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TESTCROSS PERFORMANCE FOR ANTHOCYANIN AND ANTIOXIDANT ACTIVITY IN THE EAR COMPONENTS OF PURPLE WAXY CORN LINES

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SUMMARY

Purple corn color is expected to substitute for artificial colorants in commercial use and provide health benefits while combating corn waste. The question of how to maximize the genetic gain and anthocyanin level of purple waxy corn in practical breeding arises. We investigated the mode of gene action on anthocyanin yield, phenolic yield (PY), and antioxidant activity. Also, we selected promising lines with advanced anthocyanin in whole ear components on the basis of general combining ability. Seven waxy corn genotypes were subjected to hybrid formation by following the North Carolina II mating design, and their F_1progenies together with their parents and hybrid checks were laid out in randomized complete block design with three replications in two growing seasons during 2018–2019. The anthocyanin and PY of the tested genotypes were quantified, and their antioxidant activity was assayed through the 2,2-diphenyl-1-picrylhydrazyl and trolox equivalent antioxidant capacity methods. This study revealed an overwhelming dominance genetic effect, great reciprocal cross effects, and a low heritability for targeted traits in corn husks and cobs. The promising lines, namely NSX/DKA/PF for corn husk, PF/AB and S6248/TB/KND//PF for corn cob, and PF/AB and TB/KND//PF for corn kernel, were identified. Further strategies and implications in practical breeding are discussed in this paper.

Keywords: Anthocyanin improvement, combining ability, gene action, hybrid breeding, phytochemical yield

Key findings: Several tested lines showed impressive per se performance and general combining ability for anthocyanin attributes. These lines can be exploited as potential donors through intercrossing to recombine favorable dominant alleles. This study revealed an overwhelming dominance gene effect, great reciprocal cross effects, and a low narrow-sense heritability for targeted traits in corn husks and

cobs. Proper growing time, reciprocal cross inclusion, and delaying selection to later generations are workable solutions to maximizing genetic gain and boosting anthocyanin production in purple waxy corn effectively.

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INTRODUCTION

Waxy corn is one of the main vegetable crops and is popular in most Asian countries. It is sold mainly at local markets, and a few portions of total waxy corn production are evenly distributed regularly at supermarkets throughout Thailand (Lertrat and Thongnarin, 2008). People commonly consume corn kernels only, and all husks and cobs get tossed into the bin. These nonedible parts, defined as corn waste, are still underutilized. Cobs and husks represent 8.2% (2.4 ton ha^{-1}) and 7.0% (2.0 ton ha^{-1}) of the total dry matter of corn, respectively (Pordesimo *et al.,* 2004). Corn waste has become a serious environmental issue as the global production and consumption of vegetable corn are increasing.

In purple waxy corn, the anthocyanin content of the cob and husk is three to four times as high as that of the kernel (Simla *et al.,* 2016). Anthocyanin can reduce the risk of chronic diseases, such as cancer, obesity, and diabetes (Lao *et al.,* 2017), due to its antioxidant activities (Khoo *et al.,* 2017). To date, antioxidant activity in various crops is commonly assayed on the basis of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and validated via the trolox equivalent antioxidant capacity (TEAC) methods due to the simplicity, stability, and high accuracy and sensitivity of these methods (Moon and Shibamoto, 2009). At the same time, there is an effort to seek novel natural colorants due to consumer concern regarding synthetic food colorants. Purple corn color (PCC) is an affordable colorant and has been applied in the food industries in dessert and beverages (Petroni *et al.,* 2014). Two clinical studies have independently confirmed the safety issue of PCC in accordance with no-observed-adverse-effect level (Nabae *et al. 2008*) and Chinese toxicity grading standards (Zhou *et al., 2007*). PCC has been classified as a color additive in the USA (Code of Federal Regulations, 2011) and labeled by the European Union under the code of E-163 (EFSA Panel, 2013). The use of purple corn cobs and husks is expected to replace the use of artificial colorants and provide health benefits while combating corn waste.

Crop yield is one of key factors that determine the feasibility of purple waxy corn as an economical source of anthocyanin-based natural colorant (Chatham *et al.,* 2019). Improving the anthocyanin content of corn cobs and husks through conventional breeding is a sustainable way to boost the commercial production of PCC. This strategy is possible because the pigmentation of anthocyanin in corn cobs and husks is regulated by different sets of the dominant allele *P1* (Coe *et al.,* 1988). However, its inheritance is attributed to maternal effects (Harakotr *et al.,* 2016). Thus, the first aim of this study was to investigate how much reciprocal cross effects quantitatively reside in and which genetic effect is controlling anthocyanin in corn cobs, husks, and kernels.

Hybrid breeding has been proven as an effective method in corn because it exploits natural hybrid vigor (Hallauer *et al., 2010*). Promising inbred lines are required prior to obtaining superior hybrids through the test cross procedure. One of test cross goals is to better understand the combining ability of inbred lines, enabling breeders to perform selection (Davis, 1927; Hallauer *et al., 2010*). Line × tester analysis has been widely used to estimate general combining ability (GCA), and in selection for GCA, the testers should have been known to be broad based genotypes with stable performance over environments (Matzinger, 1953) and maximum genetic gain (Hallauer, 1975). However, the parameters of our inbred lines have not been recognized. The North Carolina II scheme (Comstock and Robinson, 1948) is a similar mating scheme that enables combining ability analysis regardless of the homozygosity level of inbreds and tester backgrounds. The GCA of several aleurone-pigmented corn kernels, such as carotenoids (Egesel *et al., 2003*) and beta-carotene (Li *et al., 2013*), has been estimated. However, information on the combining ability for anthocyanin yield (AY) in corn cob and husk remains lacking. Therefore, the second aim of this study was to determine the GCA for AY and phenolic yield (PY), and the antioxidant activity in the cobs, husks, and kernels of purple waxy corn inbred lines were determined through the DPPH and the TEAC methods.

MATERIALS AND METHODS

Plant material

Seven waxy corn genotypes were used in this study (Table 1). These genotypes included five S5 inbred lines, one commercial hybrid variety (Fancy), and one improved population (KND). Five S5 inbred lines have dark purple cob and husk with diverse kernel colors (purple, white, and yellow) and were developed by the Vegetable Corn Breeding Project, Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen University, Thailand. Fancy111 has good yield, good adaptation, reddish purple kernels and cobs, and green husks, whereas KND has dark purple kernels and cobs and reddish green husks.

