



Research article

Variability of kernel carbohydrate components on commercial waxy corn hybrids in Thailand

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Abstract

Importance of the work: A specialty waxy corn with sweet kernels has been developed through hybrid breeding, focusing on palatability improvement to fulfill consumer preference. However, information on the kernel carbohydrate components of waxy corn hybrids is still lacking.

Objectives: (1) To investigate the carbohydrate composition of fresh kernels harvested at milk stage among 10 commercial waxy corn hybrids based on the contents of total starch, amylopectin, total sugar and phytoglycogen; (2) to assess the seasonal effect on given traits; and (3) to classify all test hybrids and rank them regarding kernel carbohydrate components.

Materials & Methods: Ten waxy corn hybrid cultivars currently available in the Thai markets were evaluated under field condition in the 2017/2018 dry season and in the 2018 rainy season. The fresh kernels harvested at the milk stage were analyzed for the contents of total starch, amylopectin, total sugar, and phytoglycogen.

Results: The seasonal effect on the given traits were assessed and the phenotypic variabilities of test genotypes on given traits were visualized using a dendrogram based on Ward's hierarchical clustering method. Additionally, the data were subjected to unweighted selection indexing to rank the promising genotypes regarding their kernel carbohydrate components. Growing season, genotype and their interactions significantly altered the contents of total starch, amylopectin, total sugar and phytoglycogen with varied responses. Despite a large variation in the carbohydrate profiles among varieties, the fresh kernels carbohydrate of tested waxy corn hybrids were generally composed predominantly of total starch and amylopectin, with a small portion of sugar and phytoglycogen.

Main finding: Considering the divergence in both the carbohydrate profiles and market targets, a proper waxy corn ideotype for a specific market target is imperative.

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Introduction

Waxy corn (*Zea mays* L. var. *ceratina*), a special type of corn possessing high amylopectin contents controlled by the waxy (*wx*) gene (Ferguson, 2001), has become popular due to its stickiness, particularly in Asian countries, such as China, Korea, Lao, Myanmar, Thailand and Vietnam (Lertrat and Thongnarin, 2008). Since waxy corn is harvested at the milk stage or 20 d after pollination (DAP) and traditionally cooked by either boiling or steaming (Lertrat and Pulam, 2007), consumers are demanding more delicious waxy corn with sweet-flavored, sticky kernels. One of the parameters determining the palatability of vegetable corn is the kernel carbohydrate components (Creech, 1965). In synergistic waxy corn, the attributes of the carbohydrate components are the contents of total starch, amylopectin, total sugar and phytoglycogen (Simla et al., 2016).

Palatability improvement might be achieved through breeding; thus, it is imperative to obtain fundamental information on phenotypic variation in traits of interest among available sources of germplasm. The sources could be exotic germplasm, landraces or commercial varieties. In Thailand, available commercial waxy corn hybrids are largely developed by private companies followed by the Department of Agriculture and the university sector. Combining recessive genes encoding sweetness such as shrunken *sh2* or brittle *bt* into waxy background is one of major breakthroughs to develop modern waxy corn ideotypes. For example, to date, the Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen University has developed two distinct synergistic sweet-waxy corn hybrids, namely Kaow Gaum Wan (KGW) and Kaow Neaw Wan (KNW). However, information is still lacking on variation in the kernel carbohydrate components of these cultivars and other commercial Thai waxy corn hybrids in Thailand.

The environment is another factor altering the carbohydrates properties of waxy corn, including different growing seasons in which abiotic stresses such as heat and low light intensity could exist. For example, high temperature affected the physicochemical properties of waxy corn in the early reproductive (Lu et al., 2013a) and grain formation (Lu et al., 2016) stages. Additionally, low light intensity due to shading had a detrimental effect on the starch, soluble sugar and physicochemical properties of waxy corn at the grain filling stage (Lu et al., 2013b). Thailand has a tropical savanna climate (Beck et al., 2018) that experiences two contrasting seasons: a prolonged dry season and a short, torrential rainy

season. Little is known about the seasonal effect of a tropical savanna climate on the kernel carbohydrate properties of waxy corn hybrid cultivars. Therefore, this study aimed: (1) to investigate the carbohydrate composition of fresh kernels harvested at the milk stage among 10 commercial waxy corn hybrids on the contents of total starch, amylopectin, total sugar and phytoglycogen; (2) to assess the seasonal effect on given traits; and (3) to classify all test hybrids and rank them regarding kernel carbohydrate components. The results would benefit breeders by identifying potential sources of waxy corn genotypes for breeding regarding kernel carbohydrate profiles. Furthermore, the potential genotypes in a particular season could be adopted by farmers to raise market demand, production and farmer incomes.

Materials and Methods

Plant materials

Ten commercial waxy corn hybrids existing in Thailand were used (Table 1). Two cultivars (KGW#1 and KNW) were released by the Plant Breeding Research Center for Sustainable Agriculture, Khon Kaen University, Khon Kaen, Thailand. Two cultivars (Bi-Color and Tri-Color) were released by the Pacific Seed Company. Two cultivars (Sweet Violet and Big White 852) were released by the East West Seed Company. Two cultivars (NMW 09 and White Green) were released by the Chia Tai Seed Company. Cultivar L16W03 was released by the Seed Asia Company and cultivar NMT was released by Syngenta. Among these hybrids, KGW#1, KNW, Sweet Violet, Neaw Muang Wan 09, White Green and L16W03 are synergistic sweet-waxy types which possess segregated kernels of 75% waxy kernels (*wx*) and 25% sweet kernels (*bt*) within an ear, whereas the other genotypes are normal waxy corn (*wx*).

