



Research article

Improved sugar content in a sweet corn grain mutant with high quality protein and anthocyanin

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Abstract

The kernels of red or purple sweet corn are rich in anthocyanin; however, increasing the tryptophan content in kernel via the *opaque-2* gene would confer more nutritional value. The objective of this study was to increase the tryptophan content in the kernels of red sweet corn via conventional plant breeding. A simple sequence repeat marker, *phi057*, was used in marker assisted-selection (MAS) for the *opaque-2* gene. In the S_1 segregating population, *opaque-2* plants were detected using MAS, *phi057*. Selected lines with good agronomic characters were self-pollinated until the S_6 generation. Their tryptophan content in the protein was about 1% (one-quarter higher than for the *non-opaque-2* checks), while the red kernel lines had total anthocyanin contents in the range 259–557 mg/100 g. The main anthocyanin component was pelargonidin-3-glucoside. The tryptophan content of F_1 hybrids was in the range 0.88–0.98% and their total anthocyanin contents were in the range 144–364 mg/100 g, much higher than for the check hybrid having tryptophan and anthocyanin contents of approximately 0.75% and 27 mg/100 g, respectively. The total sugars content in the F_1 hybrids was in the range 326–385 mg/g, which was the same level as in commercial hybrids. Therefore, the specific goal of a high sweetness level of triple grain mutants was achieved, and the fresh ear yield of the F_1 hybrids was at the commercial standard. In summary, new commercial sweet corn varieties can be launched soon with excellent functional food quality with high tryptophan and anthocyanin contents in the kernel.

Introduction

Three different mutations have resulted in the three most widely diffused genetic varieties of sweet corn: *sugary* (*su*), *sugary enhanced* (*se*), and *shrunk-2* (*sh2*) (Juvik et al., 2003). The most diffused and ancient sweet corn variety is *sugary*, having harvest, storage and shelf life periods slightly shorter than the others, and the sugar content is lower compared to *se* and *sh2* (Juvik et al., 2003). The *shrunk-2* homozygous recessive gene, located on chromosome 3 in sweet corn, has a high level of sweetness from sucrose and after harvesting,

it can retain higher sugar and moisture contents longer than most other mutants (Creech, 1965). Three of the four kernel colors—yellow, red and purple—are produced by pigments synthesized from one of two metabolic pathways, the carotenoid (yellow pigments) or the anthocyanin (red and purple pigments) pathway, while the fourth color, white, results from the lack of pigments produced from either pathway (Ford, 2000). The four genes, *Pr1*, *Cl*, *R1* and *Y1* represent purple aleurone, colored aleurone, colored aleurone and yellow endosperm, respectively. Although the names of two of the genes, colored aleurone1 (*Cl*) and colored1 (*R1*), are similar, their symbols are different (Ford, 2000). Since the early 20th century, the *pr1* locus has been used as a marker in maize genetics for

