *et al*., 2010). These health benefits are attributed to the phytonutrients or phytochemicals appear in edible plants. Thus, functional foods from pigmented corn especially purple corn, have received great interesting in recent years due to there are rich source of valuable nutrients. Particularly, it is rich in anthocyanins and other phenolic compounds in the aleurone layer of corn kernel and cob that have long been used by the people of the Peruvian Andes to color foods and sweet beverages (Aoki *et al*., 2002; Cevallos-Casals and Cisneros-

Zevallos, 2004). Researches show that this pigment may have potential effects in suppression the risk of cardiovascular diseases and cancers, anti-inflammatory, type 2 diabetes and chemoprotective properties (Jones, 2005). Therefore, pigmented corn breeding has become an important aspect for bio-enhanced that is improved through biological means such as conventional plant breeding.

Waxy corn (*Zea mays* L. var. *ceratina* Kulesh) is a special type of corn that is rich in anthocyanins and other antioxidant compounds, and has widely cultivated and consumed in Thailand and other Asian countries (Harakotr *et al*., 2014; Hu and Xu, 2011). This corn type is harvested prior maturity as fresh foods and matures as whole grain foods, which are sold as processed corn kernels, fresh corn kernels, and frozen corn ears in the European and US markets (Harakotr *et al*., 2014; Ketthaisong *et al*., 2014). Waxy corn have a various kernel color ranging from white to black colors that are correlated to phytochemical constituents and concentrations. Grain colors in corn naturally occur from pigments and other substances, including carotenoids, phenols and anthocyanin, which establish coloration in the pericarp, aleurone layer, and/or endosperm (Ford, 2000). Increasing the levels of these bioactive compounds and pigments in waxy corn kernels should increase the nutritional quality of corn. Dark colored corn such as purple, blue and red are found generally in the aleurone and/or pericarp of corn kernels (Mahan *et al*., 2013). The intensity of these colors depends upon the concentration of anthocyanins, presents in the corn kernel and cob. This pigment is primarily decided by genetic factors with some modifications of environmental conditions (Liang *et al*., 2009).

Corn breeders have studied the prospects for improvement of waxy corn hybrids containing various kernel colors to enhance the functional and antioxidant materials (Ji *et al*., 2010). Although, variations in the color of corn kernel have attracted geneticists since the early 1900s when studies on the inheritance patterns of kernel color helped validate classical genetics (Ford, 2001). Further understanding regarding the qualitative and quantitative genetic control of colored-kernel and cob corn and hence genetic researchers on colored-corn has attracted great attention. Generation mean analysis provides information on the relative importance of average of effects of the genes (additive effects), dominance deviations, and effects due to non-allelic genic interactions, in determining genotypic values of the individuals and, consequently, mean genotypic values of families and generation (Said, 2014). Generation mean analysis is a useful technique that provides the estimation of gene effects for quantitative traits such as yield and yield components, is great merit lying in the ability to estimate 3 types of epistatic gene effects including additive × additive, additive × dominance, and dominance × dominance (Mather and Jinks, 1977; Singh and Singh, 1992). The genetic mechanisms governing agronomic traits, yield, and yield attributes in corn have been reported (Dofing *et al*., 1991; Leng, 1963; Shahrokhi *et al*., 2013). However, there are very few reports concerning information on anthocyanin level in corn kernel and cob. Thus, this study was carried out to estimate the relative importance of additive and non-additive gene effects in controlling the inheritance of anthocyanin and its derivatives content in both purple waxy corn kernel and cob. It is hoped that results from this work would be value for corn breeders.

MATERIALS AND METHODS

Plant material

Two inbred lines ($KND_{10\text{-}4P}$ and BW) with different kernel and cob colors were use as genetic material in this study. These inbred lines were developed by the Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen University, Thailand. $KND_{10\text{-}4P}$ is a waxy corn inbred line with purple kernels and purple cobs, high anthocyanin content (Figure 1A). This inbred line was developed from the cross between field corn population with dark purple kernel and cob, and waxy corn population with white kernels, white cobs, good eating quality, stay green and high yield. The seeds of the F_1 generation showing waxy endosperm were identified by using potassium iodide (KI). The purple kernel and purple cob inbred was obtained from consecutively self-pollination for 5 generations. BW is a waxy corn inbred with

white kernels and white cobs, high general combining ability (Figure 1B).

