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Reciprocal cross effect over seasons on agronomic and yield traits in waxy corn under tropical savanna

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Abstract. *Sawangha M, Dermail A, Chankaew S, Simla S, Lertrat K, Suriharn K. 2023. Reciprocal cross effect over seasons on agronomic and yield traits in waxy corn under tropical savanna. Biodiversitas 24: 4120-4125.* The exponentially increasing number of hybrids per additional inbred lines results in a tremendous workload in crop hybrid breeding. The question arises if reciprocal crosses could be excluded in waxy corn hybrids to reduce the workload by up to 50%. In this study, we aimed to assess the importance of reciprocal cross effects for yields, yield components, and agronomic traits of sweet-waxy corn F_1 hybrids and to validate the stability of those effects over seasons. Five parental lines, comprised of one sweet corn inbred line and four waxy corn inbred lines, were mated through a top cross scheme to generate eight F_1 progenies, including the reciprocals. All genotypes, including checks, were arranged in a Randomized Complete Block Design (RCBD) with three replications in the dry season of 2021/2022 and the rainy season of 2022 in Khon Kaen, Thailand. Genotype was significant for all observed traits, and the interaction between genotype and season was significant for all traits. The reciprocal cross-effect was non-significant for most observed traits. The non-significance of the interaction between N vs. R and season confirmed the absence of reciprocal cross effects in different growing seasons. Thus, dissecting one of two possibilities in each cross combination is advisable for affordable hybrid formations. Considering the absence of reciprocal cross effects but significant G × E effect for given traits, allocating more efforts and resources for conducting multi-environment trials is advisable to reduce the gap between actual and potential yields of testing hybrids.

Keywords: Hybrid breeding, maternal effect, mating design, top cross, *Zea mays*

INTRODUCTION

The corn seed business is quite attractive in Thailand. By 2018, this commodity values 73 million USD from 24 million tons of corn seeds, ranking the highest among other crops in Thailand (Thai Seed Trade Association 2019). Although the seed demand for waxy corn is relatively smaller than other corn types, such as field and sweet corn (Napasintuwong 2014), the trends are projected to increase annually. Waxy corn is one of the important vegetable crops in Thailand and neighboring countries such as Vietnam, Laos, and China. Farmers in those regions commonly cultivate waxy corn as vegetable crops, meaning that it is harvested as immature or milk stage, and people consume it as cooked ears either by boiling or steaming (Dermail et al. 2022). When the fresh kernels are cooked, the texture becomes sticky and tender as the amylopectin, the major starch composing endosperm, experiences physicochemical changes (Ketthaisong et al. 2014; Samarakoon et al. 2020). The genetic mechanism underlying high amylopectin content is the presence of a single recessive *waxy1* gene located on chromosome 9 (Hussain et al. 2019). The rising demand for waxy corn is attributed to its unique eating quality and early maturity compared to other corn types, allowing growers to cultivate waxy corn as cash crops.

Although the adoption rate of corn hybrids has increased remarkably and almost 100% in 2011 (Napasintuwong 2014), the number of available commercial waxy corn hybrids is still limited. In tropical savanna regions, hybrid waxy corn is proposed for high yield, resistance to biotic and abiotic, vigor stems, early maturity, good ear appearance, and eating quality (Dermail et al. 2020; Dermail et al. 2022). However, performing hybrid formations followed by extended hybrid testing for consecutive years and locations is time-consuming and resource intensive. For instance, in a full-diallel scheme, each additional inbred will exponentially increase the number of hybrids. Furthermore, half of the crosses derived from the reciprocal crosses carry the same genetic materials as the corresponding crosses within the same pairwise parents. The question arises whether reciprocal crosses could be excluded during hybrid formation.

Reciprocal cross-effects are composed of maternal and non-maternal factors. Maternal effects may be caused by cytoplasmic genetic, endosperm nuclear, or maternal phenotypic. It indicates the contribution of the maternal parent to the phenotype of its offspring beyond the equal chromosomal contribution expected from each parent (Roach and Wulff 1987). Meanwhile, non-maternal is due to the interaction between cytoplasmic genetics and endosperm nuclear (Roach and Wulff 1987). The evidence of reciprocal cross effects in corn is still arguable.

