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GENETIC ANALYSIS FOR AGRONOMIC TRAITS AND KERNEL CARBOHYDRATES IN THE TROPICAL LONG-TERM SELECTION SHRUNKEN-2 SWEET CORN HYBRIDS

W. SIMMAKHAM¹, A. DERMAIL¹, S. CHANKAEW^{1,2}, S. SIMLA³, K. LOMTHAISONG⁴,
 K. LERTRAT², and K. SURIHARN^{1,2*}

¹Department of Agronomy, Faculty of Agriculture, Khon Kaen University, Thailand

²Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen University, Thailand

³Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, Thailand

⁴Department of Biochemistry, Faculty of Science, Khon Kaen University, Thailand

*Corresponding author's email: sphala@kku.ac.th

Email addresses of co-authors: weerayut7271@gmail.com, abildermail@gmail.com, somchan@kku.ac.th, sakunkan.s@msu.ac.th, kholom@kku.ac.th, kamol9@gmail.com

SUMMARY

Better understanding breeding values and their combining ability of favored traits is essential to identify potential parents and hybrids. Our study utilized *shrunken* (*sh2*) sweet corn (*Zea mays* L.) parental lines and their derived hybrids, where the parental lines have undergone long-term selections to improve adaptation, yield, and eating quality under the tropical savanna of Thailand. We aimed to estimate heterosis, combining ability, and gene action for agronomic traits and kernel carbohydrates of tropical super sweet corn hybrids carrying the *sh2* gene. All observed traits exhibited substantial interaction between hybrid and season, non-additive inherited, and low narrow-sense heritability. Long-term selections may contribute to the overwhelming dominance variance on all observed traits. Genotype TSC/H3-7-2 in the dry season was promising as a general combiner for high yield, plant stature, and early flowering. Genotype W54/Allure showed a favorable tester for kernel carbohydrates. Hybrid ws160 × W54/Allure was stable across different seasons for eating qualities. The other hybrid, TSC/H3-7-2 × W54/Allure, can be an alternative in the rainy season. The information obtained from this study can assist breeders in developing super sweet corn hybrids for better yield and eating quality.

Keywords: Sweet corn (*Zea mays* L.), combining ability, gene action, heritability, heterosis, hybrid breeding, sugar

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Key findings: Long-term selections contribute to remarkable dominance variance on the maize (*Zea mays* L.) traits observed in this study. Agronomic traits and kernel carbohydrates of *sh2*-sweet corn are non-additive inherited and exhibit negligible narrow-sense heritability estimates. We propose two hybrids, ws160 × W54/Allure and TSC/H3-7-2 × W54/Allure, as promising tropical *sh2*-sweet corn hybrids.

INTRODUCTION

Sweet corn (*Zea mays* L.) is a popular vegetable harvested at the milking stage (Dermail *et al.*, 2021). In Thailand, the demand for this vegetable is on the rise, both domestically and internationally. While people commonly consume sweet corn as a fresh product in the domestic market, Thai producers export it in frozen and canned products (Lertrat and Pulam, 2007). Super sweet corn is a type of sweet corn with an allelic mutant *shrunk2* (*sh2*) gene. This mutation can retain reducing sugars and sucrose at high levels during the milking stage. As a result, *sh2* mutants exhibit three-fold and eight-fold higher sugar levels than normal *sugary1* (*su1*) sweet corn and field corn, respectively (Singh *et al.*, 2014; Szymanek *et al.*, 2015). However, *sh2* sweet corn genotypes often experience poor seedling germination. Long-term selection for sweetness may accumulate high doses of recessive alleles and inbreeding depression, contributing to worse agronomic properties of sweet corn lines under field conditions.

Hybrid cultivars have become the predominant choice in the sweet corn seed market to overcome these challenges of inbreeding depression and poor agronomic performance (Revilla *et al.*, 2021), as they can exploit heterosis to restore vigor while maintaining desirable eating quality traits. Heterosis, the relative performance between hybrid performance and the corresponding parents (Hochholdinger and Baldauf, 2018), naturally occurs in cross-pollinated crops like corn. However, the magnitude may vary depending on traits and genetic backgrounds (Li *et al.*, 2021). For instance, in sweet-waxy corn hybrids, heterosis estimates were positive for agronomic traits, yield, and yield components, but negative for flowering times (Dermail *et al.*, 2018; Dermail *et al.*, 2020). Eating quality like sweet flavor in sweet corn

becomes critical to determine the acceptability in seed markets. While heterosis studies exist for sweet corn yield and agronomic traits, reports on sugar-related heterosis in tropical *sh2* sweet corn hybrids are unavailable, despite the known effects of tropical environments on sugar metabolism.

Combining ability, comprising general combining ability (GCA) and specific combining ability (SCA), is necessary for identifying promising parental inbred lines and hybrids, respectively. Many studies on combining ability and genetic analysis for agronomic and yield characteristics in sweet corn are available (Solomon *et al.*, 2012; Xavier *et al.*, 2019; Mahato *et al.*, 2021). Likewise, studies on combining ability for agronomic traits exist in both sweet corn parents and hybrids (Solomon *et al.*, 2012; Mahato *et al.*, 2021), but research on combining ability for eating quality traits is few. Earlier studies have reported on basic eating quality parameters like tenderness and sweetness in temperate sweet corn (Lertrat and Pulam, 2007). However, comprehensive analysis of combining ability for specific quality traits, such as, sugar profiles, texture, and flavor compounds in tropical *sh2* sweet corn genotypes remains unexplored, particularly in parent-hybrid relationships. In this study, we aimed to estimate heterosis, combining ability, and gene action for traits related to eating quality in *sh2* super sweet corn genotypes. Better understanding combining ability, heterosis, and genetic analysis will provide an efficient breeding strategy before extensive multi-environment hybrid trials.