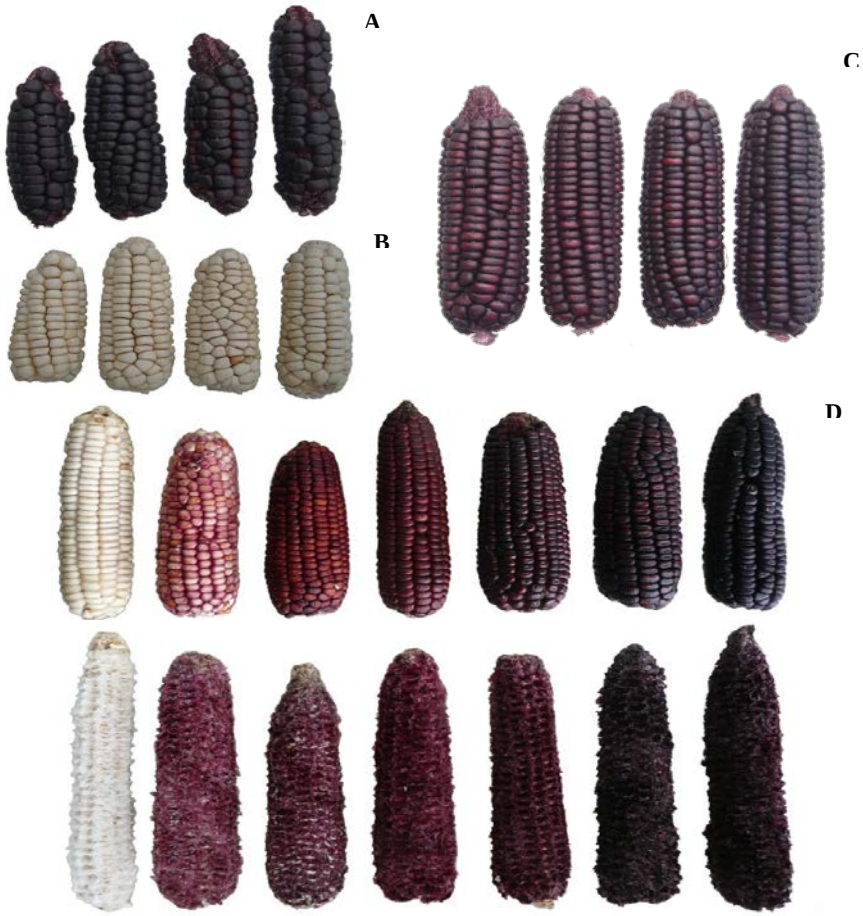

Figure 1. Kernel and cob colors for parental lines [$KND_{10\text{-}4p}$; P_1 (A) and BW; P_2 (B)], F_1 (C), and F_2 (D) generations.

This inbred line was derived from commercial waxy corn variety with good eating quality, good standing ability and high yield. This inbred line was also obtained after 5 consecutive generations of self-pollination.

The F_1 hybrid was generated by crossing $KND_{10\text{-}4P}$ and BW at the Khon Kaen University Research Farm, Thailand in the rainy season during May to August 2011. The F_1 generation was self-pollinated to produced F_2 seeds and were back-cross to both parental lines to generate backcross populations in the dry season during November 2011 to February 2012. Seeds of parental generation and F_1 generation were kept in cool room. Consequently, 6 populations (P_1, P_2, F_1, F_2, and backcross generations) were available for the evaluating experiment.

Field management

The seeds of the F_1, F_2, BC_{11}, BC_{12} generations and their patents were use in the experiment. The entries were planted in Randomized Complete Block design (RCB) with 3 replications in November 2012 at the Research Station, Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen University, Thailand. The experimental units had a varying number of rows, depending on the genetic uniformity of each generation. For non-segregating generations ($KND_{10\text{-}4P}$, BW, and F_1) the plot contained 2 rows, while F_2 generation was planted in 6 rows. First backcrosses (BC_{11} and BC_{12}) were presented by 4 rows per plots. Each row was 5m long, with 25cm spacing between plants and 75cm between rows. Recommended practices for commercial production of corn were followed. Hand-pollination of adjacent plants in each plot was practiced for parents, F_1 and backcross generations to avoid contaminated

from stray pollen and individual self-pollination of each plant was practiced for the F_2-generation. Ears were picked by hand at the mature stage (35 Day-after Pollination; DAP). Samples were harvested on two competitive ears per plot for the P_1, P_2 and F_1 generations. Similarly, 20 ears were sampled per plot for the BC_{11} and BC_{12} and on 30 ears per plot for the F_2 populations. Corn ears were dried with hot air oven at 40 ºC (moisture content less than 14%). First, ear samples of parental and F_1 generations were hand-shelled into corn kernel and cob then samples were bulked within replications in each generation prior mill into whole-grain flour as well as corncob powder. Furthermore, ear sample of the first backcrosses and F_2 populations were individually hand-shelled and milled. All milled sample were sieved through 60-mesh screen, thoroughly mixed and stored at -20°C until analysis.