The North Carolina II mating design was assigned in hybrid formation. Two genotypes (KND and Fancy) were designated as group 1, and five inbred lines were designated as group 2. In this case, normal hybrids were generated by intercrossing group 1 as a female and group 2 as a male, whereas reciprocals were derived from an opposite cross direction. Thus, 20 crosses were generated in the dry season of 2017/2018 at the Field Crop Research Station, Khon Kaen University, Thailand.

Field experiment

Seven parental lines, 20 hybrids, and three commercial check varieties (NMT, NTTm and KGW#1) were evaluated at Khon Kaen University, Thailand (16°28′27.7″ N, 102°48′36.5″ E; 190 m above sea

Table 1. Parental lines used in this study.

Lines	Name	Type	Color		
			Kernel	Cob	Husk
1	PF/AB B 4-2-1-#-B-B	Inbred	Purple	Dark Purple	Dark Purple
2	WSTS/PF//KND B1-1-1-2-#-B-B	Inbred	White	Dark Purple	Dark Purple
3	TB/KND//PF13-1-3-6-#-B-B	Inbred	Black	Dark Purple	Dark Purple
4	NSX/DKA//PF	Inbred	Yellow	Dark Purple	Dark Purple
5	S6248/TB/KND//PFB-B	Inbred	White	Dark Purple	Dark Purple
6	Fancy	Hybrid	Reddish purple	Purple	Green
7	KND	Population	Dark purple	Purple	Reddish green

level) during the 2018 rainy season and the 2018/2019 dry season. A randomized complete block design (RCBD) with three replications was assigned, and 90 plots were obtained. Each plot comprised two rows that were 5 m in length with a spacing of 0.75 m between rows and 0.25 m between hills. Thus, the plot size was 7.5 m^2, and plant density was 40 plants per plot. The recommended agricultural practices for vegetable corn production in Thailand were followed.

Sample preparation and extraction

Five representative ears from each plot were harvested at physiological maturity (35 days after pollination [DAP]) and oven-dried at 40 °C for 48 h to obtain the targeted moisture content of approximately 14%. Samples were derived from sibbing pollination to keep genetic purity from any strange pollen contamination. Each plot-based sample was in triplicate. The extraction of anthocyanin followed a previous method (Yang *et al., 2008*) with proper adjustments.

Determination of AY (kg CGE·DW ha^{-1})

The total anthocyanin content (TAC) of each husk, cob, and kernel sample was estimated through the pH differential method (Wrolstad *et al., 2005*). A UV–vis spectrophotometer (GENESYS 10S, ThermoScientific, Waltham, MA, USA) was used to measure absorbance at 510 and 700 nm. TAC was expressed as the mg of cyanidin-3-glucoside equivalents per 100 gram dry weight (mg CGE·100 g^{-1} DW) and derived from this following equation:

$$\text{TAC (cyanidin-3-glucoside equivalents, mg/L)} = \frac{(A \times MW \times DF \times 10^3)}{(\varepsilon \times 1)}$$

where A = (A510 nm − A700 nm) pH 1.0 − (A510 nm − A700 nm) pH 4.5; MW (molecular weight) = 449.2 g mol^{-1} for cyanidin-3-glucoside (cyd-3-glu); DF = dilution factor; 1 = path length in cm; ε = 26,900 molar extinction coefficient in L/mol/cm for cyd-3-glu; and 10^3 = factor for conversion from g to mg. Then, TAC was converted into AY by using the following formula:

$$\text{AY (kg CGE/.DW ha-1)} = \frac{\text{TAC (mg CGE. 100g}^{-1}\text{ DW}^{\text{mg CGE}}/_{\text{100 g DW}})}{\text{Dried matter yield (kg ha}^{-1}\text{ }^{\text{kg}}/_{\text{ha}})}$$

Determination of PY (kg GAE·DW ha^{-1})

The total phenolic content (TPC) of each sample was quantified by following Folin–Ciocalteau's phenol reagent protocol (Hu and Xu, 2011) with proper adjustments. Absorbance was read at 765 nm with a UV–visible spectrophotometer. A calibration curve was prepared on the basis of a standard solution of gallic acid (20, 40, 60, 80, and 100 µg/mL). The TPC was expressed as mg gallic acid equivalents per 100 gram dry weight (mg GAE·100 g^{-1} DW). Then, TAC was converted into PY using the following formula:

$$\text{PY (kg GAE/.DW ha-1)} = \frac{\text{TPC (mg GAE. 100 g}^{-1}\text{ DW}^{\text{mg GAE}}/_{\text{100 g DW}})}{\text{Dried matter yield (kg ha}^{-1}\text{ }^{\text{kg}}/_{\text{ha}})}$$

Determination of antioxidants

The DPPH free radical scavenging activity was assayed by measuring the capacity of bleaching a black-colored methanol solution of DPPH radicals as reported by Hu and Xu (2011). Absorbance was read at 517 nm. TEAC for each sample was assayed by following Hu and Xu (2011) with proper adjustments. The absorbance was immediately recorded at 734 nm. The results of both methods were expressed as micromole trolox equivalents (TE) per 100 gram of dry weight (µmol TE·100 g^{-1} DW).

Statistical analysis

GCA male, GCA female, and SCA effects in the North Carolina II analysis were represented by the mean squares of the male parent, the female parent, and male by female interaction (Hallauer *et al*., 2010). The statistical model for North Carolina II analysis followed Singh and Chaudhary (1979) with proper modifications with the linear mixed model of North Carolina II multienvironment (Rodríguez *et al., 2018*). Seasons were considered fixed, whereas hybrids and replications within seasons were considered as random effects. The hybrid means and combining ability estimates are best linear unbiased predictions; therefore, the Henderson method was chosen. GCA estimates, including their standard errors, were calculated by following Singh and Chaudhary's (1979) formula. Gene action was based on the proportion of additive-to-dominance variances (Singh and Chaudhary, 1979). GCA and SCA variances were estimated in accordance with Rodríguez *et al.* (*2018*) as follows:

$$\sigma^2_{\mathrm{GCA}} = \frac{(\mathrm{m}-1)\mathrm{MS_m} + (\mathrm{f}-1)\mathrm{MS_f} - (\mathrm{m}+\mathrm{f}-2)\mathrm{MS_{mf}}}{\mathrm{sr}(2\mathrm{mf}-\mathrm{m}-\mathrm{f})}$$

$$\sigma^2_{\mathrm{SCA}} = \frac{1}{\mathrm{sr}(\mathrm{MS_{mf}}-\mathrm{MS_e})},$$

Where *m, f, s,* and *r* are number of male parents, female parents, seasons, and replications, respectively, and MS_m, MS_f, MS_{mf}, and MS_e are the mean squares of male, female, male × female, and pooled error, respectively.

