KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI FACULTY OF AGRICLTURE COLLEGE OF AGRICULTURE AND RENEWABLE NATURAL RESOURCES

CONVERSION OF OKOMASA-A NORMAL OPEN POLLINATED MAIZE VARIETY TO QUALITY PROTEIN MAIZE USING THE BACKCROSS BREEDING APPROACH.

BY

TENGAN, KUUBALBANUUR MARTIN LUTHER

JUNE, 2010

CONVERSION OF OKOMASA-A NORMAL OPEN POLLINATED MAIZE VARIETY TO QUALITY PROTEIN MAIZE USING THE BACKCROSS BREEDING APPROACH

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 SCIENCE DEGREE IN AGRONOMY (PLANT BREEDING)

BY

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JUNE, 2010

DECLARATION

I hereby declare that except for the references cited in relation to other works, 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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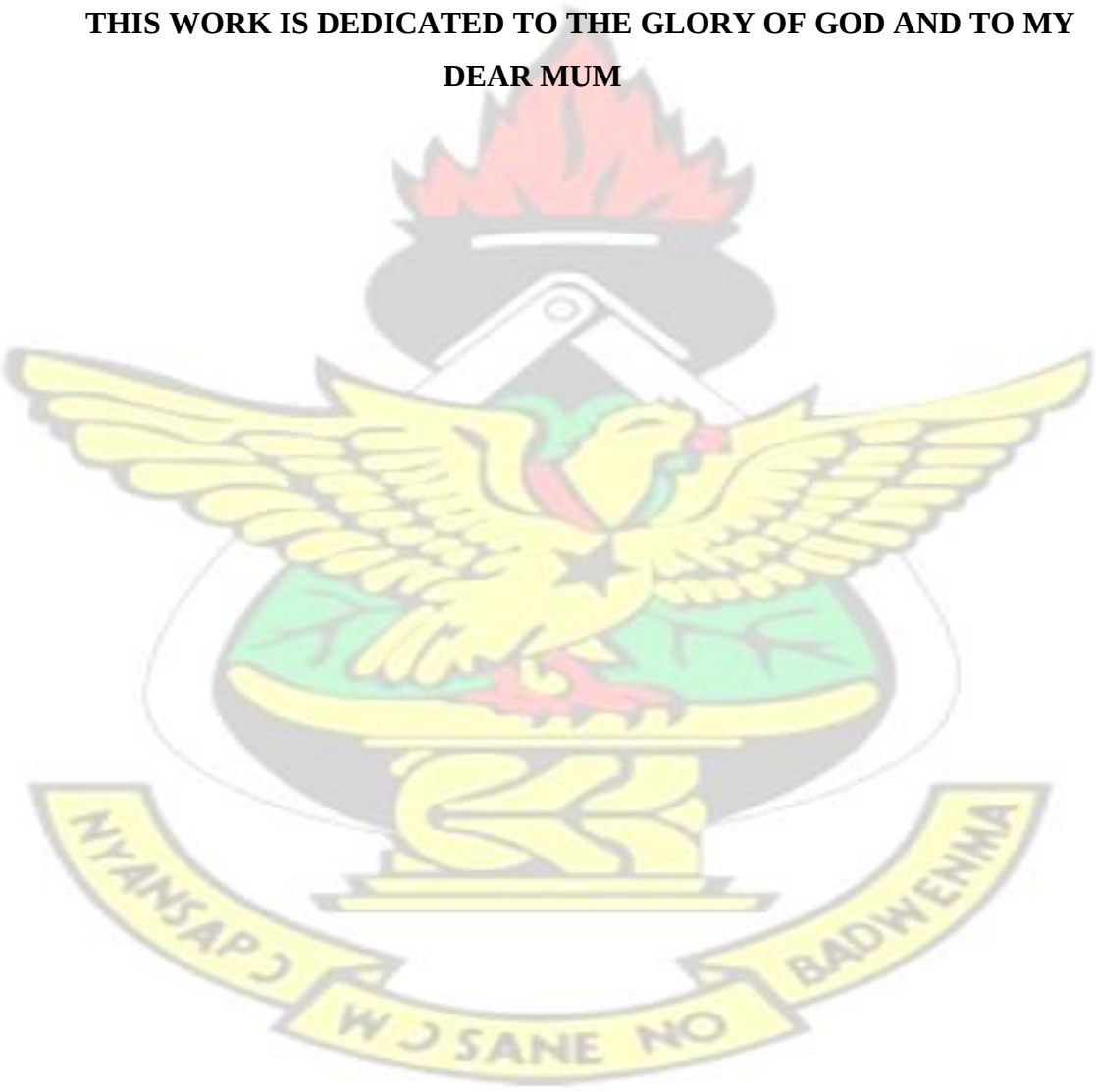
ABSTRACT