Field experiment and crop management

The 10 commercial waxy corn hybrids were grown in the 2017/2018 dry season and the 2018 rainy season at the Agronomy Field Research Station, Khon Kaen University, Thailand using a randomized complete block design (RCBD) with three replications. Standard agronomic practices for vegetable corn production published by the Department of Agriculture, Thailand were followed. The corn ears were harvested at the milk stage approximately 18 d and 22 d after pollination (DAP) in the rainy and dry seasons, respectively.

Table 1 Ten commercial waxy corn hybrids used in this study

Cultivar	Kernel pigmentation	Genotype	Source
KGW#1	Purple	Btbtwxxw	Khon Kaen University
KNW	White	Btbtwxxw	Khon Kaen University
Bi-Color	White and yellow	BtBtwxxw	Pacific Seed Company
Tri-Color	Yellow, white, and purple	BtBtwxxw	Pacific Seed Company
Sweet Violet	White and purple	Btbtwxxw	East West Seed Company
Big White 852	White	BtBtwxxw	East West Seed Company
NMW 09	White and purple	BtBtwxxw	Chia Tai Seed Company
White Green	White	BtBtwxxw	Chia Tai Seed Company
L16W03	White	BtBtwxxw	Seed Asia Company
NMT	White and purple	Btbtwxxw	Syngenta

During the complete experimental period, records from the Agricultural Weather Station, Agronomy Field Research Station, Khon Kaen University, Thailand provided weather data, such as the daily minimum and maximum temperature, precipitation rate, relative humidity and solar radiation. The growing degree days (GDD) of each growing season were calculated for every day after planting by accumulating the temperature per day using Equation (1):

$$^{\circ}\text{C}/\text{d} = ((T_{\min} + T_{\max})/2) - 10 \quad (1)$$

where $T_{\min}$ is the daily minimum temperature and $T_{\max}$ is the daily maximum temperature (both measured in degrees Celcius). The base temperature for maize is 10 °C.

Sample preparation

Five fresh corn ears of each genotype derived from selected healthy plants were harvested at the milk stage for the analysis of the kernel carbohydrate components. These samples were obtained from sib-pollination to keep genetic purity from any unintended pollen contamination. Approximately two kernel rows per ear were sliced, bulked into one sample unit, packed in a vacuum plastic bag and sealed immediately. Then, each bag was immersed in liquid nitrogen for 1 min to stop enzymatic activity and kept at -20 °C until the day of sample extraction according to Harakotr et al. (2014).

Sample extraction and quantification for kernel carbohydrate components

Each sample was trawled and homogenized in a blender. About 1 g of each sample was loaded in a micro-tube with

different solvents for all extractions (sugar, phytyglycogen, total starch, amylopectin). The sample was treated with 1 mL of 90% ethanol and kept in a water bath (80 °C). The tubes containing the sample were shaken with a vortex mixer and centrifuged at 15,000 revolutions per minute for 7 min. These steps were repeated three times to complete the sugar extraction. Further details of sample extraction procedure are available from Simla et al. (2010).

Preparation of standard solutions for sugar, phytyglycogen and total starch followed modified methods of Headrick et al. (1990) and Simla et al. (2010). The stock solution was diluted with distilled water at different concentrations of 0.02 mg/mL, 0.04 mg/mL, 0.06 mg/mL and 0.08 mg/mL. The distilled water was used as a standard solution for a concentration of 0 mg/mL. Each stock solution was determined using spectrophotometry at 490 nm and 600 nm, respectively. The values were recorded to create graphs of the standard solutions. Total sugar was quantified using the phenol-sulfuric colorimetric method (Dubois et al., 1956). Quantification of both phytyglycogen and total starch used the phenol-sulfuric colorimetric method described by Dubois et al. (1956) with absorbed wavelengths of 490 nm and 600 nm, respectively.

The preparation of standard solutions for amylose and amylopectin followed the methods of Wrolstad et al. (2005) and Simla et al. (2010). Sets of standard pure potato amylose and amylopectin at specified concentrations (0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%) of amylopectin were made and then dissolved with 10 mL of 90% dimethyl sulfoxide. The absorbance of the standard 600 nm mixture was measured against a reagent blank as a reference. The values were plotted on a standard curve and the regression equation for the standard curve was determined. Then, the total amylopectin content of each sample was obtained.

Data analysis

All observed data on the carbohydrate components were analyzed based on combined analysis of variance in the RCBD. Least significant difference (LSD) was used to determine the significance of seasonal effect toward genotype means on each given trait. Duncan's multiple range test (DMRT) was used to compare the means among tested corn varieties for significant traits (Gomez and Gomez, 1984). All above analysis were done with Statistix 10 (Statistix®, 2013) and STAR v.2.0.1 (International Rice Research Institute, 2014). Phenotypic variability of test hybrids was visualized with a dendrogram based on hierarchical Ward's clustering method by JMP Pro software (SAS Institute, 2022). The dendrogram was constructed regarding four parameters including the total starch, amylopectin, total sugar, and phytoglycogen of waxy corn hybrid varieties.

Each hybrid mean was standardized prior to performing selection index. The standardized hybrid mean (p_{ij}) was calculated, as follows Equation 2:

$$p_{ij} = \frac{x_{ij} - m_i}{s_i} \quad (2)$$

where x_{ij} is mean of each hybrid; m_i grand mean of each trait; s_i standard deviation.

The unweighted selection index used the same parameters as cluster analysis. Considering two aspects that Thai consumers demand the balance levels of both sweetness and stickiness of waxy corn hybrids (Dermail et al., 2022) and the trait associations between sweetness and stickiness (Simla et al., 2016) and total sugar and amylopectin were negative (Fuengetee et al., 2020), the index for hybrid selection was as follows Equation 3:

$$Z = -1p_1 - 1p_2 + 1p_3 + 1p_4 \quad (3)$$

where Z is selection index; p_1 is total starch of hybrid; p_2 is amylopectin of hybrid; p_3 is total sugar of hybrid; and p_4 is phytoglycogen.

