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Gene Effects of Sugar Compositions in Waxy Corn

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Abstract: To supplement the selection for qualitative traits, the quantitative study by generation means analysis was carried out. The aim of this study was to determine gene effects for sugar contents (sucrose, glucose, fructose and total sugar) in two waxy corns crosses (101*su*×101*bt* and 101*su*×216sh_2-crosses). Three inbred lines (101*su*, 101*bt* and 216sh_2) homozygous in waxy gene were used as parents to generate six basic populations (P_1, P_2, F_1, F_2, BC_{11} and BC_{12}). Eleven entries were planted in a randomized complete block design with three replications. The sugar contents were evaluated en masse from immature kernels at 21 days after pollination. The data were used in generation means analysis to understand gene effects. Dominance and epistatic gene effects explained most of genetic variation for sucrose and total sugar in both crosses. Negative dominance gene effect indicated that sugar content in the F_1 hybrids were not as high as that of their parents. Significant additive gene effect also indicated the synergistic effect of the sweet gene combinations. Based on the results, backcross or three-way cross is the best choice to increase sweetness in waxy corn and the use of gene combinations is better than single gene. This information is useful for planning breeding strategies for improving sweetness in waxy corn.

Key words: *Zea mays* L., generation means analysis, sucrose, glucose, fructose, total sugar

INTRODUCTION

Waxy or glutinous corn (*Zea mays* L. var. *ceratina*) has been grown for green corn as a cash crop for more than a century by small scale farmers in Asian countries such as Thailand, Vietnam, Loas, Myanmar, China, Taiwan and Korea (Lertrat and Thongnarin, 2008). It is popular among local people because of its stickiness. Similar to sticky rice, the stickiness in sticky corn is due to the amylopectin containing in its endosperm.

Unlike grain and sweet corn, waxy corn has received little interest for genetic improvement through breeding programs. The increase in demand for waxy corn and market potential arouses more attention from breeders to improve waxy corn. Furthermore, the incorporation of genes controlling sweetness, tenderness, different kernel colors and other useful characters such as ear size for waxy corn can diversify waxy corn products and increase ample market potential.

As a model crop, corn has been subjected to genetic studies more than other crops and genetic control of carbohydrate synthesis in corn has been reported by Creech (1965). Several mutant genes altering carbohydrate synthesis have been exploited commercially to improve eating quality of waxy corn. Four most commonly used mutants included waxy gene (*wx*), shrunken-2 (sh_2), brittle1 (*bt*) and sugary (*su*).

The *wx* gene modifies carbohydrate compositions, leading to stickiness. Mature kernels of the *wx* are opaque. The *su* gene affects the tenderness or sometimes referred to as creaminess. Mature dry kernels of the *su* are wrinkled and translucent. The *su* results in increased in sucrose concentration and the accumulation of the highly branched gluco-polysaccharide; phytoglycogen. While, the sh_2 and *bt* genes are involved in large reductions in starch and large increase in sugar. Mature dry kernels of both mutants are collapsed, angular, opaque and brittle. Both *sh2* and *bt* affect the taste of waxy corn, especially, sweetness. The *bt*, sh_2 and *su* mutants are epistatic over the *wx* genes (James *et al*., 1995; Boyer and Hannah, 2001; Tracy, 2001).

Improvement of sweetness through conventional phenotypic selection of kernel characters has been successful in sweet corn breeding, because, these characters are highly heritable with mendelian segregation. Breeding procedures for improving sweet corn quality have also been available (Boyer and Shannon, 1984). This knowledge can be applied to develop better eating quality of waxy corn.

The attempt to incorporate sweetness into waxy corn in single kernels has not been succeeded, because of, the epistatic effect of genes controlling sweetness over stickiness (Creech, 1965). However, it is possible to incorporate sweetness in one ear of waxy corn by

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segregation of genes in F_2 seeds. In most breeding practices, only, one or two sweet genes have been used and the sweetness is much lower than normal sweet corn. By incorporating *su*, *sh*$_2$ and *bt* genes into waxy background, it is possible to produce waxy corn hybrids with increased sweetness and other eating qualities such as creaminess texture in one ear to diversify the waxy corn products. In using gene combinations to increase sweetness in waxy corn while maintaining acceptable waxy taste, the questions to be asked are that which gene combinations are most suitable and what breeding strategies are to be carried out?