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identification because it signals the presence (*Pr1*) or absence (*pr1*) of a hydroxyl group in pigment, and this phenotype is controlled by a single gene (McClary, 1942; Coe, 1955). In maize, mutations in the *pr1* locus lead to the accumulation of pelargonidin (red) rather than cyanidin (purple) pigments in aleurone cells where the anthocyanin biosynthetic pathway is active; genetic mapping using singular-nucleotide polymorphism (SNP) markers has confirmed its position on chromosome 5L (Sharma et al., 2011). Similarly, the formation of anthocyanidins in the kernel aleurone requires the activity of a flavonoid 39-hydroxylase (F39H), and this activity has been attributed to the functional red aleurone1 locus, also known as purple aleurone1 and designated as *pr1* in maize (Larson et al., 1986). Purple corn has high anthocyanin levels accumulated in the aleurone layer and kernel pericarp (Li et al., 2011). Anthocyanin is useful for health due to its high antioxidant and anti-mutagenic effects; it reduces cancer cell proliferation and inhibits tumor formation (Cevallos-Casals and Cisneros-Zevallos, 2003). Differences in the health impacts of pelargonidin and cyanidin (purple) are still unknown. Currently, the protein of maize has low tryptophan and lysine in the endosperm. For the nutrition of humans, lysine is one of the essential amino acids, followed by tryptophan; however, the lysine and tryptophan contents of zeins, the main protein type in maize, are quite low (Food and Agriculture Organization, 1992). Therefore, it is essential to raise the protein quality in maize-based diets. Breeding for maize with enhanced protein quality started in the mid-1960s, when mutants with an increased lysine content, such as *opaque-2* (*o2*) and *floury2* (*fl2*), were discovered; the increase in essential amino acids resulted from a change of the relative amounts of different fractions constituting the maize endosperm proteins (Vasal, 1994). In recent years there has been success in combining two recessive traits, *waxy* and QPM (w/o), into one new kernel type with normal aspects so that it is suitable as a staple food (Dang et al., 2011; Sinkangam et al., 2011; Stamp et al., 2016). Those findings have been confirmed (Zhang et al., 2013). The new colored sugary line is a good source of anthocyanins, of other beneficial flavonoids and antioxidant potential, and thus it can be considered a good functional food. Moreover, to preserve the healthy properties of the colored sweet corn, it is better to consume it fresh, but if the processing is needed, it would be better to use a mild process, such as the steam treatment, to benefit from the best nutritional composition. Consumers could possibly choose this new product for its healthy properties, given that no differences in appreciation ability were scored (Lago et al., 2014). In Thailand, sweet corn with the *shrunk-2* gene has been used for about 95% of both fresh and processed corn consumption. At present, there is only a red sweet corn hybrid in the Thai market; however, there has been no integration with an *opaque-2* gene to increase the tryptophan content in the red kernel of the sweet corn hybrid. Therefore, the main objective of the current study was to improve the new red *opaque-2* sweet corn lines and their single cross hybrids in sweetness acceptable to consumers and to stabilize high anthocyanin and tryptophan contents in the kernel on a broadened genetic basis.

Materials and Methods

Plant materials and cultivations

The recurrent parent was the S_4 line of red *opaque-2* sweet corn from the Department of Agronomy, Faculty of Agriculture at

Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus, Nakon Pathom, Thailand. Briefly, purple *opaque-2* waxy maize was crossed with sweet corn (*sh2*). In the segregating selfed progenies of S_1 , the *opaque-2* gene was detected using the genetic marker *phi057*. The two other genes (purple and shrunk) were selected based on visual selection of the kernel. Then, selected lines were self-pollinated into S_4 to get the red kernel of sweet corn. In the current study, the S_4 line was crossed with the yellow normal sweet corn (Insee 2, a commercial hybrid from the National Corn and Sorghum Research Center, Kasetsart University, Bangkok, Thailand) to increase the sweetness and nutritional quality on a commercial level.

The experiments were conducted at the National Corn and Sorghum Research Center, Nakhon Ratchasima, Thailand, during 2015–2018 as part of a breeding program. Finally, two consecutive yield trials of parental lines and their hybrids were conducted during May–August in 2017 (early season) and from October 2017 to January 2018 (late season). The experiments were arranged in a randomized complete block design with three replications. Each plot consisted of four rows, 0.75 m apart and 5 m long, with 21 plants per row. The distance between adjacent plants in a row was 0.25 m, resulting in a population density of 5.33 plant/m². Before sowing, basal N:P:K fertilizer (15:15:15) was applied at approximately 125 kg/ha and approximately 250 kg/ha of N fertilizer (46:0:0) was added 1 mth after planting. Herbicides were used based on local recommendations.

DNA extraction and marker assay for the *opaque-2* locus

The leaves of individual plants aged 28 d were collected for DNA extraction using the method described by Sinkangam et al. (2011) with slight modifications. The simple sequence repeat (SSR) marker *phi057* was used to identify the *opaque-2* plants based on amplification products of about 140 to 160 bp (Chin et al., 1996; Sinkangam et al., 2011). The amplification was performed in 20 μ L of reaction mixture containing 3 mmol MgCl₂, 0.2 mmol dNTPs, 10 pmol of each primer, 1 U Taq DNA polymerase and 50 ng of template DNA and distilled water. Polymerase chain reaction products were separated in 6% polyacrylamide denaturing gel and stained with silver.

