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Growth Characters and Sugar Content During Grain Filling in New Hybrid, Chalok 1/Cooktail 51 Corn

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ABSTRACT: 'Chalok 1/Cocktail 51' corns, supersweet corn gene controlled by either brittle-1 (*bt1*) or shrunken-2 (*sh2*) gene introduced into waxy corn, showed agronomic characteristics between supersweet corn and waxy corn. The ears were harvested at different development stages from 15 to 35 days after silking (DAS). Ear diameter of Cocktail 51 and 'Cocktail 51'/Chalok 1 increased from 15 DAS to 30 DAS and little increased thereafter, but that of Chalok 1/Cocktail 51 and 'Chalok 1' increased until 35 DAS. Diameter of ear extension increased more in Cocktail 51 and Chalok 1/Cocktail 51 corn than Chalok 1. Ear fresh weight of Cocktail 51 decreased later 30 DAS but those of the other hybrids were vice versa. Rate of supersweet kernels per ear of Chalok 1/Cocktail 51 corns was about 38%. Development, and elongation of kernel were much more prominent in supersweet kernel than in waxy kernel, but fresh weight increased higher in waxy kernel than supersweet kernel. Moisture content in kernel decreased from 15 DAS to 35 DAS. Total sugar content of the kernel increased until 25 DAS, and that of Cocktail 51 kernel showed the highest among of them. After cooked by steam, flavor and mastication feeling rate cooked by steam of Cocktail 51 and Chalok 1/Cocktail 51 were increased from 15 DAS to 25 DAS and markedly decreased thereafter. But those of Chalok 1/Cocktail 51 and, Chalok 1 were decreased after 30 DAS. These results suggested that the optimum harvest date for fresh supersweet corn (Cocktail 51), Cocktail 51/Chalok 1 seems to be about 20 DAS and Chalok 1/Cocktail 51 and waxy corn (Chalok 1) was about 25 DAS.

Keywords **:** Chalok 1(waxy corn), Cocktail 51(supersweet corn), mastication rate, sugar content.

Corn (*Zea mays* L.) originated during the invention of New World agriculture more than 8,000 years ago. It is the most important and the highest yielding crop in the world, so it has been widely cultivated together with rice and wheat.

In the USA, corn emerged as a major feed crop for both domestic use and export, thus providing abundant, high-quality animal products for consumption in many countries. More recently, corn became an important raw material for producing industrial products, such as ethanol. After centuries of development, there remains an untapped potential in the corn crop.

Sweet corn, and waxy corn consumption are increasing in Korea. Sweet corn is a popular vegetable in the USA and ranks second in farm value for processing and fourth in commercial value (Boyer & Shannon, 1982). Shrunken-2 (*sh2*) hybrids constitute 40% of the sweet corn seed market in USA (Willson *et al.*, 1991). These hybrids are attractive to consumers because the recessive mutant, *sh2*, effectively doubles the sugar content of kernels at the "roasting ear" stage compared to the traditional vegetable corn which is homozygous for the recessive sugary-1 (*su1*) allele. Furthermore, conversion of sugars to starches by *sh2* gene is slowed at ambient temperatures thus reducing the need for refrigeration after harvest and extend the time of edible maturity (Ferguson *et al.*, 1979), and better tolerate shipping to distant markets. However, *sh2* seeds typically show poor germination and seedling vigor compared to standard sweet corn (Andrew, 1982). Low germination and low seedling vigor (Styer *et al.*, 1980) have limited the use of *sh2* maize in production for processing (Churchill & Andrew, 1984).

Waxy corn, so called because of the dull, waxy appearance of its grain, is good for starch thus, making separation into distinct components unnecessary. Waxy, a recessive mutant, affects the synthesis of endosperm starches like amylopectin-a branched chain starch (amylopectin). Waxy corn contains starch granules that are 100% amylopectin (branched fraction) compared to 72% for regular cultivar. Waxy corn is produced with 97% or higher waxy kernels. Ordinary corn is a mixture of branched chain and straight chain starches. Yellow waxy hybrids are preferred for starch processing in the USA, but white hybrids are preferred in Korea, Japan and the Philippines where waxy corn is harvested as green cob. A pure white waxy starch is preferred in Korea. Waxy corn seeds which has low suger and high starch content show high germination and seedling vigor compared to supersweet corn (Kim *et al.*, 1994).

Chalok 1/Cocktail 51 corn is developed to improve glutinous waxy corn with sweetness that Koreans prefer to eat. The ear of Chalok 1/Cocktail 51 corn, supersweet corn gene

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introdured controlled by either brittle-1 (*bt1*) or shrunken-2 (*sh2*) gene into waxy corn, is set waxy and supersweet kernels each other.

This study was conducted to investigate the agronomic trait, ear and kernel characteristics, and sugar content associated with changes during grain filling after silking of new Chalok 1/Cocktail 51 corn hybrids (Cocktail 51/Chalok 1, Chalok 1/Cocktail 51).

MATERIALS AND METHODS

Chemical properties of the experimental field at Kyungpook National University (KNU) are presented in Table 1.

