

Inbred-hybrid relationship and heterosis on agronomic traits in bi-color sweet and waxy corn

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Abstract. *Patthawaro P, Demail A, Chankaew S, Simla S, Lomthaisong K, Lertrat K, Suriharn K. 2023. Inbred-hybrid relationship and heterosis on agronomic traits in bi-color sweet and waxy corn. Biodiversitas 24: 6351-6359.* Maize hybrids become more acceptable than other cultivar types since they promise high yield and uniformity by exploiting the heterosis advantages. Numerous lines are to be crossed to obtain the promising hybrids, and the progenies must be tested in multi-environment trials. Handling large number of genotypes in routine work is resource intensive; thus, the question arises if hybrid prediction based on the mid-parent (MP) value is feasible to reduce the workload. This study aimed to investigate the reliability of MP values in hybrid prediction through simple linear correlation and to estimate the magnitude of heterosis on given traits in sweet-waxy corn. Six parental lines and 10 F₁ hybrids were evaluated in Khon Kaen in the dry and rainy seasons (2021/2022). Genotype and the interaction between genotype and season were significant for all observed traits. Waxy corn genotype 8A3-B was a good combiner for yield and yield components. Heterosis was trait-dependent, and it could imply parental adaptation when the estimation was done in contrasting environments. We found that the MP value can be used to predict the hybrid performance for flowering times only, but this approach was not effective for the rest agronomic traits. Since commonly, maize improvements are regarding multiple favorable traits, the strategy to use MP as single factor in hybrid prediction was not reliable. Other approaches, either SCA alone or the sum of mid-parent GCA and SCA, could be implemented for hybrid prediction in future studies.

Keywords: General combining ability, hybrid breeding, hybrid prediction, yield trial, *Zea mays*

INTRODUCTION

Waxy corn belongs to vegetable corn (*Zea mays* L.). It was first discovered in China and later gained popularity in other Asian countries (Dong et al. 2019). In Thailand, waxy corn is harvested at fresh stage and consumed as steamed products (Demail et al. 2022). Although waxy corn ranks three after field corn and sweet corn regarding total planting area and the number of registered varieties sold annually, the demand for waxy corn is rising in both domestic and international markets. The rising demands are attributable to the unique eating quality of steamed/boiled waxy kernels, which are sticky and tender. Stickiness happens when corn genotypes carry mutant *waxy1* gene located on chromosome 9 encoding high kernel amylopectin content (Hossain et al. 2019). In the past, growers used open-pollinated varieties that farmers could recycle the seeds for further growing seasons; however, it comes to low productivity, susceptibility to biotic and abiotic stresses, and inbreeding depression. Nowadays, farmers are increasingly turning to adopt hybrid cultivars to benefit from high yield, uniformity, and adaptability (Hallauer et al. 2010). However, the adoption rate of hybrid seeds in waxy corn is slower than that in maize in Thailand. Perhaps, a lack of promising waxy corn hybrids may explain that fact.

Breeding waxy corn for high carotenoid content has been initiated by the Plant Breeding Research Center for Sustainable Agriculture at Khon Kaen University in Thailand. In preliminary studies, we have assessed the variability of yellow small-ear waxy corn germplasm for prolificacy, total carotenoids, lutein, and zeaxanthin, and few promising populations could be further improved (Sukto et al. 2020). We also conducted recurrent selection to enhance the lutein and zeaxanthin of orange waxy corn populations (Khamkoh et al. 2019), and some of their inbreds were used in our current study. Hybrid breeding is well adopted in most cross-pollinated crop species, including corn, due to heterosis advantages. Heterosis is prominent with varying levels from low for flowering times, moderate for agronomic traits and yield components, and high for final yields in vegetable corn (Demail et al. 2020) and field corn (Demail et al. 2023). Perhaps, this hybrid vigor can be exploited in our waxy corn germplasm.

As a routine work in hybrid breeding program, it requires initial information on general combining ability (GCA) and specific combining ability (SCA) to select promising inbred lines and hybrids, respectively. The GCA describes the average performance of an inbred line in hybrid combination, while the SCA indicates which combinations relatively perform better or worse than would be expected regarding the average performance of the lines

tested (Hallauer et al. 2010). However, hybrid formation and extended multi-environment trials are resource intensive, making it not convenient for startup seed business. The question arises whether the prediction of hybrid performance without the need to generate all possible combinations is possible. One of approaches in hybrid prediction is based on the mid-parent (MP) value. In this method, the prediction accuracy of hybrid performance based on their MP values is determined by simple linear correlation and regression (Makumbi et al. 2011). In maize, conflicting reports of the hybrid-MP relation are noticed. The correlation between hybrid means and MP values for maize grain yield was significant (Li et al. 2021), while Ertiro et al. (2013) argued that the hybrid-MP relation for given traits was negligible. However, similar studies on agronomic traits of waxy corn are still lacking. Therefore, the objectives of this study were (i) to investigate the reliability of MP values in hybrid prediction through simple linear correlation analysis and (ii) to estimate the magnitude of heterosis on given traits in waxy corn hybrids. The information of this study will benefit breeders to what extent the hybrid-MP relationship on given traits will reduce the workload regarding hybrid formation and testing through multi-environment trials.