Then, additive and dominance variances were calculated on the basis of GCA and SCA variances, respectively, by following Rodríguez *et al.* (*2018*) as follows:

$$\sigma^2_A = 4\,\sigma^2_{GCA} \quad \sigma^2_D = 4\,\sigma^2_{SCA}\,.$$

Narrow-sense heritability was estimated on the basis of plot means (Holland *et al*., 2003) and was adjusted to percentage (%) units.

The reciprocal mean difference and reciprocal cross advantage of hybrid means, GCA, and SCA were subjected to ANOVA in RCBD to declare the significance of reciprocal effect. Least significant difference (LSD) at $P < 0.05$ was used to compare the effect of different two growing seasons on the hybrid means, GCA, and SCA of averaged reciprocal cross mean differences. Duncan's multiple range test (DMRT) at $P < 0.05$ was also performed to compare the means of the seven parental lines for each observed trait (Gomez and Gomez, 1984). The reciprocal cross effect was determined on the basis of the reciprocal cross mean difference (*Rd*) and reciprocal cross advantage (*Ra*). A plot based on *Rd* and *Ra* was calculated by using the following formula of Bulant *et al*. (2000) as:

$$Rd = RC - NC, \qquad Ra = \frac{Rd}{NC} \times 100$$

Where RC = reciprocal cross mean and NC = normal cross mean.

The North Carolina II analysis, variance components including additive and dominance variances, GCA estimates, and narrow-sense heritabilities for all observed traits were computed by Analysis of Genetic Designs in R (AGD) version 5.0. software (Rodríguez *et al., 2018*). DMRT at 5% of probability level was computed by using STAR version 2.0.1 software (STAR, 2014), single ANOVA on reciprocal effect, and LSD's test by Statistix 10.0 (Statistix 10, 2013).

RESULTS AND DISCUSSION

North Carolina II analysis

The results of the North Carolina II analysis of the corn cob and husk are presented in Table 2, and those for the corn kernel are presented in Table 3. The season effect was significant for all observed traits, except antioxidant activity, which was determined through the DPPH and the TEAC methods, of the corn cob in normal cross. The hybrid effect was significant for all traits, except for DPPH, of the corn kernel in normal cross and reciprocals. The effect of GCA male was significant for all traits, excluding DPPH, of the normal cross and the AY, PY, and TEAC of reciprocals of the corn kernel. The effect of GCA female was significant for all traits, except AY and PY, of the corn husk in reciprocals, TEAC of the corn cob in reciprocals, PY and DPPH of the normal cross and DPPH of the corn kernel in reciprocals. The effect of SCA was significant for all traits of the corn cob and husk, whereas this effect was not significant for all traits for the corn kernel except TEAC in normal cross. The interaction between hybrid and season (G × S) was significant for all observed traits. A similar pattern was observed for the interaction between SCA and season.

The significance of the hybrid effect indicated that genetic variation in respective traits existed. Thus, the variability in anthocyanin and antioxidant activity guarantees that breeders can evaluate and select purple waxy corn germplasm, for instance, kernels (Harakotr *et al., 2015*), anthers (Duangpapeng *et al.,*

Table 2. North Carolina II analysis of anthocyanin yield (AY), phenolic yield (PY), and antioxidant activity determined by the DPPH and the TEAC methods in corn cob and husk evaluated across two seasons between 2018 and 2019.

SOV	*df*	Corn Husk				Corn Cob			
		AY	PY	DPPH	TEAC	AY	PY	DPPH	TEAC
Normal cross									
Season (S)	1	185.9**	259.5**	762,257.7**	108,021,340.7**	27.8**	109.9**	15,847.0ns	46,800,944.7ns
Rep	4	1.4ns	1.7ns	3,855.5ns	778,825.5ns	0.1ns	0.4ns	12,000.3ns	200,198.7**
Hybrids (H)	9	25.7**	46.0**	956,086.2**	51,006,707.6**	6.0**	11.2**	653,089.2**	6,209,686.3**
GCA_M	4	32.5**	67.7**	1,392,876.5**	56,233,217.1**	2.1**	5.5**	236,934.3**	1,546,368.8**
GCA_F	1	16.2**	38.4**	1,626,972.5**	65,182,523.6**	20.4**	59.2**	3,362,702.3**	24,235,555.3**
SCA	4	21.2**	26.3**	351,574.3**	42,236,244.2**	6.4**	5.0**	391,840.9**	6,366,536.5**
H × S	9	5.2**	8.0**	112,419.5**	21,874,415.7**	4.8**	8.9**	542,882.6**	3,991,048.1**
GCA_M× S	4	4.1*	5.6ns	189,429.2**	31,039,469.0**	5.7**	5.2**	1,029,811.6**	7,039,744.4**
GCA_F × S	1	8.2**	14.1*	6,959.6ns	420,241.0ns	2.9**	26.7**	320,865.2**	558.8ns
SCA × S	4	5.6**	8.8**	61,774.7*	18,072,906.0**	4.4**	8.3**	111,458.0*	1,939,974.1**
Pooled error	36	1.0	2.2	20,695.1	438,552.5	0.3	0.9	37,801.1	36,0962.6
Reciprocal cross									
Season (S)	1	273.0**	386.5**	6,147,840.6**	258,267,784.7**	40.7**	163.4**	246,413.2**	25,599,299.9**
Rep	4	1.7ns	1.2ns	9,458.2ns	2,704,768.8**	1.5**	3.2**	108,138.1**	401,388.3ns
Hybrids (H)	9	7.4**	33.2**	478,159.2**	9,815,221.3**	11.5**	23.7**	1,305,356.3**	13,476,981.8**
GCA_M	1	0.7ns	2.9ns	8,544.3ns	10,753,852.0**	3.8**	11.6**	269,916.5**	271,367.2ns
GCA_F	4	11.2**	46.2**	687,226.7**	12,201,988.3**	15.5**	28.5**	1,864,862.2**	18,214,274.6**
SCA	4	5.2**	27.8**	386,495.6**	7,193,796.6**	9.4**	22.0**	1,004,710.4**	12,041,092.7**
H × S	9	7.2**	7.7**	147,568.3**	3,259,269.8**	2.8**	5.6**	515,810.4**	2,563,107.2**
GCA_M× S	1	12.0**	8.6*	243,206.7**	4,730,132.2**	1.4ns	3.0ns	548,189.5**	457,958.6ns
GCA_F × S	4	4.2**	4.2ns	148,285.9**	4,537,565.7**	2.9**	8.3**	711,008.0**	3,100,138.8**
SCA × S	4	9.0**	11.0**	122,941.0**	1,613,258.3**	3.1**	3.5**	312,518.0**	2,552,362.7**
Pooled error	36	0.8	1.7	16,996.3	36,9252.9	0.3	0.8	22,397.0	358,877.0

df degrees of freedom; *GCA_M* general combining ability of male parent; *GCA_F* general combining ability of female parent; *SCA* specific combining ability
** and * significant at $P < 0.01$ and $P < 0.05$, respectively; *ns* not significant.