Two corn hybrid cultivars, Cocktail 51 (supersweet corn) and Chalok 1 (waxy corn), and two double cross hybrids, Cocktail 51/Chalok 1and Chalok 1/Cocktail 51, were used in this study. The hybrid seeds were supplied from International Agriculture Research Institute in KNU. The hybrids were planted at the Agricultural Experimental Station at KNU. Planting space was 60 cm between rows and 25 cm between plants. Two seeds were planted per hill and thinned to one plant at the 7~8th leaf stage. The plots were arranged in a randomized complete block design (RCB) with three replications. The plots were fertilized at the rate of 150 kg N, 130 kg P_2O_5 and 130 kg K_2O per ha. Half of the nitrogen was applied by split application as basal dressing before planting, and the rest as top dressing at the 7~8th leaf stage. The other fertilizers were applied as basal dressing. Plant growth was measured using the standard method designed by the Rural Development Administration.

Thirty days after planting, data for emergence were collected. Percentage of emergence was determined by direct count of the emerged seedling of each hybrid in each replicate.

Individual plants were evaluated every morning for silking date. Ten days after silking, data were collected for plant height, ear height and number of tillers. The corn ears were harvested at different development stages from 15 to 35 days after silking (DAS) with 5 days interval. These were removed with husks intact and immediately brought to the laboratory. Ear characteristics such as ear diameter, length and fresh weight were measured every harvesting time. Supersweet kernel segregation ratio per ear of each hybrid was determined 40 DAS.

Each ear was cut in parts of about 3 cm in half, and kernels were removed with a sharp knife from the circumference of the cut ear, Waxy (*wx*) corn kernels were separated well form the supersweet (*sh2*) kernel. Kernels characteristics such as kernel length, width, thickness, weight and moisture content were measured in each hybrid on respective interval date. The rest of each kernel were stored in a freezer at -70°C, and these were used for sugar and starch analysis. Fructose, glucose, sucrose and starch contents were determined using Spectrophotometer (U-2001) in 340 nm absorbance. Five gram sample of kernels (*wx*:*sh2*=62 : 38) was extracted with 20 ml boiling HEPES buffer (pH 7.4) for two minutes, homogenized, then centrifuged for 10 minutes at 14,000 rpm and divided into soluble and insoluble fraction. The insoluble fraction, containing starch, was re-extracted twice with 80% methanol until the insoluble matter was free from soluble fraction. Starch was digested with amyloglucosidase (BM) and released glucose was determined enzymatically (Park & Furukawa, 1997). Standard curves were run with amylopectin.

A panel of 8~12 untrained tasters evaluated flavor and chewing texture. Five ears of each hybrid from various development stages were cut in five parts and cooked for 20 minutes in steam. Each development stage and hybrid were coded and each ear was evaluated by the individual panel members. Panelists tasted all the corn types in one session. Corn from the each development stage was cooked and evaluated in succession. Panelists were allowed to add salt, to the corn, but not to sugar. Corn taste was rated as acceptable or not acceptable (very poor or poor). If acceptable, the corn was then rated as fair, good, or excellent. Flavor and chewing texture ratings were later converted to numerical values as follow : very poor=1, poor=3, fair=5, good=7, and excellent=9. A rating of 5 was considered for minimum acceptability.

Statistical analysis was done using PC Statistical Analysis System (SAS). Differences among mean values were tested by Duncan's Multiple Range Test.

Table 1. Chemical properties of the experimental field (0-15 cm).

pH (1:5)	OM (%)	P_2O_5 (ppm)	Ex. cation (me/100 g)			EC (ms/cm)
			K^+	Ca^{++}	Mg^{++}	
6.7	1.23	62.0	0.26	7.87	2.36	0.57

RESULTS

Agronomic characteristics of each hybrid are given in Table 2. Field emergence rate, ranged from 68.8% (Cocktail 51) to 87.5% (Chalok 1) and plant height from 115.5 cm to 168.0 cm. Number of days to silking after planting of Chalok 1 was earliest among hybrids ranged from 60.0 to 61.9 days. Cocktail 51 was significantly shorter than Chalok 1 in plant and ear height, while those of the waxy/supersweet corn hybrids, Cocktail 51/Chalok 1 and Chalok 1/Cocktail 51, ranged from 135.8 cm to 150.3 cm and from 40.8 cm to 60.8 cm respectively. The number of tillers per plant exhibited similarities in all hybrids. Among the agronomic traits

Table 2. Agronomic characteristics of four maize hybrids.

Hybrids	Emergence (%)	Days to silking	Plant height (cm)	Ear height (cm)	No. of tillers per plant
Cocktail 51	68.8 b†	61.9 a	115.5 c	34.8 b	1.3 ab
Cocktail 51/Chalok 1	77.1 ab	61.5 a	150.3 ab	60.8 a	1.6 a
Chalok 1/Cocktail 51	77.1 ab	61.9 a	135.8 bc	40.8 b	1.4 ab
Chalok 1	87.5 a	60.0 b	168.0 a	70.3 a	1.2 b

†Mean followed by the same letters within a column are not significantly different at the 5% level by Duncan's Multiple Range Test.