MATERIALS AND METHODS

Plant materials and hybrid formation

Six parental lines were used in this study (Table 1). Briefly, five waxy corn inbred lines including genotypes 2A3-B, 8A3-B, 12C5-B, 13A5-B, and 99A4-B belonged to normal waxy group and had single recessive gene (*BtBt_{wxwx}*).

Those lines had orange kernel color, stiff stalk, and erect leaves and derived from two cycles of recurrent selection (Khamkoh et al. 2019). Besides, a sweet corn inbred line, 101LBW, had double recessive genes (*btbtSh2Sh2_{wxwx}*), white kernel pigmentation, and good tropical adaptation when tested in Thailand (Dermail et al. 2020; Fuengtee et al. 2020). All parental lines were developed by the Plant Breeding for Sustainable Agriculture, Khon Kaen University, Thailand.

Both normal and reciprocal crosses were generated via top cross mating design. For normal crosses, six lines were divided into two groups (Table 2), one as female (1 sweet line) and another one as male (5 waxy lines), to obtain five F₁ hybrids. For reciprocal crosses, the same number of crosses were derived with the opposite cross directions (5 waxy lines as female parents and 1 sweet line as male parents). Hence, ten crosses were obtained in the rainy season of 2021 at the Agronomy Research Station, Khon Kaen University, Thailand. Due to various maturity levels of our parental lines, three staggered-planting dates were done to ensure good nicking.

Field experiment and crop management

All genotypes including six parental lines, ten hybrids, and two check varieties were evaluated at the Agronomy Research Station, Khon Kaen University, Thailand in the dry and the rainy seasons (2021/2022). A Randomized Complete Block Design (RCBD) with 3 replications was applied in both seasons. Each plot consisted of two rows of 5 m in length with a spacing of 0.75 m between rows and 0.25 m between hills. Hence, the plot size was 7.5 m² to obtain 40 plants per plot.

Table 1. Brief description of six inbred lines used in this study

Parent	Cultivar type	Seed color	Genotype	Source
Waxy corn				
2A3-B	Inbred line	Orange	<i>BtBt_{wxwx}</i>	KKU
8A3-B	Inbred line	Orange	<i>BtBt_{wxwx}</i>	KKU
12C5-B	Inbred line	Orange	<i>BtBt_{wxwx}</i>	KKU
13A5-B	Inbred line	Orange	<i>BtBt_{wxwx}</i>	KKU
99A4-B	Inbred line	Orange	<i>BtBt_{wxwx}</i>	KKU
Sweet corn				
101LBW	Inbred line	White	<i>btbtSh2Sh2_{wxwx}</i>	KKU

Note: KKU: Khon Kaen University

Table 2. Top cross mating scheme including reciprocals to obtain 10 F₁ sweet-waxy corn hybrids

Genotype (F/M)	2A3-B	8A3-B	12C5-B	13A5-B	99A4-B
101LBW	101LBW/2A3-B	101LBW/8A3-B	101LBW/12C5-B	101LBW/13A5-B	101LBW/99A4-B
Genotype (F/M)	101LBW				
2A3-B	2A3-B/101LBW				
8A3-B	8A3-B/101LBW				
12C5-B	12C5-B/101LBW				
13A5-B	13A5-B/101LBW				
99A4-B	99A4-B/101LBW				

Note: F: Female, M: Male

The crop field management applied in this experiment was according to the Thailand agricultural recommendations including fertilization, irrigation, and pest, disease, and weed controls. Land clearing and soil preparation (two times for tillaging and one time for sloping) have been done before planting. Corn was oversown 2-3 seeds per hill and thinned in to 1 plant per hill at two weeks after planting. Fertilizer formula 15-15-15 of NPK was applied at the rate of 312.5 kg ha⁻¹ before planting, and fertilizer formula 46-0-0 at the rate of 156.25 kg ha⁻¹ was applied twice at 20 and 40 days after planting. Sprinkler irrigation system was applied for corn watering with the 6:1 ratio of plant rows and sprinkler rows, respectively. The amount of water applied was according to the corn growth stages, which were increasing following later vegetative stages from V1 to V14, and the water volume was slightly decreasing from flowering stage (R1) to milking stage (R4) when the corn ears were ready to be harvested. Weeds were controlled with pre-emergence herbicide and manual weeding at critical periods of crop whereas pest and disease were controlled if only exceeding the economic injury level (EIL). Corn ears were harvested at R4 stage or milking stage when seed moisture reached 70%.