The breeding program aiming to incorporate these genes into waxy background is undergoing at Khon Kaen University (Thoungnarin *et al*., 2005; Lertrat and Thongnarin, 2008; Thongnarin *et al*., 2008). However, most previous study has been related to one or two sweet genes with simple inheritance. Genetic variation for sugar content in sweet corn has been reported. Corn genotypes with common gene combination for sweetness also differed in sugar content (Holder *et al*., 1974a, b; Ferguson *et al*., 1978).

When multiple genes are involved, we raise the assumption that, the inheritance of sweetness is inherited quantitatively and to the best of our knowledge, this information has not been available in the literature. Thus, the aim of this study was to determine gene effects for sugar (sucrose, glucose, fructose and total sugar) in two waxy corn crosses of sweet corn inbred lines with waxy background. The information obtained will be useful for planning suitable strategies for improving sweetness in waxy corn.

MATERIALS AND METHODS

Plant materials and field management: Three inbred lines (101*su*, 101*bt* and 216*sh*$_2$) differ in genes controlling sugar content were selected in this study. These lines are promising inbreds in the pipeline of breeding program at the Plant Breeding Research Center for Sustainable Agriculture, Faculty of Agriculture, Khon Kean University, Khon Kean, Thailand (16° 28' N Lat.; 102° 48' E Long.; 200 m above sea level).

The inbred lines 101*su* and 101*bt* were developed from the cross between Sumlee Esarn population and a white kernel super sweet population (Fig. 1). The white kernel waxy corn population was derived from Sumlee Esarn population (an open-pollinated, large-eared and white kernelled population released in 1999). Sumlee Esarn population combines three kernel mutant genes namely soft sticky texture from glutinous corn (*wx*), creaminess texture form sweet corn (*su*) and sweet taste from super sweet corn (*sh*$_2$). The white kernel super sweet corn population posses the *bt* gene controlling sweetness. These populations were crossed and selection was carried out for Bt- Su- *wxwx* genotypes. Two cycles of mass selection for eating quality and other agronomic traits were completed in 2001. Three inbred lines (101*wx*; BtBt Sh$_2$Sh$_2$ SuSu *wxwx* genotype, 101*bt*; *btbt* Sh$_2$Sh$_2$ SuSu *wxwx* genotype and 101*su*; BtBt Sh$_2$Sh$_2$ *susu wxwx* genotype) were developed by using a classical pedigree scheme during 2002-2005 (Fig. 1).

The inbred line 216*sh*$_2$ was, also, developed by a similar breeding scheme, but, the base populations of this line are the white kernel waxy corn population as described previously and the yellow kernel super sweet corn population which controlled by *sh*$_2$ gene. Two inbred lined were derived from this program (216*wx*; BtBt Sh$_2$Sh$_2$ SuSu *wxwx* and 216*sh*$_2$; BtBt *sh*$_2$*sh*$_2$ SuSu *wxwx*) (Fig. 1).

The line 101*su* has lower sugar content than lines 101*bt* and 216*sh*$_2$. It is also the isogenic line of 101*bt*. The three lines are homozygous in waxy gene. Therefore, the descriptions of used inbred lines are presented in Table 1.