Biofortification of cereals especially maize which is the major cereal consumed in Ghana will help increase the health status of Ghanaians. A study was conducted with the main objective of enhancing the nutritional qualities of *Okomasa*, a normal high yielding open-pollinated variety by introgressing the opaque-2 gene, which confers high lysine and tryptophan into the said variety. The study was conducted at the breeding nurseries of the Maize Improvement Programme at the CSIR-Crops Research Institute at Fumesua which is located on Lat $6^{o}\ 45^{1}$N and Long $1^{o}\ 36^{1}$W from 2007 minor season to 2009 minor seaon. *Obatanpa*, a quality protein maize variety was used as the gene source to improve the nutritional status of *Okomasa*. The F_1 was advanced to the F_2 by self pollinating good F_1 plants. Kernels from each cob were screened under the light box. Three levels of kernel modification were observed, less than 25% opaque, between 25%-50% opaque and completely opaque or over 50% opaque. The Chi-square test was employed to test the goodness of fit of the observed ratios to the expected genetic ratios in the F_2 segregating material. The frequency of completely opaque (homozygote dominant) and heterozygotes were lower than the homozygote recessives. Additive genetic variance for plant height, ear height, cob length, cob diameter and rows per cob recorded low values of 8.76, 8.87, 0.13, 0.0, and 0.10 respectively. 95% of the phenotype was attributed to genetic variance, and 5% attributed to genetic variance which implies genetic gain will be maximized. Broad sense heritability values for plant height, ear height, and cob length were high: 0.95, 0.81 and 0.75. Narrow sense heritability values were low for the same traits which is an indication that environmental factors influenced maize production in this particular study. Correlation among parameters studied showed positive values in the two parents with plant height and ear height in both parents being significant. The F_1 generation also showed significant values between plant and ear height.

DEDICATION

THIS WORK IS DEDICATED TO THE GLORY OF GOD AND TO MY DEAR MUM



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BC	Backcross
CIMMYT	International Maize and Wheat Improvement Centre
CIDA	Canadian International Development Agency
COD	Cob diameter
COL	Cob length
CRI	Crops Research Institute
CSIR	Council for Scientific and Industrial Research
et al	And others
EHT	Ear height
FAO	Food and Agricultural Organization
FAOSTAT	Food and Agricultural organization Statistics

GGDP	Ghana Grains Development Project
H^2b	Broad sense heritability
h^2n	Narrow sense heritability
NM	Normal maize
OPV	Open-Pollinated Maize Variety
PHT	Plant height
QPM	Quality Protein Maize
QTL	Quantitative Trait Loci
ROC	Rows per cob
Va	Additive genetic variance
Vd	Dominance genetic variance
Ve	Environmental variance
Vp	Phenotypic variance

CHAPTER ONE 1.0 INTRODUCTION

Maize (*Zea mays* L.) is the third most important cereal crop after wheat and rice in terms of production in the world (Ochse *et al*., 1996). It is grown on more than 96.5 million hectares in the developing world (FAOSTAT–Agric, 2004), and many millions of people worldwide are dependent on maize as a staple food. Maize accounts for 15 to 56% of the total daily calories of people in about 25 developing countries (Prasanna *et al*., 2001).

