KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF AGRICULTURE AND NATURAL RESOURCES

FACULTY OF AGRICULTURE

DEPARTMENT OF CROP AND SOIL SCIENCES

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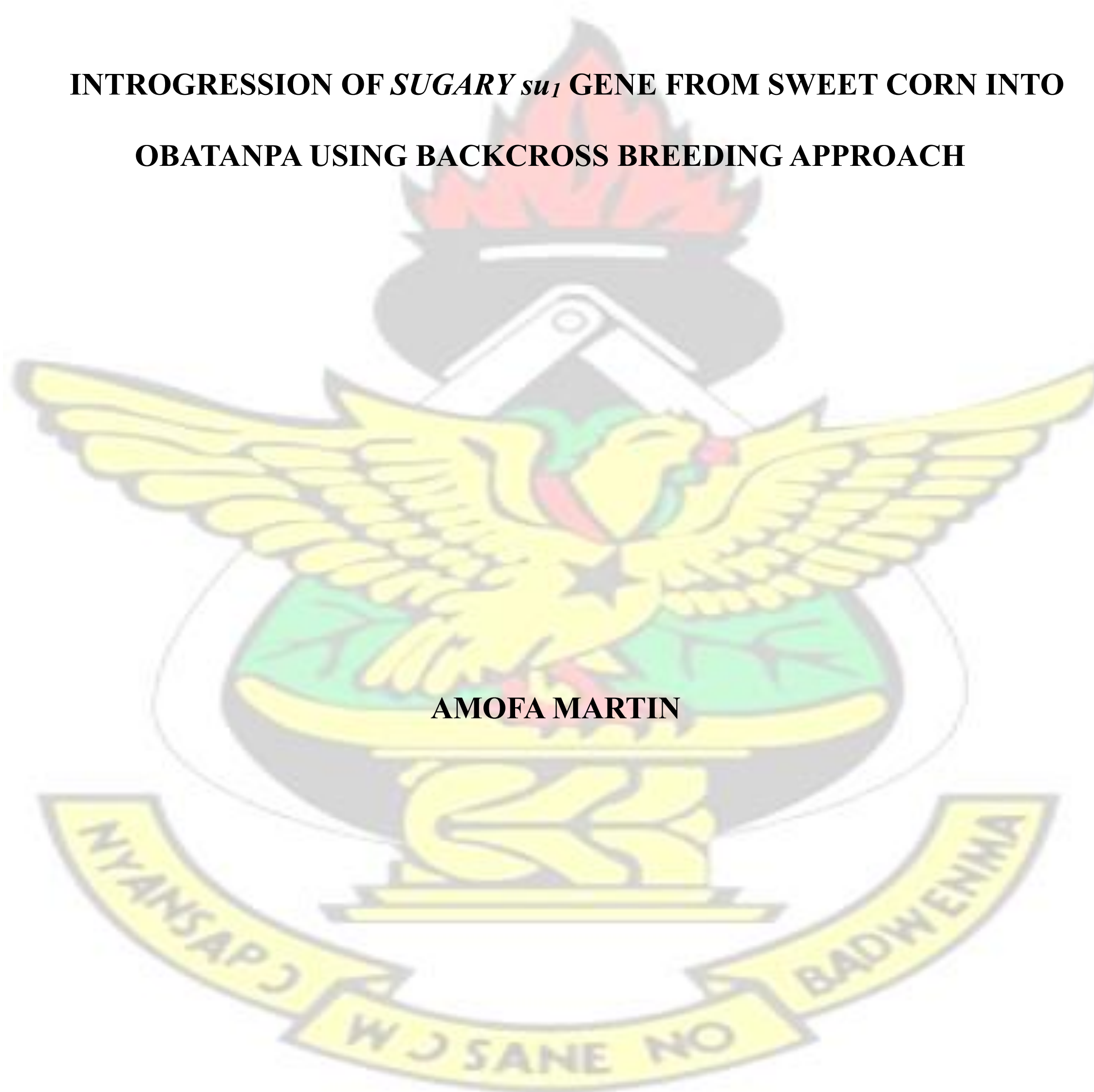
INTROGRESSION OF *SUGARY su*$_1$ GENE FROM SWEET CORN INTO OBATANPA USING BACKCROSS BREEDING APPROACH



AMOFA MARTIN

AUGUST, 2015

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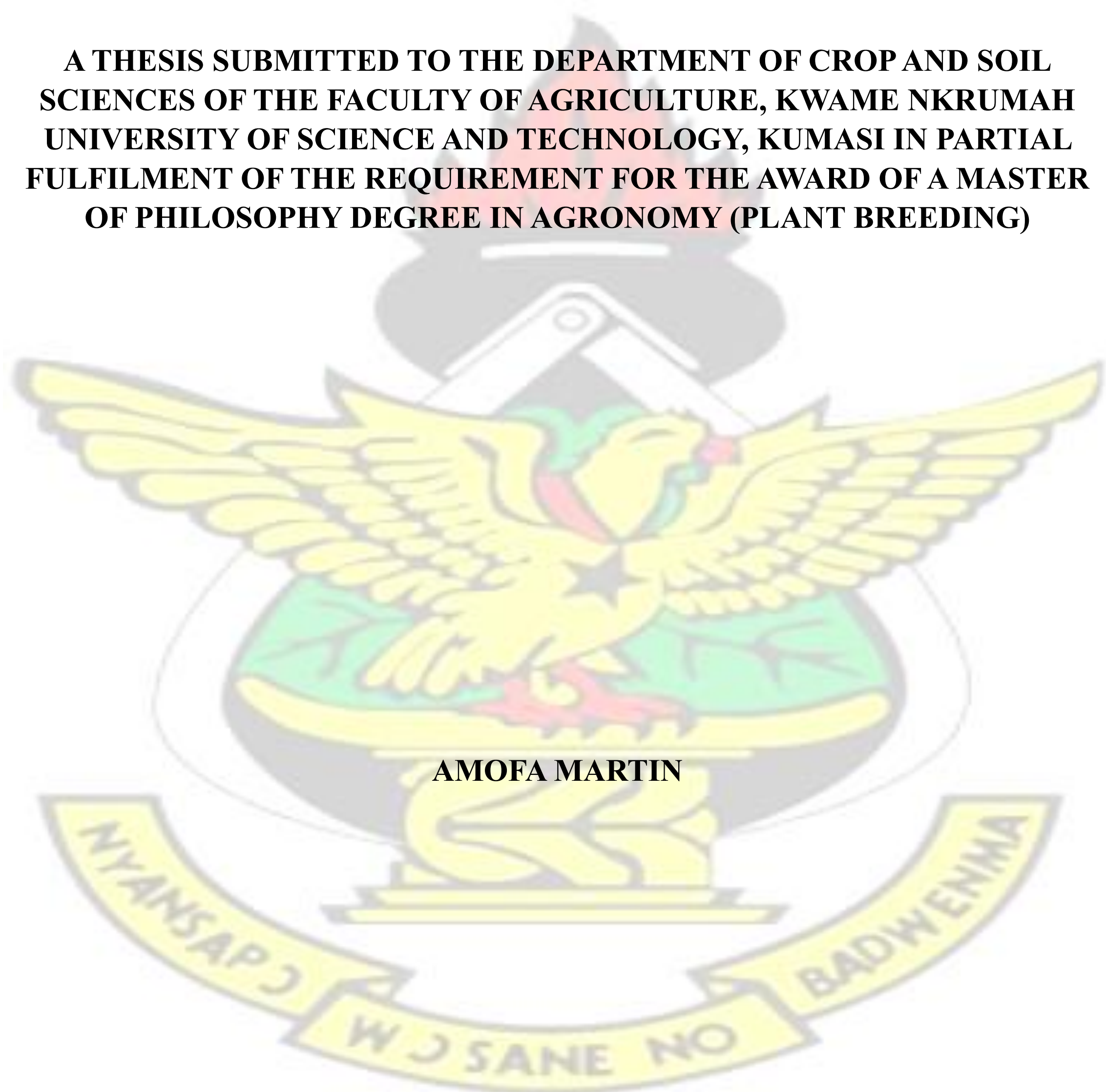

A THESIS SUBMITTED TO THE DEPARTMENT OF CROP AND SOIL SCIENCES OF THE FACULTY OF AGRICULTURE, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF A MASTER OF PHILOSOPHY DEGREE IN AGRONOMY (PLANT BREEDING)

AMOFA MARTIN

AUGUST, 2015

DECLARATION

I hereby declare that except for the references cited in relation to other works and duly acknowledged, this work is the result of my own original research and that this thesis has neither in whole nor part been presented anywhere for a degree.

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This work would not have been completed without the spiritual, physical and moral support from my sweet wife Mrs. Agatha Prempeh Amofa and child, Lawrence Kofi Amofa. I love them.

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ABSTRACT

Biofortification of maize which is the major cereal consumed in Ghana will help alleviate malnutrition primarily among children. The study was conducted to further biofortify QPM by introgression of sugary gene. It was conducted at the Finatrade field at Faculty of Agriculture, KNUST. The parental materials used were Obatanpa (source of *opaque-2* gene) as the first parent and Stowell's evergreen (source of *sugary* gene) as second parent. The F_1 produced was selfed to obtain F_2 to obtain segregation ratios. The F_1 was again backcrossed to both parents to obtain BC_1P_1 and BC_1P_2. Opaque seeds were selected and selfed to obtain BC_1S_1. Cobs of BC_1S_1 that segregated into 3:1 of non-shrunken opaque to shrunken opaque were again selected. The shrunken opaque (new) seeds were then evaluated with the two parents for both vegetative and output traits. Output traits of sucrose content, starch yield and protein content were analysed biochemically. From the study, the *sugary* gene was found to be inherited

monogenically and all the vegetative traits of percentage emergence, ear leaf width, ear height, plant height and stem girth were highly heritable. Mid parent heterosis was positive for all vegetative traits measured. Among all the three seed type, it was only the new seed that obtained positive estimate for both mid and high parent heterosis for sucrose content which suggest some synergistic influence of *opaque-2* and *sugary* genes. Harvesting should be done at 20 and 25DAP for sweet corn; 25, 30DAP for obatanpa and 25, 30 and 35DAP for new seed in similar studies. Also the best times for harvesting when sucrose content is of priority should be 20, 25 and 30DAP for sweet corn, obatanpa and new seed respectively. Sugary gene is thus fixed into the genetic background of obatanpa as the highest sucrose content obtained in sweet corn was insignificantly different from that obtained in new seed whilst maintaining their quality of protein.

DEDICATION

THIS WORK IS DEDICATED TO GOD AND MY DEAR WIFE AND BABY BOY

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NYANSAPƆ WƆSANE NO BADWENMA

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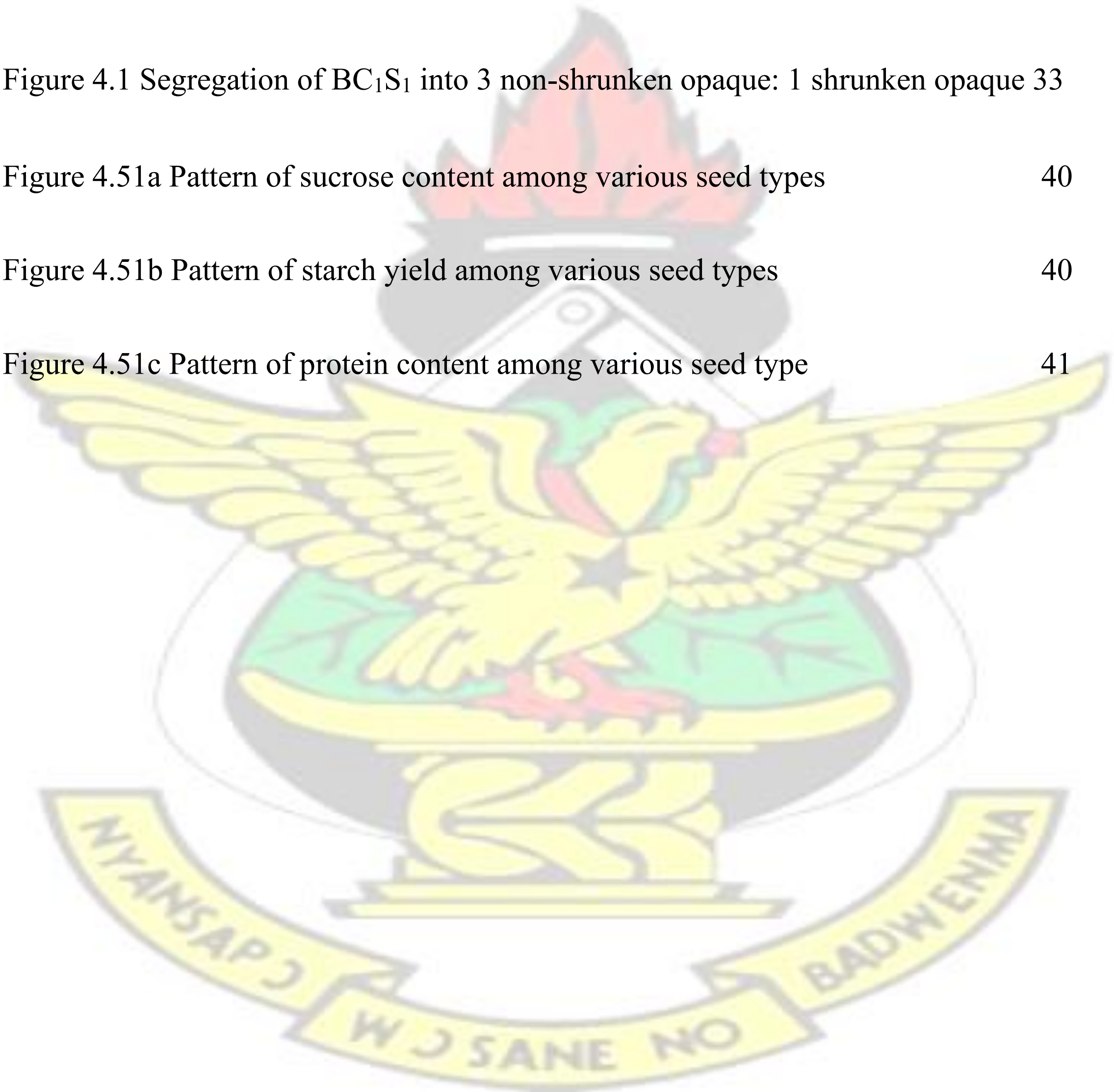