MATERIALS AND METHODS

Plant material

This study used six *shrunk2* (*sh2*) sweet corn (*Zea mays* L.) inbred lines (Table 1).

Table 1. Brief information of six *shrunken-2* (*sh2*) sweet corn inbred lines used in the study.

Corn lines	Generations	Characteristics			
		Seed size	Ear size	Sucrose (mg g ⁻¹ FW)	Eating quality
WS26	S ₇	Small	Large	42.4	Medium
WS160	S ₇	Small	Large	50.9	High
W54/Allure	S ₆	Small	Small	29.6	High
W54/Montauk	S ₆	Large	Large	37.5	High
TSC/H3-7-2	S ₁₁	Large	Small	54.6	Medium
TSC/H3-7-8	S ₁₁	Large	Small	40.6	High

Those lines experienced long-term selections for tropical adaptation and eating quality, developed by the Plant Breeding Research Center for Sustainable Agriculture, Faculty of Agriculture, Khon Kaen University, Thailand. These inbred lines were derivatives of three distinct founder parents: WS, W54, and TSC/H3. Following six selection cycles, two lines from the W54 group came from the crosses between W54 and Allure Synergistic (*sh2se+*) and Montauk Synergistic (*sh2se+*). Genotype TSC/H3 was a base population constructed from the intercrossing between temperate F₁ hybrid TSC from the U.S.A. and tropical F₁ hybrid H3 from the Pacific Seed Company, Thailand. Both TSC and H3 carried the *sh2* gene (Pairochteerakul *et al.*, 2018). It took 11 selection cycles to obtain two selected lines, TSC/H3-7-2 and TSC/H3-7-8. Those parental lines' crossing underwent partial diallel design to generate 12 F₁ hybrids without reciprocals in the rainy season of 2021 at the Agronomy Field Crop Research Station, Khon Kaen University, Khon Kaen, Thailand.

Field experiments

Hybrids and parents received a randomized complete block design (RCBD) arrangement with two replications in the dry season of 2021/2022 and the rainy season of 2022 at the Agronomy Field Crop Research Station, Khon Kaen University, Khon Kaen (16°28'27.7" N, 102°48'36.5" E; 190 masl), Thailand. Each plot consisted of two rows 4 m in length with a plant spacing of 75 cm × 25 cm. The plot size was 6 m², with 32 plants per plot. Five plants per plot reached self-pollination for kernel carbohydrates analysis at the reproductive

stage. In contrast, the remaining plants within the plot served for phenotyping agronomic and yield traits. The crop field management applied in this experiment relied on Thailand Agricultural recommendations, including fertilization, irrigation, and pest, disease, and weed controls.

On-field data collection

Ten random plants per plot underwent recording for plant height (cm). Anthesis date measuring used a plot basis. Unhusked and husked yields (t ha⁻¹) and the weight ratio between seed and ear (%) bore measuring at the fresh stage (21 days after pollination/DAP).

Sample preparation and chemical analysis

Five corn ears per plot derived from self-pollination reached harvest at the milk stage, 20 DAP in the rainy season and 22 DAP in the dry season for quantifying kernel carbohydrates. After a predetermined storage time, the samples, placed in sealable plastic bags, sustained an ice bath before transporting to the laboratory. The samples' later storage in a freezer had a -20 °C temperature. The details are available in Simla *et al.* (2010).

Sugar analysis used Ultra-Performance Liquid Chromatography (UPLC Technology) (Waters Corporation, 2004, United States). Chromatography ran at 90 °C with a flow rate of 0.5 ml min⁻¹. The quantification of phytyglycogen used the phenol-sulfuric colorimetric method following DuBois *et al.* (1956). The absorbed wavelength was 490 nanometers.

Statistical analysis

Subjecting data to partial diallel analysis in RCBD multi-season employed the AGD-R (Analysis of Genetic Designs in R) version 5.0 (Rodríguez *et al.*, 2020). Two models of analysis ensued for different purposes. Model I, where all factors assumed as fixed effects, served to estimate the general combining ability (GCA) values and the mean squares of each source variations. Model II, where all factors assumed as random effects, aided for estimating variance components to determine the Baker's ratio and heritability estimates. The Baker's ratio calculation used the formula of Baker (1978). Broad-sense heritability estimation utilized a plot basis.

Estimating heterosis used a plot basis. The formula to calculate both mid-parent heterosis (MPH) and best-parent heterosis (BPH) followed Dermail *et al.* (2018). Tukey's

Honest Significant Difference test at $P < 0.05$ proceeded for mean comparisons. Pearson linear correlation coefficient calculations estimated the relationship between line *per se* and GCA. Those analyses continued using Statistix 10.0 (Statistix 10, 2013). The dendrogram construction through hierarchical clustering based on Ward's method engaged JMP Pro software version 15.0 (SAS Institute, 2021).

RESULTS

Partial diallel analysis

The effect of sweet corn (*Zea mays* L.) hybrids was significant for all traits (Table 2). The effects of GCA and SCA were noteworthy for most traits, except for phytoglycogen (PG). The interaction between hybrid and season (H

Table 2. Partial diallel analysis for agronomic (upper) and kernel carbohydrates (lower) of 12 super sweet corn hybrids evaluated in the dry season of 2021/2022 and the rainy season of 2022.