In Africa maize supplies at least 20% of total daily calories consumed and accounts for 17 – 60% of peoples' total daily protein supplies in 12 countries as estimated by FAO food balance sheets (Krivanek *et al*., 2007). These values are average per capita estimates, specific groups within these countries such as children being weaned, sick children, sick adults and everyone when other crop production is low, are even more dependent on maize as a major source of dietary protein. Protein containing foods are necessary for the rapid growth of children (Millward and Rivers, 1989).

In Ghana, maize ranks top on the list in terms of total production and consumption among cereal crops (Twumasi-Afriyie *et al*., 1992). Ghana has a high per capita utilization of maize, as food and feed, compared to the other cereals produced in the country. For many Ghanaians, maize is one of the major sources of their dietary protein since poverty makes it impossible for such people to afford meat, milk or beans except on special occasions. It contributes an estimated 23% of national protein production compared with 12% by legumes and 2% by meat (Ministry of Agric, 1991., Twumasi-Afriyie *et al*., 1992).

Maize was first introduced into Ghana by the Portuguese in the early sixteenth century, but organized maize research started in Ghana in the 1930's (GGDP, 1986). The focus or mandate of the Institute was to develop high and stable yielding maize varieties that will perform well in all the agro-ecologies in Ghana. Over 25 improved varieties including open pollinated and hybrid maize varieties has since been developed and released to the Ghanaian farming community.

Maize breeding efforts in Ghana were further given a boost in 1979 with the inception of the Ghana/CIDA Grains Development Project (Sallah, 1986). During the period of the project, the maize improvement programme developed and released white and yellow populations with various maturity periods ranging from 80 to 120 days to suit the different agroecological zones of Ghana.

Okomasa, a normal, full-season open pollinated maize variety (OPV) has its origin from CIMMYT Population 43-SR. It was released in 1988 and has a yield potential of 6.5t/ha. It has acceptable agronomic characteristics including resistance to streak and lodging, good ear position and good stand ability, a property some of the popular Quality Protein Maize (QPM) hybrids released do not possess. Farmers prefer this variety because of the attributes enumerated.

Dependency on normal maize as protein source puts people at risk of dietary protein deficiency because normal maize protein (as most cereal protein) is deficient in two essential amino acids, lysine and tryptophan. Therefore, normal maize is a poor source of protein for

both humans and monogastric animals. Thus any maize-based diet lacking in complementary proteinaceous foods that contain greater levels of lysine and tryptophan, such as meat, pulses and dairy products, is considered protein deficient. Protein deficiency especially in children causes kwashiorkor, a potentially fatal syndrome characterized by initial growth failure, irritability, skin lesions, edema and fatty liver (Akuamoa-Boateng, 2002). Thus, for communities that rely heavily on maize as the main staple, maize cultivars with an improved amino acid profile are a must.

Genetic manipulations for increased nutritional value particularly protein content and quality is a noble goal. Realizing achievements through normal breeding efforts are difficult, complex, frustrating and require investments, sustained research efforts, patience and continuing administrative, financial and scientific support (Vivek *et al*., 2008). Penalty in yield and in other economic traits is not accepted unless the product has value added properties. Some of these value added properties include lysine, tryptophan, zinc, iron, Vitamin A and beta-carotene. Several natural maize mutants conferring higher lysine and tryptophan were identified in the 1960's and 1970's, viz opaque-2 (*o2*), floury-2 (*fl 2*), opaque-7 (*o7*), opaque-6 (*o6*) and floury-3 (*fl 3*) (Vietmeyer, 2000). Of these, the *o2* mutant, originally identified in a maize field located in the state of Connecticut, USA (Vietmeyer, 2000) was found to be the most suitable for genetic manipulation in breeding programmes aimed at developing maize high in lysine and tryptophan. Maize homozygous for the o_2 (recessive) mutation was shown to have substantially higher lysine and tryptophan content than was either heterozygous 0_2o_2 or homozygous 0_20_2 (dominant) for the opaque –2 locus (Crow and Kermicle, 2002).