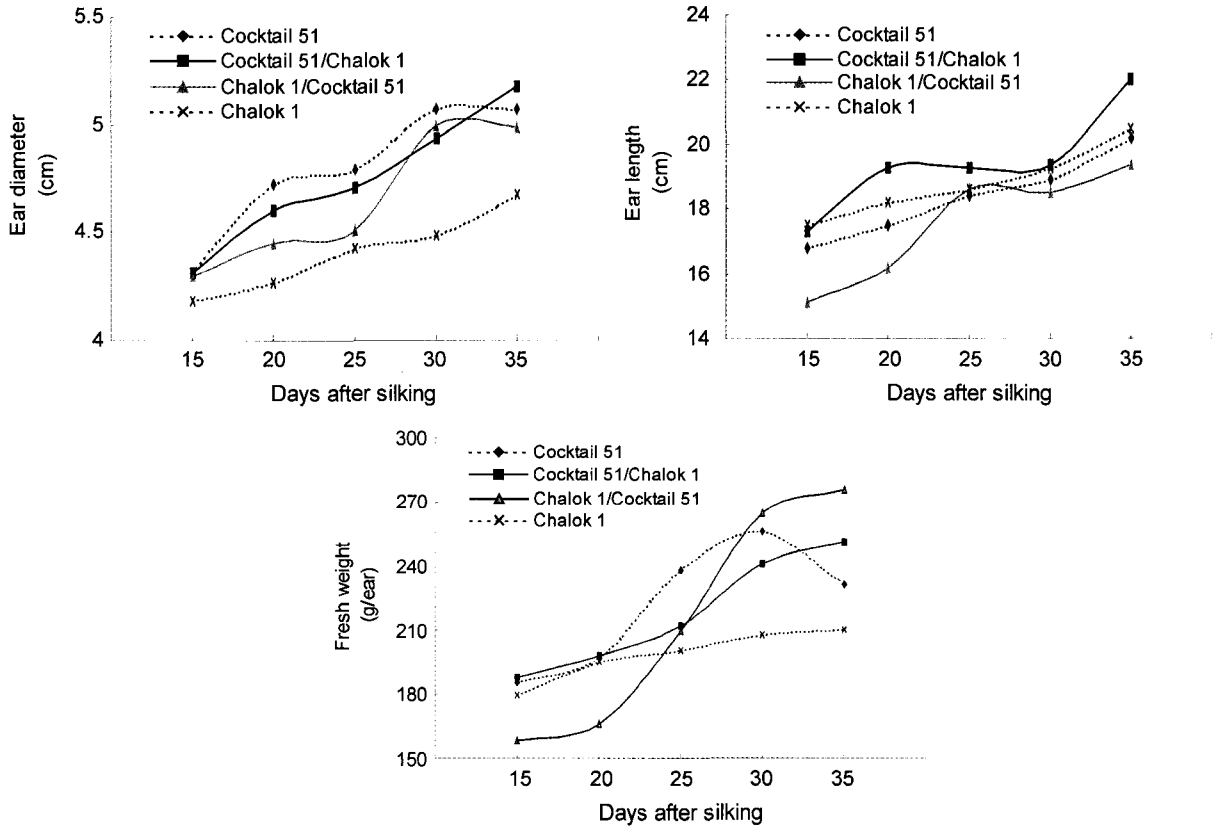

Fig. 1. Changes in ear diameter, length and fresh weight of four maize hybrids during 35 days after silking.

of the two reciprocal crosses (Cocktail 51/Chalok 1 and Chalok 1/Cocktail 51), only ear height was significantly different. Agronomic characteristics of Chalok 1/Cocktail 51 corn differed from supersweet corn (Cocktail 51) and waxy corn (Chalok 1).

Changes in ear characteristics of each hybrid are shown in Fig. 1. Ear diameter of Cocktail 51 and Chalok 1/Cocktail 51 increased from 15 to 30 DAS, and little increased thereafter, while those of Chalok 1/ Cocktail 51 and Chalok 1 increased until 35 DAS. Extension of ear diameter increased more in Cocktail 51 and Chalok 1/Cocktail 51 corn compared with Chalok 1 corn.

Ear length of each hybrid increased until 35 DAS, while that of Cocktail 51/Chalok 1 increased more than the others. Ear fresh weight of Cocktail 51 increased until 30 DAS, while it was vice versa for others. This indicates that supersweet corn must be harvested before 30 DAS. The change in fresh weight of Chalok 1/Cocktail 51 was higher than those of the other hybrids.

Changes in ear shape of four maize hybrids during 35 DAS are shown in Fig. 2. The ear of Chalok 1/Cocktail 51 corn was randomly set waxy and supersweet kernels each other. Supersweet kernel rate per ear of Chalok 1/Cocktail 51 corn hybrids ranged from 37.6% to 38.6% (Table 3). Two Chalok 1/Cocktail 51 corn hybrids were similar in the number of *sh2*, *wx*, and total kernels per ear.

Fig. 3 shows the kernel development and elongation of each hybrid during maturity. Chalok 1/Cocktail 51 corns were