Results and Discussion

Combined analysis of variance (ANOVA)

Mean squares followed by the relative percentage of sum of squares on carbohydrate components are presented in Table 2. Season, variety and the interaction between variety and season were significant for all observed traits, indicating that these factors altered the contents of amylopectin, total starch, total sugar, and phytoglycogen with varied contributions. A seasonal effect largely contributed to the variation in the total sugar content (67.4%), indicating that the sweetness of waxy corn kernels was greatly influenced by season and corroborated the finding by Wong et al. (1994) on sweet corn hybrids. Additionally, this effect was detected for the contents of total starch and amylopectin. Lu et al. (2009) reported significant responses in the amylopectin and starch properties of waxy corn cultivars due to different sowing seasons (spring or autumn). Therefore, an appropriate growing season is critical to achieve the optimum total sugar content in the commercial production of a sweet-waxy corn hybrid.

Carbohydrate components (20.0–33.8%) made only a small contribution to the variation. Simla et al. (2010) reported that variety was a major source of variation in the contents of amylopectin, total starch, total sugar and phytoglycogen of waxy corn. The current study tested both normal waxy and synergistic sweet-waxy genotypes. This elucidated the presence of different multiple gene combinations controlling the expressions of carbohydrate profiles (Simla et al., 2016).

Table 2 Combined analysis of variance based on carbohydrate components of 10 commercial waxy corn hybrids across two seasons

SOV	df	Mean square (% sum of squares)			
		Amylopectin	Total starch	Total sugar	Phytoglycogen
Season (S)	1	6,236** (15.6)	9,425** (17.4)	2,700.1** (67.4)	4.37** (45.0)
Variety (V)	9	1,019** (22.8)	1,579** (26.3)	122.1** (27.4)	0.23** (20.0)
V × S	9	2,646** (59.3)	3,404** (55.8)	22.6** (5.1)	0.27** (24.5)
Pooled error	36	26 (2.3)	8 (0.5)	0.1 (0.1)	0.02 (7.5)
CV (%)		5.2	2.6	1.9	4.8

SOV = source of variation; df = degrees of freedom

** significant at $p < 0.01$

The interaction effect between season and variety ($V \times S$) was predominant for the contents of amylopectin (59.3%) and total starch (55.8%); however, this effect was small for the contents of total sugar (5.1%) and phytylglycogen (24.5%). These result corresponded with Fuengtee et al. (2020) who studied tropical, sweet-waxy corn hybrids and reported that both amylopectin and total starch were largely contributed to by the interaction effect between hybrid and season. In the current study, all targeted traits were polygenic and highly influenced by either the environment or the interaction between genotype and environment ($G \times E$) (Acquaah, 2012).

Seasonal variation and plant phenology

The daily minimum and maximum temperatures during the rainy season (23.5 °C and 31.5 °C, respectively) were higher than during the dry season (16.5 °C and 30.0 °C, respectively), as shown in Fig. 1B. Likewise, the precipitation rate during the rainy season was about 11.5 mm, which was higher than that during the dry season (1.4 mm). Thus, the rainy season (94.1%) had a higher relative humidity than the dry season (85.3%). In contrast, solar radiation during the rainy season was 15.9 MJ m⁻¹, which was lower than during the dry season (18.0 MJ m⁻¹). These facts revealed seasonal variations between the dry and rainy seasons.