Source of variation	d.f.	Mean squares (% SS)				
		UY	HY	PH	DT	PC
Season (S)	1	2.5 (0.3)	7.6 (2.0)	7,454 (5.5)	687.0 (69.5)	2,135 (26.1)
Rep./S	2	1.1 (0.3)	0.3 (0.2)	50,422 (74.3)	0.0 (0.0)	3 (0.1)
Hybrid	11	11.7 (22.5)**	5.4 (21.9)**	421 (4.3)**	79.3 (8.0)**	60 (10.3)**
GCA	5	16.8 (11.5)**	8.9 (12.9)**	801 (2.9)**	45.3 (4.6)**	113 (6.9)**
SCA	6	8.9 (11.0)**	3.5 (9.1)**	210 (1.4)**	34.0 (3.4)**	31 (3.4)**
Hybrid × S	12	13.4 (25.6)**	6.1 (24.8)**	510 (5.3)**	68.9 (7.0)**	150 (25.7)**
GCA × S	5	15.5 (10.6)**	6.0 (8.7)**	985 (3.6)**	25.1 (2.5)**	133 (8.1)**
SCA × S	6	12.2 (15.1)**	6.2 (16.0)**	246 (1.6)**	43.8 (4.4)**	160 (17.6)**
Pooled errors	22	0.8 (3.1)	0.5 (4.2)	49 (1.0)	0.2 (0.6)	5 (1.8)
CV (%)		6.7	7.1	5.2	0.9	3.7
		SC	GC	FT	TS	PG
Season (S)	1	1,794.0 (22.5)	73.0 (16.3)	0.1 (0.0)	2,616.0 (27.9)	0.9 (17.0)
Rep./S	2	15.7 (0.4)	0.6 (0.3)	0.0 (0.0)	11.7 (0.2)	0.1 (2.8)
Hybrid	11	137.0 (23.9)**	7.5 (23.5)**	2.6 (21.8)**	149.0 (22.3)**	0.0 (8.1)**
GCA	5	211.0 (13.2)**	6.4 (7.1)**	1.8 (5.3)*	255.0 (13.6)**	0.1 (4.9)NS
SCA	6	95.3 (10.7)**	8.2 (16.4)**	3.0 (16.5)**	90.5 (8.7)**	0.0 (3.2)NS
Hybrid × S	12	57.2 (10.0)*	5.1 (16.1)**	2.9 (24.0)**	62.5 (9.3)*	0.1 (25.6)**
GCA × S	5	99.9 (6.3)**	5.7 (6.4)**	3.3 (10.0)**	131.0 (7.0)**	0.2 (19.3)**
SCA × S	6	33.4 (3.8)NS	4.8 (9.7)**	2.6 (14.0)**	24.6 (2.4)NS	0.0 (6.3)NS
Pooled errors	22	26.3 (9.2)	0.7 (4.2)	0.5 (8.3)	28.5 (8.5)	0.0 (12.8)
CV (%)		10.8	13.4	20	9.2	20.2

GCA= general combining ability; SCA= specific combining ability; UY= unhusked yield ($t\ ha^{-1}$); HY= husked yield ($t\ ha^{-1}$); PH= plant height (cm); DT= anthesis date (days after planting); PC= the ratio between seed and ear weight (%); SC= sucrose ($mg\ g^{-1}\ FW$); GC= glucose ($mg\ g^{-1}\ FW$); FT= fructose ($mg\ g^{-1}\ FW$); TS= total sugar ($mg\ g^{-1}\ FW$); PG= phytoglycogen ($mg\ g^{-1}\ FW$).

** Data significant at $P \leq 0.01$; * data significant at $P \leq 0.05$; NS= data not significant at $P \leq 0.05$.

× S) and between GCA and season (GCA × S) were significant for all traits. The interaction between SCA and season (SCA × S) was remarkable for most traits, except for sucrose (SC), total sugar (TS), and phytoglycogen (PG).

We found hybrid was the primary source of variation for unhusked yield (UY), husked yield (HY), sucrose (SC), glucose (GC), fructose (FT), and total sugar (TS), accounting for 22.5%, 21.9%, 23.9%, 23.5%, 21.8%, and 22.3%, respectively. It implied notable phenotypic variations for further hybrid evaluation for high yield and sweetness. In contrast, hybrid shared low contributions for plant height (PH) and anthesis date (DT). The Hybrid × S effect was predominant for UY, HY, the ratio between seed and ear weight (PC), FT, and PG. The coefficient of variation was relatively low, ranging from 0.9% for DT to 20.2% for PG, indicating the data was reliable, and the experiment may be repeatable.

Gene action and heritability

The SCA variance was higher than the GCA variance for all traits observed, resulting in lower estimates of the Baker ratio, ranging from 0.00 to 0.37 (Table 3). Likewise, the dominance variance was higher than the additive variance for all given traits. Broad-

sense heritability was high ($0.90 < h_{bs}^2 < 0.98$) for all traits, except for fructose (0.76) and phytoglycogen (0.65). However, all traits had poor narrow-sense heritability ($0.00 < h_{ns}^2 < 0.27$). The result implied all traits observed, including agronomic traits and kernel carbohydrates, were dominance inherited.