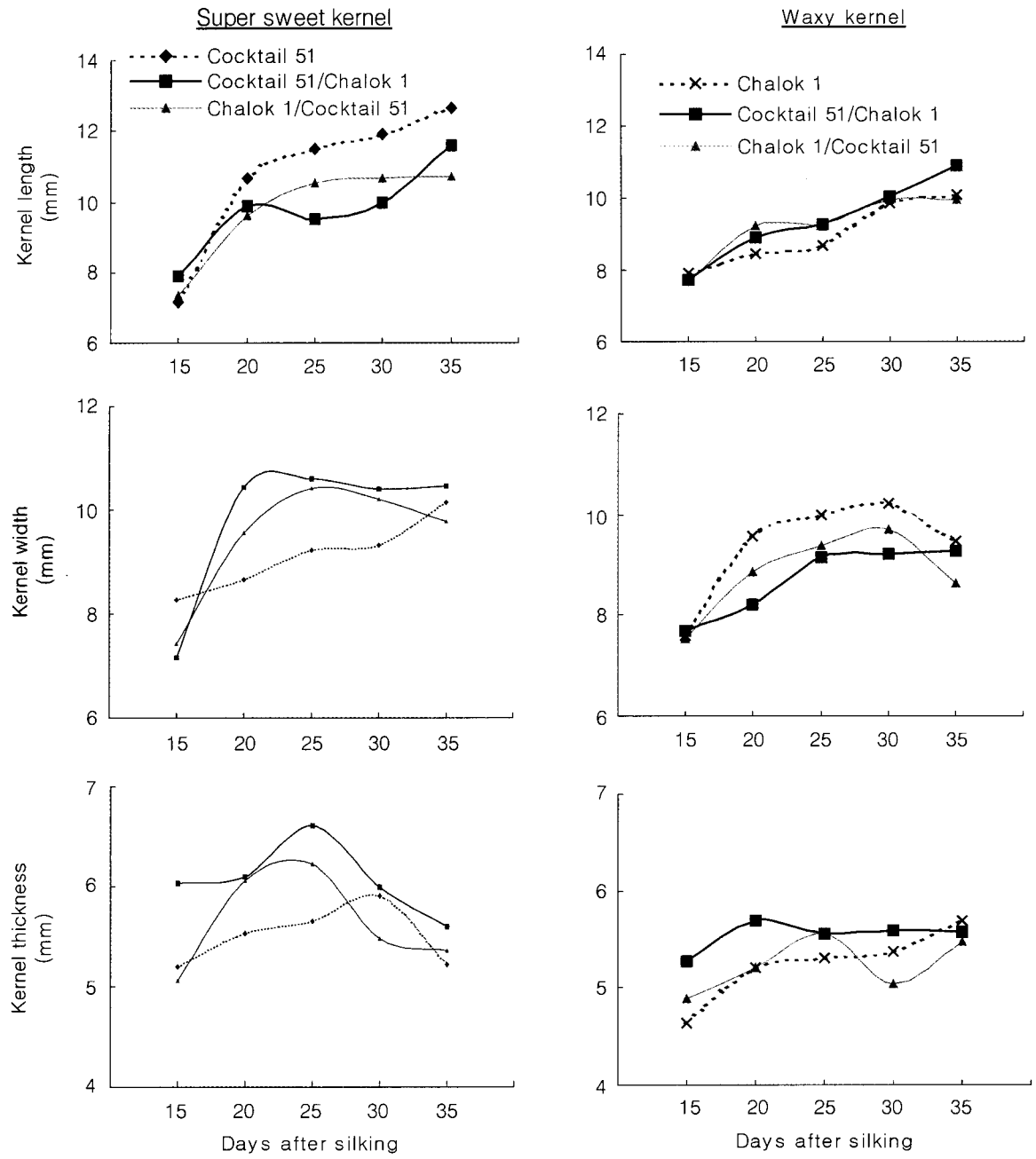

Fig. 2. Changes in kernel length, width and thickness of four maize hybrids during 35 days after silking.

separated to waxy (*wx*) and supersweet (*sh2*) kernels for kernel characteristics investigation. The change rate in kernel length of Cocktail 51 during maturity was larger than the kernel of hybrid. Also the kernel width of Cocktail 51 to maturity increased until 35 DAS. The *sh2* kernels width in Cocktail 51/Chalok 1 and Chalok 1/Cocktail 51 increased from 15 to 25 DAS and decreased thereafter. On the other hand, *wx* kernels in waxy/super-sweet corn showed a maximum width at 30 DAS. Changes in thickness of supersweet kernels during maturity were larger than those of waxy kernels. Change rate in kernel length of Cocktail 51 during maturity was larger than that of each kernel in the others. Kernel width of Cocktail 51 according to maturity increased until 35 DAS. However, *sh2* kernels in Cocktail 51/Chalok 1 and Chalok 1/Cocktail 51 increased from 15 to 25 DAS and decreased a little thereafter. On the other hand, *wx* kernels in Chalok 1/Cocktail 51 corns showed a maximum width at 30 DAS. Changes in thickness of supersweet ker-

Table 3. No. of *sh2* and normal *wx* kernels in two reciprocal crosses (Cocktail 51/Chalok 1 and Chalok 1/ Cocktail 51).

Hybrids	No. of *sh2* kernels per ear	No. of *wx* normal kernels per ear	No. of total kernels per ear	Rate of *sh2* kernels per ear (%)
Cocktail51/Chalok 1	168.3 ± 13.1†	282.3 ± 44.4	450.7 ± 45.0	37.6 ± 4.4
Chalok 1/Cocktail 51	180.7 ± 6.7	287.3 ± 21.6	468.0 ± 23.6	38.6 ± 1.9