Sample extraction

Anthocyanins in ground waxy corn kernel and cob were extracted according to the method previously described by Yang *et al*. (2008) with slight modifications. Portion of 0.5g of kernel flour and 0.25g of corncob powder were put into a conical flask containing 25 mL of acidified methanol (methanol-1% citric acid, 80:20 v/v) mixed well and stored for 24 h at 4 °C. The solution was transferred to centrifuge tube and centrifuged at 11,538 ×g for 10 min at 4°C. Consequently, the supernatants were collected and kept at -20 °C in the dark until analysis.

Determination of total monomeric anthocyanin content

The monomeric anthocyanin content was measured by the pH differential method, as described by Giusti and Wrolstad (2001). A UV-vis spectrophotometer (GENESYS 10S, Thermo Scientific, USA) was used to measure the absorbance at 510 and 700 nm. The anthocyanin content was calculated as cyanidin 3-glucoside equivalents (CGE) using an extinction coefficient of 26900 $L.cm^{-1}.mg^{-1}$ and a molecular weight of 449.2 g/L.

Determination of total phenolic content

Phenolic contents were determined using the Folin-Ciocalteu method, as described by Hu and Xu (2011). Briefly, the appropriate dilutions of extracts were oxidized with Folin-Ciocalteu reagent, and the reaction was neutralized with sodium carbonate. The absorbance of the resulting blue color was measured at 765 nm after 90 min, and the phenolic content was expressed as mg of gallic acid equivalents (GAE) per g of dry weight (mg GAE/g DW).

Quantification of anthocyanins

Reversed-phase HPLC analysis of phenolic compounds was performed using a Shimadzu LC-20AC pump and a SPD-M20A diode array detector. Chromatographic separations were performed on the Xselect CHS C-18 column (4.6 × 250 mm, i.d. 5 µm) (Agilent Technologies, USA). The composition of solvents and the gradient elution conditions used were those described by Kim *et al*. (2007), with slight modifications. The mobile phase consisted of acidified methanol (methanol: 0.1% HCl, 85:15 v/v; solvent A) and formic acid (8% formic acid; solvent B), at a flow rate of 1 mL/min. Gradient elution was performed as follows: 0-0.5 min, 0 to 80% solvent B; 0.5-9.5 min, 80% to 10% solvent B; 9.5-10 min, 10% to 15% solvent B; 10-15 min, 15% to 5% solvent B; 15-20 min, 5% to 80% solvent B; and a re-equilibration period of 1 min with 80% solvent B used between individual runs. Operating conditions were as follows: column temperature 30°C, injection volume 20 µL, and UV-vis detection at 520 nm. Solutions were injected after being filtered through a 0.20 µm nylon membrane filter. Anthocyanins in the samples were identified by comparing their relative retention times with those of standard compounds.

Statistical analysis

Analysis of variance was carried out for anthocyanin concentrations according to Randomized Complete Block design (Gomez and Gomez, 1984) by using JMP Pro software (version 10, SAS Institute Inc., USA). Duncan's multiple range test (DMRT) was used to identify significantly differences between group means. Significance levels were defined as the probability of 0.05.

A generation means analysis for each character was separately conducted to determine additive, dominance and epistatic effects following Mather and Jinks (1977) model. The joint scaling test utilizes a weighted least square (multiple linear regression), with weights that are the reciprocals of the variances of the generation means. The reason for weighting is advisable since genetic parameters are not estimated with the same precision. The six-parameter models and joint scaling test were used to determine. These gene effects were defined in Gamble's (1962) notation as mean using F_2 as a reference, [a] = pooled additive gene effect, [d] = pooled dominance gene effect, [aa] = pooled additive × additive epistatic gene effect, [ad] = pooled additive × dominance epistatic gene effect, and [dd] = pooled dominance × dominance epistatic gene effect. In the case of the six-parameter model, the individual genetic components were tested using Student's t-test at the probability ≤ 0.05. Any effect that was not significant was omitted from the model. Finally, significant parameters were fitted using the weighted least squares method as described by Rowe and Alexander (1980). All calculations for generation mean analysis were accomplished using Microsoft Excel program.