Bressani (1992) showed that increased concentrations of these two essential amino acids in the grain endosperm can double the biological value of maize protein. However, the amount of protein in such maize remains at about 10%, the same as that of common or normal maize endosperm. In other words, the amount of common maize that needs to be consumed to achieve amino acids equilibrium is more than twice as much as the amount of opaque-2 maize (FAO, 1992).

The nutritive value of milk protein is considered higher than that of maize protein. However, milk as a protein is expensive and only very few people in Ghana can afford. Maize homozygous for the *o2* mutants has a quality equivalent to 90% that of milk (Burgoon *et al*.,1992).

The objectives of the study are to:

(i) Increase lysine and tryptophan content of *Okomasa.*

(2) Determine estimates of genetic variations among F_1, F_2, BC_1, BC_2, *Okomasa* and *Obatanpa*.

CHAPTER TWO 2.0

LITREATURE REVIEW

2.1 Evolution of the maize plant.

The evolution of maize (*Zea mays* L. ssp *mays*) from its probable wild progenitor teosinte (*Zea mays* ssp *parviglumis*) provides one of the most striking and complex examples of morphological evolutions in plants. These taxa differ extensively in both plant and inflorescence architecture. The differences are so extreme that when teosinte was first discovered, taxonomists failed to recognize its close relationship to maize, placing it in a separate genus and tribe (Wilkes, 1967). After subsequent research demonstrated that teosinte and maize were fully interfertile, and in essence members of the same biological species, Beadle (1939) proposed that maize is simply a domesticated form of teosinte, and that as few as five major genes largely control the morphological evolution from teosinte.

Maize (*Zea mays* L.) is one of the most robust plants of the *Gramineae* genus. Its stover architecture is similar to that of other grasses (Surányi and Mándy, 1955). The stalk is cylindrical, dense and spongoid inside, and divided into parts called internodes by the nodes. The number of nodes is 8 to 40, depending on the variety. Below the ground, there are about 3-10 nodes close to each other and above the ground 6-30 or more. The height of the stalk is determined by the number and length of the internodes. In this way, plant height can vary from 0.3 m to 7.0 m, depending on the variety and growing conditions. Usually, early maturing varieties are shorter, and late maturing ones are taller. In a tropical climate, where the growing season may be as long as 11 months, some late maturing varieties reach a height of 7 m.

Leaves emerge from the nodes of the stalk or primary stover, and their open husks enfold the whole stalk. Secondary stovers emerge from the axils of the leaves. These are much shorter than the primary stover and carry the female inflorescences, or ears. stovers emerging from the axils of the lowest leaf are called tillers. They sometimes reach the height of the main Stover and may also have ears, but their inflorescences are usually hermaphrodites (Andrejenko and Kuperman, 1961). The upper ear is the main ear. During the growing season only the upper one or two ears are able to develop completely, except in "prolific" varieties which may have more ears. These prolific varieties could play an important part in breeding for higher yield (Motto and Moll, 1983).

2.2 Plant breeding and Crop improvement.

Plant breeding is the art and science of application of techniques for the genetic adjustment of plants for the service of man (Frankel, 1958; Sleeper and Poehlam, 1995) and thus it is a way of exploiting a species' genetic potential. It is an ancient activity dating to the time when man started tilling the soil. Scientific plant breeding, however, started around the time that the laws of genetic inheritance were recognized. The process involves the selection of economically and aesthetically desirable plants by first controlling the mating of selected individuals, followed by further selection among the progeny and repeating the process over time.