†Mean ± SD

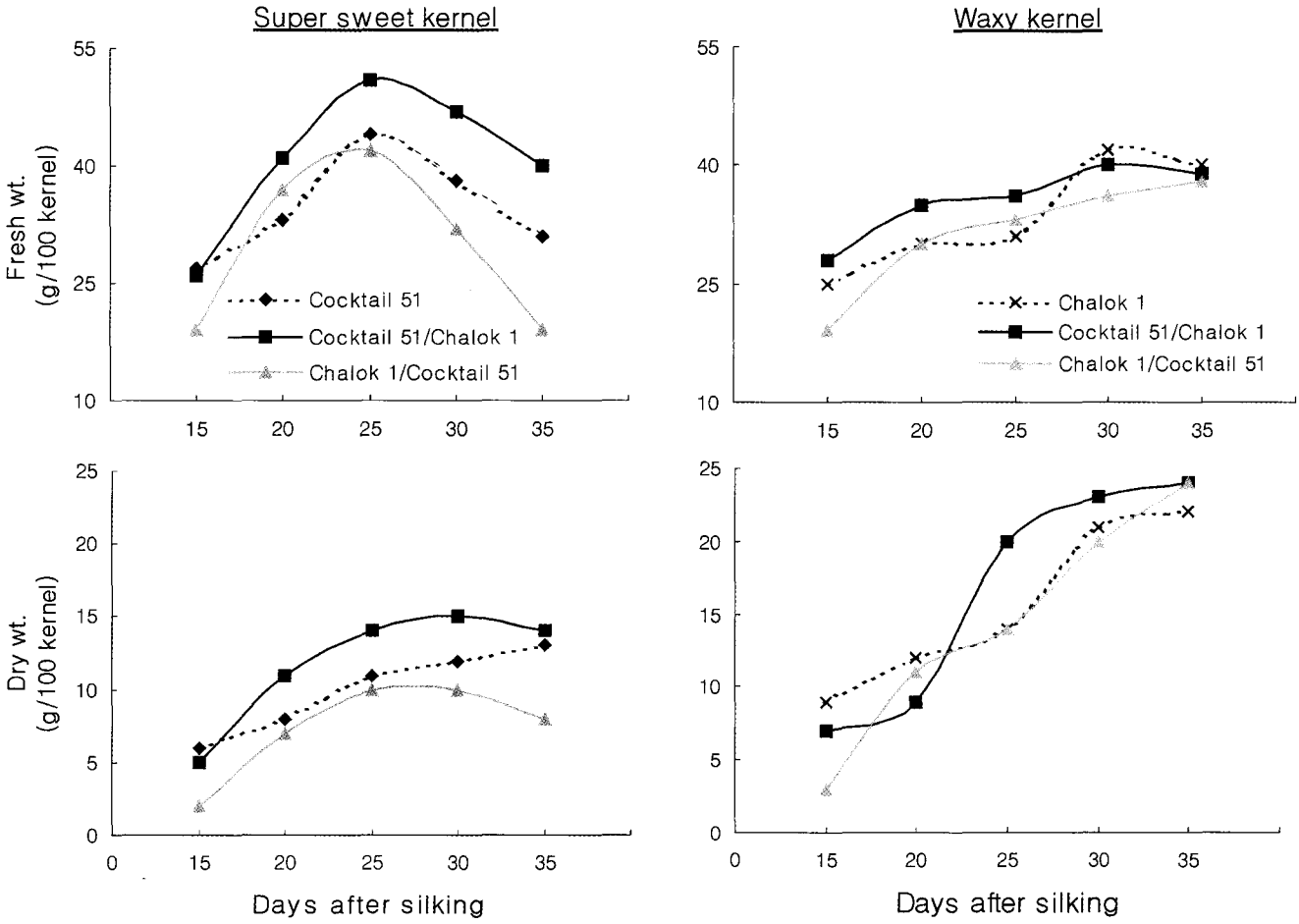

Fig. 3. Changes in kernel fresh and dry weight of four maize hybrids during 35 days after silking.

nels during maturity were larger than those of waxy kernels.

Changes in kernel shape of four maize hybrids during 35 DAS are shown Fig. 4. The ears of Chalok 1/Cocktail 51 hybrids were separated to supersweet *(sh2)* and waxy *(wx)* kernels.

The kernel development and elongation during maturity were more prominent in supersweet kernels than in waxy kernels.

The fresh weight of *sh2* kernels of each hybrid notably increased from 15 to 25 DAS, and markedly decreased thereafter (Fig. 5), while those of the *wx* kernels continuously increased until 35 DAS. The dry weight of kernels of the *wx* genotype according to maturity was larger than that of the *sh2* genotype.

Changes in each endosperm type of kernel moisture at intervals of harvest dates are shown in Fig. 4. The moisture content per hybrid markedly decreased from 35 to 15 DAS, while that of *wx* kernels decreased compared with *sh2* kernels.

Sugar content (sucrose, fructose and glucose) in each hybrid was significantly affected in each development stage (Fig. 5). Sucrose content showed the highest value at 25 DAS in the kernel in all hybrids, and decreased thereafter. Also, sucrose content of *sh2* kernels was higher than that of *wx* kernels.