Table 3. North Carolina II analysis of anthocyanin yield (AY), phenolic yield (PY), and antioxidant activity determined by the DPPH and the TEAC methods in corn kernel evaluated in the dry season 2018.

SOV	d.f.	Corn kernel			
		AY	PY	DPPH	TEAC
Normal cross					
Rep	2	0.10ns	0.60ns	54.07ns	1,576.77ns
Hybrids	9	0.24**	1.11*	68.45ns	2,972.72**
GCA_M	4	0.25*	1.58**	81.89ns	3,422.67**
GCA_F	1	0.53**	1.28ns	33.80ns	4,708.32**
SCA	4	0.17ns	0.60ns	63.69ns	2,088.86*
Error	18	0.06	0.32	30.17	549.68
Reciprocal cross					
Rep	2	0.02ns	0.34ns	177.03**	167.11ns
Hybrids	9	0.50**	2.27**	41.82ns	1,668.85**
GCA_M	1	0.11ns	1.65ns	135.99*	1,853.92ns
GCA_F	4	0.93**	3.77**	30.12ns	2,105.76**
SCA	4	0.17ns	0.93ns	29.98ns	1,185.68ns
Error	18	0.09	0.44	23.19	434.39

df degrees of freedom; *GCA_M* general combining ability of male parent; *GCA_F* general combining ability of female parent; *SCA* specific combining ability; ** and * significant at $P < 0.01$ and $P < 0.05$, respectively; *ns* not significant.

2019), and cobs (Khampas *et al.,* 2015). The significant effects of season and the interaction between hybrid and season on almost all observed traits indicated that contrasting climate profiles between dry and wet seasons significantly altered the anthocyanin pigmentation of corn cobs and husks. A previous report revealed the presence of the location effect and interaction between genotype and location on the anthocyanin content of purple waxy corn cob (Khampas *et al.,* 2015). Then, the significant effects of GCA male and GCA female suggested that the performance of each parental line on anthocyanin pigmentation in hybrid formation was divergent. The significance of the SCA effect indicated that there was at least a parental pair producing an impressive hybrid in terms of anthocyanin pigmentation.

Gene action, heritability, and reciprocal cross effect

The relative proportion of GCA and SCA variances and narrow-sense heritability are presented in Figure 1. Among normal cross hybrids, dominance variance existed for the AY (86.7%), PY (63.8%), and TEAC (87.3%) of corn husks; AY (100%), DPPH (66.2%), and TEAC (100%) of corn cobs; and AY (69.3%), DPPH (91.0%), TEAC (71.6%) of corn kernels, whereas equal proportions between dominance and additive variances were present for the remaining traits (44%–49% : 51%–56%). Among reciprocal hybrids, dominance variance was clearly predominant for all observed traits of corn husks (75.0%–87.5%) and corn cobs (82.5%–94.8%), whereas the same result was noticed for the TEAC

(69.2%) of corn kernel. A balanced proportion between dominance and additive effects was noticed for the DPPH (45.3%:54.7%) of corn kernels only. The presence of additive effect was revealed for the AY (75.7%) and PY (65.4%) of corn kernels. Hybrids derived from reciprocal crosses are a good model for explaining the mode of gene action that controls a trait of particular corn parts.