RESULTS AND DISCUSSION

Mean analysis

Mean, standard error, and variance for the 6 generations were calculated from their corresponding three selected anthocyanin concentrations, total monomeric anthocyanin content, and total phenolic content. An analysis of variance (ANOVA) indicated that the effects of generations were highly significant for all studied traits (Table 1).

Table 1. Means and standard errors of different generation for three selected anthocyanin concentrations, total monomeric anthocyanin content, and total phenolic content in the kernels and cob of waxy corn cross [a].

Generations	C3G	Pg3G	Pn3G	MAC	TPC
kernel					
P_1	315.3 ± 4.2a	106.7 ± 5.0a	133.2 ± 1.5a	1556.4 ± 7.5a	5.7 ± 0.3a
P_2	0.5 ± 0.0c	0.5 ± 0.1c	0.3 ± 0.0d	2.6 ± 0.1d	2.0 ± 0.2d
F_1	114.2 ± 3.1b	37.6 ± 2.8b	34.7 ± 2.3bc	679.8 ± 7.4b	4.6 ± 1.3ab
F_2	118.4 ± 24.8b	30.8 ± 25.3bc	27.2 ± 21.2bcd	401.2 ± 137.1c	3.7 ± 0.5bc
BC_{11}	109.7 ± 63.8b	37.1 ± 34.4b	51.0 ± 32.2b	431.1 ± 262.6c	4.3 ± 0.9b
BC_{12}	31.8 ± 36.6c	8.5 ± 2.9bc	10.2 ± 10.5cd	135.2 ± 72.8d	2.8 ± 0.5cd
MP	157.9	53.6	66.8	779.5	3.9
cob					
P_1	992.2 ± 7.5a	184.7 ± 2.0a	220.5 ± 1.6a	2881.7 ± 4.7a	27.5 ± 1.2a
P_2	0.8 ± 0.1e	0.3 ± 0.1c	0.6 ± 0.1d	3.8 ± 0.7f	8.0 ± 0.4d
F_1	902.8 ± 8.5a	164.1 ± 8.8ab	216.7 ± 2.4a	2340.2 ±6.2b	19.1 ± 1.3b
F_2	404.8 ± 115.9c	119.0 ± 34.2b	134.6 ± 27.2b	1110.7 ± 324.6d	17.0 ± 3.3b
BC_{11}	644.2 ± 153.8b	159.8 ± 42.8ab	163.4 ± 16.3b	1759.2 ± 274.5c	20.3 ± 2.3b
BC_{12}	190.0 ± 92.5d	44.7 ± 28.3c	73.8 ± 37.4c	721.3 ± 246.4e	11.8 ± 1.1c
MP	496.5	92.5	110.6	1442.8	17.8

[a] P_1; $KND_{10\text{-}4P}$, P_2; BW, F_1; First filial generation of crosses, F_2; Second filial generation of crosses, BC_{11}; First backcross generation with parental line 1 and BC_{12}; First backcross generation with parental line 2. C3G; cyanidin 3-glucoside, Pg3G; pelargonidin 3-glycoside, Pn3G; peonidin 3-glucoside and MAC; monomeric anthocyanin contents was expressed as µg of CGE/g DW and TPC; total phenolic contents was expressed as mg of GAE/g DW. Values in the same column sharing different letters are expressed as significantly different ($P \leq 0.05$).

In this cross, means of the F_1 and F_2 generations for all traits lay between parental means. The mean for cyanidin-3-glucoside for the F_1 generation of corn kernel (114.2±3.1 µg/g DW) was 27.7% less than the mid parent value, whereas in the corn cob (902.8±8.5 µg/g DW) it was 45.0% more than the mid parent value. The mean cyanidin-3-glucoside for the F_2 generation of corn kernel (118.4±24.8 µg /g DW) was 24.7% less than

the mid parent value, whereas in the corn cob (404.8±115.9 µg /g DW) it was 18.5% also less than the mid parent value. The backcross mean was between the F_1 and the recurrent parents or less than F_1 and F_2 generation means in the both kernel and cob exception for backcross with purple colored cob of corn was 40.1% more than F_2 generation mean. These results indicated that varying relative importance of additive effects and dominance deviation for cyanidin-3-glucoside in the purple colored corn kernel and cob. Moreover, the variances of the parental lines and F_1 generation were low indicating the uniformity of this anthocyanin derivative within these generations.