Analysis of total sugars

Kernels at 21 d after pollination were used for total sugar analysis following chopping, crushing and percolation through a cotton membrane. The supernatant following centrifugation at 1,200 rpm and 4°C was used to examine the percentage of total soluble solids. The final solution was analyzed using Nelson's reducing-sugar method (Nelson, 1980; Sinkangam et al., 2011), and a spectrophotometer (SPECTROstar Nano; Ortenberg, Germany) at 500 nm. The resulting absorbance value in milligrams glucose per milliliter was compared with the standard D-glucose solution.

Analysis of tryptophan and total protein

Thirty kernels per plant were harvested at 21 d after pollination and the pericarps and embryos were removed. The endosperms were dried in a hot-air oven at 40 °C for 3 d. The dried endosperms were ground in a cyclone mill and wrapped in a commercial filter-paper envelope to remove the fat with 100% hexane in a Soxhlet-type

continuous extractor. Then, the ground samples were analyzed for tryptophan content using a spectrophotometer as described by Nurit et al. (2009). The protein content was measured according to the micro-Kjeldahl method (Bailey, 1967).

Anthocyanin analysis

Sample preparation

Thirty kernels of each sample were harvested at 21 d after pollination. The kernels were dried in a hot-air oven at 40°C for 3 d. The kernels were ground in a cyclone mill into a fine powder and dried at 64°C for 16 hr.

HPLC analysis

Fine powder (1 g) from each sample was extracted using 8 mL of methanol (50% MeOH + 0.01% HCl, solvent A) mixed well and ultrasonicated for 15 min on ice. Incubating the sample without disturbing the interface under ice (4°C), the supernatant was transferred to another tube. The pellet was re-extracted using the same method as for the first extraction. All supernatant was transferred to a 50 mL centrifuge tube and centrifuged at 11,000 rpm for 15 min at 4°C. The supernatant was placed into a volume flask and methanol (solvent A) was added to obtain a final volume of 20 mL. A high-performance liquid chromatography (HPLC) system (Waters 600) equipped with a photodiode array detector (Waters 2998) and an autosampler (Waters 717 plus) from Waters (Milford, MA, USA) were used. Solutions were injected after being filtered through a 0.22 µm nylon membrane filter (Agela Technologies Cat. AS021320; Torrance, CA, USA). Anthocyanins in the samples were identified by comparing their relative retention times with those of standard compounds (cyanidin 3-O-glucoside chloride, peonidin 3-O-glucoside chloride, and pelargonidin 3-O-glucoside chloride; Sigma-Aldrich; St. Louis, MO, USA). Chromatographic separations were performed on the Gemini NX-C18 (5 µm 250 × 4.60 mm). The composition of solvents and the gradient elution conditions used were those described by Kim et al. (2007), with slight modifications. The mobile phase consisted of formic acid (5% formic acid in water) and formic acid (5% formic acid in water:ACN; 50: 50 volume per volume) at a flow rate of 1 mL/min. The operating conditions were: column temperature 30°C, injection volume 10 µL, UV-vis detection at 525 nm and runtime about 60 min.

Spectrophotometry analysis

The composition of solvents and the method for extraction of anthocyanin used were those described by Galicia et al. (2009). Briefly, dry powder (approximately 20 mg) was placed in an Eppendorf microcentrifuge tube and 1.3 ml of 1% (trifluoroacetic acid in 80% methanol) was added. Then, the vortexed samples were placed horizontally on ice (4°C) and shaken for 90 min at 150 rpm. After the ice incubation, the tubes were centrifuged at 14,000 rpm for 5 min. The supernatant was measured using a spectrophotometer (SPECTROstar Nano; Ortenberg, Germany) at 520 nm. A second extract was repeated, but the incubation on ice was done for 60 min at 150 rpm and measured using the spectrophotometer at 520 nm. The result of the absorbance value was compared with the standard pelargonidin 3-O-glucoside chloride.