The weather data including precipitation rate (mm), minimum, mean, and maximum temperatures (°C), and relative humidity (%) were monitored and collected daily during the experiment from the Agricultural Weather Station, Agronomy Field Research Station, Khon Kaen University, Thailand.

Data collection

Ten randomly plants excluding any plant surrounding a missing hill and border plants were used for observation on agronomic traits according to Dermail et al. (2020) and Dermail et al. (2023), mainly plant height (PH/cm), from ground level to the base of the tassel after milk stage; and ear height (EH/cm), from ground level to the node bearing the uppermost ear after milk stage. Plot based measurements were done on days to tasseling (DT), as the number of days from sowing to when 50% of the plants have shed the pollen and days to silking (DS), as the number of days from sowing to when silks have emerged on 50% of the plants.

Yield and yield components were measured after harvest at fresh stage (20 days after pollination) including unhusked ear weight (UW/g), husked ear weight (HW/g), husked ear diameter (ED/cm), and husked ear length (EL/cm), row number per ear (RN), and seed number per row (SN). Unhusked and husked yields (UY and HY, respectively/ton ha⁻¹) were based on averaged of ten ears per plot and then converted into (ton ha⁻¹) unit.

Statistical analysis

The combined analysis of variance over seasons in RCBD of all studied traits was done. Mean comparison through Tukey's Honest Significant Difference (HSD) test at the 5% probability level was carried out (Gomez and Gomez 1983). The general combining ability (GCA) of parental lines was estimated through Singh and Chaudhary (1979) method. Both mid-parent heterosis (MPH) and high-

parent heterosis (HPH) were estimated (Liu et al. 2022), as follows:

$$\text{MPH} = (F_1 \cdot \text{MP}) / \text{MP} \times 100$$

$$\text{HPH} = (F_1 \cdot \text{HP}) / \text{HP} \times 100$$

Where: F₁ is hybrid performance, MP is mid-parent value, and HP is high-parent value.

Linear correlation between mid-parent (MP) and hybrid performance (F₁) was performed for given traits.

RESULTS AND DISCUSSION

Analysis of variance

Season significantly affected all traits except for HY, EH, EL and RN (Table 3). Genotypic effect was significant for all traits and so did the interaction between season × genotype (G × S). The significant effect of genotype indicated that both hybrids and inbred lines evaluated had enough phenotypic variations. The significance of season implied that the presence of seasonal variations regarding different weather profiles in our testing seasons between the rainy and the dry seasons. In tropical savanna, the rainy season was indicated by relatively high precipitation rate, total rainfall, relative humidity, daily temperature, and solar radiation, while the dry season showed the contrasting evidence for each weather parameter given (Sintanaparadee et al. 2022). The significant effect of G × S indicated that the performance of our testing genotypes was not stable over growing seasons; thus, multi-season field trials are critical to avoid bias estimation in hybrid evaluations. Since field testing in the tropical regions can be done two to three seasons per year, multi-season testing would be more effective than multi-year testing to determine the stability of testing hybrids. Field trials of corn hybrids in tropical savanna Thailand were commonly conducted in at least two different growing seasons to dissect the genotype by environment interaction (GEI) effects (Dermail et al. 2020; Fuengtee et al. 2020; Dermail et al. 2022; Ja et al. 2022).

Precipitation rate in the dry season (0.70 mm) was significantly lower than that in the rainy season (10.61 mm), differing about 9.91 mm in total (Table 4). It was also noted that there were only two rainy days during the dry season. The daily minimum, mean, and maximum temperatures during the dry season were 17.89°C, 24.52°C, and 31.13°C, respectively, which were significantly lower than those during the rainy season (23.97°C, 29.21°C, and 34.45°C, respectively). Relative humidity in the dry season (89.30%) was slightly lower than that in the rainy season (91.32%). These results indicated that the dry season had lower precipitation rate, daily temperature, and relative humidity than the rainy season. It corroborated the finding of Sintanaparadee et al. (2022) which also monitored the seasonal variations under tropical savanna environments. Our weather data may explain the presence of both S and G × S effects altering corn performance for given traits.