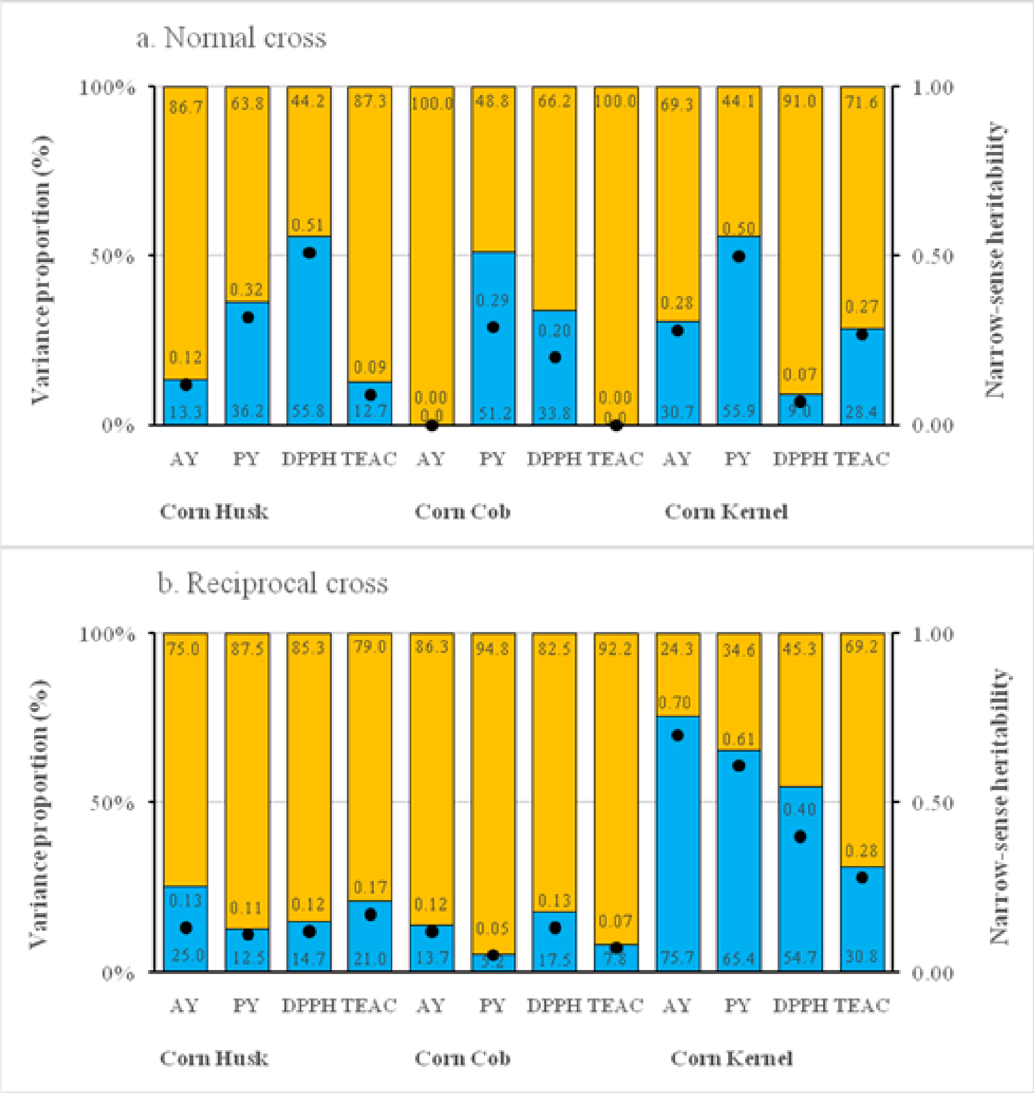


Figure 1. Proportion of additive genetic variance (lower blue bar) and dominance genetic variance (upper orange bar), and narrow-sense heritability (black dot) on anthocyanin yield (AY), phenolic yield (PY), and antioxidant activity (the DPPH and the TEAC methods) in purple waxy corn husk, cob, and kernel derived from normal cross (a) and reciprocals (b).

The GCA/SCA variance ratio can be used to identify the gene action that controls traits (Singh and Chaudhary, 1979). The reciprocal cross hybrids used in this study comprised two males and five females. These males were assigned as testers for the five tested lines. This result showed that the dominance effect predominantly controlled AY and antioxidant activity in purple waxy corn husk and cob. In contrast to those in corn husks and cobs, additive genetic effect was important for AY and antioxidant activity in purple waxy corn kernels. Previous reports also showed overwhelming additive gene effects for other phytochemicals, such as β-carotene (Li *et al., 2013*) and carotenoids (Egesel *et al., 2003*) in pigmented corn kernel. Further results showed low narrow-sense heritabilities (h^2_{ns}) for all observed traits, excluding DPPH in corn husk (0.51) and PY in corn kernel (0.50) among normal cross hybrids and AY (0.70) and PY (0.61) for corn kernels among reciprocal hybrids. The lack of additive gene effect and low heritability for corn husks and corn cobs revealed the slow progress of genetic gain. The tight intensity of selection should be better to perform on later generations on phytochemicals yield and antioxidant levels in purple waxy corn.

The reciprocal effect was significant for the AY, PY, DPPH, and TEAC of corn husks and cobs; however, this effect was absent for these traits of corn kernels (Table 4). In corn husk and cob, this effect increased in the dry season for AY and PY. For instance, on hybrid per se, the Rd increased from 1.1 kg CGE·DW ha^{-1} to 3.2 kg CGE·DW ha^{-1} and 1.7 GAE·DW ha^{-1} to 4.3 kg GAE·DW ha^{-1} for AY and PY, respectively, in corn husk. A similar trend was noticed for corn cobs. The GCAs of each parental line and SCA of each parental pair were also influenced by reciprocal cross effects with similar pattern on hybrid per se. The direct comparison of reciprocal effects on either anthocyanin or even other phytochemicals is not possible; however, a previous study revealed that this effect in hybrid means of sweet and waxy corn genotypes was significant with low-to-medium contributions to agronomic traits, including yield (Dermail *et al., 2018*). Therefore, the joint effects of reciprocal crosses and interactions between season and reciprocals altered the AY, PY, and antioxidant activity of purple waxy corn husks and cobs. To the best our knowledge, this current study was the first to report these effects on anthocyanin and antioxidant activity. High consideration for suitable planting date and reciprocal cross inclusion can help breeders maximize anthocyanin production in purple waxy corn.

GCA, parent means, and selection

The estimations of GCA and per se performance among seven parental inbred lines in corn husk, cob, and kernel are given in Tables 5, 6, and 7. Favorable genotypes with high AY, PY, and antioxidant level were represented by high parental mean and positive GCA. In corn husk, NSX/DKA/PF assigned as a tester (male) showed the highest parental mean and positive GCA for AY (4.60 kg CGE·DW ha^{-1}; 2.85, $P < 0.01$), PY (8.98 kg GA·DW ha^{-1}; 3.91, $P < 0.01$), DPPH (1,533.16 µmol TE·100 g^{-1} DW; 394.09, $P < 0.01$), and TEAC (12,637.22 µmol TE·100 g^{-1} DW; 3,448.33, $P < 0.01$). NSX/DKA/PF assigned as a tested line (female) also

Table 4. Reciprocal cross effects on hybrid performance, GCA, and SCA on anthocyanin yield (AY), phenolic yield (PY), and antioxidant activity (DPPH and TEAC) in corn husk, cob, and kernel.