It is seen as an accelerated evolution guided by humans rather than nature. Breeders replace natural selection with human selection in order to modify plants genetically to meet the needs of man. The primary goal of plant breeding programmes are commonly improved yields, with disease resistance, pest resistance and stress tolerance, enhanced nutritional qualities

contributing to yield. With new sources of agricultural land dwindling and the human population continuously growing, efforts must concentrate on improving the quantity and quality of food harvested per unit area and developing crops that can grow on low fertility land.

Plant breeding can be accomplished through many different techniques ranging from simply selecting plants with desirable characteristics for propagation, to more complex molecular techniques.

The visual selection of the best germplasm based on plant morphological traits by plant breeders and subsequent merging of such plants into one re-producible unit to obtain the desired genotype was the major approach for crop improvement prior to the use of more sophisticated biochemical and biotechnological tools (Botha & Venter, 2000).

The success of any breeding programme therefore depends on accurate evaluation of genetic potential and an effective recombination to form new gene combinations for further selection. A means to improve adapted genotypes is therefore crucial in any plant breeding programme. However, a major problem that confronts the practical plant breeder is the identification of useful genes from other sources, and combination of the identified genes into one ideal re-producible genotype without disrupting the superior gene complexes of the adapted cultivars (Briggs, 1953).

An ideal plant breeding programme should therefore guarantee the plant breeder an accurate prediction of the nature of the variety to be bred, or having bred such a variety, that the breeding operation could be repeated to produce exactly the same variety (Briggs, 1938). The backcross approach is known to offer the plant breeder such an exact method.

Two reasons advanced by Briggs and Allard (1953) for adopting the backcross breeding method are: that the method offers a scientific approach which assures success; and also, it is economical of the plant breeder's time in that the approach is devoid of laborious note taking and yield trials.

2.3 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 backrossing.

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 cross-pollinated crops. Backcrossing can, in fact, be used for both self- and cross-fertilized crops. It is especially useful in the transfer of specific, simply inherited characters (Belum and Comstock, 1976) 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, 1938). The backcross breeding method is well suited for affecting 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.

2.4 Quality Protein Maize (QPM)

Quality protein maize (QPM) is a maize variety that possesses significantly higher levels of two essential amino acids, lysine and tryptophan as compared to Normal Maize (NM) varieties. The higher levels of lysine and tryptophan are due to the presence of the opaque-2 gene in a homozygous recessive state which contributes to doubling the biological value of maize (Bressani, 1992). It is nutritionally enhanced maize that was 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.

Quality protein maize has higher lysine and tryptophan levels and these levels compared to normal maize contribute to doubling the protein content of QPM. 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 1) which is practically devoid of lysine. Lysine in maize is considered to be the first limiting amino acid and tryptophan second (Vasal, 2002). The

unfavorable 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 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	22.8
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 (fl_2) with similar effects was identified (Nelson *et al.*, 1965). Other mutants that have been identified include opaque-7 (McWhirter 1971), opaque-6 (Ma and Nelson 1975), Mucronate (Salamini *et al*., 1983) and defective endosperm B30 (Salamini

et al., 1979). 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, have shown positive impact on the lives of humans and livestock feed.

Working on the nutritive value of quality protein maize in the diets of broiler chicks, Osei *et al*. (1998) showed that the use of quality protein maize in feeding broilers reduces the amount of fishmeal required from 21%-16%, thereby reducing cost by a maximum of about USD21.00 per ton when the highest amount of QPM was incorporated into broiler diets.

Okai *et al.* (2001, 2007) assessed the growth performance and economic traits of pigs fed diets containing either normal maize or *Obatanpa*- a quality protein maize, and observed that when maize is the sole source of amino acids in the diets of pigs, quality protein maize is of higher nutritional value than normal maize. The work further revealed that QPMcontaining diets are good for pigs' growth and carcass characteristics. Such diets were cheaper because smaller quantities of the more expensive protein-rich ingredients such as fishmeal would be needed for inclusion in pig diets. Furthermore, QPM-based diets tend to provide some savings on the cost of producing pork and it is likely that such savings may ultimately benefit the consumer. The lower amounts of fishmeal that would be used by pig and poultry producers would mean that there may be more of the fishmeal for human consumption. In assessing the performance

of birds fed to normal maize and opaque-2 maize (Okai *et al.,* 2003) again showed that birds receiving QPM diet had significantly higher weights than their counterparts fed on Normal Maize (NM).