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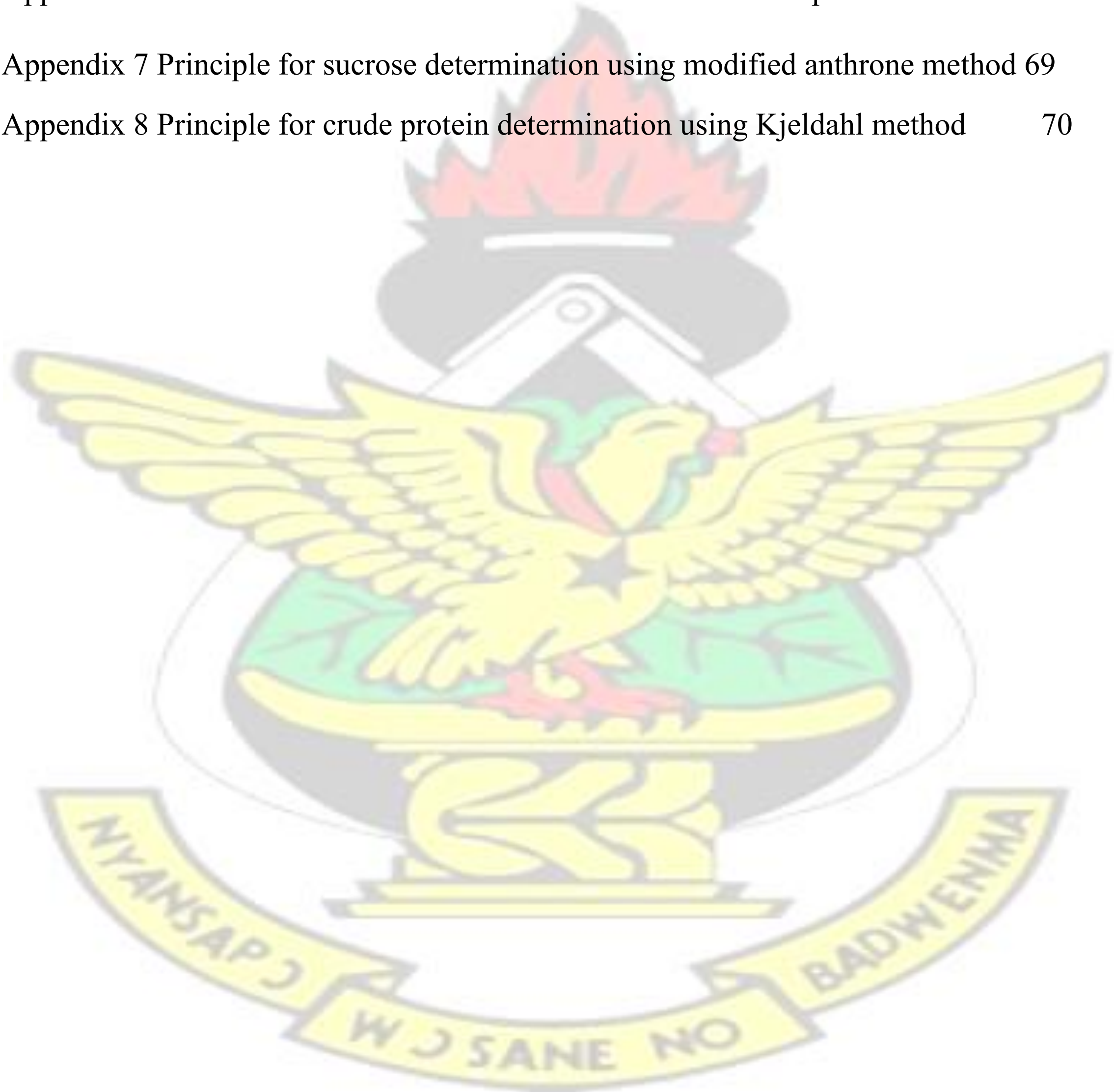

LIST OF ABBREVIATIONS

Abbreviation	Meaning
V_A	Variance of additivity
V_D	Variance of dominance
V_E	Variance of the environment
V_P	Phenotypic variance
V_G	Genotypic variance
V_{P1}	Variance of first parent (Obatanpa)
V_{P2}	Variance of second parent (Stowell's evergreen)
V_{F1}	Variance of first filial generation
V_{F2}	Variance of second filial generation
V_{B1}	Variance of first backcross to the first parent
V_{B2}	Variance of first backcross to the second parent
BC_1P_1	First backcross to the first parent
BC_1P_2	First backcross to the second parent
BC_1S_1	Selfed first backcross
MP	Mid parent
HP	High parent
LSD	Least significant difference
HCl	Hydrochloric acid
DAP	Days after pollination
SO	Shrunken opaque
NO	Non-shrunken opaque
SN	Shrunken non-opaque
NN	Non-shrunken non- opaque

ELW	Ear leaf width
SG	Stem girth
PH	Plant height
EH	Ear height
ANOVA	Analysis of variance
MS	Means squares
StSw	Starch content in sweet corn
St Ob	Starch content in Obatanpa
St Ns	Starch content in new seed
PcSw	Protein content in sweet corn
PcOb	Protein content in Obatanpa
PcNs	Protein content in new seed
ScSw	Sucrose content in sweet corn
ScOb	Sucrose content in Obatanpa
ScNs	Sucrose content in new seed
H^2bs%	Percentage heritability
FAO	Food and Agriculture Organization
CRI	Crop Research Institute
CSIR	Council for Scientific and Industrial Research
CIMMYT	International Maize and Wheat Improvement Centre
et al.	And others
PEM	Protein energy malnutrition

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CHAPTER ONE

1.0 INTRODUCTION

Maize (*Zea mays* L.), the American Indian word for corn, literally means "that which sustains life". It is, after wheat and rice, the most important cereal grain in the world, providing nutrients for humans and animals and serving as a basic raw material for the production of starch, oil and protein, alcoholic beverages, food sweeteners and more recently, fuel (FAO, 1992). Maize is the most important cereal crop produced in Ghana and it is also the most widely consumed staple food in Ghana with increasing production since 1965 (FAO, 2008). In Ghana, it is the most important cereal in terms of production and consumption (Breisinger *et al*., 2008).

There are several varieties of corn which can be categorized into four basic groups: field corn, sweet corn, popcorn and ornamental corn. For all the corn groups, kernel texture, shape and flavour are often governed by the starch and sugar content and this differs with each variety.

Although maize is mainly considered a carbohydrate source, it is also an important source of protein because of its considerable total protein yield per hectare (Bjarnson and Vasal, 2010). However, from nutritional perspective, protein of maize and that of other cereals is deficient in essential amino acids, particularly lysine and tryptophan that are essential for monogastric animals and humans (Alan *et al*., 2007). This has led to the introgression of a mutant *opaque-2 (o_2)* gene into normal maize lines to confer high lysine and tryptophan content (Bressani, 1992).

Obatanpa, quality protein maize (QPM), is a field corn which is well adapted to the tropical environment and has resulted from the mutation of *opaque-2* gene.

Sweet corn is as a result of a naturally occurring recessive mutation in the genes that control the conversion of sugar to starch inside the endosperm of the corn.

The endosperm properties called ‘sugary’ and ‘starchy’ are primarily conditioned by the sugary *su_1* gene and its normal allele *Su_1* (Correns, 1901; East and Hayes, 1911). Currently, breeding efforts in the West and Central Africa sub region have focused on improvement in maize-grain quality characteristics of organoleptic and nutritional properties. This is premised on the belief that such varieties is likely to increase utilization of maize grains for variety of dishes, improve the nutritional status of resource-limited rural communities and increase the land area cultivated to maize in the sub region. Genetic improvement of sweet corn for grain yield has been limited primarily because of its narrow genetic base, the lack of defined heterotic group and the greater effort devoted to improving yield in field corn (Tracy, 1990). Consequently, the focus in field corn and sweet corn crosses is the exploitation of hybrid vigour in the improvement of sweet corn varieties for grain yield, adaptation and genetic diversity (Tracy, 1990; Cartea *et al*., 1996).

Transfer of mutant genes (*su_1, sh_2, bt_2 and wx*) from sweet corn into field corn on the other hand, is for the improvement in nutritional contents (protein, lysine, soluble sugar, sucrose, reducing sugar, albumin, globulin and glutelin) of the grains, which has also been accompanied by a corresponding reduction in starch and zein either in the varieties per se (Li and Liu, 1994) or in the resultant F_1 hybrids (Shao and Shao, 1994).

1.1 Problem statement

Obatanpa, quality protein maize (QPM), is a highly nutritious variety of field maize. It provides the nutrients (protein) required for growth and development. QPM helps in the management of Kwashiorkor mostly in children and has a quality equivalent to 90% that of milk (Rolfes *et al*., 2009).

Furthermore, quality protein maize is limited in use specifically due to the low content of sugar (0.64g/100g of seeds) as compared to the high sugar content in sweet corn (6.26g/100g of seeds) (NDL, 2012; Manful and Osei Yaw, 1999). This is because infants prefer sweet-tasting foods and reject bitter foods such as vegetables reflecting an evolutionary response that was historically useful because the sweet taste signalled sources of energy (calories) while bitter tastes signalled foods that might be toxic (Food Today, 2012).

Economically, the rural poor would prefer maize variety with high protein and sugary content so that extra cost would not be borne in sweetening their foods.

Quality protein meals are very beneficial to children especially during the weaning phase of their lives and thus they require porridge that is highly nutritious and acceptable in taste. However, Manful and Osei-Yaw (1999) highlighted that among the eight white endosperm varieties developed by CSIR/CRI, Ghana in 1997, Obatanpa was placed second in terms of the overall suitability for porridge preparation after GH 232888; whilst porridge is the main source of food for children.

Appleton and Jacobs (2013) also reported that quality protein maize with high sugar content will be more beneficial to children, since refined sugars have undesirable effect on children such as causing tooth decay, obesity and diabetes.

Moreover, from Olaoye *et al*. (2009), there have been several works on improvement in sweet corn for grain yield and agronomic traits through introgression of genes from the field corn. However, only a handful of studies have been done on improvement in field

corn varieties for organoleptic and/or nutritional properties through the transfer of recessive alleles that condition sweetness in sweet corn into the field corn.

It is therefore very imperative to further biofortify Quality Protein Maize (Obatanpa) by improving upon its sugar content (sweetness) using sweet corn to provide high calories, good taste, lower cost of food preparation and healthy meals for children.

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1.2 Study objectives

Main objective

The main objective of the study was therefore to introgress the sugary gene from sweet corn into Obatanpa genetic background through backcrossing and selfing.

Specific objectives

1. To determine the inheritance of the su_1 gene and heritability of ear leaf width, stem girth, plant height and ear height.
2. To determine heterosis of the hybrid produced and differences between the two parents and the hybrid.
3. To determine the relationship between time of harvest and reproductive traits and the correlation between sucrose content, starch and protein content.

1.3 Significance of study

The combination of high protein content with high sugar content in Obatanpa will tend to boost appetite and enrich Obatanpa meals. This will consequently help to reduce protein-energy malnutrition in children. It will also reduce the cost of food and feed preparation to children and animals respectively. The study will also help to identify a breeding method for improving upon sweetness in a field corn such as Obatanpa and recommendations for future studies.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Biology of Zea mays

2.1.1 General description, cultivation, use as a crop plant and hybrid production

Zea is a genus of the family Graminae (Poaceae), commonly known as the grass family. Maize (*Z. mays* L.) is a tall, monoecious annual grass with overlapping sheaths and broad conspicuously distichous blades. Plants have staminate spikelets in long spikelike racemes that form large spreading terminal panicles (tassels) and pistillate inflorescences in the leaf axils, in which the spikelets occur in 8 to 16 rows, approximately 30cm long, on a thickened, almost woody axis (cob). The whole structure (ear) is enclosed in numerous large foliaceous bracts and a mass of long styles
(silks) protrude from the tip as a mass of silky threads (Hitchcock and Chase, 1971). Pollen is produced entirely in the staminate inflorescence and ovules, entirely in the pistillate inflorescence. Maize is wind pollinated and both self and cross pollination is usually possible. Shed pollen usually remains viable for 10 to 30 minutes, but can remain viable for longer durations under favourable conditions (Coe *et al*., 1988).

Maize is cultivated worldwide and represents a staple food for a significant proportion of the world's population. No significant native toxins have been reported to be associated with the genus, Zea (International Food Biotechnology Council, 1990). Maize is planted when soil temperatures are warm (greater than or equal to 10° C) usually early to mid-May (OMAF, 1994). Optimum yields occur when the appropriate hybrid maturity and population density are chosen. In addition, exogenous sources of nitrogen fertilizer are generally applied and weed and insect control measures are generally recommended. Traditional cultivation practices in maize often result in bare soil' which is susceptible to erosion by wind or water; increasingly, "no till" maize is being grown in an effort to reduce this soil loss.