Statistical analysis

Data were analyzed according to combined analysis of a randomized complete block design using the R statistical package (R Core Team, 2019). Significance was tested at the $p < 0.01$ level.

Results

I. Development of purple opaque-2 sweet corn line

The breeding program for the current study was initiated from the cross between yellow *non-opaque-2* sweet corn (Insee2, commercial hybrid, *sh2*) and the S_4 line of red *opaque-2* sweet corn (*sh2*) (Fig. 1). The F_1 plant was self-pollinated to get the S_1 generation. In the segregation of S_1 plants, the SSR marker, *phi057*, was applied to identify the *opaque-2* plants, following the self-pollination of selected plants into the S_2 generation. There were three types of S_1 plants: 115 O_2O_2 , 293 O_2o_2 and 42 o_2o_2 plants. Therefore, only 42 *opaque-2* plants from the 450 plants in the S_2 generation were produced. The fixed *opaque-2* plants with red kernels were self-pollinated in each generation from S_2 to S_6 . In the S_3 lines, they were still segregated in the kernel colors purple, red and white (Fig. 1). However, in the S_6 generation, the gene for red kernel color was fixed. All were shown to be *opaque-2* plants which were tested based on the SSR marker, *phi057*.



Fig. 1 (A) commercial hybrid (Insee2), (B) S_4 *opaque-2* sweet corn, and (C) S_3 segregated lines of *opaque-2* sweet corn

II. Expression of purple, opaque-2, and shrunken-2 genes in S_6 lines

In the S_6 generation of *opaque-2* sweet corn, five lines of the yellow kernel and three lines of the red kernel with good agronomic characters such as healthy and the same flowering date were crossed between the yellow kernel and the red kernel to make 15 F_1 hybrids. Parental lines were analyzed for chemical components in the kernel, consisting of total sugars, sucrose, cyanidin-3-glucoside (Cy-G), pelargonidin-3-glucoside (Pl-G), peonidin-3-glucoside (P-G), total anthocyanin content (TAC) and tryptophan in the protein content. For all traits, significant differences existed between genotypes, as shown in Table 1. The contents of total sugars and sucrose in the yellow kernel (Y3, Y5, Y33, Y35 and Y42) and red kernel (P50, P51 and P52) lines were significantly lower than in the two check hybrids with yellow or red color kernels, respectively. The total anthocyanin composition in the anthocyanin-containing lines was determined using HPLC. The total anthocyanin content (TAC) was in the ranges 3.9–16.1 mg/100 g of dry matter in yellow kernels and 258.9–556.6

mg/100 g in red kernels. In contrast, the total anthocyanin content in the check variety was 3.5 mg/100 g in yellow sweet corn and 101.5 mg/100 g in red sweet corn (SRQ). The cyanidin-3-glucoside (Cy-G) contents were in the ranges 0–2.3 mg/100 g in yellow kernels and 21.2–68.0 mg/100 g in red kernels while the commercial hybrids contained about 0 ppm in yellow kernels and 39.6 mg/100 g in red kernels. The pelargonidin-3-glucoside (Pl-G) contents were in the ranges 3.1–13.8 mg/100 g in yellow kernels and 122.0–523.4 mg/100 g in red kernels. Peonidin-3-glucoside (P-G) was not detected in yellow kernels, but it was detected in red kernels in the range 11.9–68.9 mg/100 g. Pelargonidin-3-glucoside predominated in red kernels, as shown in Table 1. The average amount of tryptophan in the protein

content differed among the genotypes ($P < 0.01$). The tryptophan content in the endosperm of the yellow *opaque-2* sweet corn was in the range 0.90–1.02%, while in the red *opaque-2* sweet corn, the range was 1.07–1.09%. On the other hand, *non-opaque-2* sweet corns, Insee2 (0.75%), and SRQ (0.69%) had a lower tryptophan content than the *opaque-2* sweet corn.