Starch accumulation of *sh2* kernels was nearly completed

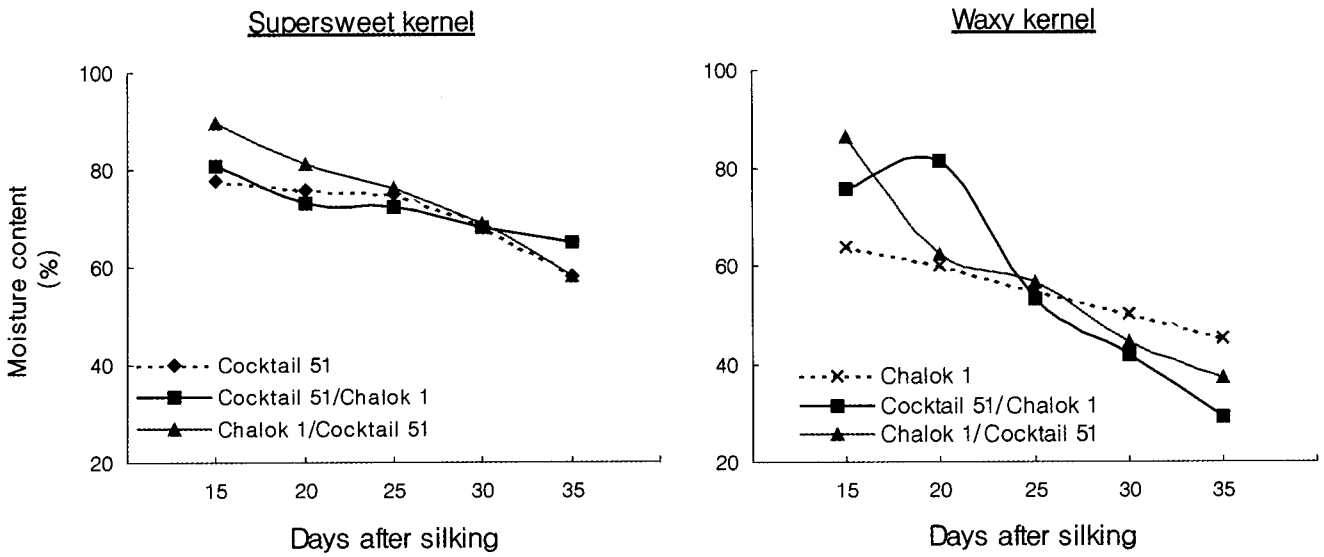

Fig. 4. Changes in kernel moisture content of four maize hybrids during 35 days after silking.

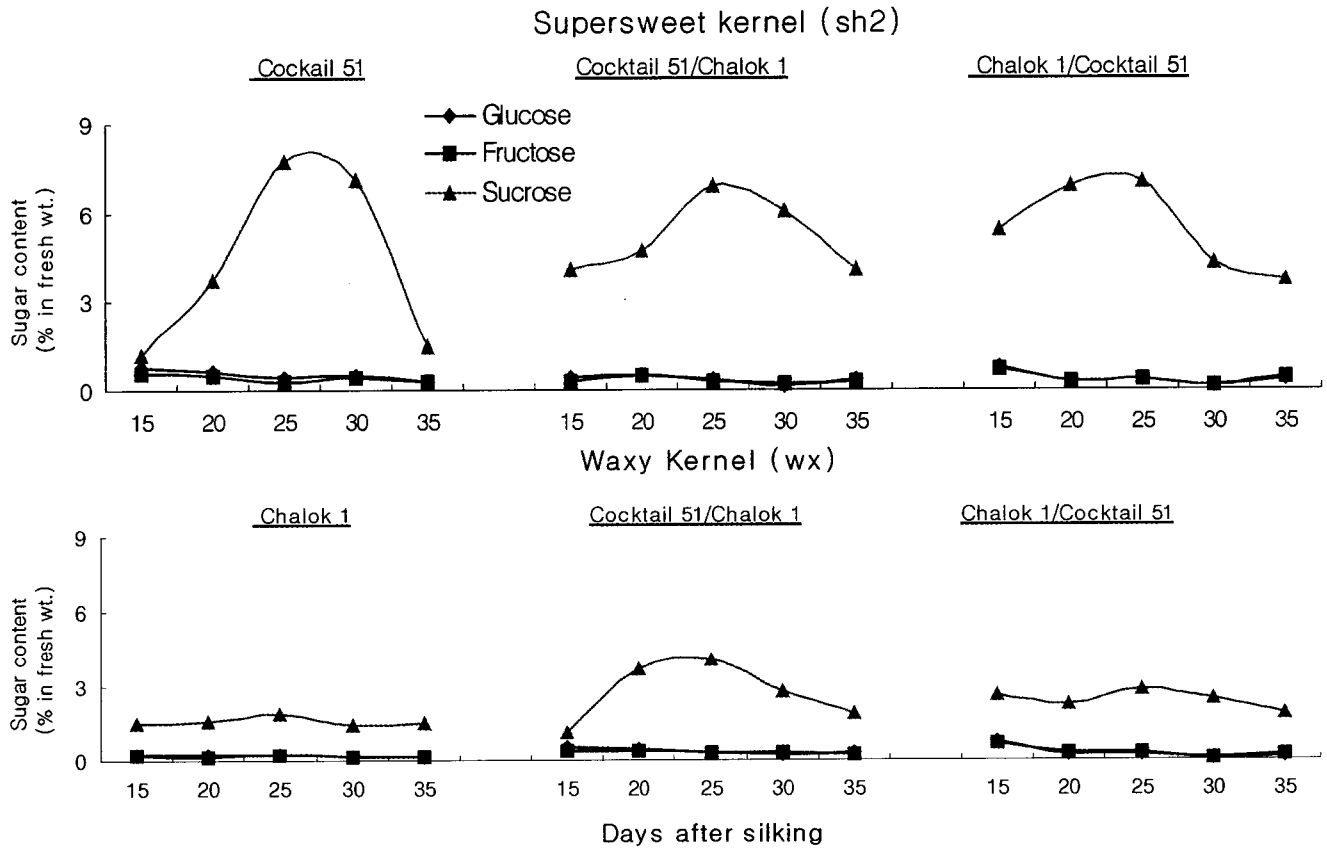

Fig. 5. Changes in sugar content of four maize hybrids during 35 days after silking.

to 25 DAS (Fig. 6), but that of *wx* kernels continued to 35 DAS. Starch content of *wx* kernels at 35 DAS was five times as high as that of *sh2* kernels.