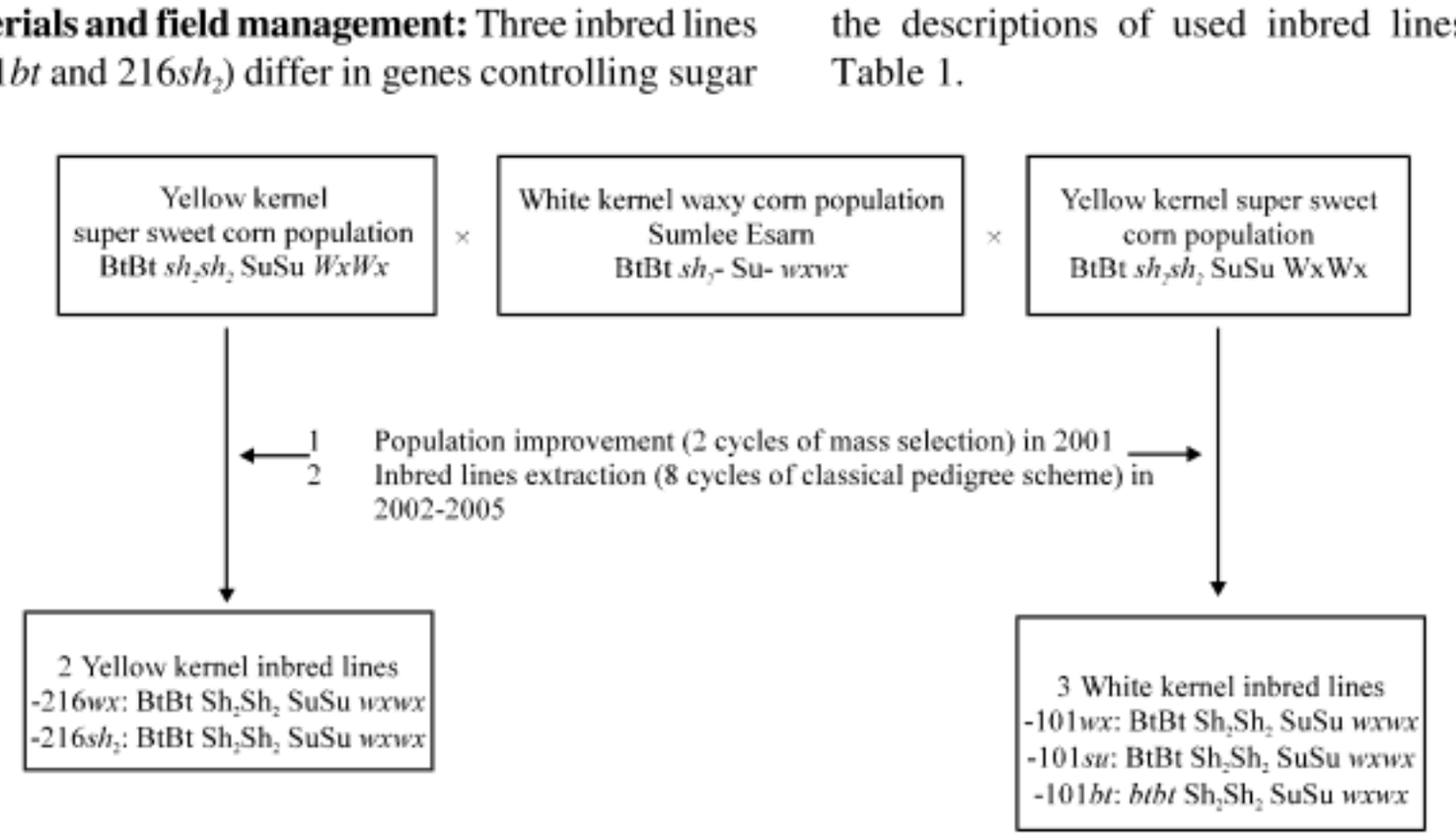

Fig. 1: Schematic diagram for three inbred lines that used as parental lines

Table 1: Descriptions of used inbred lines

Inbred lines	Genotypes	Phenotypes	Type
101*su*	*wxwx BtBt* Sh_2 Sh_2 *susu*	Wrinkled and translucent	Sweet corn
101*bt*	*wxwx btbt* Sh_2 Sh_2 *SuSu*	Mature kernel collapsed, angular often translucent and brittle	Super sweet corn
216sh_2	*wxwx BtBt* sh_2 sh_2 *SuSu*	Inflated, transparent, sweet kernels collapse on drying becoming angular and brittle	Super sweet corn

Two F_1 hybrids were generated by crossing 101*su* to 101*bt* and 216sh_2 at the Khon Kaen University Research Farm in the dry season during November 2006 to February 2007. The F_1s were self-pollinated to generate F_2 seeds and were back-crossed to both parents to generate backcross generations in the rainy season during May to August 2007. Seeds of parental generation and F_1-generation were stored in cool room. Therefore, six basic populations (P_1, P_2, F_1, F_2, BC_{11} and BC_{12}) of each cross were available for the evaluating experiment.

Parental, F_1 and backcross generations were planted in double-row plots with plastic mulching and each plot accommodated 20 plants. Because of high segregation, plots of F_2 generation were much larger, containing 80 plants. Each plot was 5 m long with spacing of 0.75 m between rows and 0.25 m between plants within row.

The experiment was carried out in the dry season at the Research Farm, Faculty of Agriculture, Khon Kean University, Khon Kean, Thailand during November 2007 to February 2008. The dry corn is commercially produced in Thailand, while, corn production in other seasons is not profitable because of severe diseases and lodging. The six generations of the two crosses were laid out in a randomized complete block design with three replications (Gomez and Gomez, 1984). Hand-pollination of adjacent plants in each plot was practiced for parental, F_1 and backcross generations to avoid contamination from stray pollens and individual self-pollination of each plant was practiced for the F_2-generation. Thereafter, five hand-pollinated ears from favour plants were harvested from each plot at the immature fresh-market stage (21 days after pollination), husked, shelled with the knife, frozen in liquid nitrogen and stored at -20°C before sugar analyses and 80 ears of F_2-generation were harvested from each plot.