Research conducted in the early part of this century proved that hybrid maize could produce a yield superior to open-pollinated varieties (Sprague and Eberhart, 1977). The production of hybrid seed requires the development and maintenance of inbred lines and subsequent controlled crosses to produce commercial seed. Self-pollination is essential for inbred development while controlled cross pollination is mandatory for hybrid seed production. Mechanisms have been developed to ensure the correct form of pollination for each process and to prevent genetic contamination of seed stocks (Wych, 1988). In breeding nurseries, receptive ear shoots are protected from unwanted pollination by ear shoot bags that cover the silks. Pollen is contained and collected in bags that cover the tassels. Controlled hand pollinations are then made by exposing the ear shoot on the selected female parent and covering it with the bag containing pollen from the selected male parent.

Hybrid seed production is accomplished by inter-planting rows of the male and female inbred parents (e.g., one row of male to four female rows). Hybrid seed production requires isolation similar to that for foundation seed. Self-pollination of the female parent is prevented through detasseling prior to pollen shed or by the use of male sterile females. Genetic conformity of inbreds and hybrids is monitored and assured through grow-outs of representative seed lots and laboratory screening using such criteria as isozyme profiles.

2.1.2 Centres of origin of the species

It is generally agreed that teosinte (*Z. mexicana*) is an ancestor of maize, although opinions vary as to whether maize is a domesticated version of teosinte, (Galinat, 1988). Teosinte is an ancient wild grass found in Mexico and Guatemala. Because it has differentiated into various races, species and plant habits, taxonomic classification is still a matter of controversy. Doebley and Iltis (1980) classified the annual teosintes into two

subspecies of *Z. mays: ssp. mexicana* (including races Chalco, Central Plateau and Nobogame) and *ssp. parviglumis-var. parviglumis* (race Balsas) and var. huehuetenangensis (race Huehuetenango) and the species Z. luxurians (race Guatemala). The perennial teosintes from Jalisco, Mexico are separated into two more species according to ploidy, *Z. perennis* and *Z. diploperennis*.

During its domestication from teosinte, maize has gained many agronomically significant attributes but it has lost the ability to survive in the wild. It has become so domesticated that seeds cannot be separated from the cob and disseminated without human intervention. Maize seeds show poor dormancy, especially in the northern ranges of the crop's distribution. Plants occasionally grow in uncultivated fields and by roadsides or occur as volunteers in cultivated crops in the year following cultivation of a maize crop. However, maize is incapable of sustained reproduction outside of domestic cultivation and is non-invasive of natural habitats (Gould, 1968). Some Zea species are successful wild plants in Central America, but they have no pronounced weedy tendencies (Galinat, 1988).

2.1.3 Varieties of maize

Six general varieties of maize or corn are differentiated by the characteristics of the kernel. Dent corn is the leading type of corn grown on Ghanaian farms. The sides of the kernel consist of hard, so-called horny starch, and the crown contains soft starch. As the grain matures, this soft starch shrinks, forming the characteristic dent.

In flint corn, the horny starch extends over the top of the kernel, so there is no denting. Popcorn, a light, highly popular snack throughout the United States, is a variant of flint corn with small kernels of great hardness. When heated, the moisture in the kernels expands, causing the kernels to pop open.

Flour corn contains a preponderance of soft or less densely packed starch, and it is readily ground into meal.

Sweet corn is the type commonly grown in the United States for human consumption. The sugar produced by the sweet-corn plant is not converted to starch during growth, as it is in other types.

Pod corn is seldom used as food but is often grown as a decorative plant—each kernel is enclosed in its own set of diminutive husks. Another decorative corn, commonly called Indian corn, consists of multi-coloured varieties of flour and flint types (Darrah *et al*., 2003).

2.2 Quality protein maize

Quality protein maize is a maize variety that possesses significantly higher levels of lysine and tryptophan, two essential amino acids, as compared to normal maize varieties. The higher levels of lysine and tryptophan are due to the presence of the *opaque-2-gene* in a homozygous recessive state which doubles the biological value of maize (Bressani, 1992). It is a nutritionally enhanced maize variety developed by researchers from CIMMYT using two genetic systems, *opaque-2* and genetic modifiers. The use of these two genetic systems overcame the highly complex problems that were inherent in the original soft endosperm opaque.

The germ and endosperm of a maize kernel constitute the two most important parts of a maize kernel. They vary in size and their relative contributions to the quantity and quality of protein. Depending on the type of maize, the germ and the endosperm may constitute 8-10% and 80-85% of the kernels' weights, respectively, while the pericarp and the aleurone layer constitute the rest of the kernel weight. The maize endosperm protein consists of four fractions (Osborne, 1897): the water soluble albumins (3%), salt soluble globulins (3%), alcohol soluble zein or prolamine (60%), and alkali soluble glutelin

(34%). In contrast, the germ protein is predominantly in the form of albumin (+60%) while containing a relatively small alcohol soluble fraction (Schnieder, 1955, Tsai 1979, Wall and Paulis, 1978). These protein fractions vary in their lysine content. In general, albumins, globulins, and glutelins are quite rich in their lysine content (> 2g/100g) compared to the very low levels in the zein or prolamine fraction (0.01%). This fraction is therefore nutritionally deficient and cannot support the growth of rats (Osborne and Mendel, 1914).

The poor nutritional quality in the endosperm protein results from the high proportions of prolamine (zein) fractions (Table 2.1) which is practically devoid of lysine. Lysine in maize is considered to be the first limiting amino acid and tryptophan second (Vasal, 2000). The unfavourable amino acid composition especially of lysine and tryptophan reduces the protein value of ordinary maize for monogastric animals and humans as they cannot synthesize these amino acids.

Table 2.1: Protein fractions in the endosperm of normal and *opaque-2* maize

Protein fraction	Normal maize	*Opaque -2* maize
Albumins	3.2	13.2
Globulins	1.5	3.9
Prolamine (zein)	49.2	22.9
Glutelin	35.1	50.0

Source: Vasal, 2000

In the absence of the specific genes and gene combinations, the genetic manipulation and breeding of high quality protein varieties and hybrids of maize faced enormous challenges. In the mid-sixties, the discovery of the first mutant allele *opaque-2* which has

twice o_2, the levels of lysine and tryptophan in normal maize lines paved the way for such breeding efforts (Mertz, Bates, and Nelson, 1964).

The search for newer and better mutants has continued and following the *opaque-2* mutant, another mutant allele*, floury-2 (fl2)* with similar effects was identified (Nelson, Mertz and Bates, 1965). Other mutants that have been identified include *opaque-7* (McWhirter 1971), *opaque-6* (Ma and Nelson 1975), defective endosperm *B30* and *Mucronate* (Salamini *et al*., 1983). Mutants with high lysine and zein have been reported (Nelson, 1981), two of which include *opaque 7749* and *opaque 11*. Unfortunately none of these two new mutants offered any real advantage over the *opaque-2* and therefore the main focus of breeders or the development of quality protein maize has involved an intensive use of the *opaque-2* mutant gene. Several researches on the efficacy or nutritive value of *opaque-2* maize, otherwise designated quality protein maize to have shown positive impact on the lives of humans and livestock feed.

2.2.1 Breeding QPM

There are two possible approaches to QPM breeding, no matter which breeding method is used: the conventional approach and the molecular approach (use of molecular markers to assist in o_2 selection). Regardless of the breeding method and approach used, there are two unique and essential steps in the development of QPM germplasm. The first is to simultaneously identify segregates in a family or population having the o_2 allele in the homozygous recessive (o_2o_2) condition with a hard endosperm. The conventional approach for this task uses light table and the molecular approach involves the use of both molecular markers and light table. The second step is to identify and confirm QPM quality, i.e. percentage of tryptophan and protein in a sample, through laboratory analysis.

2.2.1.1 Light table selection

Light table is done to pick out kernels with the o_2o_2 genotypes by using the degree of opaqueness as an indirect measure or secondary trait. Due to segregation of genes for endosperm hardness, varying degrees of softness/hardness are expressed in endosperm of segregating generation, i.e. varying levels of opaqueness are observed on a light table. A kernel with o_2o_2 genotype (soft endosperm) is seen as complete opaqueness, while kernels with O_2O_2 or O_2o_2 genotypes (hard endosperm) are translucent. Gradation in the opaqueness is visually assessed on a 1 to 5 scoring scale according to Micic-Ignjatovic (2008). The scores were as follows:

Type (modification score) 1: not opaque

Type (modification score) 2: 25% opaque

Type (modification score) 3: 50% opaque

Type (modification score) 4: 75% opaque

Type (modification score) 5: 100% opaque

Less opaqueness implies higher/more action of modifiers. Types 1 to 3 would be considered QPM, provided their protein quality is verified. It is recommended to select only types 2 and 3 in a conventional breeding approach. Type 2 kernels should be selected only in advanced generations, because O_2O_2 or O_2o_2 genotypes may have a small degree of opaqueness and the presence of o_2o_2 genotypes in early generations is the priority. Type 3 is recommended for selection in early generations as it is a compromise between the guaranteed presence of o_2o_2 (high priority) and good modification (which can be improved in subsequent generations).

2.2.2 QPM quality (percentage of tryptophan and protein in a sample)

Samples are usually first sent to the laboratory for protein content and tryptophan analysis at the F_3 or F_4 stage (before the first test cross). Both lysine and tryptophan concentrations are increased in QPM, but only tryptophan is analysed on routine basis.

This is because lysine and tryptophan are highly correlated and normally, the value of lysine is four times that of tryptophan. Due to the well-established relationship between these amino acids in the protein of *opaque-2* maize endosperm (Hernandez and Bates, 1969; Villegas *et al*., 1992), tryptophan can be used as a single parameter for evaluating the nutritional quality of the protein. When interpreting the results of laboratory analysis for making selections, the protein, tryptophan and quality index (QI - tryptophan to protein ratio in the sample) have to be above the acceptable limits described in Table 2 (Vivek *et al.,* 2008).

Table 2.2. Ready reckoner for interpreting laboratory results (all values in %)

		QPM	Non-QPM
In Protein	Protein	≥8	≥8
	Lysine	4	2
	Tryptophan	>0.65	<0.60
In sample		Whole grain	Endosperm
	Tryptophan	>0.075	>0.07
	QI	>0.8	>0.7

Source: Vivek *et al*. (2008)

2.3 Sweet corn

Sweet corn (*Zea mays convar. saccharata var. rugosa*; (Erwin, 1951)) also called sugar corn and pole corn) is a variety of maize with high sugar content. Sweet corn is the result of a naturally occurring recessive mutation in the genes which control conversion of sugar to starch inside the endosperm of the corn kernel. Unlike field corn varieties, which are harvested when the kernels are dry and mature (dent stage), sweet corn is picked when immature (milk stage) and prepared and eaten as a vegetable, rather than a grain. Since the process of maturation involves converting sugar to starch, sweet corn stores poorly and must be eaten fresh, canned, or frozen, before the kernels become tough and starchy.

2.3.1 History of sweet corn

Sweet corn occurs as a spontaneous mutation in field corn and was grown by several Native American tribes. The Iroquois gave the first recorded sweet corn (called Papoon) to European settlers in 1779 (Schultheis, 1994). It soon became a popular food in southern and central regions of the United States. Open pollinated varieties of white sweet corn started to become widely available in the United States in the 19th century.

Two of the most enduring varieties, still available today, are Country Gentleman (a 'Shoepeg' corn with small, white kernels in irregular rows) and Stowell's Evergreen. Sweet corn production in the 20th century was influenced by the following key developments:

- Hybridization allowed for more uniform maturity, improved quality and disease resistance
- Identification of the separate gene mutations responsible for sweetness in corn and the ability to breed varieties based on these characteristics: su_1 (normal sugary), *se* (sugary enhanced, originally called Everlasting Heritage) and sh_2 (shrunken-2) (Debra, 2003).

There are currently hundreds of varieties, with more constantly being developed.

2.3.2 Health benefits

- Cooked sweet corn increases levels of ferulic acid, which provides anti-cancer properties (Friedlander, 2002).
- At 86 calories per 100 g, sweet corn kernels are moderately high in calories compared to other vegetables. However, fresh kernels have been much lower in calories than field corn and other cereals like wheat, rice, etc. Their calorie mainly comes from simpler carbohydrates like glucose, sucrose than complex sugars like amylose and amylopectin as in cereals.
- Sweet corn is gluten-free cereal and may be used safely much like rice, quinoa, etc., in celiac disease individuals.
- Corn features high-quality phyto-nutrition profile comprising of dietary fibre, vitamins, and antioxidants in addition to moderate proportions of minerals. It is one of the finest sources of dietary fibres; 100 g kernels provide 2 g or 5% of dailyrequirement of dietary-fibre. Together with slow digesting complex carbohydrates; moderate amounts of fibre in the food regulate a gradual rise in blood sugar levels. However, corn, in line with rice, potato, etc., is one of the high glycaemic index food items, limiting its role as the chief food ingredient in diabetes patients.
- Yellow variety corn has significant levels of phenolic flavonoid pigment antioxidants such as ß-carotenes, and lutein, xanthins and cryptoxanthin pigments along with vitamin A. 100 g fresh kernels provide 187 IU or 6% of daily-requirement of vitaminA. Altogether; these compounds are required for maintaining healthy mucus membranes, skin and vision. Consumption of natural foods rich in flavonoids helps to protect from lung and oral cavity cancers.