Table 3. Mean squares of analysis of variance across two seasons for agronomic traits and yields in sweet and waxy corn

SOV	df	UY		HY		EH		PH		DT		DS	
Season (S)	1	6,454,023	**	397,248	ns	30.0	ns	2,296	*	631.0	**	833.0	**
Block/S	4	214,508		58,065		123.0		251		1.2		3.2	
Genotype (G)	17	4,268,048	**	2,615,665	**	1,053.0	**	3,476	**	77.2	**	87.8	**
S x G	17	110,631	**	50,820	**	259.0	**	348	**	5.4	**	5.0	**
Pooled error	68	48,921		17,890		48.4		85		0.8		1.4	
c.v. (a) %		20		15		11.9		9		2.2		3.7	
c.v. (b) %		10		8		7.5		5		1.8		2.4	
SOV	df	UW		HW		ED		EL		RN		SN	
Season (S)	1	86,683	**	6,672	*	2.9	*	13.9	ns	5.5	ns	73.7	*
Block/S	4	3,037		819		0.2		7.5		0.9		4.7	
Genotype (G)	17	58,316	**	36,113	**	3.6	**	54.5	**	9.0	**	278.0	**
S x G	17	1,773	**	756	**	0.2	**	9.2	**	0.7	*	9.7	**
Pooled error	68	718		277		0.1		1.5		0.3		2.8	
c.v. (a) %		20		15		5.3		15.2		6.5		7.4	
c.v. (b) %		10		9		4.9		6.9		3.9		5.8	

Note: df: degree of freedom; c.v.: coefficient of variation; UY: unhusked yield (ton ha⁻¹); HY: husked yield (ton ha⁻¹); EH: ear height (cm); PH: plant height (cm); DT: anthesis date (days after planting); DS: silking date (days after planting); UW: unhusked ear weight (g ear⁻¹); HW: husked ear weight (g ear⁻¹); ED: husked ear diameter (cm); EL: husked ear length (cm); RN: row number per ear; SN: seed number per row; **: 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 of five weather profiles during the experiment in the dry season of 2021/2022 and the rainy season of 2022

Season	PR (mm)	T _{min} (°C)	T _{mean} (°C)	T _{max} (°C)	RH (%)
dry	0.70±0.40	17.89±0.27	24.52±0.23	31.13±0.25	89.30±0.44
rainy	10.61±2.08	23.97±0.21	29.21±0.22	34.45±0.28	91.32±0.54
difference	-9.91 **	-6.08 **	-4.69 **	-3.32 **	-2.02 **

Note: PR: precipitation rate; T_{min}: daily minimum temperature; T_{mean}: daily mean temperature; T_{max}: daily maximum temperature; RH: relative humidity; **: data significant at $P \leq 0.01$ based on unpaired t-test. The values following the means are standard error

General combining ability (GCA)

The estimates of GCA and per se of each parental line tested across two growing seasons for all observed traits are presented in Table 5. Genotype 8A3-B had significantly positive GCA estimates and high per se for UY (527.6**; 8.6 ton ha⁻¹), HY (323.1**; 4.8 ton ha⁻¹), UW (60.8**; 132.7 g ear⁻¹), HW (37.9**; 90 g ear⁻¹), ED (0.5**; 4.3 cm), RN (1.3**; 14.1), and EH (7.5**; 105.3 cm). Two genotypes 99A4-B and 2A3-B equally had significantly positive GCA estimates and high per se for SN (1.3*; 26.9 and 0.9*; 24.2, respectively). Two genotypes 2A3-B and 8A3-B equally had significantly positive GCA estimates and high per se for PH (5.6*; 158.3 cm and 4.0*; 183.0 cm, respectively). Genotype 12C5-B had significantly positive GCA estimates and high per se for DT only (2.2**; 51.8 days after planting). We can infer that it was difficult to obtain any single waxy corn genotype possessing favorable GCA and per se for all targeted traits. However, genotype 8A3-B seemed to be the closest ideal tester, as it showed favorable GCA for improving yield components, final yield, plant stature, and ear position of waxy corn.

Farmers in the tropics favor corn cultivars performing high yield, early maturity, and lodging tolerance (Abadassi 2015; Dermail et al. 2022); thus, parental selection was directed to genotypes showing significantly positive GCA for yield and yield components but negative GCA for flowering dates and plant architecture. In this study, we proposed genotype 8A3-B as a new tester for a routine waxy corn breeding program because it performed positive

GCA for UY, UW, HY, HW, ED, RN, but negative GCA for AD. Since we could not find a single genotype showing favorable GCA and per se in all observed traits, further recombinations and selections are required to fix all favorable alleles within individual waxy corn genotype.