Sugar analysis: Sugars were extracted following a modified procedure of Headrick *et al*. (1990). For parental, F_1-and-Backcross generations, balanced fresh kernels of five ears from each plot were mixed into a single sample and 200 g of the sample was homogenized in a blender. 1 g of the homogenized sample was dissolved with 1 mL solvent containing 80 ethanol and 20% water at 80°C. Then, the sample was centrifuged for 10 min at 13,000 rpm at 20°C. The supernatant was collected in a container. The extraction procedure was repeated four times for complete sugar extraction from the sample. The supernatant was added with the solvent to give a sample of 5 mL and the sample was further centrifuged and filtered thought 0.45 µm nylon syringe filter and the final sample was stored in a freezer at -20°C until sugar analysis. For F_2-generation, the similar procedure was carried out for sugar extraction, for selected 80 individual ears in each plot.

The HPLC consisted of a Shimadzu™ LC10AT pump, a Shimadzu™ RID 10A reflextive index detector, a Shimadzu™ SIL $10AD_{VP}$ auto injector, a Water™ Temperate Control Module II and a Water™ Column Heater Module. The column is 300×6.5 mm, Sugar Pak™ with Guard Pak Holder and Guard Column insert. The mobile phase was deionized water previously filtered thought 0.45 µm nylon membrane filter and degassed prior to use. Chromatography was carried out at 90°C with a flow rate of 0.5 mL min^{-1}.

Standard solution of sucrose, glucose and fructose were prepared and filtered as described above and injected into the HPLC instrument to determine the standard curve. A 20 µL portion of sample extract was injected to determine the amount of each sugar component. The total sugar content was estimated by summing the amount of such component.

Data analysis: Analysis of variance was carried out for sucrose, glucose, fructose and total sugar content according to a randomized complete block design (Gomez and Gomez, 1984) using MStat-C software program. Least Square Difference (LSD) was used to compare means at 0.05 and 0.01 probability levels.

A generation means analysis for each character was separately conducted for each cross to determine additive, dominant and epistatic effects following Hayman (1958) model. The joint scaling test (Cavalli, 1952) was, also, performed to provide the best estimates of the genetic parameters. As the various generation means did not have equal variances, they were weighted using the inverse of the variance (Nigam *et al*., 2001; Suriharn *et al*., 2005). The regression analysis was used to find the best fit model as suggested by Torres *et al*. (1993), including the parameters m, a, d, aa, ad and dd, sequentially. Any effect that was not significant at 5% level of probability was omitted from the model. Finally, only, significant parameters were fitted using the weighted least squares method as described by Rowe and Alexander (1980). All calculations for generation means analysis were accomplished using Microsoft Excel program.

RESULTS AND DISCUSSION

Generation mean: Significant ($p \leq 0.05$) or highly significant ($p \leq 0.01$) differences among generations for sucrose, glucose, fructose and total sugar were observed in both crosses of waxy corn inbred lines differing in genes controlling sugar content (Table 2). Most characters were highly significant ($p \leq 0.01$) except for glucose content in 101*su*×101*bt*-cross which showed rather low genetic variability for this character.

Among parental lines, 216sh_2 had the highest content of glucose (31.40) fructose (18.70) and total sugar (115.00), but, sucrose content was intermediate (64.90). In contrast, 101bt, had the highest sucrose content (81.90), but, it was intermediate for glucose, fructose and total sugar (20.30, 13.30 and 112.90, respectively). Otherwise, the lowest performance for all sugar characters was given by line 101*su* as summarized in Table 2.

When 101*su* was crossed to 101*bt* and 216sh_2, 101*su*×101*bt*-cross was superior for sucrose and total sugar at F_1-generation. Whereas, the 101*su*×216sh_2-cross was superior for glucose and fructose because their parental lines were more diverse for these characters. In general, sucrose contributed to most part of total sugar and these characters seemed to be inter-related, whereas, glucose and fructose constituted a smaller portion of total sugar.

Sucrose and total sugar in the F_1-generation of both crosses were significantly lower than its mid parent values, whereas, the values were not significantly different for glucose and fructose. Successive self-pollination in the F_2-generation tended to reduce sucrose, glucose and total sugar, but, fructose was significantly increased. These observations were consistent in both crosses.