The mean pelargonidin-3-glucoside for the F_1 generation of corn kernel (37.6±2.8 µg/g DW) was 29.8% less than the mid parent value, whereas in the corn cob (164.1±8.8 µg/g DW) it was 43.8% more than the mid parent value. The mean pelargonidin-3-glucoside for the F_2 generation of corn kernel (30.8±24.8 µg /g DW) was 42.5% less than the mid parent value, whereas in the corn cob (119.0±34.2 µg/g DW) it was 22.3% more than the mid parent value. The backcross mean was between the F_1 and the recurrent parents or less than F_1 and F_2 generation means in the both kernel and cob exception for backcross with purple colored cob of corn was 42.1% more than F_2 generation mean. These results indicated that varying relative importance of additive effects and dominance deviation for pelargonidin-3-glucoside in the purple colored corn kernel and cob.

The mean peonidin-3-glucoside for the F_1 generation of corn kernel (34.7±2.3 µg/g DW) was 40.1% less than the mid parent value, whereas in the corn cob (216.7±2.4 µg/g DW) it was 49.0% more than the mid parent value. The mean peonidin-3-glucoside for the F_2 generation of corn kernel (27.2±21.2 µg /g DW) was 59.3% less than the mid parent value, whereas in the corn cob (134.6±27.2 µg/g DW) it was 178% more than the mid parent value. The backcross mean was between the F_1 and the recurrent parents or less than F_1 and F_2 generation means in the both kernel and cob exception for backcross with purple colored cob of corn was 32.3% more than F_2 generation mean. These results indicated that varying relative importance of additive effects and dominance deviation for peonidin-3-glucoside in the purple colored corn kernel and cob.

The mean total monomeric anthocyanin content for the F_1 generation of corn kernel (679.8±7.4 µg of CGE/g DW) was 12.8% less than the mid parent value, whereas in the corn cob (2340.2±6.2 µg of CGE/g DW) it was 38.3% more than the mid parent value. The mean total monomeric anthocyanin content for the F_2 generation of corn kernel and cob (401.2±137.1 and 1110.7±324.6 µg of CGE/g DW, respectively) was 48.5 and 23.0% less than the mid parent value, respectively. The backcross mean was between the F_1 and the recurrent parents or less than F_1 and F_2 generation means in the both kernel and cob exception for backcross with purple colored cob of corn was 36.9% more than F_2 generation mean. These results indicated that varying relative importance of additive effects and dominance deviation for total monomeric anthocyanin content in the purple colored corn kernel and cob.

The mean total phenolic content for the F_1 generation of corn kernel and cob (4.6±1.3 and 19.1±1.3 mg of GAE/g DW, respectively) were 15.2 and 6.8% more than the mid parent value, respectively. The means total phenolic content for the F_2 generation of corn kernel and cob were very similar to the mid parent value (3.7±0.5 and 17.0±3.3 mg of GAE/g DW, respectively). The backcross means was between the F_1 and the first backcross generation with white colored parental line or less than F_1 and F_2 generation means in the both kernel and cob. However, the backcross means between the F_1 and the first backcross generation with purple colored parental line was more than F_2 generation mean and similar to the F_1 generation mean in the both kernel and cob. These results indicated that less pronounced role for dominance deviations and a more pronounced role for additive effects for total phenolic content in the purple colored corn kernel and cob.

Genetic components

As shown in Figure 1C, in the F_1 generation, when using purple-colored kernel and cob corn as a female parent and white-colored kernel and cob corn as a male parent, it is obtained all purple-colored kernel and cob progeny (data not shown). The result suggests that the color

inheritance of purple colored corn follows a maternal inheritance pattern. However, a few (0.1%) showed striped yellow kernel; therefore, the occurrence of kernel with purple and yellow colored may be related to the kernel color genes interactions between purple-colored and white-colored parents. The inheritance patterns, pericarp pigmentation, or kernel color did not fit Mendelian rules due to unstable alleles at the *P* locus (Hu *et al*., 1991). As shown in Figure 1D, the kernel and cob colors of the F_2 generation ranged from white to purplish black with different intensities and were difficult to categorize into distinct classes. The segregation pattern did not fit either a single- or two-gene model, but showed continuous variation indicating a quantitative mode of inheritance (Zewie and Bosland, 2003). Moreover, the kernel color of the backcross generations had an intensity to shift towards the parents. This result indicated that a change (increase or decrease) of color tendency depend on which parent is chosen as P_1.