Heterosis

The estimates of mid-parent heterosis (MPH) in the dry season were high on UY (47%-216%, 122% on average), HY (59%-270%, 144% on average), UW (47%-216%, 122% on average), and HW (59%-270%, 144% on average), moderate on ED (5%-61%, 28% on average), EL (6%-56%, 30% on average), RN (-9%-25%, 5% on average), SN (16%-57%, 44% on average), EH (-35%-24%, 1% on average), and PH (-11%-37%, 19% on average), and low on DT (-20%--2%, -13% on average) and DS (-19%-0%, -12% on average) (Figure 1A). A similar pattern was noticed for best parent heterosis (BPH) in that season although the BPH estimates on averages were slightly lower than MPH on each respective trait. The estimates of BPH were high on UY (35%-168%, 104% on average), HY (51%-209%, 122% on average), UW (35%-168%, 104% on average), and HW (51%-209%, 122% on average), moderate on ED (0%-48%, 21% on average), EL (-3%-36%, 16% on average), RN (-16%-19%, -1% on average), SN (6%-36%, 27% on average), EH (-36%-7%, -12% on average), and PH (-14%-23%, 9% on average), and low on DT (-22%-5%, -16% on average) and DS (-20%--2%, -14% on average) (Figure 1B).

Table 5. Per se and general combining ability estimates for agronomic traits and yields in six parental lines of sweet-waxy corn hybrids

Parent	Unhusked yield (ton ha ⁻¹)			Husked yield (ton ha ⁻¹)			Unhusked ear weight (g ear ⁻¹)			Husked ear weight (g ear ⁻¹)			Husked ear diameter (cm)			Husked ear length (cm)		
	Per se	GCA		Per se	GCA		Per se	GCA		Per se	GCA		Per se	GCA		Per se	GCA	
2A3-B	7.5	-195.3	*	4.6	-153.0	*	136.1	-23.9	*	85.5	-17.9	*	4.3	-0.1	ns	13.8	-0.2	ns
8A3-B	8.6	527.6	**	4.8	323.1	**	132.7	60.8	**	90.0	37.9	**	4.3	0.5	**	12.7	-0.2	ns
12C5-B	9.5	-153.9	*	4.6	-55.3	ns	135.2	-19.0	*	86.1	-6.5	ns	4.2	-0.2	*	12.8	0.3	*
13A5-B	7.3	2.1	ns	3.0	17.8	ns	112.1	4.2	ns	56.9	2.1	ns	3.9	0.0	ns	16.6	0.6	**
99A4-B	12.7	-180.5	*	4.8	-132.5	*	173.7	-22.1	*	84.2	-15.5	*	4.2	-0.2	*	15.3	-0.6	*
101LBW	10.4	26.1	ns	6.2	12.3	ns	163.4	4.0	ns	115.8	1.4	ns	4.5	0.0	ns	14.2	0.2	ns
mean	9.3			4.7			142.2			86.4			4.2			14.2		
HSD 5%	654.8			396.0			79.3			49.3			0.8			3.7		
SE		149.6			92.4			18.1			10.8			0.2			0.3	

Parent	Row number per ear			Seed number per row			Ear height (cm)			Plant height (cm)			Anthesis date (days after planting)			Silking date (days after planting)		
	Per se	GCA		Per se	GCA		Per se	GCA		Per se	GCA		Per se	GCA		Per se	GCA	
2A3-B	13.9	-0.5	*	24.2	0.9	*	83.8	1.6	ns	158.3	5.6	*	54.0	-0.5	ns	53.0	-0.1	ns
8A3-B	14.1	1.3	**	17.9	-2.2	**	105.3	7.5	**	183.0	4.0	*	54.3	-0.2	ns	52.2	1.7	**
12C5-B	13.4	-0.2	ns	21.8	0.1	ns	65.6	-13.1	**	144.6	-14.0	**	51.8	2.2	**	50.7	-1.6	**
13A5-B	12.6	0.0	ns	14.0	-0.1	ns	75.6	3.7	*	143.6	2.1	ns	55.8	-1.6	*	54.5	0.3	ns
99A4-B	14.0	-0.6	*	26.9	1.3	*	96.9	0.4	ns	160.1	2.4	ns	55.7	-0.2	ns	53.8	-0.2	ns
101LBW	13.8	0.1	ns	19.0	0.1	ns	70.5	0.4	ns	153.0	0.6	ns	55.7	0.5	ns	55.7	0.4	ns
mean	13.6			20.7			82.9			157.1			54.6			53.3		
HSD 5%	1.7			5.0			20.6			27.3			2.7			3.5		
SE		0.4			0.6			3.7			3.8			0.8			0.7	

Note: GCA: general combining ability; HSD: Tukey’s Honest Significant Difference; SE: standard error; HSD 5% was used as critical value for per se; SE was assigned as critical value for GCA estimates; ** and * GCA estimates significantly different from zero at $\geq 2SE$ and $\geq SE$, respectively; ns GCA estimates not significantly different from zero at $\geq SE$