When the F_1-hybrid of 101*su*×101*bt*-cross was either crossed to low parent (P_1) or high parent (P_2) based on sucrose and total sugar, sucrose and total sugar of the backcross generation in both directions were significantly higher than those of their F_1-hybrid. Similar patterns were, only, observed for sucrose and total sugar in 101*su*×216sh_2-cross at BC_{12} level. In the backcross to low parent (Line-101*su*), the sucrose and total sugar contents were increased, however, exceeded those in the F_2-generation, but, did not exceed those in the F_1-generation. However, sucrose and total sugar of backcross to high parent were surprisingly higher than those of the F_1-hybrid, but, they did not exceed those of their high parent.

The results of glucose and fructose in the backcross generation were rather confounding and cross-dependent. In 101*su*×101*bt*-cross, glucose and fructose in the backcross generation that was backcrossed to either low or high parent were not significantly different from those of the F_1-hybrid. However, significant reductions in glucose and fructose were found in the backcross to low parent in 101*su*×216sh_2-cross, whereas, glucose and fructose of the backcross to high parent were not significantly different from those of the F_1-hybrid and much lower than its high parent.

Table 2: Means and standard errors of different generations for sucrose, glucose, fructose and total sugar content (mg g^{-1} fresh weight) for two crosses of waxy corn

Generation[a]	Sucrose	Glucose	Fructose	Total sugar
101*su*×101*bt*-cross				
P_1	47.8±0.8^{e}	17.8±0.1^{b}	7.4±0.3^{c}	73.0±1.0^{d}
P_2	81.9±0.6^{a}	20.3±0.7^{ab}	13.3±0.3^{a}	112.9±4.8^{a}
F_1	55.8±0.6^{d}	20.2±0.3^{ab}	10.5±0.1^{b}	86.5±0.9^{c}
F_2	37.7±23.9^{f}	16.4±5.7^{b}	12.9±3.2^{a}	66.5±27.9^{d}
BC_{11}	62.4±1.4^{c}	24.2±2.1^{a}	10.4±1.2^{b}	97.0±3.9^{b}
BC_{12}	70.4±21.2^{b}	17.8±5.3^{b}	11.9±2.6^{ab}	100.0±14.3^{b}
MP	64.9	19.1	10.3	93.0
F-ratio[b]	389.2**	3.9*	9.9**	39.9**
$LSD_{0.05}$	2.5	4.4	2.2	8.7
101*su*×216sh_2-cross				
P_1	47.8±0.8^{c}	17.8±0.1^{d}	7.4±0.3^{e}	73.0±1.0^{d}
P_2	64.9±1.5^{a}	31.4±0.8^{a}	18.7±0.4^{a}	115.0±2.8^{a}
F_1	48.6±0.5^{c}	22.8±0.3^{b}	12.7±0.1^{c}	84.2±0.9^{c}
F_2	25.3±10.7^{e}	19.5±4.5^{c}	15.0±3.0^{b}	58.7±13.4^{f}
BC_{11}	37.1±4.6^{d}	17.4±4.8^{d}	8.7±0.9^{d}	63.1±2.2^{e}
BC_{12}	61.0±22.5^{b}	21.9±1.1^{b}	12.9±0.5^{c}	95.8±23.1^{b}
MP	56.4	24.6	13.0	94.0
F-ratio	348.6**	101.1**	232.8**	311.7**
$LSD_{0.05}$	2.5	1.6	0.8	3.8

[a]P_1: Parental line 1, P_2: Parental line 2, F_1: First filial generation of crosses, F_2: Second filial generation of crosses, BC_{11}: First backcross generation with parental line 1, BC_{12}: First backcross generation with parental line 2 and MP: Mid-parent value. *Significant at $p \leq 0.05$, **Significant at $p \leq 0.01$

Gene effect: The reduced model with three parameters did not adequately explain the variation of sugar compositions of corn in waxy endosperm background. The model was extended to six parameters and it was fitted to the data. Dominance and epistatic gene effects explained most of genetic variation for sucrose and total sugar in both two crosses under investigation (101*su*×101*bt* and 101*su*×216sh_2-crosses) as shown in Table 3.

Dominance and additive×additive gene effects were negative in both crosses, whereas, dominance×dominance gene effect was positive in both crosses. Additive×dominance gene effect was negative in 101*su*×101*bt*-cross, but it was positive in 101*su*×216sh_2-cross.

Significant additive gene effect was, also, found for sucrose and total sugar in 101*su*×101*bt* and 101*su*×216sh_2-crosses, but, its effect was much smaller compared to those of dominance gene effect and epistatic gene effects.