Flavor and chewing feel rate of kernels (Cocktail 51 and Chalok 1/Cocktail 51) cooked by steam were favored during 20 to 25 DAS, and those of Cocktail 51 significantly dropped at 30 DAS (Table 4). Flavor and chewing feel rates of Chalok 1/Cocktail 51 and Chalok 1 were favored at 25 to 30 DAS.

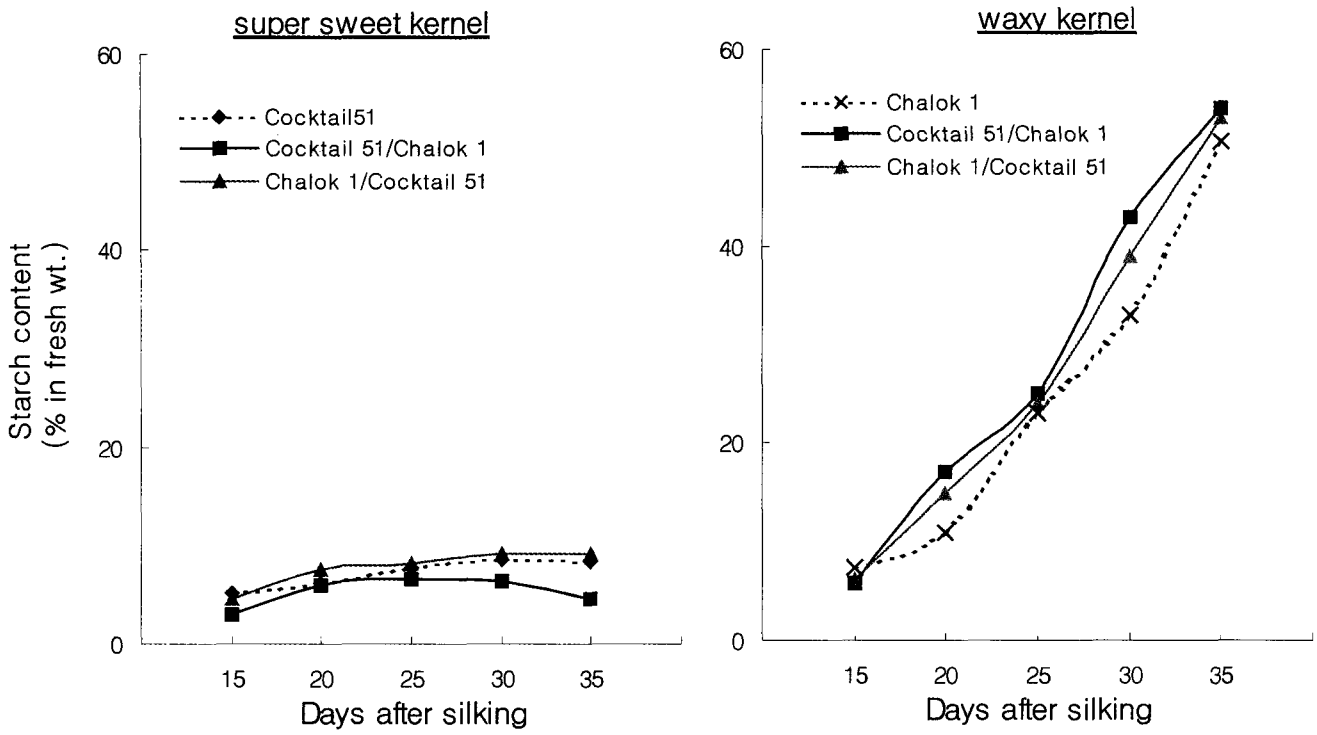

Fig. 6. Changes in starch content of four maize hybrids during 35 days after silking.

Table 4. Palatability test rate during 35 days after silking of four maize hybrids.

Hybrids	Days after silking									
	15		20		25		30		35	
	Flavor	Chewing feel	Flavor	Chewing feel	Flavor	Chewing feel	Flavor	Chewing feel	Flavor	Chewing feel
Cocktail 51	6.6†	5.4	7.6	7.6	7.7	7.4	6.0	5.9	2.3	2.7
Cocktail 51/Chalok 1	5.0	4.7	6.5	5.9	7.0	6.2	3.5	2.4	2.4	1.9
Chalok 1/Cocktail 51	5.7	5.4	6.6	5.9	6.1	6.1	6.1	5.7	3.4	2.8
Chalok 1	5.4	5.1	5.6	5.4	6.6	7.1	6.9	5.7	4.1	3.9

†Rating score (19) : 1=very poor 3=poor 5=fair 7=good 9=excellent

DISCUSSION

Results of this study showed that the emergence percentage differed for each hybrid. As expected, supersweet corn hybrid exhibited lower emergence percentage than other hybrids. Accumulation prediction for field emergence is difficult. However, this is essential if the seed industry is to produces high-quality maize seeds and sells to growers (Martin *et al.*, 1988). Low emergence percentage is associated with *sh2* gene. Hybrids with *sh2* is characterized with high seed borne pathogene infection(Anderegg & Guthrie, 1981; Berger & Wolf, 1974), small endosperm(Wann, 1980), and cracks in the pericarp (Wilson & Trawatha, 1991). The waxy corn hybrid exhibited a high emergence percentage of 87.5% and the accumulation of starch continued during maturity. Chalok 1/Cocktail 51 corn hybrids showed 77.5% emergence percentage. The development of Chalok 1/Cocktail 51 hybrid not only replaced the seeds of the introduced supersweet corn (Cocktail 51) but also solved the main problem of low emergence of supersweet hybrid corn.