Yield trials of F_1 hybrids were conducted in two consecutive environments (early- and late-rainy seasons) at the National Corn and Sorghum Research Center during 2017–2018. The quality traits (total sugars, total anthocyanin content and tryptophan content) in the protein of kernels were all significantly different among the genotypes (Table 2). Total sugars were in the range 326–385 mg/g in F_1 hybrids,

Table 1 Mean values ($\pm$ SD) for total sugars, sucrose, cyanidin-3-glucoside (Cy-G), pelargonidin-3-glucoside (Pl-G), peonidin-3-glucoside (P-G), total anthocyanin content (TAC) and tryptophan (Tryp) in protein content of the inbreeding lines produced in this study as compare with two commercial F_1 hybrids

Line	Kernel color	Total sugars mg/g	Sucrose mg/g	TAC mg/100 g	Cy-G mg/100 g	Pl-G mg/100 g	P-G mg/100 g	Tryp %
Inbreeding line (S_n)								
Y3	Yellow	215 $\pm$ 13	193 $\pm$ 6	8.5 $\pm$ 0.01	1.9 $\pm$ 0.01	6.6 $\pm$ 0.01	0.0 $\pm$ 0.0	1.02 $\pm$ 0.08
Y5	Yellow	238 $\pm$ 6	201 $\pm$ 6	16.1 $\pm$ 0.11	2.3 $\pm$ 0.05	13.8 $\pm$ 0.05	0.0 $\pm$ 0.0	0.96 $\pm$ 0.09
Y33	Yellow	216 $\pm$ 5	200 $\pm$ 0.3	3.9 $\pm$ 0.06	0.0 $\pm$ 0.0	3.9 $\pm$ 0.06	0.0 $\pm$ 0.0	0.90 $\pm$ 0.11
Y35	Yellow	224 $\pm$ 7	199 $\pm$ 2	5.0 $\pm$ 0.57	1.9 $\pm$ 0.45	3.1 $\pm$ 0.64	0.0 $\pm$ 0.0	0.98 $\pm$ 0.14
Y42	Yellow	225 $\pm$ 9	188 $\pm$ 2	5.2 $\pm$ 0.28	1.6 $\pm$ 0.22	3.6 $\pm$ 0.15	0.0 $\pm$ 0.0	0.97 $\pm$ 0.05
Average		223.6 $\pm$ 8	196.2 $\pm$ 3.2	7.74 $\pm$ 0.21	1.5 $\pm$ 0.15	6.2 $\pm$ 0.18	0.0 $\pm$ 0.0	0.97 $\pm$ 0.09
P50	Red	213 $\pm$ 2	176 $\pm$ 7	337.7 $\pm$ 3.61	21.2 $\pm$ 0.17	301.4 $\pm$ 2.91	15.1 $\pm$ 0.88	1.07 $\pm$ 0.13
P51	Red	227 $\pm$ 14	186 $\pm$ 9	556.6 $\pm$ 2.61	21.3 $\pm$ 0.11	523.4 $\pm$ 1.78	11.9 $\pm$ 0.38	1.08 $\pm$ 0.14
P52	Red	224 $\pm$ 5	191 $\pm$ 6	258.9 $\pm$ 0.26	68.0 $\pm$ 0.33	122.0 $\pm$ 0.52	68.9 $\pm$ 0.01	1.09 $\pm$ 0.05
Average		221.3 $\pm$ 7	184.3 $\pm$ 7.3	384.4 $\pm$ 2.16	36.8 $\pm$ 0.20	315.6 $\pm$ 1.74	32.0 $\pm$ 0.42	1.08 $\pm$ 0.11
Commercial F_1 hybrid								
Insee2	Yellow	377 $\pm$ 22	252 $\pm$	3.5 $\pm$ 0.10	0.0 $\pm$ 0.0	3.5 $\pm$ 0.70	0.00 $\pm$ 0.0	0.75 $\pm$ 0.04
SRQ	Purple	345 $\pm$ 12	239 $\pm$	101.5 $\pm$ 12.35	39.6 $\pm$ 7.8	44.0 $\pm$ 10.7	17.9 $\pm$ 0.58	0.69 $\pm$ 0.09
F-test		**	**	**	**	**	**	**
LSD _{0.05}		32.43	7.94	6.5	2.4	3.7	0.4	0.11
CV (%)		11.15	3.35	19.3	13.14	3.08	2.78	10.56