Table 3: Estimates of different gene effects for sucrose, glucose, fructose and total sugar content for two crosses of waxy corn

Gene effect[a]	Sucrose	Glucose	Fructose	Total sugar
101*su*×101*bt*-cross				
m	49.8±1.0**[b]	-	17.6±1.7**	35.0±0.4**
a	17.1±0.6**	-	1.6±0.5**	19.9±0.3**
d	-244.5±3.3**	-	9.4±3.4**	-284.5±1.4**
aa	-114.7±0.8**	-	7.3±1.0**	-128.0±0.3**
ad	-18.2±1.5**	-	ns	-33.9±0.7**
dd	138.9±2.8**	-	ns	163.1±1.5**
101*su*×216*sh*$_2$-cross				
m	38.4±0.5**	24.2±5.1**	29.7±3.1**	11.0±0.6**
a	8.5±0.4**	6.8±5.0**	5.6±1.3**	21.0±0.3**
d	-167.8±1.8**	17.2±15.4**	41.9±11.4**	-117.7±1.9**
aa	-94.8±0.3**	-0.4±1.0**	16.7±2.8**	-83.0±0.5**
ad	30.7±0.9**	-4.5±10.0**	-2.8±3.8**	23.4±0.8**
dd	80.8±2.4**	-15.9±10.7**	-24.9±14.8**	44.5±1.7**

[a]m: Mean, a: Sum of additive effects, d: Sum of dominance effects, aa: Sum of additive×additive epistatic effects, ad: Sum of additive×dominance epistatic effects, dd: Sum of dominance×dominance epistatic effects. **Significant at p≤0.01, ns: Non significant

Significant additive gene effect, dominance gene effect and additive×additive gene effect were found for fructose in 101*su*×101*bt*-cross, but neither of them was significant for glucose. All significant gene effects were positive, and, again, additive gene effect was still much smaller than dominance and epistasis effects.

For glucose and fructose in 101*su*×216*sh*$_2$-cross, all genetic parameters were significant. However, dominance and dominance x dominance gene effects accounted for most genetic variation for glucose and fructose, whereas, other gene effects including additive gene effects accounted for a small portion of genetic variation.

The incorporation of sweet genes into waxy genotypes is unsuccessful because waxy phenotype is masked by sweet phenotype in single seeds (Creech, 1965). However, this is possible when these kernel mutant genes are segregated in F_2-generation (Lertrat and Thongnarin, 2008). The questions underlying this study are that, if two sweet genes are incorporated into waxy background, these gene combinations are better than the incorporation of single sweet gene or not and if so, which gene combinations are most suitable. In this study, *su*, *bt* and *sh*$_2$ were incorporated into waxy background in two combinations (*subt* and *sush*$_2$) and their effects were studied quantitatively by generation means analysis.

It is well recognized that, these kernel mutant genes are inherited qualitatively (Creech, 1965). This might be the first report on quantitative inheritance of sugar contents in waxy corn. The reasons for this quantitative study are that at least three mutant genes controlling endosperm characters were incorporated, and, as multiple genes controlling the same characters together with environmental variation, these characters should be inherited quantitatively. Another reason is that the sugar characters were measured en masse instead of single seeds, because, the observation of segregation of the kernels is not possible in immature seeds.

The ultimate goal of this research is to develop waxy corn hybrids with sweetness and tenderness, while, the hybrids can maintain waxy taste. As mentioned previously, waxy phenotype was masked by sweet genes, when, they are homozygous and waxy phenotype can be restored in segregating kernels. The research project can diversify waxy corn products by adding sweetness, creaminess and different endosperm colors as the product samples shown in Fig. 2a-d.

The results conformed to the research objectives. The incorporation of either *bt* or *sh*$_2$ genes could clearly increase total sugar from 73.0 mg g^{-1} FW for *su* alone to 86.5 0 mg g^{-1} FW for *subt* and 84.2 mg g^{-1} FW for *sush*$_2$. When backcrossed to high parent, total sugar could be increased to 100 mg g^{-1} FW for *subt* and 95.8 mg g^{-1} FW for *sush*$_2$. The patterns were quite similar for sucrose but rather different for glucose and fructose. Glucose and fructose contributed a small portion to total sugar compared to sucrose content.

In a parallel study conducted by the same authors, the highest total sugar was obtained from the combinations of *sh*$_2$×*su* (reciprocal) (88.2 mg g^{-1} FW) followed by *bt*×*sh*$_2$ (86.1 mg g^{-1} FW) (unpublished data). The results were comparable to those in this study. A similar work conducted in normal endosperm background reported that total sugar was increased from 4.53% DW for *su* to 18.82% DW for *sh*$_2$ and 32% DW for *sush*$_2$ (Laughnan, 1953).