Days to silking of all hybrids ranged from 60 to 62 days. The four hybrids used in this study showed early maturity, and can be harvested from 80 DAS. Lodging tolerance of Cocktail 51 and Chalok 1/Cocktail 51 was stronger than those of the others. Variation of ear characteristics of Cocktail 51/Chalok 1 during 15 to 35 DAS was larger than those of other hybrids, while those of Chalok 1 slightly increased from maturity (Kim *et al.*, 1994). Ear characteristics influ-

ence commercial value of fresh corn. Average supersweet kernels of Chalok 1/Cocktail 51 corn hybrids showed 38%, which is 13% higher than the average of a simple recessive gene expected. The differences between observed (62:38) and expected ratio (75:25) might be due to each of isolation of each testing entry. The hybrids have good quality, high sugar and starch content which Koreans perfer eat waxy corns. The kernel development and elongation during maturity differed in supersweet and waxy kernels (Kim *et al.*, 1994). Changes in fresh and dry weight of supersweet kernels confirm earlier work (Garwood & Cheech, 1972). Also fresh weight of *sh2* kernel markedly decreased after 25 DAS, which explains why the commercial value of supersweet corn decreased after 25 DAS. On the other hand, that of the waxy kernels according to grain filling slightly increased until 35 DAS. Dry weight of *sh2* kernels slightly deceased while that of *wx* kernels prominently increased until 35 DAS. Changes in the fresh and dry weights during maturity of Cocktail 51/Chalok 1, with supersweet corn as seed parent, were more prominent than those of Chalok 1/ Cocktail 51 waxy corn as seed parent. The kernel moisture content of *sh2* and *wx* continuously decreased from 15 DAS to 35 DAS.

Although much is known about the enzymes and pathway involved in the conversion of sugars to starch (Bear *et al.*, 1958; Goodwin & Mercer, 1985; Nelson, 1980; Pridham, 1974; Shannon, 1978; Vineyard & Bear, 1952), the application of this knowledge in producing the modified starch hybrids (waxy and amylose-extender) was limited. The Chalok 1/Cocktail 51 hybrid developed by Kim's team at KNU is the first attraction of the glutinous supersweet corn for marketing to the world. Without isolation plots of each combination and pedigree in the experiment observed ratio of 62% for normal/waxy and 38% for supersweet grains might be influenced. However our target breeding for wx/sh double mutant is to achieve 75% of pure waxy and 25% of supersweet. We can achieve this expected ratio. When sh/sh parent has waxy background and wx/wx parent has shrunken background, respedtively.

Some associations between mutant endosperm types that differ in starch composition and related enzyme activity have been observed (Whistler *et al.*, 1984). The major pathway of amylose synthesis is by the transfer of glucose from adenosine diphosphate glucose (ADPG) to the nonreducing end of a preexisting primer molecule (short-chain glucose polymer with -1-4 bonds). With appropriate enzymes, uridine diphosphate glucose (UDPG) and glucose-1-phosphate can also act as glucose donors for amylose synthesis. Amylose is considered to be the precursor of amylopectin. Branching enzymes (transferase) cleave (breaks an α-1,4 glucosidic bond) segments from an amylose molecule making a branch chain. For amylose synthesis, four ADPG-type enzymes have been identified. Two are bound to the starch granule and another two are soluble. For amylopectin synthesis, three branching enzymes have been identified (Coine & Poehlman, 1983). Sugar content of *sh2* kernels was higher than that of wx kernels, but starch content of *wx* kernels was about five times as high as that of *sh2* kernels (Michaels & Andrew, 1986). Although this study did not measure the total sugar content in dry matter of *sh2* kernel, approximate content would be 30 to 40%, while estimated at 8 to 10%. It is only in fresh matter.

Flavor and chewing feel rate in the cooked by steamed Cocktail 51, Chalok 1/Cocktail 51 were increased from 15 to 25 DAS and markedly decreased thereafter. Sugar and moisture content of super sweet kernels prominently decreased after 25 DAS. However flavor and chewing feel of Chalok 1/Cocktail 51 and Chalok 1 were decreased after 35 DAS. This is positively correlated with sugar, starch and moisture content (Wann *et al.*, 1971; Culpepper and Magoon, 1924), because waxy kernels synthesized amylopectin during maturity, as shown by low sugar and high starch (amylopectin) content, but not softness and sweetness after 30 DAS.

These results suggest that the optimum harvest date, and the considered duration of storage and marketing for fresh supersweet corn (Cocktail 51), Cocktail 51/Chalok 1 is about 20 to 25 DAS. However, for Chalok 1/Cocktail 51 and waxy corn (Chalok 1), it is about 25 to 30 DAS.

By tradition, Koreans prefer to eat waxy corn. However, the new Chalok 1/Cocktail 51 corn will add sweetness and flavor characteristics to the waxy corn. The results of a new corn of this study support the importance and need for the development.

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