TAC = sum of Gy-G, Pl- G and P-G; SRQ = Siam Ruby Queen; ** = significant at the 0.01 level; LSD = least significant difference; CV = coefficient of variation III. Yield trial of F_1 hybrids and kernel chemical compositions

Table 2 Mean values ($\pm$ SD) for total sugar, sucrose, total anthocyanin (TAC), tryptophan in protein (Tryp), ear weight with husk and ear weight without husk of the hybrids produced in this study as compare with two commercial F_1 hybrids

Cross	Kernel color	Total Sugar (mg/g)	Sucrose (mg/g)	TAC (mg/100 g)	Tryp (%)	Ear weight with husk t/ha	Ear weight without husk t/ha
F_1 -hybrid							
Y3 $\times$ P50	Red	339 $\pm$ 10	284 $\pm$ 9	364 $\pm$ 7	0.93 $\pm$ 0.08	20.0 $\pm$ 2.3	13.6 $\pm$ 1.7
Y3 $\times$ P51	Red	357 $\pm$ 12	278 $\pm$ 14	144 $\pm$ 9	0.98 $\pm$ 0.17	22.1 $\pm$ 3.8	15.4 $\pm$ 2.2
Y3 $\times$ P52	Red	339 $\pm$ 6	252 $\pm$ 12	198 $\pm$ 10	0.91 $\pm$ 0.16	21.4 $\pm$ 2.2	13.6 $\pm$ 3.4
Y5 $\times$ P50	Red	339 $\pm$ 9	242 $\pm$ 10	204 $\pm$ 7	0.89 $\pm$ 0.14	18.7 $\pm$ 2.6	13.2 $\pm$ 1.8
Y5 $\times$ P51	Red	334 $\pm$ 13	234 $\pm$ 15	164 $\pm$ 18	0.88 $\pm$ 0.13	19.7 $\pm$ 2.7	13.5 $\pm$ 2.2
Y5 $\times$ P52	Red	348 $\pm$ 12	256 $\pm$ 11	210 $\pm$ 6	0.91 $\pm$ 0.08	23.1 $\pm$ 1.0	16.5 $\pm$ 0.9
Y33 $\times$ P50	Red	332 $\pm$ 1	290 $\pm$ 1	178 $\pm$ 7	0.95 $\pm$ 0.09	21.0 $\pm$ 2.1	14.9 $\pm$ 1.5
Y33 $\times$ P51	Red	354 $\pm$ 4	240 $\pm$ 3	312 $\pm$ 9	0.92 $\pm$ 0.03	23.2 $\pm$ 3.9	17.1 $\pm$ 3.2
Y33 $\times$ P52	Red	380 $\pm$ 9	284 $\pm$ 10	164 $\pm$ 6	0.90 $\pm$ 0.03	24.3 $\pm$ 1.1	17.7 $\pm$ 0.9
Y35 $\times$ P50	Red	337 $\pm$ 1	249 $\pm$ 2	258 $\pm$ 8	0.91 $\pm$ 0.05	17.3 $\pm$ 2.4	12.1 $\pm$ 3.7
Y35 $\times$ P51	Red	376 $\pm$ 11	268 $\pm$ 4	364 $\pm$ 12	0.93 $\pm$ 0.02	21.3 $\pm$ 2.2	14.5 $\pm$ 2.1
Y35 $\times$ P52	Red	351 $\pm$ 17	257 $\pm$ 4	322 $\pm$ 4	0.91 $\pm$ 0.06	22.8 $\pm$ 1.0	15.8 $\pm$ 0.8
Y42 $\times$ P50	Red	385 $\pm$ 14	325 $\pm$ 4	202 $\pm$ 3	0.92 $\pm$ 0.09	21.8 $\pm$ 3.2	15.4 $\pm$ 2.0
Y42 $\times$ P51	Red	326 $\pm$ 13	297 $\pm$ 14	348 $\pm$ 9	0.93 $\pm$ 0.09	22.8 $\pm$ 3.8	15.4 $\pm$ 1.5
Y42 $\times$ P52	Red	342 $\pm$ 9	255 $\pm$ 3	232 $\pm$ 9	0.90 $\pm$ 0.07	24.0 $\pm$ 2.6	17.0 $\pm$ 1.8
Average		349 $\pm$ 9.4	267 $\pm$ 7.7	244 $\pm$ 8.2	0.91 $\pm$ 0.09	21.6 $\pm$ 2.46	15.1 $\pm$ 1.98
Commercial F_1 hybrid							
Insee2	Yellow	371 $\pm$ 10	279 $\pm$ 7	27 $\pm$ 6	0.75 $\pm$ 0.04	22.6 $\pm$ 1.8	15.4 $\pm$ 1.2
SRQ	Red	351 $\pm$ 8	238 $\pm$ 8	160 $\pm$ 5	0.72 $\pm$ 0.04	21.7 $\pm$ 0.3	15.5 $\pm$ 0.3
F-test		**	**	**	**	**	**
LSD _{0.05}		13.69	14.50	20.88	0.11	3.06	2.37
CV (%)		3.38	4.72	7.94	10.27	12.26	13.60