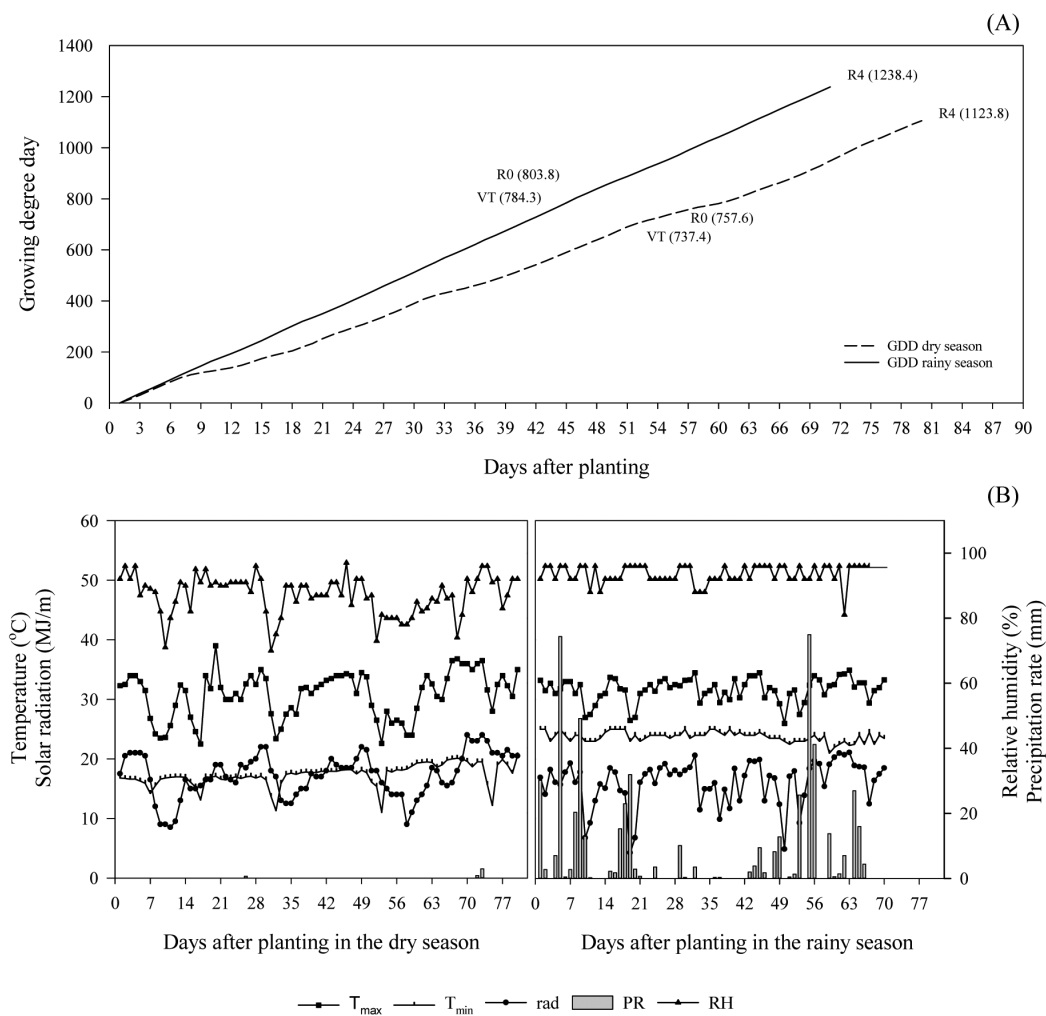


Fig. 1 Phenology of overall tested waxy corn hybrids in each growing season: (A) in days after planting and growing degree days; (B) weather conditions during experiment in two growing seasons, where VT, R0 and R4 = tasseling date, silking date and milk stage, respectively, T_{max} , T_{min} , rad, PR and RH = daily maximum temperature, daily minimum temperature, solar radiation, precipitation rate and relative humidity, respectively

The phenology of the 10 waxy corn hybrids in each season is presented in Figure 1a. The phenology was expressed in days after planting (DAP) and growing degree days (GDD). Waxy corn hybrids grown in the rainy season required higher GDD (1,238.4) and a shorter growing period (70 DAP) to reach the milk stage (R_4) than in the dry season, which required lower GDD (1,123.8) and a longer growing period (80 DAP). This seasonal effect then impacted the kernel carbohydrate composition of the waxy corn hybrids. For example, the hybrid mean for the total starch content in the dry season (166.20 mg g⁻¹) was higher than in the rainy season (96.77 mg g⁻¹), as shown in Table 3. Similarly, the hybrid mean for the amylopectin content in the dry season (107.24 mg g⁻¹) was higher than in the rainy season (86.81 mg g⁻¹). However, the hybrid means for the contents of total sugar and phytoglycogen in the dry season (13.55 mg g⁻¹ and 2.68 mg g⁻¹, respectively) were lower than in the rainy season (26.97 mg g⁻¹ and 3.21 mg g⁻¹, respectively).

The contents of total starch and amylopectin of the tested genotypes grown in the rainy season were lower than for those grown in the dry season, while the contents of total sugar and phytoglycogen in the rainy season were higher than in the dry season. As illustrated in Figure 1, the rainy season had relatively a higher GDD with a shorter duration of the vegetative stage than the dry season. This might have limited photosynthesis accumulation due to certain reductions in the growth duration

(Chowdhury and Wardlaw, 1978); consequently, the total starch content as a final product would be hampered in the rainy season. The first product after photosynthesis is sucrose (Zimmerman, 1960), a free sugar that acts as the main transport substrate for starch synthesis in cereals, including maize (Rufty and Huber, 1983). Two key enzymes in plant starch metabolism are sucrose synthase (SS) and sucrose phosphate synthase (SPS) that are responsible for sucrose formation (Li et al., 2013). This metabolic pathway could be altered by environmental disturbance. Other reports found that high temperature during the grain-filling stage reduced the activities of the enzymes involved in starch metabolism in normal and waxy maize, including SS and SPS, which restricted starch accumulation (Singletary et al., 1994; Yang et al., 2018). However, these reduced activities of SS and SPS could still retain sucrose synthesis before 15 d after pollination (Li et al., 2013). Thus, a higher content of total sugar in the rainy season in the current study was due to early harvest (70 DAP or 20 d after pollination).

Cluster analysis and interaction effects of variety and season (V×S) on carbohydrate components

Regarding individual hybrid performance across seasons, the genotypes KGW#1, KNW and Bi-Color had the highest contents of amylopectin (114.55 mg/g, 113.70 mg/g and 110.00

Table 3 Contents (mean ± SD) of waxy corn hybrids averaged across varieties based on carbohydrate components

Season	Amylopectin (mg/g)	Total starch (mg/g)	Total sugar (mg/g)	Phytoglycogen (mg/g)
Dry	107.24±31.75**	166.20±38.84**	13.55±3.07 **	2.68±0.37*
Rainy	86.81±14.59**	96.77±11.93**	26.97±6.21 **	3.21±0.17*

**, * = values in same column are significantly different by least significant difference at $p \leq 0.01$ and $p \leq 0.05$, respectively.

Table 4 Contents (mean ± SD) of 10 waxy corn hybrids averaged across dry and rainy seasons based on carbohydrate components

Variety	Amylopectin (mg/g)	Total starch (mg/g)	Total sugar (mg/g)	Phytoglycogen (mg/g)
KGW#1	114.55±46.88 ^a	128.15±53.81 ^a	22.10±9.33 ^b	2.95±0.49 ^{ab}
KNW	113.70±52.61 ^a	133.85±67.81 ^a	21.15±10.54 ^b	3.20±0.28 ^a
Bi-Color	110.00±64.35 ^a	131.65±73.33 ^a	19.45±8.70 ^{bc}	3.10±0.28 ^a
Tri-Color	89.45±2.62 ^b	100.60±3.11 ^b	16.55±10.25 ^c	2.65±0.64 ^b
Sweet violet	80.70±14.42 ^c	91.10±9.19 ^c	23.50±11.88 ^b	3.10±0.28 ^a
BW852	101.40±18.67 ^a	110.55±13.08 ^{ab}	16.25±7.71 ^c	2.80±0.42 ^{ab}
NMW03	81.75±9.69 ^c	91.50±3.25 ^c	28.90±14.00 ^a	3.20±0.14 ^a
White Green	95.40±13.29 ^b	99.45±5.02 ^b	17.25±4.60 ^c	2.85±0.78 ^{ab}
L16W03	83.35±4.03 ^{bc}	99.55±5.59 ^b	23.65±11.38 ^b	2.90±0.57 ^{ab}
NMT	99.95±7.85 ^b	106.75±6.01 ^{ab}	13.80±6.51 ^d	2.70±0.42 ^b

Means with different lowercase superscripts in the same column are significantly ($p < 0.05$) different.

mg/g, respectively) and total starch (128.15 mg/g, 133.85 mg/g and 131.65 mg/g, respectively), as shown in Table 4. Genotype NMW03 had the highest contents of both total sugar (28.90 mg/g) and phytyglycogen (3.20 mg/g).