In the rainy season, the estimates of MPH were high on UY (113%-236%, 186% on average), HY (112%-311%, 224% on average), UW (113%-259%, 188% on average), and HW (166%-508%, 344% on average), moderate on ED (31%-61%, 49% on average), EL (12%-83%, 56% on average), RN (3%-33%, 16% on average), SN (19%-98%, 55% on average), EH (1%-63%, 36% on average), and PH (19%-68%, 43% on average), and low on DT (-22%--10%, -15% on average) and DS (-25%--13%, -18% on average) (Figure 1C). Meanwhile, the estimates of BPH were high on UY (85%-202%, 161% on average), HY (77%-254%, 184% on average), UW (85%-232%, 162% on average), and HW (77%-478%, 256% on average), moderate on ED (20%-57%, 42% on average), EL (-1%-76%, 46% on average), RN (-1%-29%, 13% on average), SN (11%-39%, 24% on average), EH (-5%-45%, 25% on average), and PH (9%-58%, 35% on average), and low on DT (-23%--13%, -17% on average) and DS (-26%--13%, -19% on average) (Figure 1D).

The result above showed that both MPH and BPH estimates were slightly higher in the rainy season than in the dry season for all observed traits. It implied that different levels of parental adaptation occurred when they were evaluated in different growing seasons. The actual husked yield of hybrids averaged across ten combinations was not significantly different between two seasons, representing 12.64 ton ha⁻¹ in the dry season and 12.51 ton ha⁻¹ in the rainy season (data not shown). However, per se of averaged six parental lines were significantly different

where they demonstrated higher husked yield in the dry season (5.47 ton ha⁻¹) than in the rainy season (3.72 ton ha⁻¹) (data not shown). That contrasting adaptability over different seasons illustrated by parents and hybrids resulted in inflating estimates of heterosis in the rainy season as shown in Figure 1.

Makumbi et al. (2011) noticed higher heterosis for grain yield in drought conditions than in the well-watered condition. Perhaps during the rainy season, parental lines become more susceptible to biotic and abiotic stresses than hybrids for two reasons, as follows: (i) high inbreeding depression since those lines were inbreeds experiencing long-term selections and (ii) poor environmental conditions during the rainy season such as high precipitation, relative humidity, and daily temperature as proven in Table 4. The parental lines in poor environmental conditions, for instance in the rainy season regarding this study, will contribute to dropped MP values eventually leading to elevated heterosis estimates. Previous studies reported that the rainy season had higher means and ranges of heterosis than the dry season under tropical savanna climate (Dermail et al. 2020; Dermail et al. 2023). They noticed that higher heterosis in the rainy season contrasted with lower hybrid means (Dermail et al. 2020). Despite poor environmental issues, high daily temperature in the rainy season led to a higher accumulation of the growing degree days (GDD) and faster corn phenology such as flowering and maturing times in tropical and subtropical regions (Hou et al. 2014; Jiang et al. 2020; Sintanapadee et al. 2022).