General combining ability

In the presented study, the breeding objective was to provide super sweet corn hybrids appealing to farmers and food industries. Farmers are favorable with high yielding (UY and HY), good plant stand (PH), and early maturing (DT) corn plants, whereas food industries prefer high-edible corn ears (PC, SC, GC, FT, TS, and PG). Therefore, the parental selection was leaning toward positive and high GCA for all traits, except for DT, which should be a negative GCA.

In the dry season, three genotypes, TSC/H3-7-2, W54/Montauk, and TSC/H3-7-8, had the highest GCA for UY and HY, respectively (Table 4). Two genotypes, ws160 and TSC/H3-7-2, had the highest positive GCA for PH. Genotype W54/Allure had a highly positive GCA and was consistently in the top two for SC, GC, FT, TS, and PG. Genotype W54/Montauk had the utmost positive GCA for PG.

Table 3. Variance components, the Baker ratio, and heritability estimates for agronomic traits and kernel carbohydrates of 12 super sweet corn hybrids through partial diallel evaluated in the dry season of 2021/2022 and the rainy season of 2022.

Traits	Variance components				Baker Ratio	Heritability	
	σ_{GCA}^2	σ_{SCA}^2	σ_A^2	σ_D^2		H_b^2 (%)	H_n^2 (%)
Unhusked yield	0.99	8.01	1.98	8.01	0.20	0.96	0.19
Husked yield	0.68	4.19	1.36	4.19	0.24	0.96	0.23
Plant height	73.80	376.00	148.00	376.00	0.28	0.96	0.27
Anthesis date	0.66	4.44	1.32	4.44	0.23	0.98	0.23
Seed and ear weights ratio	10.30	54.10	20.60	54.10	0.28	0.97	0.27
Sucrose	14.50	92.40	28.90	92.40	0.24	0.90	0.22
Glucose	0.00	3.07	0.00	3.07	0.00	0.90	0.00
Fructose	0.00	0.80	0.00	0.80	0.00	0.76	0.00
Total sugar	20.60	113.00	41.10	113.00	0.27	0.92	0.24
Phytoglycogen	0.00	0.01	0.00	0.01	0.37	0.65	0.24

σ_{GCA}^2 , σ_{SCA}^2 , σ_A^2 , σ_D^2 are variance of GCA, SCA, additive, and dominance, respectively.

H_b^2 and H_n^2 are broad-sense and narrow-sense heritability, respectively.

Table 4. Estimates of GCA and *per se* for agronomic (upper) and kernel carbohydrates (lower) of six *shrunk-2* sweet corn inbred lines evaluated in the dry season of 2021/2022.

Parents	UY (t ha ⁻¹)		HY (t ha ⁻¹)		PH (cm)		DT (days after planting)		PC (%)	
	<i>per se</i>	GCA	<i>per se</i>	GCA	<i>per se</i>	GCA	<i>per se</i>	GCA	<i>per se</i>	GCA
ws26	13.60	-2.08**	10.30	-1.45**	83.00	0.97NS	45	-0.83**	57.0	5.53**
ws160	13.00	-1.84**	9.30	-1.18**	131.00	13.60**	50	0.92**	66.9	4.55**
W54/Allure	8.50	-0.38**	5.60	-0.41**	65.70	0.93NS	48	-0.21**	50.7	-1.16**
W54/Montauk	9.50	1.71**	7.10	1.21**	84.10	-3.23*	50	1.92**	68.2	-2.01**
TSC/H3-7-2	9.05	2.41**	6.50	1.53**	90.20	7.18**	43	-1.33**	66.5	-7.97**
TSC/H3-7-8	7.90	0.18*	5.95	0.30**	98.40	-19.40**	50	-0.46**	63.8	1.06*
HSD 5%	0.83		1.51		34.22		2		17.4	
SE		0.13		0.06		2.08		0.96		0.54
Parents	SC (mg g ⁻¹ FW)		GC (mg g ⁻¹ FW)		FT (mg g ⁻¹ FW)		TS (mg g ⁻¹ FW)		PG (mg g ⁻¹ FW)	
	<i>per se</i>	GCA	<i>per se</i>	GCA	<i>per se</i>	GCA	<i>per se</i>	GCA	<i>per se</i>	GCA
ws26	49.62	-7.73**	6.70	-1.35**	2.95	-0.89**	59.27	-9.97**	0.82	0.02NS
ws160	56.93	-1.47*	6.78	1.69**	3.33	1.04**	67.04	1.25*	0.81	0.04*
W54/Allure	29.84	6.08**	6.21	0.99**	3.08	0.46**	39.13	7.53**	1.02	0.06*
W54/Montauk	39.45	-1.90*	13.50	-0.37**	8.17	-0.07NS	61.10	-2.35*	1.20	0.21**
TSC/H3-7-2	65.35	7.25**	5.18	-0.69**	2.71	-0.66**	73.24	5.90**	1.02	-0.20**
TSC/H3-7-8	47.86	-2.22*	8.46	-0.26*	4.13	0.12*	60.44	-2.36*	0.77	-0.14**
HSD 5%	23.70		6.28		1.74		20.56		0.22	
SE		1.22		0.18		0.12		1.19		0.04

Per se and GCA are parental lines performance-based entry means and general combining ability, respectively.

HSD and SE are honestly significant difference and standard error, respectively, and used as a critical value for *per se* and GCA estimates, respectively.

** and * = GCA estimates significantly different from zero at $\geq 2SE$ and $\geq SE$, respectively. NS = GCA estimates not significantly different from zero at $\geq SE$.