Moreover, the inclusions of reciprocal crosses were important in the yield of waxy corn (Dermail et al. 2018). On the other hand, the effects were negligible for the anthesis date, silking date, plant height, ear height, and yield components in field corn (Dermail et al. 2023). However, none of the above studies reported on the impact of reciprocal crosses in waxy genetic background, focusing on yields, yield components, and agronomic traits. Furthermore, Thailand is a tropical savanna where seasonal variations often alter corn performance under yield trials (Sintanaparadee et al. 2022). While this effect may also affect the magnitudes of reciprocal cross effects, information on the stability of reciprocal effects over different growing seasons is lacking. Therefore, this study aimed (i) to assess the importance of reciprocal effects for yields, yield components, and agronomic traits of sweet-waxy corn F_1 hybrids and (ii) to validate the stability of those effects over seasons. Information obtained in this study will facilitate breeders whether reciprocal crosses should be included in routine maize hybrid testing. If not, the workload in further steps, such as field trials and phenotyping, would be reduced.

MATERIALS AND METHODS

Plant material and mating design

Five parental lines comprised of one sweet corn inbred line and four waxy corn inbred lines were used in this study (Table 1). A sweet corn line, 101LBW, has two recessive genes (*btbtwxwx*) (Fuengtee et al. 2020). Four waxy corn lines, including WSTS/IDL//KND-B, TB/KND//IDL-B, NSX/DKA/IDL-B, and S6248/TB/KND//PF-B have dark purple cob and husk with diverse kernel colors (purple, white, and yellow) (Kalapakdee et al. 2020). The Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen University, Thailand, developed sweet and waxy lines. Those lines were top crossed, assigning sweet corn lines female and waxy corn lines male to produce four cross combinations. Besides, the reciprocal crosses of those combinations were included to assess the reciprocal cross effects. All eight F_1 progenies were expected to possess sweet and waxy kernels in segregation modes. Two commercial F_1 hybrids, KGW and Sweet Violet, developed by the Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen University, Thailand, and East West Seed Company, respectively, were used as checks. Genotype KGW had complete purple pigmentation of the aleurone kernels both at the fresh and dried harvest stages, while genotype Sweet Violet had 25% purple kernels at the fresh stage and became 50% at the dried stage. Both checks are synergistic sweet-waxy types that possess segregated kernels of 75% waxy kernels (*wx*) and 25% sweet kernels (*bt*) within an ear (Ja et al. 2022).

Experimental design and crop management

Fifteen corn genotypes, including five parents, eight F_1 hybrids, and two checks varieties, were arranged in a Randomized Complete Block Design (RCBD) with three replications in the dry season of 2021/2022 and the rainy season of 2022 at Agronomy Field Crop Station, Khon Kaen University (16•28'27.7" N, 102•48'36.5" E; 190 masl), Thailand. Each plot consisted of 2 rows 5 m long with 75 cm × 25 cm of plant spacing. The plot size was 7.5 m^2, with 40 plants per plot.

The crop field management followed the Thailand agricultural recommendations, including fertilization, irrigation, pest, disease, and weed control. Cattle manure at the rate of 1,875 kg ha^{-1} and a combination of chemical fertilizers (15-15-15 of N, P2O5, and K2O) at 156.25 kg ha^{-1} was incorporated into the soil during soil preparation. Sowing 2-3 seeds per hill and thinning a plant per hill was done two weeks after planting. A mixed chemical fertilizer with the formula 15-15-15 of N, P2O5, and K2O was incorporated into the soil at 125 kg ha^{-1} on the planting day. Fertilizer urea (46-0-0) was applied to the crop at 320 kg ha^{-1} twice at 14 days after planting (DAP) and 30 DAP. At 50 DAP, a mixed chemical fertilizer with the formula 13-13-21 of N, P2O5, and K2O was applied to the crop at the rate of 160 kg ha^{-1}. Both herbicide and manual weeding controlled weeds at critical periods, whereas pest and disease were controlled if only exceeding the economic injury level (EIL).

Data collection

Ten random plants (5 from each row in a plot in each replication), excluding the border plants, were observed on plant height (cm), from ground level to the base of the tassel after milk stage and ear height (cm), from ground level to the node bearing the uppermost ear after milk stage. Measurements on a plot basis were performed on days to tasseling (DT), number of days from sowing up to 50% of the plants have shed the pollen and days to silking (DS), number of days from sowing up to silks have emerged on 50% of the plants.