The QPM fed birds weighed 708g after eight weeks as against 538g for birds fed on normal maize.

Similar results were recorded elsewhere also with poultry (Fernandez *et al.,* 1974, Bond *et al*., 1991) as well as with pigs (Marroquin *et al*., 1973, Sullivan *et al*.,1989). In all cases animals fed on opaque-2 maize significantly outperformed their counterparts fed on normal maize.

Singh (1977) in a seventeen month supplementation trial conducted in India showed an improvement in growth of children that were fed to QPM over those that were fed to normal maize.

Akuamoa-Boateng (2002), conducted three sets of feeding trials in the Sekyeredumasi district in the Ashanti region of Ghana with weaning children between the ages of four and seven months from 1994 to 2000 to determine the effect of feeding QPM *'koko'* and normal maize (NM) *'koko'* on their growth and development. She observed significant differences in the growth and nutritional status of the children fed with QPM *'koko'*. Mortality rates were three times higher in children fed on Normal Maize *'koko'* and was also significantly less in QPM subjects as compared to NM *'koko'*.

2.5 Genetic analysis.

2.5.1 Phenotypic selection for kernel modification in the F_2 generation

Ravindra *et al.* (2004) in their work on combining high protein quality and hard endosperm traits through phenotypic marker assisted selection in maize reported that kernels from F_2 plants segregated for hardness and levels of modification. The modifications observed on light box screening showed three classes of kernel modifications: less than 25%, 25 to 50% and above 50%. They further observed that the frequency of the fully opaque and completely modified kernels was very low; indicating that there could be several minor genes controlling kernel modification in quality protein maize.

Earlier studies by Lopes and Larkins (1995) revealed the existence of two additive modifier genes that significantly influenced endosperm modification in the population they studied. The biochemical analysis of each of the classes showed that tryptophan and lysine content has recorded up to 100% increase in all the three categories of modifications. The tryptophan content varied from 0.88% to 1.10% in the converted lines of all the categories of modification. The native recipient lines contained 0.38% to 0.41% tryptophan. However, it had been reported earlier that kernel modification of more than 50% opaque had poor storage owing to high grain moisture content and susceptibility to storage pests (Larkins *et al.*,1992). Hence only those kernels that displayed less than 50% opaqueness and contained significant enhancement in the lysine and tryptophan content were selected.

2.5.2 Genetic analysis of endosperm modification in Quality Protein Maize

Lopes and Larkins (1995) again worked on genetic analysis of endosperm modification in Quality Protein Maize and reported that opaque-2, modified opaque-2 , their reciprocal $F_{1's}$ and segregating progenies indicated that the increased deposition of gamma-zein, a cysteine rich storage protein that contains no lysine is dependent on the dosage of opaque modifier genes and directly correlated to seed modification.

Further work by Lopes and Larkins (1991), Geetha *et al*. (1991), and Larkins *et a*l. (1992) showed that the degree of endosperm modification and the increase in the synthesis of gamma-zein in the mRNA and protein are all correlated and dependent on the dosage of modifier genes in the triploid endosperm

Mertz *et al.* (1964) earlier on reported on the biochemical effects of the opaque-2 mutation on protein quality. They observed that mutant seeds were found to accumulate twice the normal concentration of lysine and tryptophan. They attributed this increase concentration of these amino acids to the drastic reduction in storage protein (zein) accumulation and the increase accumulation of non-zein endosperm protein which is high in lysine and tryptophan.