The contents of amylopectin and total starch in the rainy season reduced in each genotype, excluding Sweet Violet and Neaw Maung Tam (Table 5) due to the high temperature. Lu et al. (2013a) found similar patterns in the reduction of both the starch and amylopectin contents in fresh waxy corn due to heat stress during grain filling periods. These losses could be explained by either malfunction of endosperm cell division, a reduction in amyloplast numbers or a restriction on starch granule formation (Commuri and Jones, 2001). In particular, the amylopectin content was more sensitive to heat stress than the amylose content (Wang et al., 2008). Furthermore, solar radiation was also suspected as a plausible factor to explain that reduction; Yang et al. (2016) noticed a significant reduction in starch deposition for fresh waxy corn kernels due to shading related to low solar radiation. Light deprivation restricted photosynthate flux and stimulated abscisic acid concentration (Setter et al., 2001) and then decreased enzyme activity responsible for starch synthesis (Zhang et al., 2008).

The 10 commercial waxy corn hybrids evaluated in each growing season could be clustered into three groups according to their kernel carbohydrate profiles (Fig. 2). In the 2017/18dry season, the first group comprised three genotypes (KGW#1, KNW and Bi-Color) that had very high contents of total starch and amylopectin, moderate total sugar contents and high phytyglycogen contents. The second group comprised five genotypes (Tri-Color, NMT, BW852, White Green and L16W03) that had low to moderate contents of total starch and amylopectin but poor total sugar and phytyglycogen contents. The third group comprised two genotypes (Sweet Violet and NMW03) that had low total starch and amylopectin contents but high total sugar and phytyglycogen contents. In the 2018 rainy season, the first group comprised seven genotypes (KGW#1, KNW, Sweet Violet, L16W03, NMW03, Tri-Color and White Green) that had moderate total starch and amylopectin contents and high total sugar and phytyglycogen contents. The second group (Bi-Color) had low total starch, amylopectin and phytyglycogen contents and a moderate total sugar content. The third group comprised two genotypes (BW852 and NMT) that had excellent total starch and amylopectin contents but low total sugar and phytyglycogen contents.

Table 5 Contents (mean $\pm$ SD) of 10 waxy corn hybrids in each growing season based on carbohydrate components

Season	Variety	Amylopectin (mg/g)	Total starch (mg/g)	Total sugar (mg/g)	Phytyglycogen (mg/g)
Dry	KGW#1	147.7 $\pm$ 6.6 ^b	166.2 $\pm$ 3.1 ^b	15.5 $\pm$ 2.5 ^{hi}	2.6 $\pm$ 0.8 ^{bcd}
	KNW	150.9 $\pm$ 6.3 ^b	181.8 $\pm$ 2.4 ^a	13.7 $\pm$ 2.4 ^{ji}	3.0 $\pm$ 0.6 ^{abc}
	Bi-Color	155.5 $\pm$ 5.9 ^a	183.5 $\pm$ 4.5 ^a	13.3 $\pm$ 1.6 ^{jk}	3.3 $\pm$ 0.7 ^{ab}
	Tri-Color	91.3 $\pm$ 5.5 ^f	98.4 $\pm$ 2.5 ^{fg}	9.3 $\pm$ 2.0 ^m	2.2 $\pm$ 0.7 ^{de}
	Sweet violet	70.5 $\pm$ 8.0 ^j	84.6 $\pm$ 3.2 ^k	15.1 $\pm$ 2.9 ^h	2.9 $\pm$ 0.6 ^{abcde}
	BW852	88.2 $\pm$ 7.4 ^g	101.3 $\pm$ 4.9 ^{ef}	10.8 $\pm$ 2.5 ^{kl}	2.5 $\pm$ 0.8 ^{cde}
	NMW03	88.6 $\pm$ 6.2 ^g	93.8 $\pm$ 4.0 ^h	19.0 $\pm$ 2.3 ^g	3.1 $\pm$ 0.9 ^{ab}
	White Green	104.8 $\pm$ 7.1 ^d	103.0 $\pm$ 2.9 ^e	14.0 $\pm$ 1.4 ^{hij}	2.3 $\pm$ 0.5 ^e
	L16W03	80.5 $\pm$ 7.1 ^h	103.5 $\pm$ 4.0 ^e	15.6 $\pm$ 2.5 ^{hi}	2.5 $\pm$ 0.6 ^{abcde}
	NMT	94.4 $\pm$ 7.9 ^e	102.5 $\pm$ 3.6 ^e	9.2 $\pm$ 2.0 ^{lm}	2.4 $\pm$ 0.5 ^e
Rainy	KGW#1	81.4 $\pm$ 6.7 ^h	90.1 $\pm$ 3.9 ⁱ	28.7 $\pm$ 1.9 ^c	3.3 $\pm$ 0.5 ^a
	KNW	76.5 $\pm$ 6.1 ⁱ	85.9 $\pm$ 4.5 ^{jk}	28.6 $\pm$ 1.5 ^c	3.4 $\pm$ 0.2 ^a
	Bi-Color	64.5 $\pm$ 8.4 ^k	79.8 $\pm$ 4.6 ^l	25.6 $\pm$ 3.1 ^d	2.9 $\pm$ 0.2 ^{abcde}
	Tri-Color	87.6 $\pm$ 7.5 ^g	102.8 $\pm$ 2.2 ^e	23.8 $\pm$ 1.7 ^{de}	3.1 $\pm$ 0.6 ^{ab}
	Sweet violet	90.9 $\pm$ 7.2 ^f	97.6 $\pm$ 3.3 ^{gh}	31.9 $\pm$ 2.8 ^b	3.3 $\pm$ 0.3 ^{ab}
	BW852	114.6 $\pm$ 6.5 ^c	119.8 $\pm$ 3.0 ^c	21.7 $\pm$ 2.9 ^{ef}	3.1 $\pm$ 0.6 ^{abcd}
	NMW03	74.9 $\pm$ 7.4 ⁱ	89.2 $\pm$ 4.7 ^{ij}	38.8 $\pm$ 2.5 ^a	3.3 $\pm$ 0.6 ^{abc}
	White Green	86.0 $\pm$ 6.7 ^g	95.9 $\pm$ 3.2 ^h	20.5 $\pm$ 1.4 ^f	3.4 $\pm$ 0.3 ^{ab}
	L16W03	86.2 $\pm$ 7.5 ^g	95.6 $\pm$ 4.2 ^h	31.7 $\pm$ 3.3 ^b	3.3 $\pm$ 0.3 ^{ab}
	NMT	105.5 $\pm$ 6.9 ^d	111.0 $\pm$ 3.6 ^d	18.4 $\pm$ 2.7	3.0 $\pm$ 0.8 ^{abc}