The incorporation of sweet genes in combination to increase sweetness is practical in sweet corn. The combination of hybrids is homozygous for one gene and heterozygous for another. For example, the combinations of hybrids would be *susu Sh*$_2$*sh*$_2$ or *Susu sh*$_2$*sh*$_2$ and, by this way, the ears will have different types of kernels (Boyer and Shannon, 1984; Lertrat and Plulum, 2007; Lertrat and Thongnarin, 2008). The hybrid has very high in both sugars (approximately 50% more sucrose, 33% more total sugars). There are several hybrids that derived from this method, such as Sweet Gene hybrid and Sweetie. In this study, the hybrids are homozygous for *wx* and heterozygous for both sweet genes e.g., *Susu Btbt* and *Susu Sh*$_2$*sh*$_2$, therefore, there are different kernel types in the ears.

For breeding viewpoint, acceptable levels of sweetness could be attained from the F_1-hybrids. Although, the sweetness in the F_1-hybrids was lower than normal sweet corn hybrids. Then, the F_1-hybrids could maintain high levels of typical waxy taste. The best way to increase total sugar as high as possible and maintain

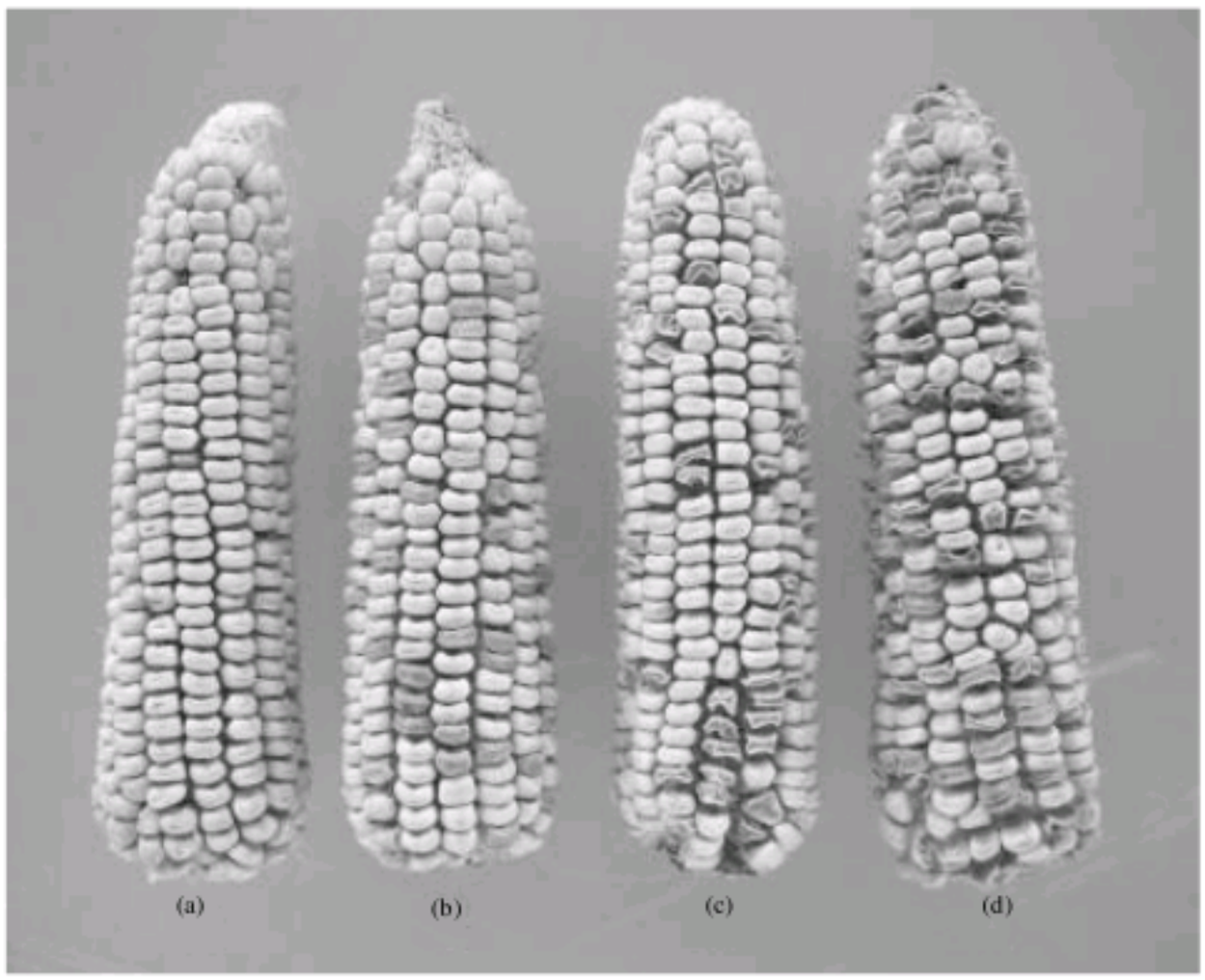

Fig. 2: The dry ears of waxy corn containing different kernel phenotypes; a: 100% waxy kernels, b: 75% waxy kernels with segregation of 25% sugary kernels, c: 75% waxy kernels with segregation of 25% shrunken-2 kernels or 25% brittle kernels and d: 56.25% waxy kernels with segregation of 18.75% sugary kernels and 25% shrunken-2 kernels or 25% brittle kernels

typical waxy taste is to use backcross or three-ways crosses. The backcrosses to high parents in this study had total sugar as high as that of a normal sweet corn hybrid commercially available in Thailand (data not reported). However, the increased sugar is on the expense of waxy taste, because, the proportion of waxy kernels in the ears is inevitably reduced up to 50%. Therefore, the research on the consumers' preference is still required. The production of three-way crosses is rather difficult compared to F_1-hybrids and the uniformity of segregating kernels is lower. However, the three-way cross hybrids can be produced profitably if female parent inbred lines have high seed vigor and high seed yield.

Generation means analysis revealed predominance of dominance gene effect for sucrose glucose, fructose and total sugar. The results indicated the presence of dominance effect for sweetness that can be exploited in single-cross hybrids. The negative sign associated with dominance effect, especially, for sucrose and total sugar also indicated that, the dominance effect reduced sugar content in hybrid combinations in which the sugar content was close to their low parent. As the sugar content was measured en masse, the lower sugar content in hybrids would be possibly, due to, the dilution effect of low sugar kernels or waxy kernels that segregated in the hybrid ears. However, as mentioned previously, waxy kernels are important for maintaining waxy taste.