TAC = sum of Gy-G, Pl- G and P-G; SRQ = Siam Ruby Queen; ** = significant at the 0.01 level; LSD = least significant difference; CV = coefficient of variation

while in the commercial sweet corn total sugars contents were approximately 371 mg/g in Insee2 and 351 mg/g in SRQ. Moreover, sucrose was in the range 234–325 mg/g. These results indicated that F_1 hybrids of red *opaque-2* sweet corn had the same sweetness as commercial sweet corn hybrids, for both yellow and red kernels. The anthocyanin content of hybrids was in the range 144–364 mg/100 g with an average of 244 mg/100 g, which was more valuable than in the red commercial sweet corn (160 mg/100 g). The percentage of tryptophan content differed among the groups ($P < 0.01$). The tryptophan content in the endosperm of the red *opaque-2* group was in the range 0.88–0.98%, while the *non-opaque-2* plants had 0.75% for yellow kernels of Insee2, and 0.72% for red kernels of SRQ. It is important to consider not only good nutrient quality in the kernels but also fresh ear weight with husk or fresh ear weight without husk to develop a successful new commercial hybrid of red *opaque-2* sweet corn. In the current study, the average fresh ear weight with husk was in the range 17.3–24.3 t/ha, whereas commercial hybrids, Insee2 and SRQ yielded about 22.6 t/ha and 21.7 t/ha, respectively. The fresh ear weight without husk of F_1 hybrids was in the range 12.1–17.7 t/ha with an average of 15.1 t/ha. The selected cross, Y42 $\times$ P52, was high yielding and had good agronomic characters including high total sugars, sucrose, anthocyanin content and tryptophan content in the kernels.