There is interested in developing corn with an increased level of anthocyanin in corn, because high activities of antioxidants and health promoting of them. To increase the levels of anthocyanin using breeding methods common to hybrid breeding program, it is important to understand the gene effects in anthocyanins. One consequence of the different gene effects on choice of a breeding strategy is that developed hybrid following crossed to high parent would be expected to raise the concentration of antioxidant content in corn due to the predominant additive gene effects. The reduced model with 3 parameters was adequate to explain the variation for total phenolic content only (data not show). The model was extended to 6 parameters and it was fitted to the data. Additive [a], dominance [d], and epistasis were significant for most studied traits, indicating that both additive and dominance effects were important in the inheritance of these traits (Table 2). Additive [a] effects seemed to have a major contribution of the inheritance for pelargonidin-3-glucoside, peonidin-3-glucoside, and total monomeric anthocyanin content in the corn cob, and so indicate that selection in early generation is effective (Hayman, 1954).

Table 2. Estimates of different gene effects with standard errors for three selected anthocyanin concentrations and total monomeric anthocyanin content in the kernel and cob of waxy corn cross.

Parameters	C3G	Pg3G	Pn3G	MAC
kernels				
m	348.5 ± 10.8**	53.6 ± 1.1**	66.8 ± 2.0**	779.5 ± 2.0**
[a]	157.4 ± 2.0**	53.1 ± 1.1**	66.5 ± 2.0**	776.9 ± 2.0**
[d]	-686.2 ± 32.4**	-97.6 ± 14.5**	-117.2 ± 14.2**	-1729.8 ± 127.5**
[aa]	-190.6 ± 10.1**	NS	NS	NS
[ad]	-158.9 ± 21.6*	-41.7 ± 15.5**	-54.8 ± 16.4**	-877.9 ± 148.1**
[dd]	451.9 ± 39.9**	81.7 ± 14.5**	85.1 ± 14.3**	1630.1 ± 127.6**
cob				
m	496.5 ± 1.6**	92.5 ± 5.6**	110.6 ± 1.8**	893.8 ± 0.4**
[a]	495.7 ± 1.6**	92.2 ± 1.6**	110.0 ± 1.1**	1438.9 ± 1.5**
[d]	-741.9 ± 117.0**	NS	NS	-578.7 ± 33.1**
[aa]	NS	NS	NS	548.9 ± 24.5**
[ad]	NS	NS	NS	-833.2 ± 49.7**
[dd]	1148.3 ± 117.1**	78.9 ± 34.7*	89.0 ± 37.0**	2025.1 ± 313.1**

[a] m; mid-parent, [a]; sum of additive effects, [d]; pooled of dominance effects, [aa]; pooled of additive × additive epistatic effects, [ad]; pooled of additive × dominance epistatic, and [dd]; pooled of dominance × dominance epistatic effects. C3G; cyanidin-3-glucoside, Pg3G; pelargonidin-3-glycoside, Pn3G; peonidin-3-glucoside, and MAC; Total monomeric anthocyanin contents. *, ** Significant from zero at $p \leq 0.05$ and 0.01 respectively, based on normal derivate table. NS; non-significant.

Higher value additive [a] comparing with dominance [d] that is observed for total monomeric anthocyanin content in the corncob showing the gene correlation. In other words, a parent has genes with high performance while another one has gene with low performance (Fatechi *et al*., 2008). On the other hands, dominance [d] effects were significantly

negative for all studied traits in the corn kernel and cyanidin-3-glucoside in corncob indicated that dominance was towards direction of decreasing the anthocyanin levels, depend on which parent is chosen as P_1. The sign of dominance effect is a function of the F_1 generation mean value in relation to the mid parent value and it indicated that the genes from the high anthocyanin content parental line ($KND_{10\text{-}4P}$) contributed it.