UY = unhusked yield (t ha⁻¹); HY = husked yield (t ha⁻¹); PH = plant height (cm); DT = anthesis date (days after planting); PC = the ratio between seed and ear weight (%); SC = sucrose (mg g⁻¹ FW); GC = glucose (mg g⁻¹ FW); FT = fructose (mg g⁻¹ FW); TS = total sugar (mg g⁻¹ FW); PG = phytyglycogen (mg g⁻¹ FW).

In the rainy season, two genotypes, TSC/H3-7-2 and TSC/H3-7-8, had the highest GCA for UY and HY, respectively (Table 5). Two genotypes, TSC/H3-7-2 and W54/Montauk, had the topmost positive GCA for PH. Another two genotypes, W54/Allure and W54/Montauk, had a highly positive GCA for SC. Two genotypes, ws160 and W54/Montauk, had an exceedingly positive GCA for GC. Genotype TSC/H3-7-2 held the highest positive GCA for FT. Genotypes, W54/Montauk, ws160, W54/Allure, and TSC/H3-7-2, had positive GCA for TS. Two genotypes, TSC/H3-7-2 and W54/Allure, had a highly positive GCA for PG.

The GCA of each inbred was unstable over seasons, suggesting breeders apply specific testers in different growing seasons. For instance, breeders can use genotype TSC/H3-7-2 in the dry season and genotype TSC/H3-7-8 in the rainy season as testers in

hybrid breeding for high yield, plant stand, and early maturity. Regarding kernel carbohydrate properties, genotype W54/Allure was a promising tester for improving sugar contents, while genotype W54/Montauk was suitable for enhanced phytyglycogen in the dry season.

Heterosis

In the dry season, the ranges of both mid-parent heterosis (MPH) and best-parent heterosis (BPH) were high (>100%) for UY, HY, PH, GC, and FT, moderate (50%–100%) for SC, TS, and PG, and low (<50%) for DT and PC (Figure 1). In the rainy season, the ranges of both MPH and BPH were high (>100%) for UY and HY, moderate (50%–100%) for PC, SC, GC, FT, and PG, and low (<50%) for PH, DT, and TS.

Table 5. Estimates of GCA and *per se* for agronomic (upper) and kernel carbohydrates (lower) of six *shrunken-2* sweet corn inbred lines evaluated in the rainy season of 2022.

Parents	UY (t ha ⁻¹)		HY (t ha ⁻¹)		PH (cm)		DT (days after planting)		PC (%)	
	<i>per se</i>	GCA	<i>per se</i>	GCA	<i>per se</i>	GCA	<i>per se</i>	GCA	<i>per se</i>	GCA
ws26	4.95	0.10NS	3.90	0.25*	91.0	-11.90**	38	-0.13*	28.5	-2.10**
ws160	10.30	0.59**	7.40	0.03NS	105.0	-7.44**	45	0.13*	62.8	-0.44NS
W54/Allure	7.80	-1.44**	5.50	-1.06**	99.8	-4.00**	38	0.63**	45.4	3.23**
W54/Montauk	4.40	-0.44*	2.95	-0.27*	137.0	10.30**	45	0.38**	60.8	3.12**
TSC/H3-7-2	6.10	0.78**	3.60	0.70**	101.0	13.00**	41	0.00NS	37.5	-2.39**
TSC/H3-7-8	9.55	0.41*	5.85	0.35*	107.0	0.06NS	41	-1.00**	33.0	-1.42**
HSD 5%	0.83		0.71		19.8		1.2		6.4	
SE		0.30		0.22		0.84		0.11		0.49
Parents	SC (mg g ⁻¹ FW)		GC (mg g ⁻¹ FW)		FT (mg g ⁻¹ FW)		TS (mg g ⁻¹ FW)		PG (mg g ⁻¹ FW)	
	<i>per se</i>	GCA	<i>per se</i>	GCA	<i>per se</i>	GCA	<i>per se</i>	GCA	<i>per se</i>	GCA
ws26	35.25	-0.97NS	6.00	-0.18NS	4.96	0.04NS	46.20	-1.11NS	0.60	-0.14**
ws160	44.95	0.91NS	4.55	0.55**	2.99	0.14NS	52.50	1.60*	0.50	0.02*
W54/Allure	29.45	2.61**	5.90	-0.82**	3.57	-0.40**	38.90	1.39*	1.02	0.12**
W54/Montauk	35.60	1.52*	9.70	0.35*	6.15	0.11NS	51.40	1.98*	1.04	-0.15**
TSC/H3-7-2	43.80	0.72NS	3.80	0.04NS	2.69	0.55**	50.35	1.30*	1.00	0.20**
TSC/H3-7-8	33.30	-4.79**	6.35	0.07NS	3.69	-0.44**	43.35	-5.16**	0.83	-0.05**
HSD 5%	40.53		2.74		2.94		38.25		0.37	
SE		1.13		0.19		0.19		1.25		0.01

Per se and GCA are parental lines performance-based entry means and general combining ability, respectively.

HSD and SE are honestly significant difference and standard error, respectively, and used as a critical value for *per se* and GCA estimates, respectively.

** and * = GCA estimates significantly different from zero at $\geq 2SE$ and $\geq SE$, respectively. NS = GCA estimates not significantly different from zero at $\geq SE$.

UY = unhusked yield (t ha⁻¹); HY = husked yield (t ha⁻¹); PH = plant height (cm); DT = anthesis date (days after planting); PC = the ratio between seed and ear weight (%); SC = sucrose (mg g⁻¹ FW); GC = glucose (mg g⁻¹ FW); FT = fructose (mg g⁻¹ FW); TS = total sugar (mg g⁻¹ FW); PG = phytoglycogen (mg g⁻¹ FW).