Table 1. Parental lines were used in this study

Name	Type	Genotype	Color		
			Kernel	Cob	Husk
Sweet corn					
101LBW	Inbred	*btbtwx*wx	White	White	Green
Waxy corn					
WSTS/IDL//KND-B	Inbred	*BtBtwxwx*	White	Purple	Dark purple
TB/KND//IDL-B	Inbred	*BtBtwxwx*	Black	Dark purple	Purple
NSX/DKA/IDL-B	Inbred	*BtBtwxwx*	Yellow	Dark purple	Dark purple
S6248/TB/KND//PF-B	Inbred	*BtBtwxwx*	White	Dark purple	Dark purple

Yield and yield components were measured after harvest at the fresh stage (approximately 20 days after pollination). The traits included husked ear weight (g), using digital weight without the husk; husked ear diameter (cm), using digital caliper without the husk; and husked ear length (cm), using ruler without the husk. The yield was averaged from the best ten ears per plot and converted to (t ha^{-1}) unit.

Data analysis

Data were subjected to the combined analysis of variance in RCBD over seasons. Paired t-test was done to declare the significance of the reciprocal effect. Tukey's Honest Significant Difference (HSD) at 5% was used for comparing the means of tested and checked hybrids (Gomez and Gomez 1984). All statistical analyses were done by using Statistix 10.0 program.

The reciprocal cross effect was determined based on the reciprocal cross mean difference (Rd) and reciprocal cross advantage (Ra). The Ra estimates were calculated following the below formula (Dermail et al. 2018):

$$Ra = (RC - NC) / NC \times 100$$

Where Ra: reciprocal crosses advantage (%), RC: reciprocal cross mean, NC: normal cross mean.

RESULTS AND DISCUSSION

Analysis of variance and the minor importance of reciprocal cross effects over seasons

The season was significant for all observed traits except for husked ear length (EL), seed number per row (SN), and the ratio between seed weight and ear weight (PC) (Table 2). Genotype was significant for all observed traits. The normal cross was significant for all observed traits except for silking date (DS). The reciprocal cross was significant for all observed traits except EL, SN, PC, anthesis date (DT), and DS. The comparison between normal and reciprocal crosses (N vs. R) was significant only for DT. Interaction between genotype and season (G × S) was significant for all observed traits. Interaction between normal cross and season was significant for unhusked yield (UY), husked yield (UW), PC, and DS. Interaction between reciprocal cross and season was significant for UY, UW, and PC. Interaction between reciprocal cross and season was significant for UY, UW, and PC. Interaction between reciprocal cross effect and season ((N vs. R) × S) was significant only for PC.

The significance of season on most observed traits and the G × S interaction on all observed traits indicated the presence of seasonal variations between the dry and the rainy seasons in Thailand. At least a few inbred lines and F_1 progenies showed unstable performance over different growing seasons. Therefore, multi-environment trials are required to avoid selection bias due to environmental variations, regardless of agroecology and climatic conditions, to reduce the gap between potential and actual yields. Farfan et al. (2013) applied a linear mixed model for field corn multi-environment trials in Texas. They found that the largest G × E variation for grain yield was three-way interaction between genotype, location, and year. In temperate Iowa, the presence of two-way interaction between genotype and year was also important for plant architecture and tassel attributes in field corn (Trentin et al. 2023). Meanwhile, the two-way interaction between genotype and location in tropical rainforest Indonesia was important for flowering times, plant architecture, yield components, and grain yield in field corn (Azrai et al. 2022). In tropical savanna Thailand, however, the common G × E variation for agronomic traits and yields in field corn and vegetable corn was two-way interaction between genotype and season (Dermail et al. 2020; Fuengtee et al. 2020; Dermail et al. 2022; Ja et al. 2022; Dermail et al. 2023). Under a tropical savanna climate, the dry season was more suitable for corn growth and development to obtain a better seed set than the rainy season (Sintanaparadee et al. 2022). They noticed the dry season had lower daily minimum and maximum temperatures, relative humidity, and total rainfall but higher solar radiation and longer photoperiod per day.