Means with different lowercase superscripts in the same column are significantly ($p < 0.05$) different.

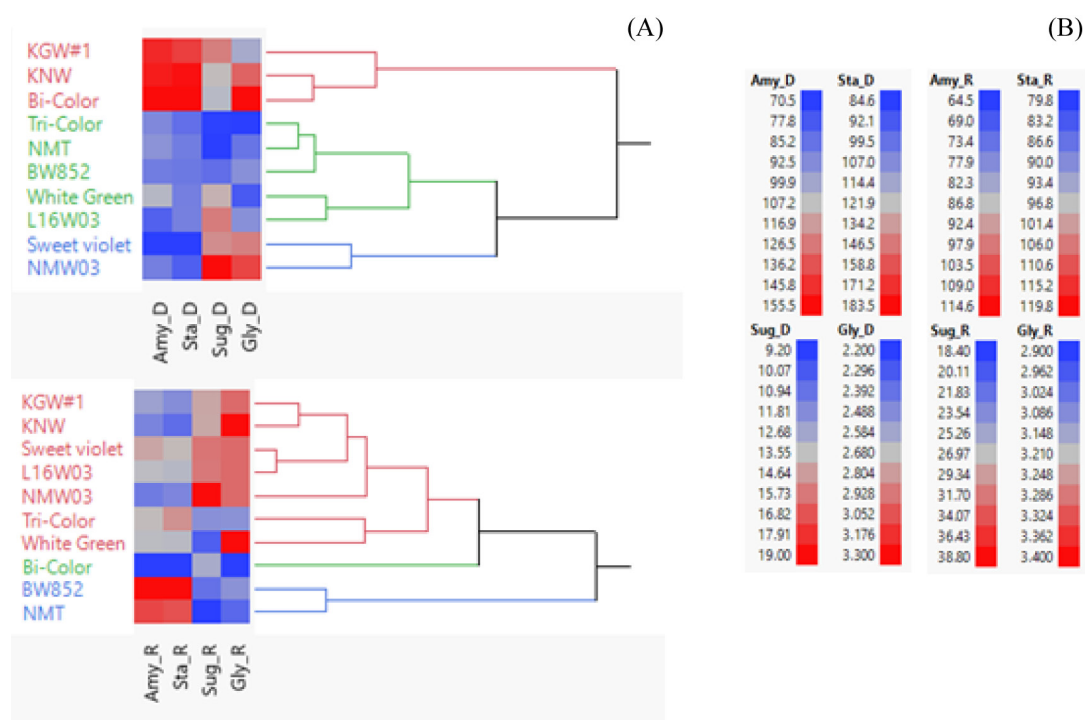


Fig. 2 Kernel carbohydrate profile-based dendrogram revealing the phenotypic variabilities of 10 Thai commercial waxy corn hybrids in response to: (A) different growing seasons under tropical savanna climate; (B) level of carbohydrate components, where blue and red represent low and high values, respectively, Amy_D, Sta_D, Sug_D and Gly_D = contents of amylopectin, total starch, total sugar and phytoglycogen, respectively, in dry season, Amy_R, Sta_R, Sug_R and Gly_R = contents of amylopectin, total starch, total sugar and phytoglycogen, respectively, in rainy season

Bi-Color (155.5 mg/g), KNW (150.9 mg/g), and KGW#1 (147.7 mg/g) had the highest amylopectin contents in the dry season, whereas BW852 (114.6 mg/g) and NMT (105.5 mg/g) had the highest amylopectin contents in the rainy season (Table 3). Bi-Color (183.5 mg/g) had the highest total starch contents followed by KNW (166.2 mg/g) in the dry season, while BW 852 (119.8 mg/g) had the highest total starch contents followed by NMT (111.0 mg/g) in the rainy season. NMW09 (19.0 mg/g) had the highest total sugar contents followed by L16W03 (15.6 mg/g) and KGW#1 (15.5 mg/g) in the dry season, whereas NMW09 (38.8 mg/g) had the highest total sugar content followed by Sweet Violet (31.9 mg/g) and L16W03 (31.7 mg/g) in the rainy season. In the dry season, Bi-Color (3.3 mg/g) had the highest phytoglycogen content followed by NMW09 (3.1 mg/g) and KNW (3.0 mg/g), respectively. White Green (3.4 mg/g) and KNW (3.4 mg/g) had the highest phytoglycogen contents in the rainy season. In addition, several varieties were notable different, such as KGW#1, KNW and Bi-color, while others showed only slight changes.