- It also contains good levels of some of the valuable B-complex group of vitamins such as thiamine, niacin, pantothenic acid, folates, riboflavin, and pyridoxine. Many of these vitamins function as co-factors to enzymes during substrate metabolism.
- Further, it contains healthy amounts of some important minerals like zinc, magnesium, copper, iron, and manganese (Rudrappa, 2009).

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2.3.3 Varieties of sweet corn

There are several cultivated varieties of sweet corn based on the type of sugary gene they possess. These include:

1. Standard (su_1)

The oldest type of sweet corn, which contains more sugar and less starch than field corn intended for livestock. It begins converting sugar into starch after peak maturity or harvest, and as such is best when harvested and eaten immediately. Examples of standard sweet corn varieties are Earlivee, Early Golden Bantam, Stowell's Evergreen, Double Standard and Hookers. These su_1 varieties at immature milking stage (20 days after pollination) contain 10.2% sucrose and 22.8% of water soluble polysaccharide (WSP), a creamy texture about 3 and 8 times the sugar and WSP contents of field corn respectively (Creech, 1965). The sugar varieties have a creamy texture and good corn flavour and are known for their good germination and seedling vigour but their kernels can lose their sucrose from 14.4% to 5.7% (about 2.5 times) at room temperature (27^0C)

24 hours after harvest due to sucrose rapidly converting to starch (Garwood *et al.*,1976). As a result, the harvest and storage periods for the su_1 varieties are short and are suitable for processing, e.g. canning and freezing. Thus sweet corn should be pre-cooled to as close as 0^oC as possible using hydro-cooling or package icing (Westerfield, 2012).

2. Sugary Extender (*se*)

Contains even more sugars in relation to starch than su_1 types, and as such is able to retain sweetness for 2 to 4 days with proper refrigerated handling. Somewhat less hardy than

su_1 types, it is known as a "tender" kernel and as such does not lend itself to mechanical handling. Examples include Precocious, Spring Snow, Sugar Baby and Ruby Queen (Westerfield, 2012).

3. Supersweet (*sh_2*)

Supersweet or shrunken-2 types contain four to ten times the sugar content of normal sugar (*su_1*) types and with proper handling are able to be stored for up to 10 days. It is less hardy than even se types, requiring higher germination temperatures, precise planting depth and isolation from all other corn pollen for optimum results. The name derives from the shrunken, shrivelled appearance of the dried kernel which is low in starch. Examples include Extra Early Super Sweet, Summer Sweet White and Aloha (Westerfield, 2012).

4. Synergistic (*sy*)

Synergistic varieties combine differing genetics on the same ear. Some varieties have 25% *sh_2*, 25% *se* and 50% *su_1* kernels on the cob but each type varies. A common trait of all *sy* types is that isolation from other su_1 and se varieties pollinating at the same time is not required to prevent starchy kernels, though isolation may still be recommended for maximum sweetness. Examples include Applause, Cinderella and Revelation (Westerfield, 2012).

5. Augmented Supersweet

Varieties of the augmented supersweet type combine multiple gene types on top of *sh_2*. These varieties have 100% of the kernels containing the *sh_2* gene, but also have *se* and *su_1* genes in some portion of the kernels. The augmented supersweet varieties have tender kernels like the *se* varieties, therefore mechanical picking is not recommended. As with other supersweets, these varieties must be isolated from *su, se* and *sy* types pollinating at the same time to prevent starchy kernels. Examples include Vision,

Devotion and Obsession (Westerfield, 2012).

2.4 Inheritance of *su_1* gene

The endosperm properties called “sugary” and “starchy” are primarily conditioned by the gene *su_1* and its normal allele *Su_1* (Correns, 1901; East and Hayes, 1911). Kernels homozygous for *su_1* are translucent, wrinkled, and glassy in texture, while *Su_1* kernels are opaque, smooth, and starchy in appearance. The gene pair behaves in simple Mendelian fashion, with *Su_1* exhibiting essentially complete dominance (Cameron, 1947). While the term starchy quite accurately describes the endosperm types to which it refers, sugary kernels differ at maturity not primarily by their higher content of simple sugars, but in that a large fraction of their reserve carbohydrate occurs as water-soluble polysaccharides rather than as granular starch.

2.5 Heritability

Heritability is formally defined as a ratio of variances, specifically as the proportion of total variance in a population for a particular measurement, taken at a particular time or age, that is attributable to variation in additive genetic or total genetic values — termed the narrow-sense heritability (or just heritability, h^2) and the broad-sense heritability (H^2) (Visscher *et al*., 2008).

It is suggested that heritability of a quantitative trait is one of its important characteristics (Falconer, 1981). Twelve maize genotypes were evaluated for character association study during Kharif 2013 at forage research farm, Ludhiana and from the study; there were high heritability with high genetic advances for plant height, leaf with and stem girth (Kapoor and Batra, 2015). During evaluation of 8 maize hybrids in 19931994 in the Antalya-Manavgat region, Tusuz and Balabanli (1997) observed high heritability estimates (H^2bs)

for days to 50% silking (0.93) and low for plant height (0.12), ear height (0.31), moisture percentage at harvest (0.03) and grain yield (0.06).

High levels of heritability estimates of 96.8%, 98.5%, 94.5%, 97.2%, 89.4%, 97.0%, 98.8%, 88.1%, 99.2% and 98.7% were observed, respectively for days to 50% flowering, days to 50% silking, plant height, ear height, number of kernel rows/ ear, number of kernels/ row, number kernels/ ear, 100-seed weight, grain yield and shelling percentage in a set of 47 diverse maize genotypes collected from CIMMYT, Mexico (Sumathi *et al*., 2005).

2.6 Heterosis

Heterosis is the increased vigour or other superior qualities arising from the cross breeding of genetically different plants. It can also be defined as the tendency of a crossbred organism to have a quality superior to those of the parents (American Heritage, 2011). Work on the combining ability and heterosis estimate of extra-early quality protein maize (QPM) single cross hybrids by Ofori *et al.* (2015) obtained both positive and negative heterotic estimates for plant height and ear height. Works also done on the cultivar heterosis between sweet and Spanish field corn by Revilla *et al*. (2000) revealed a highly positive heterotic effect when Stowell's evergreen was crossed to Spanish field corn. Another work by Revilla *et al*. (2006) on comparison among sweet corn heterotic patterns revealed that crosses between Stowell's evergreen and Golden Bantam had poorer agronomic performance and better ear quality than cross between 'NE-HY-13A' and NE-HY-13B'. Olaoye *et al*. (2009) determined the heterosis for percent emergence and ear height in populations derived from Obatanpa and sweet corn crosses. From the study, mid parent and high parent heterotic estimates for percent emergence were 13.20% and 7.08% respectively. Ear height also recorded mid parent and high parent heterotic estimates of -8.15% and -22.12%. For reproductive traits measured, crude protein recorded -3.08% and

23.59% for mid parent and high parent heterosis respectively. Estimates of mid parent and high parent for carbohydrates were -

4.99% and -8.12% respectively.

2.7 Backcross breeding

Backcross breeding is a breeding method whereby a hybrid is crossed to one of its parents for one or more progeny generations (Leininger and Frey, 1962). The method was first suggested by Harlan and Pope (1921), and was extensively studied by Briggs (1941 and 1958). The use of backcrossing in crop improvement has been widely documented, and two main objectives including the transfer of desirable traits from a genotype into another where that trait is lacking in the genotype under improvement, and secondly the recovery of traits of the recurrent parent in a series of backcrossing.

Briggs and Allard (1953) noted that for a successful backcross breeding programme, three requirements need to be met: (a) a satisfactory recurrent parent must exist; (b) it must be possible to retain a worthwhile intensity of the trait or character under transfer through several backcrosses; and (c) the genotype of the recurrent parent must be reconstituted by a reasonable number of backcrosses executed with populations of manageable size.

Briggs and Allard (1953) observed that in spite of the advantages pointed out by Harlan and Pope (1921) and the successes of plant breeders who had used the method, the system initially did not gain wide acceptance. A number of reasons why people did not want to accept this breeding method were advanced. One was the conviction that many plant breeders did not have a satisfactory recurrent parent. Another was the view that the system could not be used for improving a variety with respect to a number of characters and that it was laborious and ineffective in dealing with quantitative characters. There was also a suspicion that the system could not work with crosspollinated crops. Backcrossing can, in fact, be used for both self- and cross-fertilized crops and is based on the simple fact that a heterozygous population backcrossed to either homozygous parent will become more like the genotype of the recurrent parent with each recurrent backcross while the

desired alleles from the donor parent can be maintained in the backcross progeny (Briggs, 1958). The backcross breeding method is well suited for effecting a small number of gene substitutions necessary to increase the usefulness of successful varieties, without the risk of breaking up the existing combinations of desirable genes which have made them outstanding in many respects (Grafius *et al*., 1976).

Although backcrossing is most effective in transferring qualitative traits, Briggs and Allard (1953) argued that empirical data were needed to determine the effectiveness of backcrossing for the improvement of quantitative characters where success requires the transfer of very large number of minor genes. Since then, many scientists including Duvick (1974), Kuhn and Stucker (1995) have reported successes in dealing with quantitative characters, indicating that the backcross method is not, as many originally thought, limited to the transfer of inherited characters.

Successful use of the backcross method for the improvement of a quantitative trait was also demonstrated by Knott and Talukdar (1971) and Duvick (1974). Duvick, for example, used four generations of continuous backcrossing to modify a maize inbred line to a greater number of ears per plant. Hybrid yields of three selections were significantly greater than hybrid yields of the original inbred. Hoffbeck *et al*. (1995) studied backcrossing and inter mating as techniques for the incorporation of exotic germplasm. Traits investigated included grain yield, grain moisture, stalk lodging, plant height, ear height, and days to 50% silk emergence and pollen shed. They conducted analysis on trait means, genetic variances, correlated responses, selection differentials and frequency distributions. The analysis showed that backcrossing generally shifted means and resulted in smaller variances. The study also showed backcrossing to be useful for the incorporation of exotic germplasm.

2.8 Combinations involving *opaque-2* gene and *sugary* gene

Graham *et al.* (1979) reported that the *opaque-2* gene increases lysine and tryptophan contents of maize and its protein quality, the *sugary-2* gene (similar to *sugary-1* gene) improves vitreousness and density but decrease lysine; the double mutant *sugary-2 opaque-2* (su_2o_2) has the improved kernel characteristics and an even higher protein quality than *opaque-2*.

Hallauer (2000) reported that the su_2o_2 segregates are equal to or better than their counterpart's o_2 kernel in protein quality. Some general characteristics based on work conducted in Pardue University, CIMMYT and some other research institutions can be made on this double mutant combinations are as follows:

a. Homozygous su_2o_2 ears tended to shell more easily.
b. Rows of su_2o_2 lacked compactness. The open spaces were observable between and within rows.
c. The protein content and quality were not very different, though in some backgrounds the lysine values in protein were slightly higher.
d. The su_2o_2 ears generally had more number of kernel rows.
e. The su_2o_2 can withstand breakage and mechanical stress better than the soft o_2 kernels and are even superior to normal.
f. The incidence of ear rot was reduced but was still inferior to normal.
g. The su_2o_2 kernels have higher oil content because of higher percentage oil values in both germ and endosperm tissue and slightly higher germ to endosperm ratio but not as a result of increased germ weight.
h. In su_2o_2, the glutelin synthesis is increased which may thus contribute to higher yield of lysine per endosperm than their mutant o_2 kernels.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Maize varieties used

Two sources of open-pollinated maize varieties, Obatanpa and Stowell's evergreen developed in 20th and 19th centuries respectively were used as parentals.

Figure 3.1. Obatanpa cobs

Figure 3.2. Stowell's evergreen cobs

Obatanpa from figure 3.1, is an intermediate maturing, white and dent endosperm maize variety that was developed from GH8363SR. GH8363SR had its source from EV8363, an IITA streak conversion from CIMMYT Population 63 and released in Ghana under its current name. On the national maize program it serves as a source of the *opaque-2* gene which confers high lysine and tryptophan on normal maize varieties. It has a yield potential of 5.22 t/ha at on station and 4.28t/ha at farmer fields (Sallah *et al*., 2007).

Stowell's evergreen from figure 3.2, is a standard sweet corn variety containing the su_1 gene. It is a white variety. This is an heirloom, open pollinated sweet corn variety. It was introduced in the 19th century. A number of improved strains exist with 12 or more rows of kernels on the ear. In its cultivation, Stowell's evergreen requires full sun; direct sowing method should be employed and it takes an average of 98 days to mature.

Ears may be picked for a month or more, hence the name 'evergreen' (Weaver, 2013).

3.2 Experimental location

The study was carried out at the Finatrade Field at the Animal Science department of the Faculty of Agriculture, KNUST, Kumasi, Ghana. It is located at Lat. $6^{o}43^{1}$N and $1^{o}36^{1}$W and falls within the forest zone of Ghana. The location experiences two rainy seasons; the major season (April –July) and minor season (August –November).

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3.3 Field work

3.3.1 Land preparation

Conventional tractor was used to plough and harrow the land.