Traits	Season	Reciprocal cross mean differences (% advantages)					
		Hybrid per se		GCA		SCA	
Corn husk							
AY (kg CGE·DW ha^{-1})	Wet	1.1 **b	(72.1 *a)	0.6 *b	(138.9 **a)	0.7 *b	(107.3*b)
	Dry	3.2 **a	(116.5 **a)	1.5 **a	(183.1 **a)	1.9 **a	(440.4 **a)
PY (kg GAE·DW ha^{-1})	Wet	1.7 **b	(66.6 *a)	0.7 *b	(100.7*b)	1.1*b	(186.1*b)
	Dry	4.3 **a	(73.6 *a)	1.7 **a	(252.6 **a)	2.6 **a	(224.2 **a)
DPPH (µmol TE·100 g DW)	Wet	433.4 **a	(32.5 *a)	218.9 *a	(142.8 **a)	224.4 **a	(155.4*a)
	Dry	315.3 **a	(26.5 *a)	161.8 *b	(93.85*b)	168.6 *b	(114.6*a)
TEAC (µmol TE·100 g DW)	Wet	3,241.5 **a	(81.8 **a)	1,532.2 **a	(129.1 **a)	2,737.7 **a	(118.4*a)
	Dry	1,568.4 **b	(22.0 *b)	605.5 **b	(71.5 *b)	1,086.1 *b	(108.5*a)
Corn cob							
AY (kg CGE·DW ha^{-1})	Wet	1.1 **b	(54.7 *b)	0.6 *b	(99.2 *b)	0.6 *b	(936.1 **a)
	Dry	2.1 **a	(169.9 **a)	1.1 **a	(218.8 **a)	1.5 **a	(147.9 *b)
PY (kg GAE·DW ha^{-1})	Wet	1.2 *b	(37.6 *b)	0.6 *b	(95.5 *b)	0.8 *b	(533.6 **a)
	Dry	2.9 **a	(65.9 **a)	1.6 **a	(297.7 **a)	1.7 **a	(184.1*b)
DPPH (µmol TE·100 g DW)	Wet	520.3 **a	(56.1*a)	270.4 **b	(118.5 *b)	298.3 *a	(256.5*a)
	Dry	470.3 **a	(40.2*a)	320.1 **a	(226.9 *a)	285.8 *a	(172.7 *b)
TEAC (µmol TE·100 g DW)	Wet	1,516.5 **b	(27.4*a)	918.4 **a	(192.4 *a)	1,065.6 **a	(482.3 **a)
	Dry	1,850.1 **a	(30.1*a)	854.6 **a	(155.1 *a)	1,138.6 **a	(271.8*b)
Corn seed†							
AY (kg CGE·DW ha^{-1})		0.4ns	(30.9ns)	0.2ns	(179.0 ns)	0.2ns	(163.0 ns)
PY (kg GAE·DW ha^{-1})		0.9**	(23.5*)	0.5*	(204.9ns)	0.4ns	(231.0 ns)
DPPH (µmol TE·100 g DW)		6.6ns	(7.1ns)	1.7ns	(113.8ns)	3.1ns	(185.4ns)
TEAC (µmol TE·100 g DW)		33.7ns	(7.6ns)	10.9ns	(71.5ns)	16.2ns	(163.0ns)

†evaluated in the dry season only
** and * significant at $P < 0.01$ and $P < 0.05$;*ns* not significant
number in parentheses is reciprocal cross advantages (%); values followed by different letters within the same column and the same trait represent significant season effect on reciprocal cross effects based LSD 5%.

Table 5. Parental means and general combining ability (GCA) estimates on anthocyanin yield (AY), phenolic yield (PY), and antioxidant activity determined by the DPPH and the TEAC methods in corn husk evaluated across two seasons between 2018 and 2019.

Lines	AY (kg CGE·DW ha^{-1})		PY (kg GAE·DW ha^{-1})		DPPH (μmol TE·100 g DW)		TEAC (μmol TE·100 g DW)	
	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA
Normal cross								
Female								
Fancy111	1.03c	−0.52*	1.57c	−0.80*	245.27e	−164.67**	1,821.78 e	−1,042.29**
KND	2.57b	0.52*	3.40b	0.80*	1,152.54c	164.67**	4,571.88 cd	1,042.29**
Male								
PF/AB	2.17b	−1.13*	3.31b	−1.95**	1,331.40b	−397.53**	4,945.42bc	−1,038.40**
WSTS/PF//KND	1.94b	−0.96*	3.12b	−1.40*	534.82c	−123.46*	5,404.03b	−1,194.59**
TB/KND//PF	2.30b	−0.75*	3.14b	−1.10*	1,033.68d	−189.20**	4,683.89bcd	−1,951.10**
NSX/DKA/PF	4.60a	2.85**	8.98a	3.91**	1,533.16a	394.09**	12,637.22a	3,448.33**
S6248/TB/KND//PF	0.77c	−0.01ns	1.76c	0.55ns	1,085.35c	316.10**	4,044.03d	735.76**
P value	**		**		**		**	
SE females		0.37		0.54		52.53		241.81
SE males		0.59		0.85		83.06		382.34
Reciprocal cross								
Female								
PF/AB	2.17b	0.38*	3.31b	−0.39ns	1,331.40b	67.46ns	4,945.42bc	−195.52ns
WSTS/PF//KND	1.94b	−0.12ns	3.12b	−0.18ns	534.82c	−266.41**	5,404.03b	−663.20*
TB/KND//PF	2.30b	−1.45**	3.14b	−2.34**	1,033.68d	−235.21**	4,683.89bcd	−1,061.80**
NSX/DKA/PF	4.60a	1.21**	8.98a	3.11**	1,533.16a	261.66**	12,637.22a	1,515.99**
S6248/TB/KND//PF	0.77c	−0.02ns	1.76c	−0.20ns	1,085.35c	172.51**	4,044.03d	404.53*
Male								
Fancy	1.03c	0.11ns	1.57c	0.22ns	245.27e	11.93ns	1,821.78 e	−423.36**
KND	2.57b	−0.11ns	3.40b	−0.22ns	1,152.54c	−11.93ns	4,571.88 cd	423.36**
P value	**		**		**		**	
SE females		0.51		0.75		75.27		350.83
SE males		0.32		0.47		47.60		211.89

Means followed by the same letter in the same column are not significantly different based DMRT 5%*SE* standard error as a critical value for GCA estimates

** and * GCA estimates are significantly different from zero at ≥2SE and ≥SE, respectively.

Table 6. Parental means and general combining ability (GCA) estimates of anthocyanin yield (AY), phenolic yield (PY), and antioxidant activity determined by the DPPH and the TEAC methods in corn cob evaluated across two seasons between 2018 and 2019.

Lines	AY (kg CGE·DW ha^{-1})		PY (kg GAE·DW ha^{-1})		DPPH (µmol TE·100 g DW)		TEAC (µmol TE·100 g DW)	
	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA
Normal cross								
Female								
Fancy	0.69e	−0.58**	1.91d	−0.99**	306.14e	−236.74**	3,247.08c	−635.55**
KND	3.54a	0.58**	5.75a	0.99**	1,307.09ab	236.74**	6,074.44b	635.55**
Male								
PF/AB	2.35b	0.19ns	3.90b	0.03ns	1,443.69a	101.84ns	7,567.50a	234.90ns
WSTS/PF//KND	1.15de	−0.49*	2.51cd	−0.92*	733.42d	−167.35*	5,864.66b	−473.11*
TB/KND//PF	2.09bc	−0.26ns	3.08bc	−0.34ns	1,074.12c	−95.18ns	5,629.43b	150.18ns
NSX/DKA/PF	1.82bc	0.60*	3.43b	0.37ns	1,163.86bc	176.86*	7,102.94a	368.07*
S6248/TB/KND//PF	1.49cd	−0.04ns	3.34bc	0.85*	986.67c	−16.17ns	5,717.08b	−280.04ns
P value	**		**		**		**	
SE females		0.21		0.34		70.99		219.38
SE males		0.34		0.54		112.25		346.87
Reciprocal cross								
Female								
PF/AB	2.35b	0.93**	3.90b	1.22**	1,443.69a	305.64**	7,567.50a	1,334.74**
WSTS/PF//KND	1.15de	−1.36**	2.51cd	−1.73**	733.42d	−549.68**	5,864.66b	−1,484.90**
TB/KND//PF	2.09bc	−0.38*	3.08bc	−0.71*	1,074.12c	−56.80ns	5,629.43b	−854.15**
NSX/DKA/PF	1.82bc	−0.59*	3.43b	−0.76*	1,163.86bc	−147.56*	7,102.94a	−150.18ns
S6248/TB/KND//PF	1.49cd	1.40**	3.34bc	1.98**	986.67c	448.40**	5,717.08b	1,154.49**
Male								
Fancy	0.69e	0.25*	1.91d	0.44*	306.14e	67.07*	3,247.08c	−67.25ns
KND	3.54a	−0.25*	5.75a	−0.44*	1,307.09ab	−67.07*	6,074.44b	67.25ns
P value	**		**		**		**	
SE females		0.33		0.51		86.40		345.86
SE males		0.21		0.32		54.64		218.74