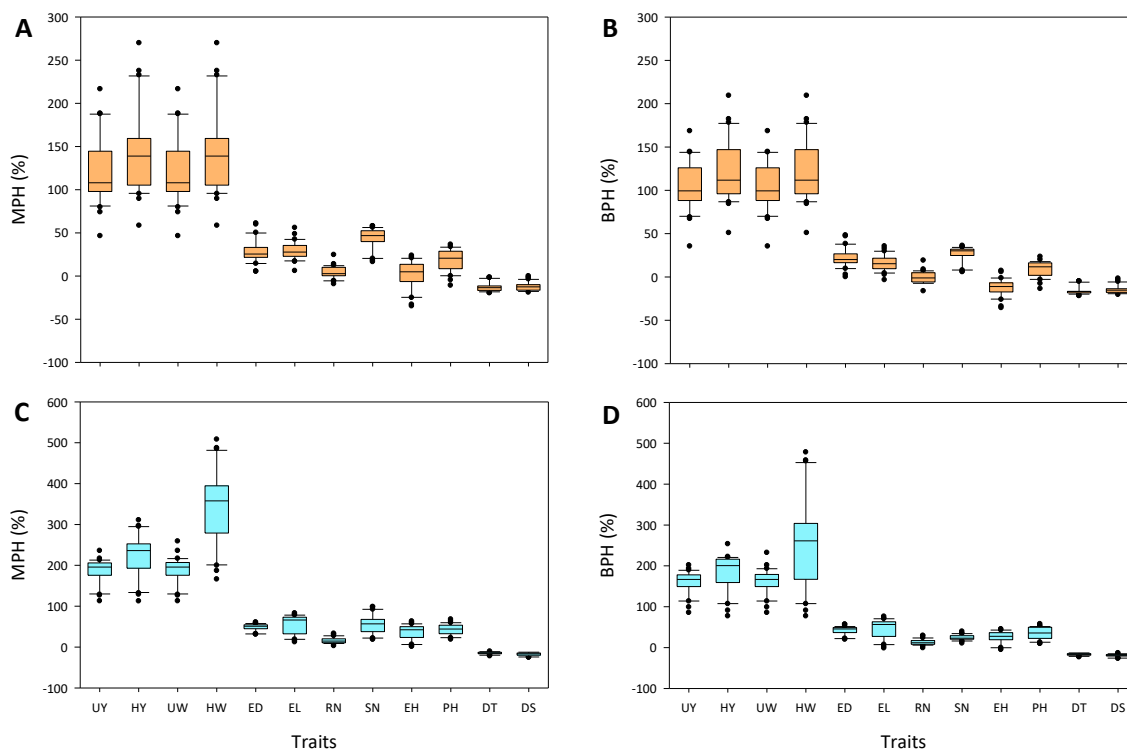


Figure 1. Heterosis of ten hybrids in the rainy (A and B) and the dry (C and D) seasons. MPH mid-parent heterosis; BPH best-parent heterosis; UY: unhusked yield (ton ha⁻¹); HY: husked ear weight (ton ha⁻¹); EH: ear height (cm); PH: plant height (cm); DT: anthesis date (days after planting); DS: silking date (days after planting); UW: unhusked ear weight (g ear⁻¹); HW: husked ear weight (g ear⁻¹); ED: husked ear diameter (cm); EL: husked ear length (cm); RN: row number per ear; SN: seed number per row

Early flowering implied that the vegetative growth period was shorter; thus, it may reduce aboveground biomass, yield components, and grain yield (Liu et al. 2013; Shim et al. 2017; Wei et al. 2017; Lizaso et al. 2018; Sah et al. 2020). High temperature and relative humidity triggered greater severity of tropical diseases such as stalk and ear rots (Prasanna et al. 2021), becoming another major constraint for maize growth and development. Since inbred lines were more susceptible to diseases than hybrids (Abera et al. 2016; Beyene et al. 2017), per se of inbreds were more depressed than the hybrids; therefore, the estimates of heterosis would be inflated.

Heterosis describes the relatively better performance of hybrids as compared to their corresponding parents. The evidence of this phenomenon has been utilized to develop superior maize hybrids with high yield, early maturity, and drought tolerance (Adebayo et al. 2017). It was high (>100%) for ear yield and ear weight, moderate (50%-100%) for ear components and plant stature, and low (<50%) for flowering times. Previous studies in maize reported that the MPH was high and positive for grain yield (200%-500%), low and positive for plant height (10%-50%) and ear height (40%-60%), but low and negative for silking date (-11% - -5%) (Owusu et al. 2017). Likewise, Dermail et al. (2023) noticed that the estimates of MPH were high and positive for ear

weight, total kernel weight per ear, and total kernel number per ear, but it was low to poor for plant height, ear height, anthesis date, and silking date. In sweet-waxy corn, the ranges and the means of MPH were high and positive for ear yields (100%-180%), low and positive for plant stature (10%-60%), and negative for flowering times (-16% - -6%) (Dermail et al. 2020). Regarding our findings and brief literature review, we concluded that the magnitudes and the ranges of heterosis estimates were genotype and trait dependent. It might be due to different genetic effects underlying given traits. Li et al. (2021) found that dominance effects led to higher estimates of heterosis. Previous studies in maize reported that flowering times (AD and SD) and plant architecture (PH and EH) were additively inherited, while ear length was under dominance effect and yield components had equal importance of additive and dominance effects (Dermail et al. 2023).

The relationship between hybrid performance and mid-parent (MP)

The coefficients of simple linear correlation between hybrid performance and MP were significant for DT, DS, UY, UW, and SN (Figure 2).