Table 2. Mean squares of four combinations over two cross directions evaluated in two seasons of 2021 and 2022

SOV	*df*	UY	HY	UW	HW	EL	RN	SN	PC	EH	PH	DT	DS
Season (S)	1	113**	33.0*	39,548**	11,582**	29.4ns	11.4**	27.8ns	90ns	197**	9,205**	462.0**	1,152.0**
Rep/S	4	0.3	0.3	100	82	4.9	0.0	11.3	14	5	41	4.5	7.7
Genotype (G)	14	94.6**	62.2**	33,247**	21,866**	39.1**	11.6**	242.0**	106**	695**	3,821**	48.8**	55.7**
Normal (N)	3	42.6**	18.8**	14,916**	6,512**	11.9*	4.0*	36.9**	88**	195**	665**	3.2*	1.8ns
Reciprocal (R)	3	19.3**	6.1**	6,799**	2,147**	3.6ns	7.0**	7.3ns	16ns	106*	307**	1.0ns	1.2ns
N vs. R	1	4.2ns	1.1ns	1,486ns	357ns	0.7ns	1.7ns	0.1ns	1ns	0ns	16ns	7.5**	5.3ns
G × S	14	22.2**	1.3**	554**	443**	9.6**	1.7*	25.6**	122**	372**	477**	2.7**	6.1**
N × S	3	2.4**	0.7ns	851**	227ns	6.8ns	0.6ns	1.4ns	89**	25ns	148ns	0.7ns	8.8**
R × S	3	1.9*	0.7ns	698*	233ns	0.8ns	0.1ns	6.8ns	40**	34ns	16ns	0.9ns	1.5ns
(N vs. R) × S	1	1.2ns	1.5ns	438ns	553ns	1.6ns	0.0ns	0.1ns	275**	34ns	126ns	0.0ns	0.1ns
Pooled error	56	0.2	0.3	87	96	2.9**	0.8	6.1	30	29	53	1.1	2.1
cv (a) (%)		4.5	5.6	4	5	12.4	2.2	11.9	6	3	4	4.4	5.7
cv (b) (%)		4.0	5.8	4	6	9.7	6.7	8.8	9	7	4	2.1	2.9

Note: UY: unhusked yield (ton ha^{-1}); HY: husked yield (ton ha^{-1}); UW: unhusked ear weight (g ear^{-1}); HW: husked ear weight (g ear^{-1}); EL: husked ear length (cm); RN: row number per ear; SN: seed number per row; PC: the ratio between seed weight and ear weight (%); EH: ear height (cm); PH: plant height (cm); DT: anthesis date (days after planting); DS: silking date (days after planting); **data significant at $P \leq 0.01$; *data significant at $P \leq 0.05$; ns: data non-significant at $P \leq 0.05$

The significant effect of genotype indicated that tested hybrids showed phenotypic variability on all observed traits as both hybrids and their parents were included. It also may be due to the different genetic backgrounds of the pairwise parents (sweet × waxy corn lines). Earlier studies reported that introducing subtropical waxy corn lines and temperate sweet corn lines into tropical corn background was effective in broadening the genotypic variance and enhancing genetic gains of sweet-waxy corn hybrids on agronomic traits (Dermail et al. 2020; Dermail et al. 2022). The lack of significant effect of *N vs. R* for most traits observed indicated that either normal or reciprocal cross will produce a similar hybrid performance for agronomic traits. Furthermore, using similar source germplasm of purple waxy corn, Kalapakdee et al. (2020) found that reciprocal cross effects were significant for anthocyanin yield, phenolic yield, and antioxidant activities of corn husk and corn cob, but the effects were not significant for given traits of corn seed. The non-significance of the interaction between *N vs. R* and season validated the absence of reciprocal cross effects in different growing seasons.

In the dry season, the reciprocal cross differences were significant only for PC (Table 3). In addition, the reciprocal cross advantage ranged from -8.1% to 6.4% over traits observed. Likewise, in the rainy season, the reciprocal cross differences were significant for PC, DT, and DS. The reciprocal cross advantage ranged from -2.0% to 7.5% over traits observed. Based on the results above, choosing only one cross-direction for affordable hybrid formations is advisable. However, efficient hybrid seed production remains the priority; thus, selecting one out of two possible crosses (e.g., A × B or B × A) from each combination regarding better MP values on traits related to hybrid seed production is important. Both sides should have good nicking, for instance, low anthesis-silking interval (ASI). In addition, each side has unique properties. Male parents should be taller, shed pollen longer, have big tassels, and produce abundant pollen. Female parents, in contrast, should be lower than male plants and have a high seed set (Dermail et al. 2022). Contrasting findings were noticed on whether reciprocal cross effects are important in corn. Some studies reported that those effects were significant and important on agronomic performance such as kernel yield, ear diameter, silking date, plant height, ear height (Khehra and Bhalla 1976) of field corn, and unhusked and husked yields of sweet and waxy corn (Dermail et al. 2018). Besides, reciprocal cross effects significantly impacted heterosis for yields; thus, superior sweet and waxy corn hybrids could be obtained when reciprocal crosses were included (Dermail et al. 2018). In contrast, other similar studies using three gene pools of field corn (temperate inducers, tropical inducers, tropical non-inducers) under different growing seasons concluded that reciprocal effects, including maternal and non-maternal were significant, but it could be negligible for anthesis date, silking date, plant height, ear height, yield components, and final seed set (Dermail et al. 2023).