Means followed by the same letter in the same column are not significantly different based DMRT 5% SE standard error as a critical value for GCA estimates

** and * GCA estimates are significantly different from zero at ≥2SE and ≥SE, respectively.

Table 7. Parental means and general combining ability (GCA) estimates of anthocyanin yield (AY), phenolic yield (PY), and antioxidant activity determined by the DPPH and the TEAC methods in corn kernels evaluated in the dry season 2018.

Lines	AY (kg CGE·DW ha^{-1})		PY (kg GAE·DW ha^{-1})		DPPH (μmol TE·100 g DW)		TEAC (μmol TE·100 g DW)	
	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA
Normal cross								
Female								
Fancy	0.82b	−0.13*	3.47a	−0.21ns	91.00a	−1.06ns	416.67c	−12.53*
KND	1.23a	0.13*	2.65b	0.21ns	98.63a	1.06ns	484.43a	12.53*
Male								
PF/AB	0.55c	0.03ns	1.85c	0.61*	91.00a	3.14ns	451.93b	21.28*
WSTS/PF//KND	0.10d	0.19**	0.60d	0.10ns	49.07c	1.12ns	321.37e	12.39ns
TB/KND//PF	0.45c	0.16**	1.59c	−0.25ns	93.53a	1.03ns	409.73c	15.58*
NSX/DKA/PF	0.08d	−0.31**	0.60d	−0.73**	40.23c	−6.42**	292.23e	−35.11**
S6248/TB/KND//PF	0.09b	−0.07ns	0.91d	0.27ns	67.40b	1.13ns	359.00d	−14.14*
P value	**		**		**		**	
SE females		0.08		0.20		2.00		8.56
SE males		0.14		0.32		3.17		13.53
Reciprocal cross								
Female								
PF/AB	0.55c	0.16*	1.85c	−0.02ns	91.00a	1.16ns	451.93b	8.50*
WSTS/PF//KND	0.10d	0.05ns	0.60d	−0.19ns	49.07c	1.16ns	321.37e	7.53ns
TB/KND//PF	0.45c	0.51**	1.59c	0.73**	93.53a	0.29ns	409.73c	22.25**
NSX/DKA/PF	0.08d	−0.55**	0.60d	−1.21**	40.23c	−3.94**	292.23e	−24.00**
S6248/TB/KND//PF	0.09b	−0.18*	0.91d	0.68**	67.40b	1.33ns	359.00d	−14.28*
Male								
Fancy	0.82b	0.06ns	3.47a	0.23ns	91.00a	2.13ns	416.67c	7.86ns
KND	1.23a	−0.06ns	2.65b	−0.23ns	98.63a	−2.13ns	484.43a	−7.86ns
P value	**		**		**		**	
SE females		0.11		0.24		1.75		7.61
SE males		0.17		0.38		2.78		12.03

Means followed by the same letter in the same column are not significantly different based DMRT 5% SE standard error as a critical value for GCA estimates

** and * GCA estimates are significantly different from zero at ≥2SE and ≥SE, respectively.

showed a high, positive GCA for AY (1.21, $P < 0.01$), PY (3.11, $P < 0.01$), DPPH (261.66**), and TEAC (1,515.99, $P < 0.01$). Thus, NSX/DKA/PF was a good combiner to enhance AY, PY, and antioxidant activity in corn husk.

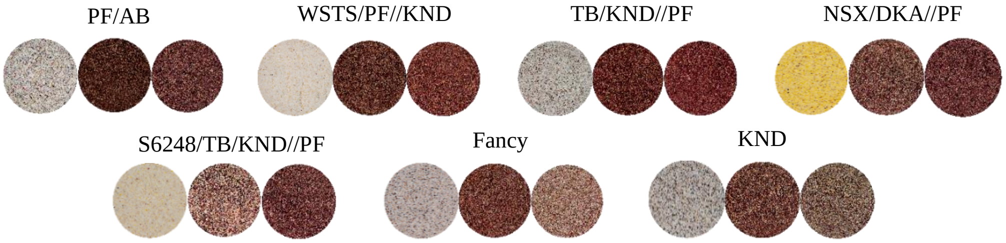
In corn cob, PF/AB was assigned as a female and exhibited a high parental mean and a high positive GCA for AY (2.35 kg CGE·DW ha^{-1}; 0.93, $P < 0.01$), PY (3.90 kg GAE·DW ha^{-1}; 1.22, $P < 0.01$), DPPH (1,443.69 μmol TE·100 g^{-1} DW; 305.64, $P < 0.01$), and TEAC (7,567.50 μmol TE·100 g^{-1} DW; 1,334.74, $P < 0.01$). Another tested line, S6248/TB/KND//PF, had medium-to-high parental means and the highest positive GCA for AY (1.49 kg CGE·DW ha^{-1}; 1.40, $P < 0.01$), PY (3.34 kg GA·DW ha^{-1}; 1.98, $P < 0.01$), DPPH (986.67.69 μmol TE·100 g^{-1} DW; 448.40, $P < 0.01$), and TEAC (5,717.08 μmol TE·100 g^{-1} DW; 1,154.49, $P < 0.01$). Thus, PF/AB and S6248/TB/KND//PF were good combiners for enhancing the AY, PY, and antioxidant activity of corn cobs. In corn kernels, PF/AB assigned as a female and exhibited a high parental mean and a high, positive GCA for AY (0.55 kg CGE·DW ha^{-1}; 0.16, $P < 0.01$), DPPH (91.00 μmol TE·100 g^{-1} DW; 1.16), and TEAC (451.93 μmol TE·100 g^{-1} DW; 8.50, $P < 0.05$). Another tested line, TB/KND//PF, had a high parental mean and an appreciable, positive GCA for AY (0.45 kg CGE·DW ha^{-1}; 0.51, $P < 0.01$), PY (1.59 kg GAE.DW ha^{-1}; 0.73, $P < 0.01$), and TEAC (409.73 μmol TE·100 g^{-1} DW; 22.25, $P < 0.01$). Thus, PF/AB and TB/KND//PF were desirable combiners for enhancing the AY, PY, and antioxidant activity of corn kernels.