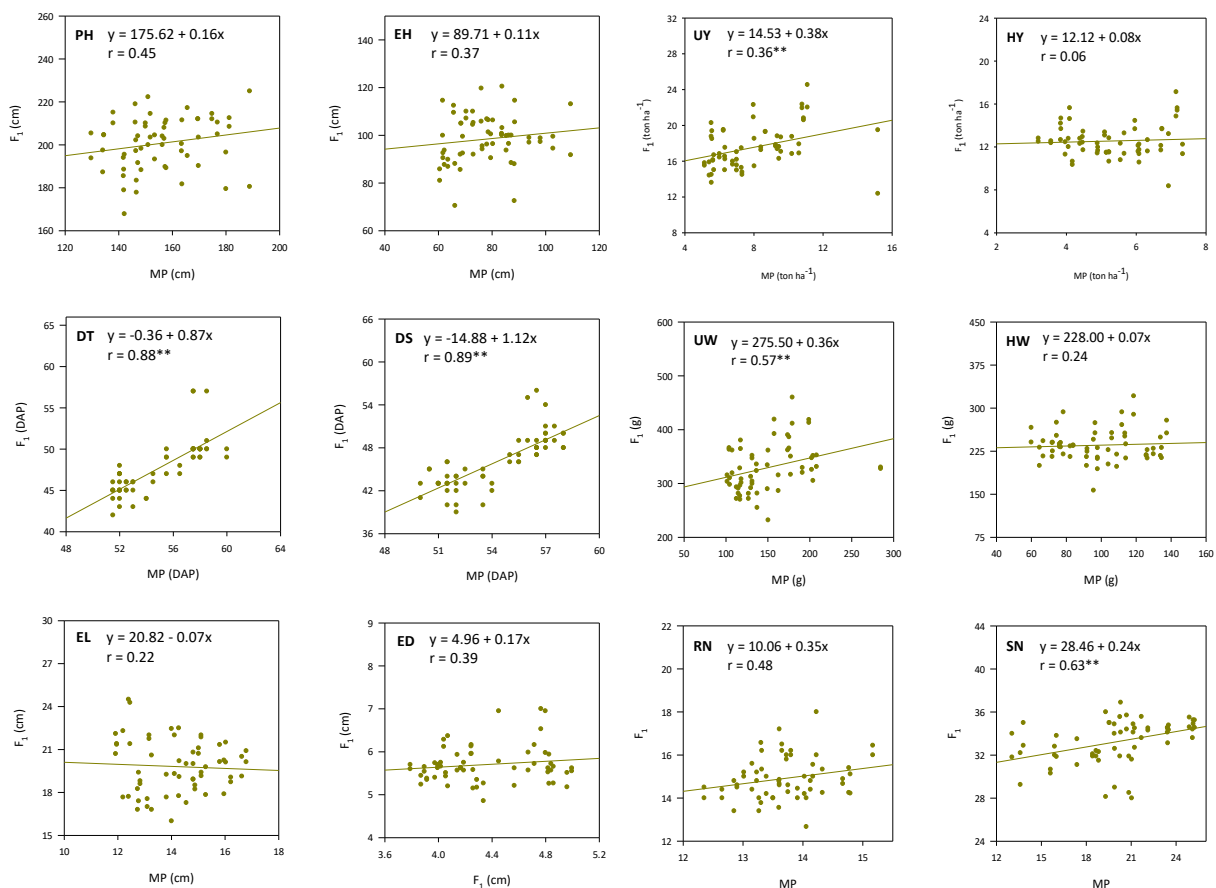


Figure 2. Linear regression between mid-parent (MP) and hybrid performance (F1) of ten combinations evaluated across two seasons. Note: EH: ear height (cm); PH: plant height (cm); DT: anthesis date (days after planting); DS: silking date (days after planting); EL: husked ear length (cm); ED: husked ear diameter (cm); 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); RN: row number per ear; SN: seed number per row; r: coefficient of correlation; **significant at P≤0.01

However, only DT and DS had high correlation coefficients ($r > 0.80^{**}$), indicating that MP value can be used to predict the hybrid performance for flowering times only, but the prediction approach was not effective for the rest agronomic traits. Gowda et al. (2013) noticed that MP value accurately predicted the hybrid performance for less complex traits such as plant height and silking date, while this approach was not effective for more complex traits like grain yield. Demail et al. (2023) mentioned five factors contributing the effectiveness of MP value to predict hybrid performance, namely gene action underlying the targeted traits, the nature of traits, genetic distance between parents, the inbreeding levels, and environments.

Since it is common that maize improvements are regarding multiple favorable traits, the strategy to use MP as single factor in hybrid prediction was still not reliable. Hybrid performance is manifestation of the sum of the MP and the MPH. Li et al. (2021) concluded that the additive model to some extent was not sufficient to explain hybrid performance. Instead, they suggested including both MP and MPH altogether in hybrid prediction. As the MP-hybrid correlation was significant and high for flowering times only, the best efforts were that first pre-screening of our wide parental lines on best MP values for anthesis and silking dates according to the breeding objectives whether early or late maturity. Then, classic hybrid formations still need to be carried out among selected lines.

In conclusion, the seasonal effect was significant for partial observed traits, while both effects of genotype and the interaction between genotype and season were significant for all observed traits. We proposed genotype 8A3-B as a new tester for hybrid testing on yield and yield components in waxy corn germplasm. The estimates of heterosis depend upon traits and can be biased by poor environments. We found that mid-parent value can be used to predict the hybrid performance for flowering times only, but this approach limited users to predict hybrid performance for the rest of the agronomic traits. Although our approach can help breeders for eliminating unfavorable pairwise parents for flowering times resulting in slightly reduced workload, selected parents having favorable flowering times still require further intercrossing and hybrid testing under multi environment trials due to lacking abilities of mid-parent values to predict the hybrid yields.

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