Selection of sweet-waxy corn hybrids for good tropical adaptation and high-yielding and biomass

Farmers in tropical savanna regions favor maize cultivars showing early maturity, short plant, and high yield since their agro-ecology often encounters a prolonged dry season and short, extreme rainfall (Dermail et al. 2020). This study emphasized hybrid selection on high yields, the ratio between seed and ear weights, shorter plant stature, and early maturity. Averaged over two seasons, two hybrid genotypes, NSX/DKA/IDL/101LBW, and 101LBW/NSX/DKA/IDL, were promising because they belonged to top four hybrids regarding husked yield (Table 4). In the dry season, hybrid NSX/DKA/IDL/101LBW weighed 229 g per ear and yielded 12.2 t ha^{-1}, while the reciprocal 101LBW/NSX/DKA/IDL weighed 222 g per ear and yielded 11.8 t ha^{-1}. In the rainy season, hybrid NSX/DKA/IDL/101LBW weighed 231 g per ear and yielded 12.3 t ha^{-1}, while the reciprocal 101LBW/NSX/DKA/IDL weighted 199 g per ear and yielded 10.6 t ha^{-1}. The result indicated that those hybrids had stable, high yielding over seasons. However, those two candidates had the tallest plant stature (NSX/DKA/IDL/101LBW ~ 209 cm in the dry season and 187 cm in the rainy season; 101LBW/NSX/DKA/IDL ~ 200 cm in the dry season and 187 cm in the rainy season) yet early flowering (50-51 days after planting in the dry season and 45 days after planting in the rainy season).

Table 3. Mean squares of four combinations over two cross directions evaluated in two seasons of 2021 and 2022

	n	UY	HY	UW	HW	EL	RN	SN	PC	EH	PH	DT	DS
Dry season													
Normal (N)	12	15.1	10.9	283	205	18.0	14.8	30.4	63.2	86.3	200	50.3	51.6
Reciprocal (R)	12	16.0	11.6	301	217	17.9	14.6	30.6	58.1	84.6	188	49.4	50.8
(R-N) difference		0.9ns	0.7ns	18ns	12ns	-0.1ns	-0.2ns	0.2ns	-5.1*	-1.7ns	-2ns	-0.9ns	-0.8ns
$\hat{R}$ (%)		5.6	6.4	6	6	-0.6	-1.4	0.7	-8.1	-2.0	-1	-1.8	-1.6
Rainy season													
Normal (N)	12	13.2	10.4	247	194	20.1	14.4	30.9	60.2	88.4	176	45.7	44.2
Reciprocal (R)	12	13.4	10.3	252	193	20.7	14.2	31.1	64.7	89.9	180	44.9	43.6
(R-N) difference		0.2ns	-0.1ns	5ns	-1ns	0.6ns	-0.3ns	0.3ns	4.5*	1.5ns	5ns	-0.9*	-0.6*
$\hat{R}$ (%)		1.5	-0.1	2	-1	3.0	-1.7	0.8	7.5	1.7	3	-2.0	-1.4

Note: n: sample size; (R-N): the difference between reciprocal cross mean and normal cross mean; $\hat{R}$: relative reciprocal cross advantage [(reciprocal cross mean-normal cross mean)/normal cross mean] x 100; UY: unhusked yield (t ha^{-1}); HY: husked yield (t ha^{-1}); UW: unhusked ear weight (g ear^{-1}); HW: husked ear weight (g ear^{-1}); EL: husked ear length (cm); RN: row number per ear; SN: seed number per row; PC: the ratio between seed weight and ear weight (%); EH: ear height (cm); PH: plant height (cm); DT: anthesis date (days after planting); DS: silking date (days after planting); **data significant at $P \leq 0.01$; *data significant at $P \leq 0.05$; ns: data non-significant at $P \leq 0.05$

Table 4. Means for yield and agronomic traits of waxy corn hybrids evaluated across two seasons