GCA estimates for particular traits were helpful for identifying promising lines in directed breeding programs because they possessed the targeted desirable alleles (Yong *et al., 2013*). In this study, we did not observed that any of the tested inbred line had impressive performance in terms of phytochemical yields and antioxidant activity in whole targeted corn parts, namely corn husk, cob, and kernel. Instead, promising lines for particular corn parts were identified. NSX/DKA/PF was a good combiner for corn husks. PF/AB and S6248/TB/KND//PF were good combiners for corn cobs. PF/AB and TB/KND//PF were good combiners for corn kernels (Figure 2). In addition, this study confirmed the genetic control of anthocyanin biosynthesis in practical breeding. Anthocyanin biosynthesis is regulated by several dominant alleles, and differences in allele combinations are responsible for differently pigmented corn parts (Coe *et al., 1988*; Hossain *et al., 2019*). Favorable alleles residing in these lines can be further exploited as potential donors by intercrossing these lines to recombine the dominant alleles in segregated gene combinations. Hopefully, a favorable genotype of purple waxy corn with impressive anthocyanin production in whole targeted corn parts can be developed in the near future.

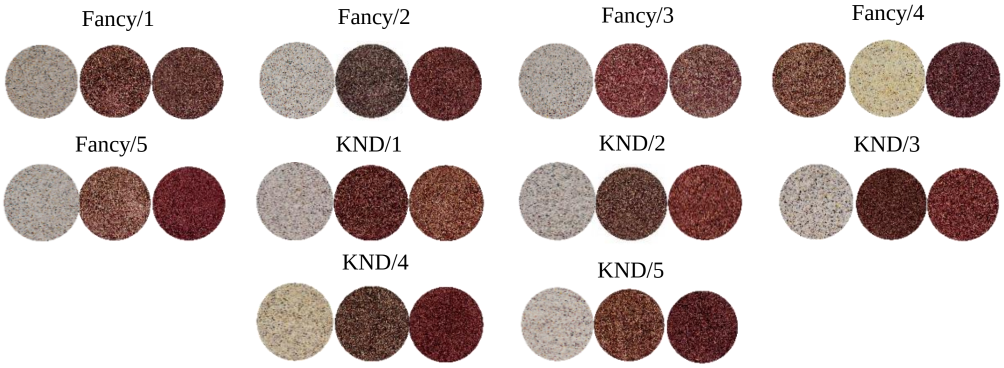
CONCLUSIONS

The AY, PY, and antioxidant activity of different parts of purple waxy corn were controlled by different modes of gene action. The dominance gene effect was predominant in the corn husk and corn cob, whereas the

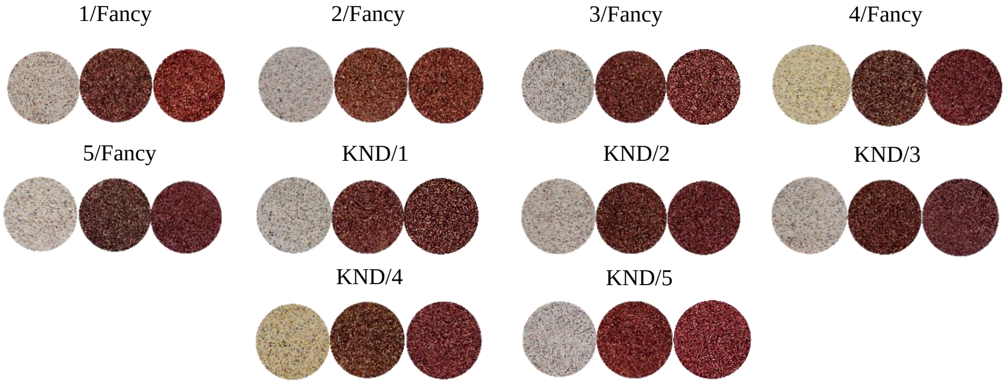
(a) Parental Inbreds



(b) Normal Cross F_1



(c) Reciprocal Cross F_1



(d) Check varieties

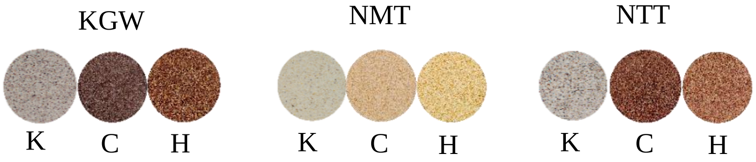


Figure 2. Powdered samples of corn kernel (K), cob (C), and husk (H) of (a) parental inbred lines, (b) normal cross F_1, (c) reciprocal cross F_1, and (d) commercial check varieties. 1 is PF/AB; 2 is WSTS/PF//KND; 3 is TB/KND//PF; 4 is NSX/DKA/PF; and 5 is S6248/TB/KND//PF.

additive gene effect was important in corn kernels. This study revealed an overwhelming dominance gene effect, great reciprocal cross effects, and a low narrow-sense heritability for targeted traits in corn husks and cobs. Proper growing time, reciprocal cross inclusion, and delaying selection to later generations are a workable solution to maximizing genetic gain and boosting anthocyanin production effectively. No tested inbred line had impressive performance and GCA for targeted traits in whole corn parts. Instead, promising lines for a particular corn part, namely NSX/DKA/PF for corn husks, PF/AB and S6248/TB/KND//PF for corn cobs, and PF/AB and TB/KND//PF for corn kernels, were revealed. These selected lines can be further exploited as potential donors through intercrossing to recombine the dominant alleles in segregated gene combinations. Hopefully, a favorable genotype of purple waxy corn with appreciable amount of anthocyanin in whole targeted corn parts can be developed in near future.

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