Discussion

The *shrunk-2* (*sh2*) gene increase the quality of the sweet corn because it had a high percentage of sucrose and less starch than kernels of the *sugary 1* (*su1*) mutation. At the level of test hybrids, the red *opaque-2* sweet corn had total sugars contents in kernels (average 349 mg/g) that were comparable to those in single grain mutants (348 mg/g for *shrunk-2*) provided by Creech (1965). These data indicated that the new red *opaque-2* sweet corn had normal grain development despite adding two further grain mutants. Interestingly, the total sugars contents seemed to be subject to hybrid vigor, as all inbred lines had more than 33% lower values (Table 1). The red kernels of S_4 *opaque-2* sweet corn used as the male parent in the study may have been controlled by $pr1/pr1$; $C_1/-$; $R_1/-$. Sharma et al. (2011) reported that the purple and red kernels were due to $Pr1/-$; $C_1/-$; $R_1/-$, and $pr1/pr1$; $C_1/-$; $R_1/-$, respectively. The selected S_6 lines had five yellow *opaque-2* sweet corn plants that were top-crossed with three red *opaque-2* sweet corn plants to obtain 15 F_1 hybrids. For yellow *opaque-2* sweet corn, the kernel color came from the color of the starchy endosperm because the aleurone layer was colorless. In the red kernel *opaque-2* sweet corn, the kernel color came from the aleurone layer because anthocyanin produces an intense color in the aleurone layer (Ford, 2000). The F_1 hybrids had all red kernels. The genotype that controlled the kernel color of three testers would be only $pr1pr1CC$ because $pr1pr1$ had red aleurone in the kernels (Sharma et al., 2011). Therefore, if the kernel color of an F_1 hybrid is not red, then the genotype controlling kernel color must come from the other line. The current result indicated that all lines had $pr1pr1$. The $pr1$ mutants produced the red pigment, pelargonidin, due to the failure to hydroxylate the B ring of dihydrokaempferol (Forkmann, 1991) to yield the purple cyanidin pigment produced in maize lines carrying a functional *Pr1* allele (Sharma et al., 2011). The total content of anthocyanin present in the purple corn has been reported to be notably high. For example, for the whole kernel of Andean purple

corn, the content was 681 mg/100 g in the humid base; however, the highest concentration of anthocyanin reported in purple corn was 1,642 mg cyanidin-3-glucoside/100 g dry weight (Cevallos-Casals and Cisneros-Zevallos, 2003) with 290–1,333 mg/100 g of dry matter (Jing et al., 2007). The anthocyanin was characterized as cyanidin-3-glucoside, pelargonidin-3-glucoside, peonidin-3-glucoside and their respective malonate counterparts (Pascual-Teresa et al., 2002). In addition, the amounts of cyanidin-3-glucoside, pelargonidin-3-glucoside and peonidin-3-glucoside were approximately 44%, 11% and 5%, respectively (Escribano-Bailon et al., 2004). In the current study, pelargonidin-3-glucoside predominated in red corn kernels, with the amount in the range 122–523 mg/100 g, while the cyanidin-3-glucoside content was in the range 21–68 mg/100 g. These results showed that in the current study, the red *opaque-2* sweet corn pigment came from the pelargonidin-3-glucoside rather than cyanidin-3-glucoside in the purple kernels (Moreno et al., 2005). The differences could have been due to a variety of factors, including the cultivar, growing conditions and even the method used for anthocyanin quantification (Jing et al., 2007). Lago et al. (2014) reported the development of a new colored sugary line (*su1*) and the results of some analyses concerning flavonoid content before and after two different cooking treatments. Mild cooking seemed to not significantly change the metabolites analyzed in the colored kernels, while the stronger treatment seemed to substantially decrease the amounts of pigments without changing the structure of the leftover molecules. Furthermore, to preserve the healthy properties of the colored sweet corn, they suggested that it was better to consume it fresh, but that if processing is needed, it would be better to use a mild process, such as steam treatment, to benefit from the best nutritional composition. As was hoped for in the findings from field corn (Vasal, 1994; Prasanna et al., 2001; Jompuk et al., 2011) and waxy corn (Sinkangam et al., 2011), both S_6 of *opaque-2* sweet corn lines and their F_1 hybrids increased the tryptophan contents in the kernel compared to the commercial sweet corn hybrid (*non-opaque-2*). At this early stage of development, 20 d after pollination, the difference between the red *opaque-2* sweet corn genotypes was less pronounced than in the mature genotypes reported in the literature above. An accumulation of grain mutants might have decreased the vitality of the plant as a whole. But this was not the case in the current study, as the best test hybrid even out yielded the commercial single cross hybrid.

In conclusion, the nutritional improvement of the sweet corn kernel by increasing the tryptophan content in the protein and anthocyanin contents was confirmed without losing the sweetness of the product. Therefore, the red *opaque-2* sweet corn hybrid should be suitable to encourage consumers to choose this new product.

Conflict of Interest

The authors declare no conflict of interest.

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