Significant epistatic gene effects were observed for all these traits in both corn kernel and cob, but at varying degrees (Table 2). All the 3 kinds of epistatic effects, Additive × additive [aa] (-190.6±10.1), additive × dominance [ad] (-158.9±21.6), and dominance × dominance [dd] (451.9±39.9) epistatic effects were important for cyanidin-3-glucoside in the corn kernel while merely additive × additive [aa] and dominance × dominance [dd] epistatic effects were predominant in the corn cob. However, only the 2 types of epistatic effects, additive × dominance [ad] (-41.7±15.5) and dominance × dominance [dd] (81.7±14.5) were important for pelargonidin-3-glucoside in the corn kernel; whereas in the corn cob only one kind of epistatic effect dominance × dominance [dd] (78.9±34.7) was predominant for this trait. Similarly, for peonidin-3-glucoside in the corn kernel, only 2 types of epistatic effects (additive × dominance [ad] and dominance × dominance [dd]) were predominant in the corn kernel while only dominance × dominance [dd] epistatic effect was predominant for this anthocyanin derivative in the corncob. On the other hands, only 2 types of epistatic effects, additive × dominance [ad] (-877.9±148.1) and dominance × dominance [dd] (1630.1±127.6) epistatic effects were important for total monomeric anthocyanin content in the corn kernel that is according to pelargonidin-3-glucoside and peonidin-3-glucoside. Moreover, all of 3 epistatic effects, additive × additive [aa] (548.9±24.5), additive × dominance [ad] (-833.5±49.0), and dominance × dominance [dd] (2025.1±313.1) epistatic effects were important for total monomeric anthocyanin content in the purple corn cob. The preponderance of dominance [d] and dominance × dominance [dd] epistatic effects for cyanidin-3-glucoside, pelargonidin-3-glucoside, peonidin-3-glucoside and total monomeric anthocyanin content in purple waxy corn kernel and some traits in corn cob, revealed that the expression of these traits was largely controlled by genes having dominance action. Moreover, the importance of dominance and its epistatic effects for these traits in corn kernel and some studied traits in corncob indicated that the selection and breeding procedures in purple waxy corn could be modified to exploit this flexible epistatic effect by delaying the selection to later generations to fixed additive genes. This breeding procedure can be maintaining large populations prior to selection to provide maximum opportunities for advantageous combinations of gene occur (Nigam *et al*., 2001). Dominance × dominance [dd] epistasis contributed anthocyanin and its derivatives levels suggest it could be expected since the F_1 generation indicated that considerable heterosis; whereas, additive × additive [aa] and additive × dominance [ad] epistatic effects do not contribute to heterosis action. This result offer guideline to corn breeders interested in improve populations or hybrids with increased anthocyanin and it derivative levels. Epistatic effect in the form of duplicate gene interaction appeared for all traits in the both corn kernel and cob, where they had the opposite signs for the estimates of dominance [d] and dominance × dominance [dd] effects. Opposite sign between additive [a] and additive × additive epistatic effects for cyanidin-3-glucoside in the corn kernel suggested that the oppositional nature of interaction in this traits (Table 2). Epistatic effects and linkage may upwardly bias the dominance, and even partial-dominance may become pseudo-over-dominance (Falconer and Mackay, 1996). These results were obtained from only one season and may be biased to some degree by environment and their interactions, so needs for further study. However, our previously study found that genotype was the most important source of variations in anthocyanin levels of colored waxy corn germplasm (Harakotr *et al*., 2015).

CONCLUSION

In summary, most of additive [a], dominance [d], and their interaction effects were significant for all studied traits in the corn kernel while dominance [d] was not significant for pelargonidin-3-glucoside and peonidin-3-glucoside in the corncob. These results

indicated that the importance of the additive [a], dominance [d], and epistatic modes of gene action in controlling the inheritance of anthocyanin levels in waxy corn. Additive effects are more important than dominance effects for pelargonidin-3-glucoside and peonidin-3-glucoside in the corncob suggest that effective selection for these traits could be practiced even in the early generations. With the overwhelming predominance of non-additive gene effects obtained in this study for all studied traits in the kernel and cyanidin-3-glucoside in corn cob, this situation was concluded that the selection process in corn can be modified to fixed additive genes by delaying the selection to later generations. Additionally, the best way to increase anthocyanin levels as high as possible is to used purple kernel and cob inbred as maternal parent for producing purple color hybrid waxy corn. Although, additive gene effect are more important than dominance effects on anthocyanin content in corn kernel, however, dominance and its interaction is more important in corncob. Therefore, commercial single cross hybrid waxy corn was crossed by inbred with different color of kernel and cob that are regarded as private property for seed companies.

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