Negative dominance effect for sugar content, also, suggested that, the increase in sweetness in single cross hybrids of waxy corn would be possible for some extent, resulting in the hybrids having sugar content higher than normal waxy corn. When crossed to high parent, the backcross hybrids gave higher sugar content comparable to those of normal super sweet corn.

If seed production is not a problem, the use of backcross hybrids or three-way cross hybrids open a possibility to diversify waxy corn products by incorporating different kernel colors and other eating qualities such as sweetness and tenderness into hybrid combinations.

In qualitative study, the expected ratio of segregating kernels in the F_1-generation would be 9:3:3:1 for *wxwx*, *wxwxsusu*, *wxwxbtbt* or *wxwxsh$_2$sh$_2$* and *wxwxsususbtbt* or *wxwxsusush$_2$sh$_2$*, respectively and the expected ratio in the backcross generation would be 1:1 for *wxwx* and *wxwxsusu*, *wxwxbtbt* or *wxwxsh$_2$sh$_2$*, respectively (Stoskopf *et al*., 1993). The increased segregating ratio in the backcross generation was associated with the reduction or the increase in sugar content depending on which high or low parent was backcrossed. These findings of quantitative study support the Mendelian inheritance of sweetness characters in corn.

For the implications for breeding, quantitative measurement of sugar content in immature grain, may be,

used in regular breeding activities for improving sweetness in corn for genotypes homozygous for sweet genes or known segregating ratio of the kernels. Variation for sweetness in corn has been reported even in materials with common sweet genes (Laughnan, 1953; Cameron and Cole, 1959; Creech, 1965; Creech and McArdle, 1966; Holder *et al*., 1974a, b; Ferguson *et al*., 1978). However, the high operation cost associated with this measurement my preclude its use in practical breeding programs and surrogate traits associated with these characters such as total soluble solid is valuable as it has high correlation with total sugar content (Campbell and Hume, 1970).

Phenotypic selection of seed characters is, generally, used in practical breeding of sweet corn and these criteria are effective in identifying superior genotypes. However, if the variation in genotypes with common kernel characters exists, the quantitative measurement can be used as supplement criterion and can increase the effectiveness in selection programs.

The main objective of this research was to increase sugar content as high as possible in waxy corn. The results suggested that the best way to obtain the highest sugar content in waxy corn while maintaining typical waxy taste is to use backcross or three-way cross hybrids. The use of backcross hybrids gives a possibility to combine useful characters such as sweetness and tenderness into hybrid combinations and to diversify waxy corn products. This information is of importance for waxy corn breeders to improve waxy corn quality and diversify waxy corn products.

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