3.3.2 Soil analysis

The soil was analysed for nutrient composition of N, P and K and other trace minerals. The results were as follows:

Table 3.1. Nutritional composition of soil from finatrade field

	% Org. C	% Org. Matter	%Total N	Avail. P (mg/kg)	pH	ECEC
Soil	1.24	2.13	0.07	6.48	4.70	6.99

	Exchangeable cations (cmol/kg)				Exchangeable acidity (cmol/kg)	
	K	Na	Ca	Mg	Al^{3+}	H^{+}
Soil	0.37	0.33	4.80	0.82	0.17	0.50

3.3.3 Climatic data

Table 3.2. Mean monthly temperature, rainfall and relative humidity during the growing period at Ayeduase, Kumasi from January, 2014 to March, 2015

Month	Temp. (^{0}C)		Rainfall (mm)	Rel. Humidity (%)	
	Min.	Max.		Min.	Max.
January	19.6	33.7	20	4	10
February	21.6	35.0	57	26	68
March	22.5	34.5	139	38	80
May	22.2	31.6	184	60	90
June	21.3	29.5	234	69	91
July	20.9	27.9	125	75	93
September	20.7	28.7	173	81	93
October	20.9	30.5	201	81	85
November	22.4	32.1	134	23	83
Jan	17.7	33.5	12	16	62
February	22.2	33.4	89	23	83
March	23.1	35.2	125	40	78

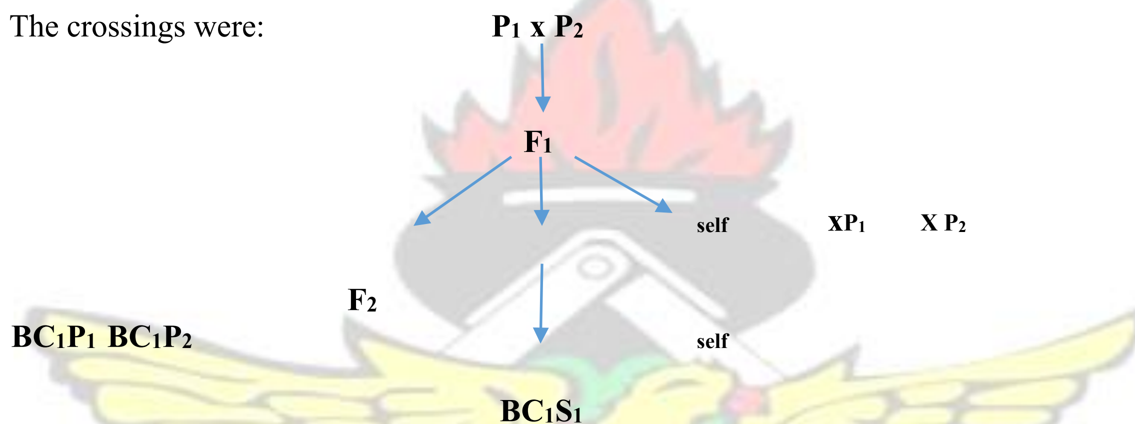
Source: Ghana Meteorological Agency, Ayeduase, Animal Science DepartmentKNUST, 2014.

3.3.4 Planting/ Hybridization

Planting was done in four planting times/seasons and these were:

1st season:	1	Development of F_1 seeds
2nd season:	2.	Development of F_2 seeds
	3.	Development of BC_1P_1 and BC_1P_2
3rd season:	4.	Development of and BC_1S_1 seeds
4th season	5.	Evaluation stage (P_1, P_2 and BC_1S_2)

The crossings were:



Key: P_1 and P_2 are Obatanpa and Stowell's Evergreen respectively.

1st Season: Development of F_1 seeds

During the dry season in 2014 (January to March), the development of F_1's was carried out. 10 rows of Obatanpa (source of opaque-2 gene) were planted one seed per hill as a female block at a spacing of 75cm x 40cm alongside 10 rows of Stowell's evergreen (source of sugary gene) as a male block at a spacing of 90cm x 50cm and also at one seed per hill. Before the appearance of the silk, developing ears were protected with a transparent plastic bag to ensure that emerging silks were not contaminated with unknown pollen grains. At anthesis, pollens were collected using brown paper bags from

agronomically good plants in the sweet corn variety. They were then bulked and used to pollinate good plants in the Obatanpa block to generate the F_1 seeds.

2nd Season: Development of F_2, BC_1P_1 and BC_1P_2

Thirty F_1 families were planted ear to row during the major season in 2014 (May –July). The first ten family lines were selfed to produce F_2, the next ten family lines were backcrossed to Obatanpa to produce BC_1P_1 and finally the last ten family lines were backcrossed to Stowell's Evergreen. At harvest 50 clean cobs each of F_2, BC_1P_1 and BC_1P_2 were obtained. Ten cobs each of these generations were shelled and bulked to be used for opaque-2 analysis and planting the next season.

3rd Season: Planting of F_2, BC_1P_1, BC_1P_2 and development of BC_1S_1 generation

In the third season (from September to November), twenty 5cm row was planted for F_2 and ten 5cm row was planted each for BC_1P_1 and BC_1P_2. 140 opaque BC_1P_1 seeds were grown to generate the BC_1S_1.

4th season: Evaluation Stage

In the fourth season (from January to March), the two parentals (Obatanpa and Stowell's evergreen) were evaluated together with the new type of seed containing both opaque-2 genes and sugary genes (New seed). These three types of seeds were planted using RCBD with 4 replications. After harvest (20, 25 and 30 days after pollination), the seeds were analysed biochemically.

3.4 Data collection

3.4.1 Vegetative data

Twenty plants were randomly selected from each generational field and the following agronomic traits were measured. All measurements were done in centimetres.

1. Plant height: This was done using graduated stick from the ground level to the flag leaf node.
2. Ear height: The height of the topmost ear node from ground level was measured to represent the ear height.
3. Ear leaf width: The width of the widest part of the ear leaf is measured using the meter rule. This widest part is normally found at the central part of the leaf.
4. Stem girth: Vernier callipers were used to take the thickness of the maize stems at second internode from the ground.
5. Emergence percentage: These were obtained by finding the percentage of the emerged seeds to the total seeds planted.

3.4.2 Output data

1. Sucrose content was determined using modified anthrone method (Finley and Fellers, 1973)

Reagents

1. 80% ethanol 2. Fehling solution according to Shriner *et al*. (1956).

Solution A: 36.4g copper sulphate pentahydrate is dissolved and diluted with water to 500ml. Solution B: 173g sodium acid tartrate and70g of sodium hydroxide are dissolved and diluted with water to 500ml. Equal parts of solution A and B are mixed daily to meet the daily needs. 3. Anthrone reagent according to Van Handel (1968). Carefully add 760ml of sulphuric acid to 300ml of water; cool to room temperature and dissolve

1.5g anthrone in the diluted acid.

Procedure

1. 1.0g of resultant product was weighed into a 100ml volumetric flask. 50ml of 80% ethanol was added and boiled gently for 15minutes. Reagent blank and a standard which is 1ml of a 15%w./v. sucrose solution (15g sucrose made up to 100ml with water) was concurrently run.
2. It is then cooled to 20^0C and diluted to 100ml with 80% ethanol.
3. It is filtered through S&S 576 filter paper or equivalent.
4. 1ml of filtrate is added to 9ml of Fehling solution in a test tube in boiling water bath for 15minutes and cooled to 20^0C.
5. To 1ml of this solution in a clean test tube, 10ml of anthrone reagent is rapidly added and held for 30minutes at 40^0C.
6. Absorbance is read at 610m, against a reagent blank.
7. Percent total apparent sucrose was calculated as follows:

Percent total apparent sucrose =A sample/A standard X 15

2. Crude Protein determination by Kjeldahl Method (1990)

Reagents

1.Sulphuric acid, concentrate, A.R grade or equivalent 2. Catalysts: Potassium sulphate and or Copper sulphate with or without Selenium (IV) dioxide 3. Sodium hydroxide solution, 50%. Weigh 500g sodium hydroxide, dissolve in water and make up to 1L with water 4. Boiling chips 5. Ammonium Sulphate, A.R. 6. Hydrochloric acid, 0.1N standard solution 7. Standardized hydrochloric acid solution 8. Methyl red indicator solution 9. Boric acid solution, 4% with indicator.

Apparatus

Kjeldahl method consists of 3 units namely, the digestion, distillation and titration units. Apparatus used include the following: 1.Digestion tubes 2. Erlenmeyer flask, 250ml, 500ml 3. Magnetic stirrer and magnetic bar 4. Drying oven, capable of being controlled at 100-120^{0}C and equipped with suitable thermometer 5. Balance, analytical, 200g capacity with 0.1ml sensitivity.

Procedure

1. Preparation of sample: Sample was ground/blended until homogenous.
2. Analysis

3.Blank: Two reagent blanks (containing all reagents used in nitrogen analysis except the sample) was included in every batch of analysis to subtract reagent nitrogen from thee sample nitrogen.

Test sample: - Sample was thawed out to room temperature and mixed thoroughly. -2-10g sample (depending on the nitrogen content of the sample) was weighed in duplicates into the digestion tube.

-5-7g catalyst was added and 1 glass bead to prevent solution from bumping and 1020ml sulphuric acid.

-Digestion tube was placed in the digester. Digestion of the mixture was initially done at low temperature to prevent frothing and boiled briskly until the solution is clear and is free of carbon or until oxidation is complete.

- Digestion is continued until a clear digestion is obtained.
- The already clear liquid is heated for another one hour to ensure complete breakdown of all the organic matter.
- 250-500ml Erlenmeyer flask containing 50ml of 4% boric acid with indicator is used as a receiver on the distillation unit.
- 100ml of water and 70ml of 50% sodium hydroxide is added to the digests to begin

distillation.

- Distillation is done until all ammonia has been released or approximately ≥ 150ml distillate is obtained.
- The receiver flask is lowered so that the delivery tube is above the liquid surface and distillation is continued for 1-2minutes.
- Finally, the delivery tube is rinsed with water and the washings are allowed to drain into the flask.

-The distillate is titrated with the standardized 0.1N Hydrochloric acid until the first appearance of the pink colour.

- The volume of acid used is recorded to the nearest 0.05ml.

$$N\ (g\%) = \frac{(mL\ 0.1N\ HCl\ sample - mL\ 0.1N\ HCl\ blank\ X 0.0014\ X\ N\ HCl\ \ X\ 100}{Weight\ of\ sample}$$

Protein (g per100g) = % Total N X Appropriate Nitrogen conversion factor (6.25).

4. Starch yield determination

The starch yield of the flour was determined based on wet extraction method as described by Ellis *et al*. (2003). To 5g of flour, 100ml distilled water was added to dissolve the starches present in the flour. The slurry was then filtered through a cheese cloth. Excess water was added to the retentate to wash all the starch into the filtrate. The filtrate was allowed to settle for 30 minutes, decanted and the sediment spread on prewashed petri dish. The starch sediment in the petri-dish was dried in the oven at 50^0C for 24 hours and weighed. Percentage starch yield was expressed as starch recovered after extraction from 5g flour.

3.5 Screening methods

3.5.1 Background selection

Background selection for the opaque -2 gene in the recurrent parent was done using the light table selection method.

Figure 3.3. Light table

3.5.2 Foreground selection

The selection for the gene in the donor variety is done using the morphology of the seed which appear wrinkled and glassy.

Figure 3.4. Wrinkled and glassy appearance of Stowell's evergreen sweet corn

3.6 Study design

Randomized complete block design was used for the field evaluation for vegetative traits whilst factorial CRD was used for the evaluation of the output traits.

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3.7 Statistical analysis

1. Statistical analyses were carried out using Genstat package, Version 16.

   - Chi-square test was employed to determine the segregation ratios of F_2, BC_1P_1, BC_1P_2 and BC_1S_1
   - Sample means and variances were computed using the statistical package and genetic analyses were performed as follows:

$V_A = 2V_{F2} - (V_{B1}+V_{B2})$ ………………………………………… 1

$V_D = [(V_{B1} + V_{B2}) - F_2 - (V_{P1} + V_{P2} + V_{F1})] / 3$ …………….......... 2

$V_E = V_{P1} + V_{P2} + V_{F1} / 3$ ………………………………………….. 3

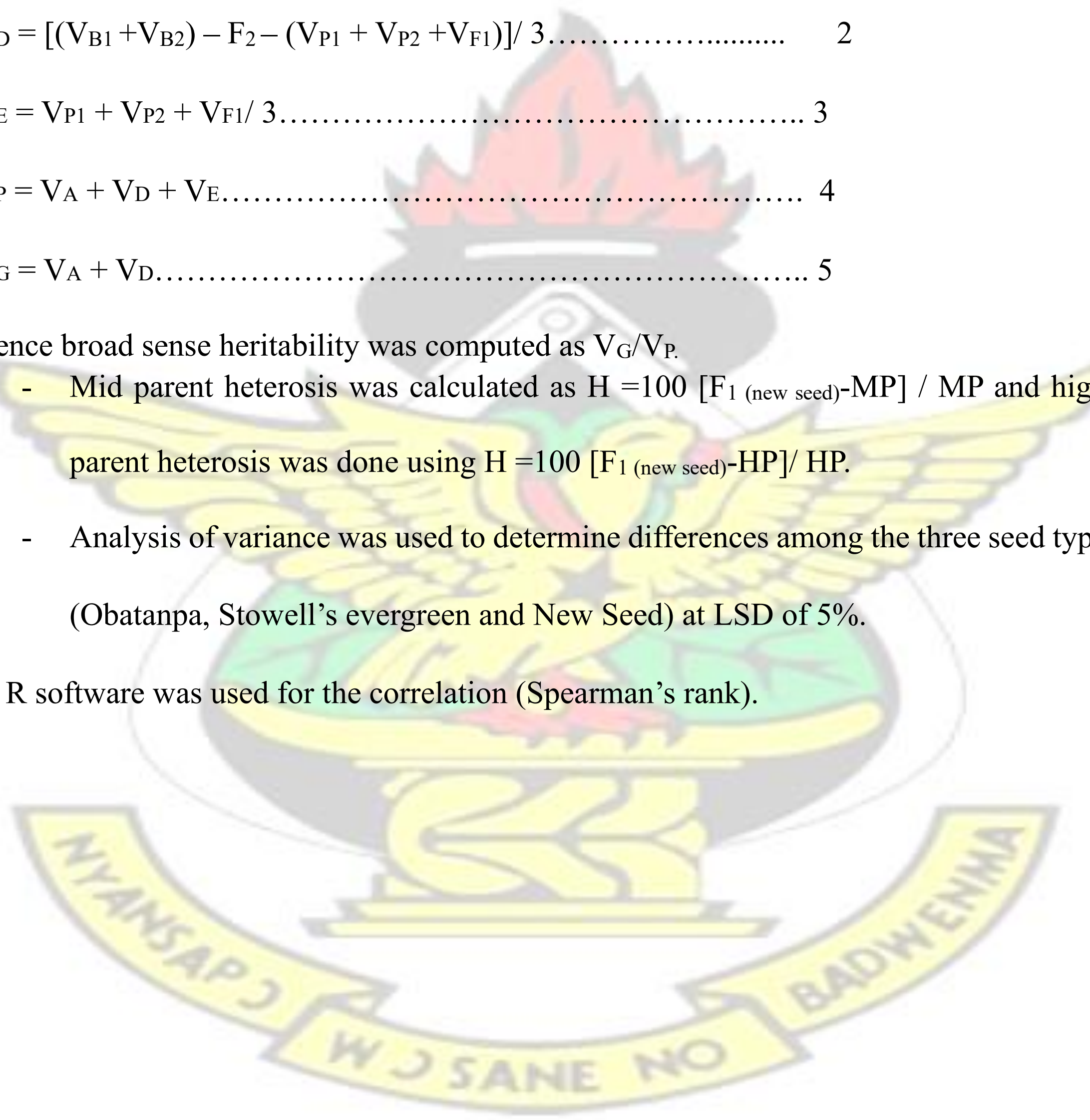
$V_P = V_A + V_D + V_E$ ……………………………………………… 4

$V_G = V_A + V_D$ ……………………………………………………….. 5

Hence broad sense heritability was computed as V_G/V_P.

   - Mid parent heterosis was calculated as $H = 100 [F_{1\ (new\ seed)} - MP] / MP$ and high parent heterosis was done using $H = 100 [F_{1\ (new\ seed)} - HP] / HP$.
   - Analysis of variance was used to determine differences among the three seed type (Obatanpa, Stowell's evergreen and New Seed) at LSD of 5%.

2. R software was used for the correlation (Spearman's rank).



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CHAPTER FOUR

4.0 RESULTS

4.1 Inheritance of sugary gene

Inheritance of the sugary gene was determined using the segregation ratios obtained from the chi-square test. The results from the test were as follows:

Table 4.1. Generational analysis for inheritance study

Generation	**Segregations**					Chi-square	
	SO	NO	SN	NN	Total	value	p-value
F_2	63	186	189	562	0.090*		0.759
BC_1P_1	-	497	-	503	0.04*		0.850
BC_1P_2	-	-	499	501	0.004*		0.950
BC_1S_1	252	748	-	-	0.02*		0.884

* Significant at 0.05 probability level

From table 4.1, chi-square calculated values were less than the corresponding tabulated (P-value) at 5%, 1 d.f which indicates that the observed values shows a goodness of fit to the expected genetic (Mendelian) ratio. This occurred for all the generations under consideration.

Figure 4.1. Segregation of BC_1S_1 into 3 non-shrunken opaque : 1 shrunken opaque

4.2 Heritability of vegetative characteristics

Estimates for heritability were obtained from the variances at the various generations.

Table 4.2. Variances in vegetative traits at the various generations

Characteristic (cm)	Measure	P_1	P_2	F_1	F_2	BC_1	BC_2
		Generations					
Ear leaf width	Mean	10.92	8.33	10.54	10.42	10.95	10.88
	Variance	**0.0026**	**0.0013**	**0.0021**	**1.015**	**0.010**	**0.050**
Stem girth	Mean	2.27	1.83	2.19	2.16	2.28	2.20
	Variance	**0.0013**	**0.00091**	**0.0034**	**0.048**	**0.0029**	**0.0027**
Plant height	Mean	204.6	140.0	173.2	175.5	197.8	150.7
	Variance	**72.41**	**36.83**	**21.65**	**681.7**	**275.8**	**205.2**

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Ear height	Mean	74.07	54.15	60.58	63.13	71.14	55.61
	Variance	**2.1**	**2.37**	**1.07**	**59.07**	**35.34**	**16.64**

Table 4.21. Heritability estimates of vegetative traits

Characteristics	V_A	V_D	V_E	V_P	V_G	**H_{2bs}%**
Ear leaf width	1.97	-0.32	0.002	1.652	1.65	**99**
Stem girth	0.09	-0.01	0.0018	0.076	0.074	**97**
Plant height	882.4	-110.53	43.63	815.5	771.87	**95**
Ear height	66.16	-4.21	1.85	63.8	61.95	**97**

As shown in table 4.21, all the vegetative traits exhibited high broad sense heritability (Kapoor and Batra, 2015; Ali *et al*., 2006) with ear leaf width obtaining the highest at 99%. This means that ear leaf height is 99% controlled by the genes and only 1% by the environment.

4.3 Heterosis for the vegetative and output traits of the new seed

These were determined based on the means of the various seeds, that is, Obatanpa, sweet corn and the new seed.

Table 4.3a. Heterosis for vegetative traits

Trait	Mean of trait in Obatanpa	Mean of trait in sweet corn	Mean of trait in new seed	**% Mid parent heterosis**	**% High parent heterosis**
	----------------------	cm	----------------------		
Percent emergence	93.70	68.70	89.30	**10.0**	**-4.7**
Ear leaf width	10.91	8.14	10.48	**10.0**	**-3.9**

Stem girth	2.28	1.81	2.14	**4.7**	**-6.1**
Ear height	73.17	54.30	66.52	**4.3**	**-9.1**
Plant height	210.8	141.7	179.7	**2.0**	**-14.8**

Estimates of mid parent heterosis for vegetative traits though positive, were low (Table 4.3a). There were also negative high parent heterosis in the hybrid for all the traits measured.

Table 4.3b. Heterosis for output traits

Trait	Mean of trait in obatanpa	Mean of trait in sweet corn	Mean of trait in new seed	% **Mid parent heterosis**	% **High parent heterosis**
	------------------cm----------------------				----
Sucrose	14.86	16.80	17.27	**9.1**	**2.8**
Starch	23.93	14.98	13.67	**-29.8**	**-42.9** yield
Protein	11.82	17.65	13.39	**-9.2**	**-24.1**

From table 4.3b, there was negative mid-parent heterosis for starch yield and protein content in the hybrid but not for sucrose content. There was also positive high parent heterosis for sucrose content in the hybrid but not for protein and starch yield.

4.4 Difference between the two parents and the new seed

The differences between Obatanpa, sweet corn and the new seed were determined using the ANOVA at 0.05 probability level.

Table 4.41a. ANOVA mean square values for vegetative traits in the 3 seed type

Source of variation	% Emergence	ELW	SG	PH	EH
		(cm)			
Seed type	711.10*	8.87*	0.24*	4787.11*	366.63*
Error	12.40	0.009	0.001	28.61	1.72

*Significant at 0.05 probability level

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Table 4.41b. Means of vegetative traits in the 3 seed type

Seed Type	% Emergence	ELW	SG	PH	EH
		(cm)			
Obatanpa	93.7	10.91	2.28	210.8	73.17
Sweet corn	68.3	8.14	1.81	141.7	54.3
New seed	89.3	10.49	2.14	179.7	66.52
$LSD_{0.05}$	6.09*	0.17*	0.057*	9.25*	2.269*

*Significant F test at 0.05 probability level

There were significant difference among all parameters measured for the different seed type (Table 4.41b).

Table 4.42a. ANOVA mean square values for output traits in the 3 seed type and DAP

Source of variation	Sucrose content	Starch yield	Protein content (g/100g)
	%	%	
Seed type	9.82*	54.54*	187.24*
DAP	0.22	2.79*	39.90*
Seed type	3.69*	2.70*	76.49*

X DAP			
Error	0.20	0.006	0.73

*Significant at 0.05 probability level

Table 4.42b. Mean of output traits in the 3 seed type and days after pollination

Seed type (ST)	Sucrose content	Starch yield	Protein content (g/100g)
	--------------------- % -------------------		
Obatanpa	14.86	23.93	11.82
Sweet corn	16.8	14.98	17.65
New seed	17.27	13.67	13.39
LSD $_{0.05}$	0.55*	1.11*	0.13*
DAP			
20	16.48[a]	20.20[a]	13.94[a]
25	16.33[b]	17.34[b]	13.85[a]
30	16.11[b]	15.05[c]	15.07[b]
LSD $_{0.05}$	0.55	1.11*	0.13
Interaction effect LSD $_{0.05}$			
ST X DAP	0.96*	1.93*	0.22*

*Significant F test at 0.05 probability level

Means across a column with the same alphabet is not significant.

From table 4.42a, there were significant differences among the three seed type in terms of their sucrose, starch and protein content. There was also significant difference between the days after pollination (DAP) in terms of their protein and starch yield but not sucrose

content. Again, there were significant differences in the interaction among the three seed type and days after pollination for all the output traits measured.

From table 4.42b, it can be seen that the difference among the different seeds were significant at 5% lsd for all the output traits measured. There was however significant difference at 5% lsd for interaction effect between seed type and DAP of all the output traits.

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Table 4.43. Interactive means between sucrose content and DAP and their comparison at 5% LSD

Treatments	Means	Groups
Sw: 20DAP	18.52	a
Ns: 30DAP	18.36	a
Ns: 25DAP	17.25	b
Sw: 25DAP	16.34	bc
Ns: 20DAP	16.20	c
Sw: 30DAP	15.55	cd
Ob: 25DAP	14.74	de
Ob: 30DAP	14.42	e
$Lsd_{0.05}$	**0.96**	

From table 4.43, the mean of sweet corn at 20DAP was statistically similar to New seed at 30DAP.

4.5 Relationship between time of harvest and output traits

This was done using regression analysis and spearman's rank correlation. The analyses were performed at 95% confidence level.

Table 4.5. Correlation analysis of sucrose content in relation to time of harvest

Seed type	rho value	p-value
Obatanpa	-0.5	1
Sweet corn	-1.0	0.33
New seed	1.0	0.33

Spearman's p-value of 1 for Obatanpa meant that there was a failure to reject the null hypothesis that there was a relationship between the two variables. In addition, Spearman's p-value of 0.333 for sweet corn signified that there was a relationship between days after pollination and sucrose content. The Spearman's p-value of 0.333 for new seed again signifies that there was a relationship between days after pollination and sucrose content (Table 4.5).

4.5.1 Patterns of output traits in relation to time of harvest

The patterns of reproductive traits in relation to time of harvest were presented using charts as follows:

From figure 4.51a, it can be seen that the sucrose content in Obatanpa is somewhat static, neither decreasing nor increasing. The sucrose content was the least recorded at 14.74% (20 DAP) and was still 14.42% at 30 DAP. Furthermore, the starch yield in

Obatanpa was the highest at 34% (20 DAP) but declined to 15% (56% decrease) at 30 DAP (Figure 4.51b). Moreover, the crude protein content in Obatanpa was least and never increased with DAP (Figure 4.51c).

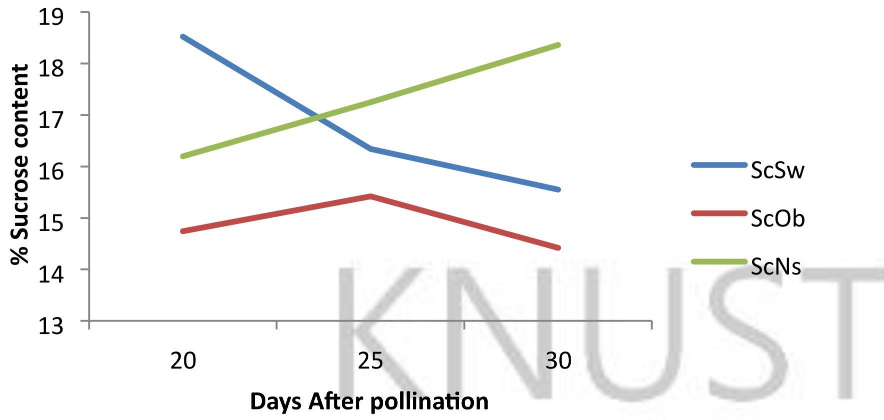

Figure 4.51a. Pattern of sucrose content among the various seed types in relation to time of harvest