In the rainy season, we noted some hybrids exhibited remarkable heterosis on yield parameters, such as, UY and HY, as indicated by high MPH and BPH, surpassing 200%. All hybrids showed positive heterosis on PH in this season. The upper limit of heterosis among hybrids in the rainy season was slightly higher than in the dry season for SC and TS. The result implied the rainy season led to higher heterosis estimates than the dry season for yield parameters, plant stature, and total sugars.

Cluster analysis

All *shrunken2* sweet corn genotypes, including hybrids and parents, gained clustering according to their phenotypic similarity of sucrose (SC) and phytoglycogen (PG) in the dry season (Figure 2A) and the rainy season (Figure 2B). Those two parameters were

options to represent two major eating qualities: sweetness and tenderness.

In the dry season, all genotypes' clustering can be into five major groups (A – E). Group A comprised two inbred lines and three hybrids with moderate to poor SC and PG. Meanwhile, group B comprised one inbred and three hybrids with moderate to high SC, but low PG. Group C included one inbred lines and one hybrid with excellent SC and moderate to high PG. Group D contained two inbred lines with poor SC, but high to exceptional PG. Finally, group E comprised five hybrids with low to moderate SG, but high PG.

In the rainy season, hybrids and parents' clustering formed four groups (A – D). Group A consisted of one inbred and three hybrids with low SC and poor to moderate PG. The second group B contained one inbred and four hybrids with moderate to high SC, but low to moderate PG. Group C comprised three

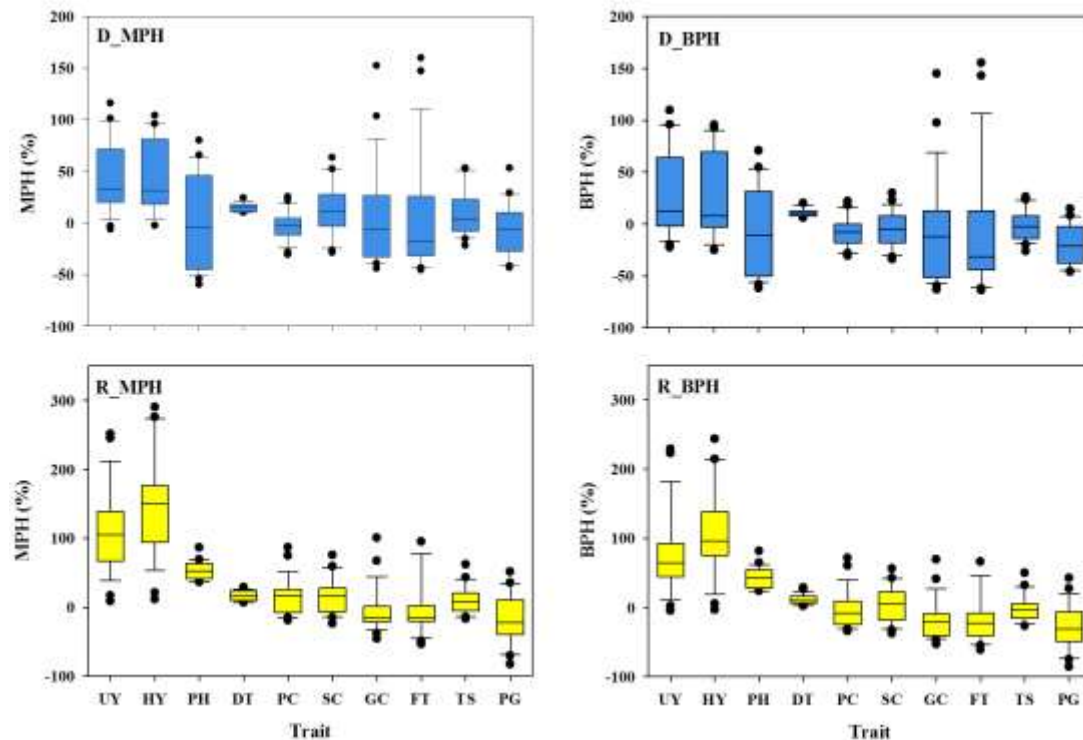


Figure 1. Heterosis performance on agronomic and kernel carbohydrates of 12 *shrunked-2* sweet corn hybrids evaluated in the dry (D) and the rainy (R) seasons. MPH= mid-parent heterosis; BPH= best-parent heterosis; D_MPH= mid-parent heterosis in the dry season; R_MPH= mid-parent heterosis in the rainy season; D_BPH= best-parent heterosis in the dry season; R_BPH= best-parent heterosis in the rainy season; UY= unhusked yield ($t\ ha^{-1}$); HY= husked yield ($t\ ha^{-1}$); PH= plant height (cm); DT= anthesis date (days after planting); PC= the ratio between seed and ear weight (%); SC= sucrose ($mg\ g^{-1}\ FW$); GC= glucose ($mg\ g^{-1}\ FW$); FT= fructose ($mg\ g^{-1}\ FW$); TS= total sugar ($mg\ g^{-1}\ FW$); PG= phytyloglycogen ($mg\ g^{-1}\ FW$).

inbred lines and one hybrid with low to poor SC, but high to excellent PG. Last group D comprised one inbred and four hybrids with moderate to outstanding SC and moderate to high PG.