The unstable performance regarding the carbohydrate components among the tested waxy corn varieties across

seasons is presented in Table 3, while the two different dendrograms in Figure 2 revealed the presence of an interaction effect between genotype and environment. The presence of this effect for given traits indicated that waxy corn breeding would be challenging for the stable performance of kernel carbohydrate components. Although stable waxy corn hybrids across two seasons could be identified, there would be a chance of a genotype with low stability. Thus, multi-environmental trials were required for genotype selection.

Carbohydrate composition of yield-based waxy corn groups

All tested waxy corn hybrids were categorized into four groups by 25% of the difference in each yield group, based on total unhusked yield (t/ha) evaluated in the dry season (Fig. 3). In general, all waxy corn groups had similar compositions of carbohydrate components with total starch making up the predominant component in the kernel carbohydrates, followed by amylopectin, total sugar, and phytoglycogen. Total starch and amylopectin were two major components, while total sugar and phytoglycogen were minor fractions in the composition of the kernel carbohydrate.

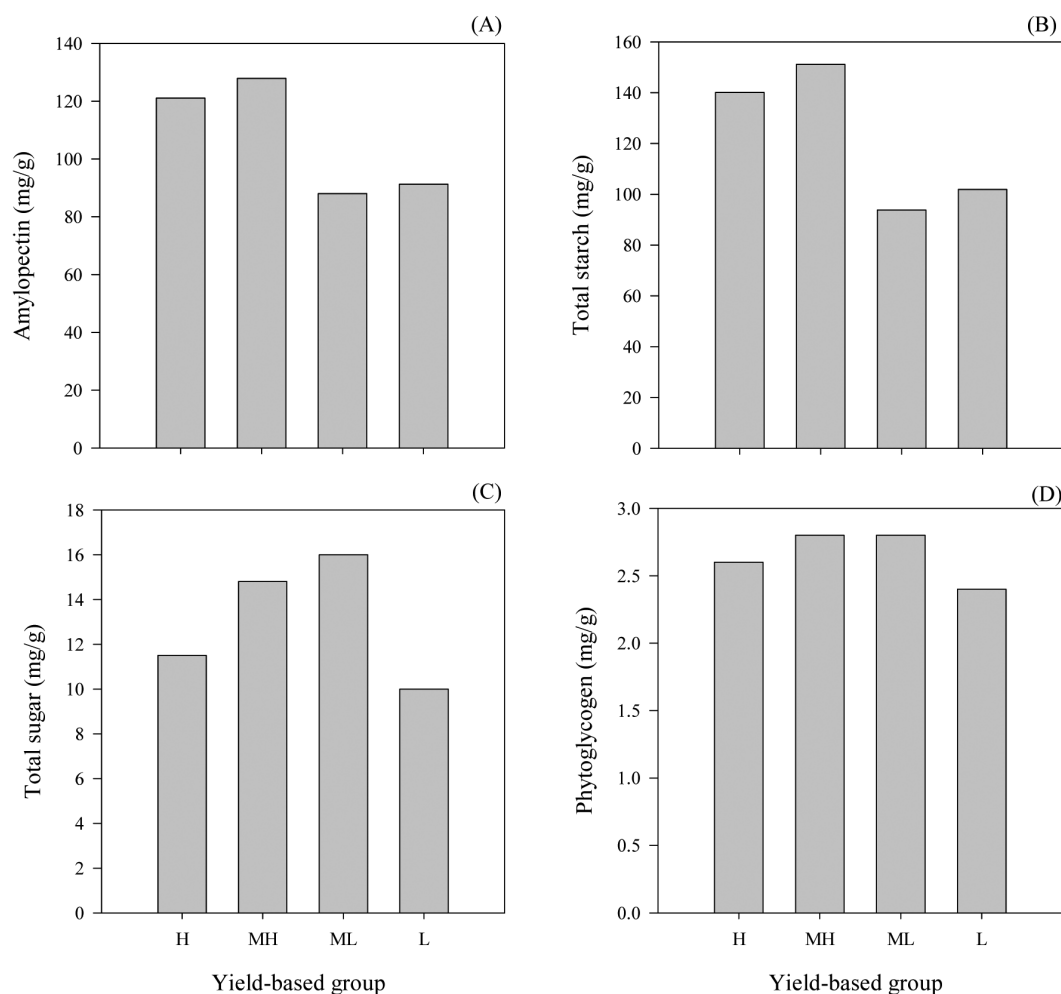


Fig. 3 Dry season evaluation of hybrid performance of yield-based, waxy corn groups on contents of: (A) amylopectin; (B) total starch; (C) total sugar; (D) phytoglycogen, where H = “high yield” group (KNW, Tri-color), MH = “high-to-moderate yield” group (L16W03, KGW#1, Bi-color) group; ML = “moderate-to-low yield” group (White Green, Sweet Violet, Neaw Maung Wan 09) and L = “low yield” group (Big White 852, Neaw Maung T)

The total starch contents in the high, high-to-moderate, moderate-to-low and low yield groups were 140.1 mg/g, 151.1 mg/g, 93.8 mg/g, and 101.9 mg/g, respectively (Fig. 3B). The amylopectin contents in the high, high-to-moderate, moderate-to-low and low yield groups were 121.1 mg/g, 127.9 mg/g, 88.0 mg/g and 91.3 mg/g, respectively (Fig. 3A). The total sugar contents in the high, high-to-moderate, moderate-to-low and low yield groups were 11.5 mg/g, 14.8 mg/g, 16.0 mg/g, and 10.0 mg/g, respectively (Fig. 3C). The phytoglycogen contents in the high, high-to-moderate, moderate-to-low and low yield groups were 2.6 mg/g, 2.8 mg/g, 2.8 mg/g and 2.4 mg/g, respectively (Fig. 3D).