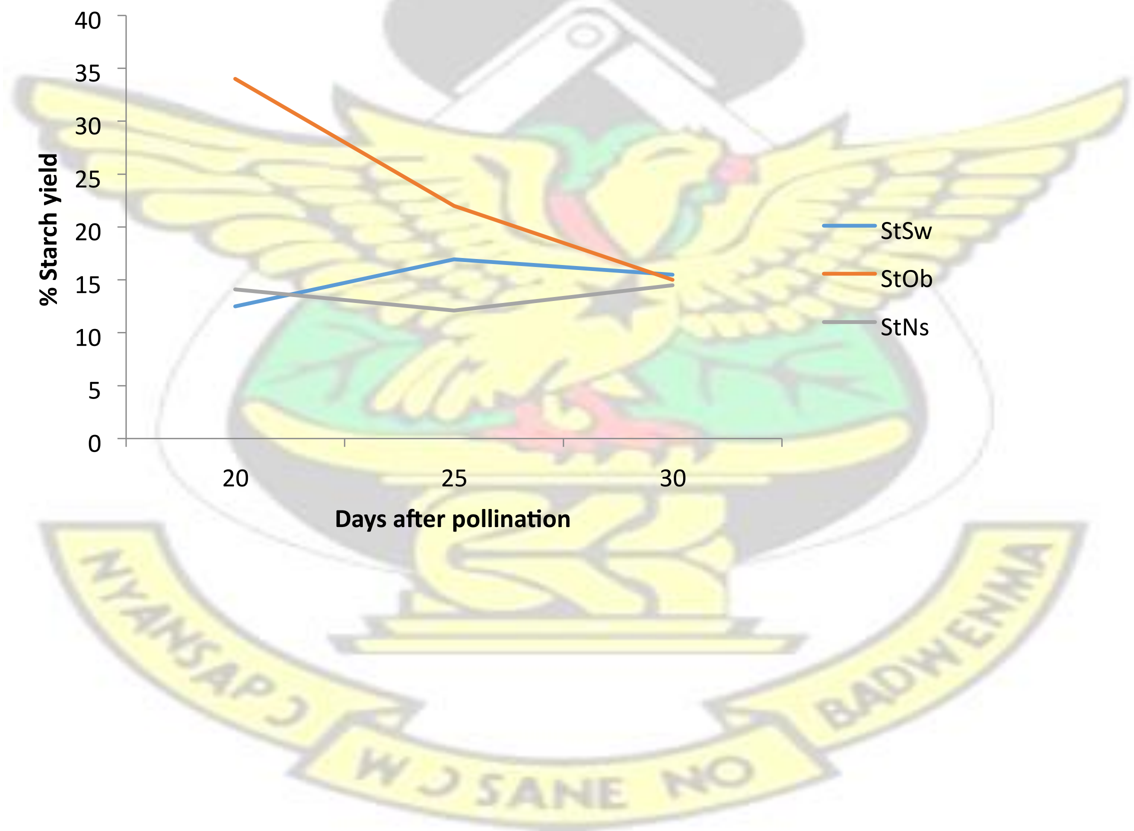

Figure 4.51b. Pattern of starch yield among various seed types in relation to time of harvest

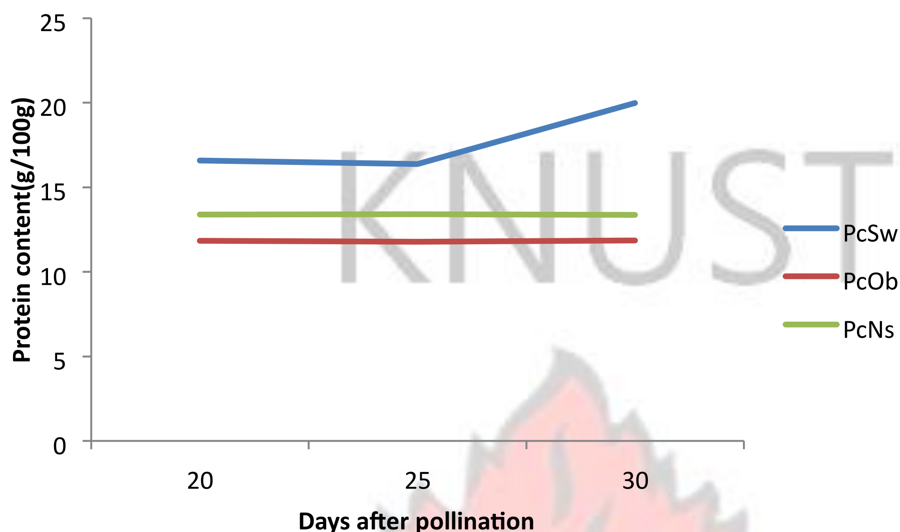

Figure 4.51c Pattern of protein content among various seed type in relation to time of harvest

4.52 Correlation between sucrose content and starch and protein yield

Table 4.52. Correlation analysis of sucrose content in relation to starch yield and protein content

	Starch yield		Protein content	
Seed type	rho	p-value	rho	p-value
Obatanpa	0.5	1	-1.0	0.33
Sweet corn	-0.5	1	-0.5	1
New seed	0.5	1	-0.5	1

From table 4.52, the p-value for all the relations were greater than the 0.05 significance level, hence fails to reject the null hypothesis that there is a relationship between the variables under consideration.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Inheritance of *sugary* su_1 gene

The segregations observed in the F_2 population according to the chi-square test showed significance and hence fitted to the expected ratio of 9:3:3:1 (Table 4.1). This signifies that the *sugary* su_1 gene together with the *opaque-2* gene behaves in a simple Mendelian fashion with Su_1 exhibiting complete dominance over the su_1 gene (Cameron, 1947). This was further confirmed by the segregations of BC_1P_1 and BC_1P_2 populations where they both fitted to 1:1 ratio. Hence the *sugary gene* conferring the sugary trait is monogenically inherited. This means that the gene is either present or absent and is qualitative trait without any influence of the environment.

5.2 Heritability of vegetative traits

Ear height obtained the least among them with heritability estimate of 93%. This suggests that all the vegetative traits measured are highly heritable and can be used to distinguish between the various populations and to select promising lines (Roham *et al*., 2003). Heritability estimates obtained in this study however contrasted with estimates obtained by Tusuz and Balabanli (1997) where they obtained lower broad sense heritability estimates for plant height and ear height.

5.3 Heterosis for vegetative and output traits of the new seed

However, since they were positive, they suggest a modest increase in heterotic response of such characters in the hybrid. This result confirms the definition by American Heritage (2011) because the hybrid was superior to the lower parent. The negative values obtained for the high parent heterosis by all the vegetative traits suggest that the hybrid could not

surpass their better parent. Though there is hybrid vigour, they are not superior to their better parent. Similar results were also reported by Ofori *et al*. (2015), where they also recorded both positive and negative heterotic estimates for single crosses between QPMs. It can therefore be conclusively said that if percent emergence, plant height, stem girth and ear leaf width are needed to be improved in a hybrid, then the two parents were promising (Olaoye *et al*. 2009).

From table 8, it can be deduced that the hybrid was only heterotic with sucrose content but not starch yield and crude protein content. Though positive heterosis was obtained for sucrose content, as similarly reported by Revilla *et al*., 2000, they were quite low. These low estimates were not unexpected since the trait actually being transferred is conditioned by recessive *sugary* su_1 gene.

There was a very low mid parent heterosis for starch yield at -30.0% and high parent heterotic value of -43.0%. This may probably be as a result of the lack of Su_1 gene in the hybrid. This is because starch content is conditioned by the Su_1 gene (Correns, 1901; East and Hayes, 1911) and since this is found in Obatanpa, selection to only include the recessive su_1 genes in the hybrid means that there will be lowered estimate for the starchy trait.

Moreover, although the mid parent and high parent heterotic estimates for crude protein were negative, they were far better than that for starch yield. The reason may be due to the fact that *opaque-2* gene does not improve upon the quantity of protein in the maize seed but rather the quality by improving upon the lysine and tryptophan content (Bressani, 1992). Hence the presence of the *opaque-2* gene in the new seed together with the *sugary* gene was not enough to increase the crude protein content above any of the two parents.

5.4 Differences between the two parents and the new seed

The main objective of the study was to obtain a variety that combines the *sugary* gene from sweet corn and the *opaque-2* gene from Obatanpa, producing a hybrid. This hybrid being produced termed 'new seed' was always at the midline of all the traits being measured. This affirms the contribution of the two parents in the hybrid formation. The new seed was significantly different from the two parents in all the vegetative traits measured, which means that the differences were not due to chance but as a result of the differences in the genetic make-up of the different seed types. With percent emergence, Obatanpa recorded the highest mean value of 93.7% whilst sweet corn recorded the lowest of 68.3% which is in consonance to a report by Hallauer (2000) that high sugary mutants of maize generally reduce field emergence and seedling vigour compared to field corn. The difference might also come from the environment where Lerner and Dana (2001) reported that pH of 6-6.5 was desired for optimal performance of sweet corn and that a pH of 4.7, in the study soil might be slightly acidic to impede germination and emergence of sweet corn. These results were not in agreement with that obtained by Olaoye *et al*. (2009), who compared the percent emergence of Obatanpa and sweet corn. From the work, it was reported that the sweet corn was recording higher percentage in terms of percentage emergence compared to Obatanpa. This is really less surprising because the soil conditions might had been favourable to the sweet corn and also the sweet corn varieties used were not the same (su_1 sweet corn versus su_2 sweet corn).

The BC_1 new seed performed better in terms of the percentage germination. This is because it surpassed the expected recovery rate for BC_3 (93.7%) with 95.3% recovery of the recurrent parent. This suggests that less backcross would be needed to recover most of the traits from the recurrent parent. All the vegetative traits ranked Obatanpa first, followed by the new seed and then sweet corn. This was probably due to the genetic

background of the three seed types and also these traits have been known to be highly heritable from the study.

New seed stood tall in sucrose content among all the seed type and the difference between it and the high parent was highly significant and not due to chance. This therefore underscores the synergistic contribution of the two genes in the hybrid. There was also significant difference in the means of the different DAP for starch yield but not for protein content and sucrose content. This means that a difference in just the DAP could not differentiate between the sucrose and protein content.

The new seed used as many as 30DAP to produce similar sucrose content as sweet corn harvested at 20DAP. These sucrose contents were however significantly different from all the other treatments. This therefore predicted the best harvesting times for the two seed types; 20DAP for sweet corn and 30DAP for new seed if sucrose content is the priority trait. The new seed at the various fixed duration after pollination were all seen to significantly produce different interaction means. This clearly justifies the durations for the various harvesting times. The sucrose content in sweet corn on the other hand at 25DAP was not different from that at 30DAP. More so, Obatanpa at 25DAP was significantly higher than Obatanpa at 30DAP but not 20DAP. Hence in order to study the effect of harvesting times on sucrose contents, harvesting should be done at 20DAP and 30DAP (10 days interval) for sweet corn; 20DAP, 25DAP and 30DAP (5 days interval) for new seed and 25DAP and 30DAP for Obatanpa to clearly ascertain significant difference. These also suggest that when sucrose content is a priority, then sweet corn should be harvested at 20DAP, new seed at 30DAP and Obatanpa should be harvested at 25DAP (Table 4.43).

5.5 Relationship between time of harvest and output traits

The rho was -0.5 for Obatanpa which indicated that there was moderate negative relationship between days after harvesting and sucrose content. The rho was -1 which meant that there existed a strong negative relationship between sucrose content and days after pollination. The rho was 1 which means that there existed a strong positive relationship between sucrose content and days after pollination.

5.51 Pattern of output traits in relation to time of harvest

The sucrose content in sweet corn however recorded the highest of 18.52% at 20 DAP which is consistent with earlier reports by Creech (1965) that *su_1* varieties at 20 DAP contains the peak amount of sucrose of as high as 10.2%. Comparing to that of Obatanpa, there was 80% increase in sucrose content. From the highest value of 18.52%, it started declining to 16.4% (12% decrease) at 25 DAP, then to 15.5% (16.3% decrease) which is in consonance to earlier report by Creech (1965) that sucrose content peaks at 20 DAP but declines steadily afterwards.

In direct contrast to sweet corn, sucrose content in the double mutant new seed kept on increasing with DAP without an assurance that it has even reached its peak. This might be due to the presence of the *opaque-2* gene which is preventing the conversion of the sucrose into starch and still fostering continual production of sucrose. This is implying a synergistic interaction between *opaque-2* gene and *sugary su1* gene in sucrose sustenance and improvement.

With sweet corn, it started with 12.5% at 20 DAP but rose to 15.5% at 30 DAP (24% increase). This means that the sugar was actually being converted into starch. The starch content in the new seed started at 14.09% (20 DAP), but declined and rose again to 14.5%

(3% increase). This means that as the sucrose content is increasing, only a very small percentage is converted into starch.

The crude protein content in the sweet corn was the highest at 20 DAP and rose to 19.98 g/100g (20.4% increase) at 30 DAP. Also the protein content in the new seed though never increased with DAP, was higher than protein content in Obatanpa. It can therefore be said here that, when sucrose content is a character of interest, one must pick new seed at 30 DAP and when starch yield is of interest, then one must go in for Obatanpa at 20 DAP. However, when crude protein is desirable, then sweet corn would be the best. In conclusion, the new seed is a very promising seed and at 30 DAP, the sucrose content is the highest, starch content is higher and protein content is also higher comparatively.

5.52 Correlation between sucrose, starch and protein content

Meanwhile a rho of 0.5 for Obatanpa and new seed signifies that there is a moderate positive relationship between sucrose content and starch yield whilst a rho of -0.5 for sweet corn means that there is a moderate negative relationship between sucrose content and starch yield. A -0.5 rho value for sweet corn and new seed also signifies that there is a moderate negative relationship between sucrose content and protein content. However, rho value of -1 suggests a strong negative correlation between sucrose content and protein content in Obatanpa.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

In conclusion, the mode of inheritance of sugary trait was simple Mendelian inheritance as the F_2 populations segregated into 3:1 and the backcross into 1:1 ratio, hence controlled by a single gene.

Also, ear leaf width, stem girth, plant height and ear height were all highly heritable and exhibit positive heterotic response in the hybrid. These vegetative traits would therefore facilitate selection for sugary trait because they move along with the gene.
More so, sucrose content was the only character among the output traits measured that proved both mid and high parent heterotic response in the hybrid seed developed.