Since the breeding program aimed to improve sweetness and tenderness, genotypes with high SC and PG were favorable. We found hybrid ws160 \times W54/Allure was promising, as it showed high and stable SC and PG across different seasons. In addition, hybrid TSC/H3-7-2 \times W54/Allure was also favorable because it had high sweetness and tenderness in the rainy season, although its tenderness slightly reduced in the dry season.

DISCUSSION

Environmental conditions significantly influence carbohydrate metabolism in sweet corn kernels. The presented study demonstrated notable variations in kernel sugar content between dry and rainy seasons, with higher sugar accumulation observed during the dry season. The interaction between temperature and sweetness in sweet corn affects both chemical composition and overall kernel quality through multiple pathways. Low-temperature storage enhances sweetness by increasing soluble sugars and reducing starch content (Xiao *et al.*, 2024), while temperature stress

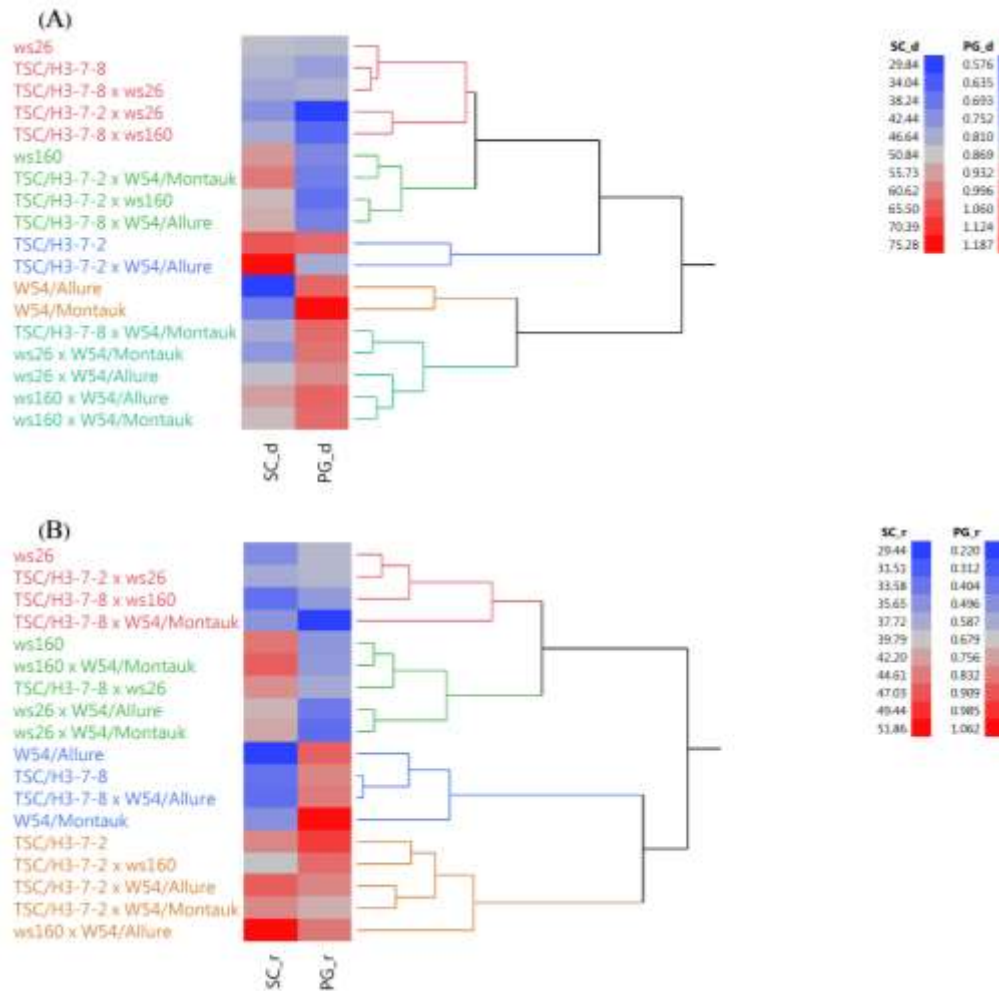


Figure 2. Dendrogram showing phenotypic relationship among 18 *shrunken-2* sweet corn genotypes (6 parents and 12 hybrids) in the dry season (A), and the rainy season (B). The Ward’s clustering method relied on sucrose (SC) and phytyglycogen (PG). The values range from poor (dark blue) to excellent (dark red).

during growth can alter biochemical pathways and metabolite accumulation, influencing sweetness levels (Xiang *et al.*, 2020; Xiang *et al.*, 2021). High-temperature stress reduces beneficial compounds like ascorbic acid and folates through oxidative responses, while lower temperatures enhance folate-related gene expression, improving kernel quality (Xiang *et al.*, 2020).

Our study examined 12 *sh2* sweet corn hybrids derived from six corresponding parents. These parents had undergone long-term selections, ranging from six to 11 cycles, where routine selections for good plant stand

and early flowering transpired in each cycle. As a result, it narrowed down the phenotypic variations for plant stand and early flowering (Table 2). However, substantial phenotypic variations in kernel sugar properties remained high (>20%). Although all the parents were *sh2* mutants, the remarkable variations of sweetness-related parameters observed could be due to the presence of allelic diversity in the entire *sh2* gene responsible for kernel sweetness. Chhabra *et al.* (2022) examined the entire *Sh2* gene and identified 25 alleles and 47 haplotypes of *sh2*. Regarding yield components, remarkable phenotypic variations

(>20%) (Table 2) may refer to the polygenic nature. Previous studies have reported the presence of substantial effects of genotype on yield (Patthawaro *et al.*, 2023; Sawangha *et al.*, 2023), kernel carbohydrates (Ja *et al.*, 2022), and eating quality of sweet-waxy corn hybrids (Dermail *et al.*, 2021).