Interestingly, total starch and amylopectin were clearly concentrated in both the high and high-to-moderate groups. In contrast, total sugar and phytoglycogen were highly accumulated in both the high-to-moderate and moderate-to-low yield groups.

Waxy corn varieties comprising the high yield group with high total starch and amylopectin contents and high yield but a poor sugar content were favorable for wet milling industries (Ferguson, 2001) since these starch products commanded a higher price, were easy to wet mill and produced a higher specialty starch yield with a low residual protein content (Rausch et al., 2019). Considering the close association between amylopectin and stickiness (Simla et al., 2016), the high-to-moderate yield group possessing high stickiness of the cooked kernels would be suitable for the local market because local people commonly consume waxy corn as a staple food. Similarly, a moderate-to-low yield group having a better total sugar content would be suitable for supermarkets, as urban people are prefer to consume waxy corn with sweet-flavored sticky kernels. Therefore, every scale of production of the waxy corn varieties should consider the specific demands of end users, including retailers, distributors and consumers.

In the current study, the relationship between each carbohydrate component and husked yield varied from not significant to poor (data not shown). This was supported by other studies in maize. For example, Setter and Parra (2010) reported that kernel set was not associated with several sugar fractions (such as glucose and sucrose) and starch concentrations during stress or recovery in the bulk tissues of the pedicel-placenta, pericarp or endosperm of maize. Paponov et al. (2020) found a negative correlation between kernel set and sucrose/total sugar ratios in the kernels of maize. They suggested that the potential kernel weight was independent of carbohydrate availability in maize.

Unweighted selection index

The top-three hybrids with the highest unweighted selection index (*Z*) evaluated in the 2017/2018 dry season were NMW03 (4.22), Sweet Violet (3.21) and L16W03 (1.50), while the top-three hybrids with the highest *Z* scores evaluated in the 2018 rainy season were NMW03 (3.88), KNW (2.98) and KGW#1 (1.73), as shown in Table 6. The genotype NMW03 had the highest unweighted selection index in the dry and the rainy seasons, indicating that this genotype was quite stable across different growing seasons under a tropical savanna climate in Thailand and may be further recommended to growers.

Eating quality is one of the major factors considered in breeding specialty corns, with the degree to which the released variety would be accepted by the consumers relying on quality factors and eating cultures (Lertrat and Pulam, 2007). Today Thai people prefer steamed waxy corn with balanced levels of stickiness, tenderness and sweetness (Dermail et al., 2022); however, there will be a slight shift in consumer preferences, especially for young people for whom their future ideotype of waxy corn should be sweeter. Therefore, in the current study, the ideotype was identified as waxy corn with excellent total sugar and phytoglycogen contents. The stable genotype NMW03 was consistent with this breeding objective, while other top-ranked genotypes, such as Sweet Violet, L16W03, KNW and KGW#1, were superior only in a certain growing season. The top-three genotypes based on the unweighted selection index in each season were sweet-waxy corn genotypes possessing segregated kernels of 75% waxy kernels (*wx*) and 25% sweet kernels (*bt*) within an ear. The presence of a minor percentage of sweet kernels in the ears of those genotypes was expected to enhance the total sugar content and this was confirmed by the current results. In addition, the current study confirmed that the unweighted selection index was reliable and flexible for genotype selection since the equation could be modified according to a certain breeding objective.

Table 6 Top-three genotypes based on unweighted selection index among 10 Thai commercial waxy corn hybrids evaluated in 2017/2018 dry season (upper table) and in 2018 rainy season (lower table)

Variety	Kernel carbohydrate component (mg/g)				Z
	Amylopectin	Total starch	Total sugar	Phytoglycogen	
Dry season 2017/18					
NMW03	88.6	93.8	19	3.1	4.22
Sweet violet	70.5	84.6	15.1	2.9	3.21
L16W03	80.5	103.5	15.6	2.5	1.50
Grand mean ¹	107.24	166.20	13.55	2.68	
LSD 5% ¹	0.55	0.72	0.67	0.72	
Rainy season 2018					
NMW03	74.9	89.2	38.8	3.3	3.88
KNW	76.5	85.9	28.6	3.4	2.98
KGW#1	81.4	90.1	28.7	3.3	1.73
Grand mean ²	86.81	96.77	26.97	3.21	
LSD 5% ²	0.76	0.51	0.83	0.64	

Z = unweighted selection index; LSD 5% = least significant difference, 5% as critical value of mean comparison between individual top-three hybrids and the grand mean.

¹ Both grand mean and LSD 5% were derived from 10 hybrids in the 2017/2018 dry season.

² Both grand mean and LSD 5% were derived from 10 hybrids in the 2018 rainy season.

Conclusion

The growing season, genotype and their interaction significantly altered the contents of total starch, amylopectin, total sugar and phytoglycogen in the tested waxy corn hybrid varieties with varied responses. Seasonal variation existed, with the dry season leading the tested genotypes with possess higher total starch and amylopectin contents but lower total sugar and phytoglycogen contents than in the rainy season. Predominantly total starch and amylopectin with small portions of sugar and phytoglycogen made up the general composition of the fresh kernel carbohydrates of the tested Thai, commercial, waxy corn hybrid varieties. Moderate-to-high yielding waxy corn varieties possessed relatively high contents of total starch and amylopectin while the moderate-to-low yield group had better sugar and phytoglycogen contents. Genotype NMW03 had the highest unweighted selection index across growing seasons, was stable regarding kernel carbohydrate profiles and could be further recommended to growers.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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