All the vegetative traits were significantly different among the seed types in a decreasing order Obatanpa > New seed > Sweet corn. There was also synergistic effect between the two genes (*opaque-2* and su_1) in their contribution to sucrose content in the new seed. A combination of different seed type and DAP could significantly
differentiate all the reproductive traits measured particularly the sucrose content. Hence for similar studies, sweet corn should be harvested at 20, 30, 40 DAP (10 days interval), Obatanpa at 25, 30, 35, 40 DAP (5 days interval) whilst new seed should be harvested at 20, 25, 30, 35, 40 DAP (5 days interval) to identify significance. It was also found that the best harvesting times for sweet corn, Obatanpa and new seed were 20, 25 and 30 DAP respectively.

There was no corresponding decrease in starch yield as the sucrose content in the new seed was increasing.

It was again found that sucrose content in Obatanpa was strongly negatively correlated to its protein content. On the other hand, there was moderate positive relationship between sucrose content in the new seed and its starch yield whilst there was moderate negative relationship between new seed and protein content.

It can therefore be said that the sugary gene has been fixed into the genetic background of Obatanpa with all the accompanying modifications in sugary and protein contents as the highest sucrose content obtained by sweet corn was insignificantly different from that obtained by the new seed.

6.2 RECOMMENDATIONS

Since only morphological traits (vegetative and output) were used to identify the presence of the gene which may be deceptive sometimes, it is recommended that similar research in future should include molecular markers to clearly distinguish between the parents and the first backcross population for the presence of the *sugary* gene.

If possible, there should be protein quality test performed on the new seed to confirm the integrity of the seed in terms of levels of lysine and tryptophan.

The nutritional composition of the new seed should also be looked at, to find out if important traits such as high ferulic acid concentration, gluten-free seed and high fiber content were transferred from the sweet corn into the new seed.

Studies into the mechanism of action of the two genes in contributing to the increase in sucrose content without significant effect on their starch content in the new seed must be conducted.

Finally, since the peak sucrose content in the new seed could not be obtained in the study, similar studies should focus on increasing the times of harvest from 20DAP to about 50DAP.

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APPENDICES

Appendix 1. Generational counts

Generational Counts											
		F2								BC1P1	
F2	SO	NO	SN	NN	Total				NN	NO	Total
Observed	63	186	189	562	1000			Observed	503	497	1000
Expected	61	188	188	563	1000			Expected	500	500	1000
		BC1P2								BC1S1	
	NN	SN	Total						NO	SO	Total
Observed	501	499	1000					Observed	748	252	1000
Expected	500	500	1000					Expected	750	250	1000

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Appendix 2. ANOVA for vegetative traits

Variate: %_Germination

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	21.26	7.09	0.57	
Rep.*Units* stratum					
Trt	2	1422.19	711.10	57.34	<.001
Residual	6	74.40	12.40		
Total	11	1517.86			

Tables of means

Grand mean 83.9

Trt	nws	obt	swc
	89.3	93.7	68.7

Least significant differences of means (5% level)

Table	Trt
rep.	4
d.f.	6
l.s.d.	6.09

Stratum standard errors and coefficients of variation

Stratum	d.f.	s.e.	cv%
Rep	3	1.54	1.8
Rep.*Units*	6	3.52	4.2

Variate: Ear Leaf Width

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.072433	0.024144	2.43	
Rep.*Units* stratum					
Trt	2	17.739650	8.869825	892.69	<.001
Residual	6	0.059617	0.009936		
Total	11	17.871700			

Tables of means

Variate: LW

Grand mean 9.845

Trt	nws	obt	swc
	10.488	10.905	8.143

Least significant differences of means (5% level)

Table	Trt
rep.	4
d.f.	6
l.s.d.	0.1725

Stratum standard errors and coefficients of variation

Variate: LW

Stratum	d.f.	s.e.	cv%
Rep	3	0.0897	0.9
Rep.*Units*	6	0.0997	1.0

Variate: Stem Girth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.002758	0.000919	0.85	
Rep.*Units* stratum					
Trt	2	0.469817	0.234908	216.28	<.001
Residual	6	0.006517	0.001086		

	d.f.	s.s.
Total	11	0.479092

Tables of means

Variate: STG

Grand mean 2.0742

Trt	nws	obt	swc
	2.1425	2.2750	1.8050

Least significant differences of means (5% level)

Table	Trt
rep.	4
d.f.	6
l.s.d.	0.05702

Stratum standard errors and coefficients of variation

Variate: STG

Stratum	d.f.	s.e.	cv%
Rep	3	0.01751	0.8
Rep.*Units*	6	0.03296	1.6

Variate: Ear Height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	2.140	0.713	0.41	
Rep.*Units* stratum					
Trt	2	733.252	366.626	213.26	<.001
Residual	6	10.315	1.719		
Total	11	745.707			

Tables of means

Variate: EHT

Grand mean 64.67

Trt	nws	obt	swc
	66.52	73.17	54.30

Standard errors of means
Table Trt
rep. 4 d.f. 6
e.s.e. 0.656

Least significant differences of means (5% level)

Table Trt rep. 4 d.f.
6
l.s.d. 2.269

Stratum standard errors and coefficients of variation

Variate: EHT

Stratum	d.f.	s.e.	cv%
Rep	3	0.488	0.8
Rep.*Units*	6	1.311	2.0

Variate: Plant height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	98.54	32.85	1.15	
Rep.*Units* stratum					
Trt	2	9574.22	4787.11	167.31	<.001
Residual	6	171.67	28.61		
Total	11	9844.43			

Tables of means

Variate: PHT
Grand mean 177.4

Trt	nws	obt	swc
	179.7	210.8	141.7

Standard errors of means

Table Trt rep. 4 d.f.
6
e.s.e. 2.67

Standard errors of differences of means

Table	Trt
rep.	4
d.f.	6
s.e.d.	3.78

Least significant differences of means (5% level)

Table Trt rep. 4 d.f.
6
l.s.d. 9.25

Stratum standard errors and coefficients of variation

Variate: PHT

Stratum	d.f.	s.e.	cv%
Rep	3	3.31	1.9
Rep.*Units*	6	5.35	3.0

Appendix 3. ANOVA for reproductive traits

Variate: Sucrose Content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	2	19.6437	9.8219	54.70	<.001
DAP	2	0.4315	0.2158	1.20	0.345
Trt.DAP	4	14.7524	3.6881	20.54	<.001
Residual	9	1.6159	0.1795		
Total	17	36.4435			

Tables of means

Grand mean 16.308

Trt nws obt swc

	17.267	14.855	16.802

DAP	20	25	30
	16.483	16.333	16.107

Trt	DAP	20	25	30
nws		16.195	17.250	18.355
obt		14.735	15.415	14.415
swc		18.520	16.335	15.550

Least significant differences of means (5% level)

Table Trt DAP Trt
DAP rep. 6 6 2 d.f. 9 9
9
l.s.d. 0.5534 0.5534 0.9585

Stratum standard errors and coefficients of variation

Variate: SC

d.f.	s.e.	cv%
9	0.4237	2.6

Variate: Protein Content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	2	109.072878	54.536439	5885.23	<.001
DAP	2	5.574711	2.787356	300.79	<.001
Trt.DAP	4	10.811056	2.702764	291.67	<.001
Residual	9	0.083400	0.009267		

Total	17	125.542044

Tables of means

Variate: PC

Grand mean 14.284

Trt	nws	obt	swc
	13.385	11.822	17.647

DAP	20	25	30
	13.933	13.850	15.070

Trt	DAP	20	25	30
nws		13.385	13.400	13.370
obt		11.830	11.775	11.860
swc		16.585	16.375	19.980

Least significant differences of means (5% level)

Table	Trt	DAP	Trt DAP
rep.	6	6	2
d.f.	9	9	9
l.s.d.	0.1257	0.1257	0.2178

Stratum standard errors and coefficients of variation

Variate: PC

d.f.	s.e.	cv%
9	0.0963	0.7

Variate: Starch Yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	2	374.4811	187.2406	257.07	<.001

DAP	2	79.7932	39.8966	54.77	<.001
Trt.DAP	4	305.9792	76.4948	105.02	<.001
Residual	9	6.5554	0.7284		
Total	17	766.8089			

Variate: StY

Grand mean 17.53

Trt	nws	obt	swc
	13.67	23.93	14.98

DAP	20	25	30
	20.20	17.34	15.05

Trt	DAP	20	25	30
nws		14.09	12.41	14.50
obt		34.00	22.65	15.15
swc		12.50	16.95	15.50

Least significant differences of means (5% level)

Table	Trt	DAP	Trt DAP
rep.	6	6	2
d.f.	9	9	9
l.s.d.	1.115	1.115	1.931

Stratum standard errors and coefficients of variation

Variate: StY

d.f.	s.e.	cv%
9	0.853	4.9

Appendix 4: Mean, variances and coefficient of variation of the various generations

Parameter	Generation	Mean	Variance	CV%
Leaf Width	P_1	10.92	0.00265	0.471

	P_2	8.334	0.0138	1.412
	F_1	10.54	0.00212	0.437
	F_2	10.42	1.015	9.666
	BC_1	10.95	0.0101	0.916
	BC_2	10.88	0.0504	2.063
Stem Girth	P_1	2.273	0.00135	1.616
	P_2	1.839	0.000915	1.645
	F_1	2.194	0.00347	2.684
	F_2	2.169	0.0489	10.20
	BC_1	2.286	0.00297	2.385
	BC_2	2.202	0.0279	7.590
Plant Height	P_1	92.72	1.199	1.181
	P_2	81.97	0.781	1.078
	F_1	88.71	0.481	0.782
	F_2	89.67	19.73	4.954
	BC_1	92.52	0.328	0.619
	BC_2	92.38	0.440	0.718
Ear Height	P_1	46.16	0.918	2.076
	P_2	40.47	0.219	1.157
	F_1	46.31	0.677	1.776
	F_2	45.39	6.572	5.647
	BC_1	46.22	0.975	2.136
	BC_2	46.28	1.363	2.523

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Appendix 5. Correlation between days after planting and sucrose content

Spearman's rank correlation rho

data: SWEETCORN$SCSW and SWEETCORN$DAP

S = 8, p-value = 0.3333

alternative hypothesis: true rho is not equal to 0

sample estimates:

rho -

1

Spearman's rank correlation rho data:

NS$SCNS and NS$DAP

S = 0, p-value = 0.3333

alternative hypothesis: true rho is not equal to 0

sample estimates: rho

1

Spearman's rank correlation rho data:

OB$SCOB and OB$DAP

S = 6, p-value = 1

alternative hypothesis: true rho is not equal to 0 sample

estimates:

rho -0.5

Appendix 6. Correlation between sucrose content, starch and protein content

> cor.test (SWEETCORN$SCSW,SWEETCORN$STYDSW,method="spearman")

Spearman's rank correlation rho

data: SWEETCORN$SCSW and SWEETCORN$STYDSW

S = 6, p-value = 1

alternative hypothesis: true rho is not equal to 0 sample

estimates:

rho

-0.5

cor.test(OB$SCOB,OB$STYDOB,method="spearman") Spearman's

rank correlation rho

data: OB$SCOB and OB$STYDOB

S = 2, p-value = 1

alternative hypothesis: true rho is not equal to 0 sample

estimates:

rho 0.5

> cor.test(NS$SCNS,NS$STYDNS,method="spearman")

Spearman's rank correlation rho data: NS$SCNS and

NS$STYDNS

S = 2, p-value = 1

alternative hypothesis: true rho is not equal to 0 sample

estimates:

rho 0.5

> cor.test(SWEETCORN$SCSW,SWEETCORN$PCSW,method="spearman")

Spearman's rank correlation rho

data: SWEETCORN$SCSW and SWEETCORN$PCSW

S = 6, p-value = 1

alternative hypothesis: true rho is not equal to 0
sample estimates:

rho

-0.5

> cor.test(OB$SCOB,OB$PCOB,method="spearman")

```
Spearman's rank correlation rho data:  OB$SCOB and
OB$PCOB
S = 8, p-value = 0.3333
alternative hypothesis: true rho is not equal to 0 sample
estimates:
rho
 -1
> cor.test(NS$SCNS,NS$PCNS,method="spearman")
Spearman's rank correlation rho data:  NS$SCNS and
NS$PCNS
S = 6, p-value = 1 alternative hypothesis: true rho is not equal to 0
sample estimates:
rho  -0.5
```

Appendix 7. Principle for sucrose determination using modified anthrone method

The method is based on extraction of sugars with 80% ethanol followed by destruction of reducing sugars with Fehling solution. The use of Fehling solution eliminates the need to evaporate to dryness as reported in the procedure of Van Handel (1968) where reducing sugars are destroyed by Alkali. Upon destruction of reducing sugars, the remaining sucrose is reacted with anthrone in sulphuric acid and absorbance read at 610nm. There are two method involved; manual and automated method. In this work, the manual method was employed.

Appendix 8. Principle for crude protein determination using Kjeldahl method (1990)

The method is based on the digestion of proteins and other organic food components in the sample with sulphuric acid in the presence of catalyst. E.g. Sodium or potassium sulphate to release nitrogen from protein and retain it as ammonium salt. Ammonia gas is liberated upon addition of excess alkali (concentrated sodium hydroxide) and is distilled

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into a boric acid solution to form ammonium borate complex. The ammonia liberated from the complex is titrated with standardized hydrochloric acid. The amount of N in the sample is determined from the milligram equivalent of the acid used. Crude protein is determined by multiplying the nitrogen content with a conversion factor specific to the food matrix.

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