In this study, non-additive effects predominantly controlled all traits observed. It resulted in a negligible narrow-sense heritability of each parameter (<0.30), although the broad-sense heritability was high (>0.80). The Baker ratio revealed low estimates (<0.30), indicating the importance of non-additive effects on targeted traits. The Baker ratio measures the proportion between GCA and SCA and indicates the importance of additive effect over non-additive effects underlying traits. A higher ratio closer to one indicates the hybrid performance on a given trait could be predictable regarding GCA alone (Baker, 1978). The results imply fixing favorable alleles within a given trait in sweet corn are unfeasible at the early segregated generations. Thus, it could continue at later generations (Dermail *et al.*, 2023). We argued long-term selections played a role in determining the degree of genetic effects of respective traits. For instance, after 13 cycles of selection of the maize population Iowa Corn Borer Synthetic No. 1 (BSCB1), Wardyn *et al.* (2007) found the dominance variance was larger than the additive variance for grain yield. The relative importance of dominance indicates long-term selection within a heterotic group may alter the structure of genotypic variance, eventually hindering breeders to obtain favorable genetic gain of a targeted population (Wardyn *et al.*, 2007; Nadeem *et al.*, 2023). Therefore, it is common in commercial Thai seed industries that breeders often recycle their genetic materials after consecutive four years of population improvements.

The substantial interaction between hybrid and season ($H \times S$), general combining ability and season ($GCA \times S$), and specific combining ability and season ($SCA \times S$) (Table 2) indicated the presence of seasonal variations between the dry and rainy seasons in Thailand. In the rainy season, the regions

experienced high air relative humidity and daily temperature (Sintanaparadee *et al.*, 2022). Multienvironment trials are necessary to avoid selection bias due to environmental variations, regardless of agroecology and climatic conditions, to reduce the gap between potential and actual yields.

The presence of $GCA \times S$ interaction in most observed traits (Table 2) signified the distinct performance of the inbred lines as parents in hybrid combinations (Makumbi *et al.*, 2011) and implicated the parental selection in hybrid breeding (Table 4). We suggest breeders apply different testers in different growing seasons for establishing superior hybrids regarding agronomic performance, for instance, genotype TSC/H3-7-2 in the dry season and genotype TSC/H3-7-8 in the rainy season. However, if the breeding objective aimed at enhancing kernel carbohydrate properties, genotype W54/Allure was a promising tester for improving overall sugar contents in both seasons, while genotype W54/Montauk was suitable for high phytyloglycogen in the dry season.

Heterosis reflects the relative performance of hybrids compared with their corresponding parents when evaluated in the same environment (Dermail *et al.*, 2023). Heterosis for kernel carbohydrates in sweet corn was lower than that for yield-related parameters. Khanduri *et al.* (2010) reported negligible mid-parent heterosis (MPH) for kernel sugar in Indian *su1* and *sh2* sweet corn, ranging from -15% to 30%. Heterosis or hybrid vigor can also be an indication of how adaptable the parental lines are to different environments (Dermail *et al.*, 2020). Our study indicated hybrids evaluated during the rainy season exhibited a greater increase in heterosis estimates for yield parameters than those evaluated in the dry season. This greater heterosis expression likely occurred because parental inbred lines are more susceptible to environmental stresses than their hybrids. The rainy season's challenging conditions—including high humidity, fluctuating temperatures, and disease pressure—typically depress parental line performance more severely than hybrid performance, resulting in larger parent-hybrid differences and, thus,

higher heterosis estimates. Makumbi *et al.* (2011) found parental lines are more susceptible to biotic and abiotic stresses than the corresponding hybrids during the rainy season. We contended the rainy season was unfavorable for inbred lines due to the heavy rainfall, high relative humidity, and large temperature differences between day and night.

When evaluating a sweet corn hybrid, consumers find specific characteristics are important. In sweet corn, its sweetness and soft texture determines eating quality. Sucrose is the primary sugar found in corn and is responsible for its flavor. In addition, the soft texture results from phytyglycogen, which is the main substance in the water-soluble polysaccharide group. Grouping sweet corn hybrids based on the relationship between sucrose and phytyglycogen can indicate consumer preferences for sweetness and tenderness. Fuengtee *et al.* (2020) studied sweet and waxy corn and found this grouping is crucial for selecting sweet corn hybrids with balance sweetness and tenderness. Our study identified the ws160 × W54/Allure hybrid as a potential super sweet corn hybrid due to its high and stable sucrose and phytyglycogen content over two contrasting seasons. Further extended yield trials are essential to evaluate promising hybrids before commercialization.

CONCLUSIONS

Long-term selections influenced genetic effects and *per se* of tropical super sweet corn (*Zea mays* L.) lines on traits related to agronomic performance and kernel carbohydrates. All observed traits were non-additive inherited with low narrow-sense heritability estimates. Selection of super sweet corn for good agronomic performance and kernel carbohydrates can continue at later stages. In general, heterosis on kernel sugars was lower than yield parameters, but higher than the anthesis date. W54/Allure and W54/Montauk had genetic potential for improving kernel sugars and phytyglycogen, while TSC/H3-7-2 and TSC/H3-7-8 shared favorable alleles for high yield, plant stand, and early maturity.

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