Hybrid	Dry season					Rainy season				
	HY	HW	PC	PH	DT	HY	HW	PC	PH	DT
WSTS/IDL//KND/101LBW	8.2d	155d	67.0ab	182b	51.3a	8.1d	153d	53.8d	166c	46a
TB/KND//IDL/101LBW	11.9ab	224ab	55.3de	181b	50.3ab	11.0ab	207ab	61.3a-c	181ab	46a
NSX/DKA/IDL/101LBW	12.2ab	229ab	68.3a	209a	50.3ab	12.3a	231a	65.8ab	187a	45a
S6248/TB/KND//PF/101LBW	11.3bc	212bc	62.0a-d	187b	49.0b	10.0bc	188bc	60.1c	169bc	45a
101LBW/WSTS/IDL//KND	10.2c	192c	58.3c-e	180b	49.3ab	9.2cd	172cd	63.8a-c	174a-c	44a
101LBW/TB/KND//IDL	11.1bc	208bc	60.3b-d	183b	49.0b	10.5bc	197bc	65.9ab	177a-c	46a
101LBW/NSX/DKA/IDL	11.8abc	222ab	61.3a-d	200ab	50.0ab	10.6a-c	199a-c	62.9a-c	187a	45a
101LBW/S6248/TB/KND//PF	13.2a	248a	52.3e	188b	49.3ab	11.0ab	206ab	66.3a	181ab	44a
Grand mean	11.5	216	60.7	189	49.7	10.5	197	62.2	179	45
KGW	11.8a-c	220a-c	65.7a-c	190ab	49.3ab	11.3ab	212ab	62.2a-c	187a	46a
Sweet Violet	13.1a	246a	56.7de	186b	48.7b	10.9ab	205ab	60.4bc	177a-c	45a
HSD%	1.6	30	7.7	20	2.2	1.7	33	5.7	15	3

Note: HY: husked yield (t ha^{-1}); HW: husked ear weight (g ear^{-1}); PC: the ratio between seed weight and ear weight (%); PH: plant height (cm); DT: anthesis date (days after planting). Means followed by different letters within the same column are significantly different based on Tukey's Honest Significant Difference (HSD) at 5%

Tall plant stature yet early maturity is still accepted by farmers rather than a tall plant with late maturity. Early maturity is an important agronomic trait in field corn for avoiding water deficit and heat tolerance (Badu-Apraku et al. 2013; Oyekunle et al. 2015; Nelimor et al. 2019). Another advantage of early maturity is that growers may receive more income annually if they cultivate the crop twice to triple yearly. The taller corn plant has been reported to be correlated with higher corn biomass (Infante et al. 2018). Perhaps our two promising hybrids had higher fresh biomass. The fresh biomass derived from those two hybrids, including corn stalk, husks, and leaves, could be utilized as by-products for cattle feeds. Previous studies reported that the inclusion of fresh corn silage in cattle diet resulted in improved carcass characteristics of beef cattle (Johnson et al. 2019). In addition, it can improve the gain of body weight of cross-bred cattle by providing the best nutritive composition with the lowest concentration of highly indigestible fiber and the highest concentration of organic matter and energy (Nazli et al. 2018). Those reports indicated that high corn biomass is economically beneficial and could add to the animal's nutritional values. Those two hybrids possessed purple stems, leaves, and husks. Perhaps those intense purple colorations are due to genetic contribution from purple waxy corn genotype NSX/DKA/IDL-B. In the previous report, waxy corn genotype NSX/DKA/IDL-B was a good combiner and had high anthocyanin yield, phenolic yield, and antioxidant activities in corn cob and husk (Kalapakdee et al. 2020). Since purple pigmentation correlated with anthocyanin content in many plant species (Khoo et al. 2017), including in purple waxy corn (Harakotr et al. 2015), the animal feed industry will benefit from those purple-rich materials (Khamphasan et al. 2020).

In conclusion, the effects of genotype, season, and their interaction (G × S) on agronomic traits, including yield components, were significant in waxy corn. We confirmed preliminary studies in waxy corn that the reciprocal cross effects were negligible and stable over seasons. Pre-screening among inbred lines having good mid-parent values for traits related to hybrid seed production is encouraged. Thus, the ideal mating design for hybrid testing in waxy corn should be partial diallel, instead of either full or half, for flexibility to dissect one out of two possibilities in each cross combination. Considering the absence of reciprocal cross effects but significant G × S effect for given traits, we suggested breeders allocate more efforts and resources for conducting multi-environment trials to reduce the gap between actual and potential yields of testing hybrids.

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