Ortega and Bates (1983), and Lopes (1993) again reported of a consistent biochemical alteration associated with endosperm modification which leads to a slight decrease in the content of lysine and tryptophan in the seed. Habben *et al*. (1993) concluded in a study of the nutritional quality of opaque-2 maize that the quality is a function of the content of nonzein

proteins in the endosperm, and increased accumulation of this protein fraction occurs in opaque-2 compared to normal maize.

2.5.3 Environmental variance

Genetic causes are not the only reason for the resemblance between relatives; there are also environmental circumstances that tend to make relatives resemble each other; some sorts of relatives more than others. Environmental variance, which by definition embraces all variation of non-genetic origin, can have a great variety of causes and its nature depends very much on the character and the organism studied (Falconer, 1960). The environmental variance is dependent on the conditions of culture or management as well as natural conditions such as those that are controlled by the climate. Maize genotypes vary in their response to the environment in which they are grown (Mosisa *et al*., 2001).

2.5.4 Genetic variance

Generally variation may be defined as the differences among parents and their offspring or among individuals within a population. Genetic variation specifically is the phenotypic differences that result from the presence of genotypes in a population. Two types of genetic variation are identifiable, continuous variation (quantitative) and discontinuous (qualitative or discrete). In continuous variation, a character exhibits a continuous range of phenotypes in the population. Most of the economically important characters in plants, such as grain yield, quality, protein or oil content as well as height, maturity, weight and color intensity are examples of continuous variations. This distribution is the result of interaction between many genes and the crop production environment. In continuous variation, statistical parameters like

means and variances are used to measure the response to selection. Progress in phenotypic selection for quantitative traits is difficult unless their heritability (h^2) is high.

Falconer and Mackley (1996), defined heritability as the proportion of the variance of the phenotypic variance that may be attributable to genotypic effects. In discontinuous variations a character is found in two or more distinct and separate forms. Examples of discontinuous variations are discrete phenotype variables such as grain color and texture. A measure of genetic variation is given by the amount of heterozygosity at a locus in a population which is given by the total frequency of heterozygotes at that locus. Variability constitutes an important source of genetic variation which in turn is a major prerequisite for genetic enhancement. Evidence of successful utilization of genetic variation for crop improvement has been reported.

Ahloowalia *et a*l. (2004) used induced mutation in groundnuts *(Arachis hypogaea)* to generate over 2000 mutants of groundnuts that were released into the world. Landraces and introductions from other programmes (Zenglu and Nelson, 2001) may also serve as a good source of genetic variability.

Akanvou *et al*. (1996), working on the estimation of genetic variance and interrelationship of traits associated with *striga* resistance in maize, reported that date of flowering, plant height, ear height, number of *striga* plants emerged per row at 8 weeks after planting showed that estimates of additive variance were lower than dominance variance. Plant height and ear height of infected plant were genetically correlated (rg = 0.89 ± 0.52). Similar results were

reported by Sturber and Moll (1971).Their study revealed the presence of genetic interrelationships between yield and number of ears, plant height, and ear height.

Genetic correlations between plant height and ear height showed high and positive value.

2.6 Heritability

Heritability of a metric character is one of its important properties, and this expresses the proportion of the total variance that is attributable to the average effects of genes, and this is what determines the degree of resemblance between relatives (Falconer, 1960). The most important functions of heritability in the genetic study of metric characters are its predictive role, expressing the reliability of the phenotypic value as a guide to the breeding value. Only the phenotypic values of individuals can be directly measured, but it is the breeding value that determines their influence on the next generation. If a breeder therefore chooses parents according to their phenotypic values, his success in changing the characteristics of the population can be predicted only from the knowledge of the degree of correspondence between the phenotypic values and breeding values.

This degree of correspondence is measured by the heritability which is defined in broad sense as the ratio of genetic variance to phenotypic variance [H^2=Vg/Vp]. The narrow sense heritability which is of importance to the breeder is defined as the ratio of additive variance to the phenotypic variance [h^2= Va/Vp].

Heritability estimates allow breeders to develop more efficient selection strategies

(population structure and size, selection differential) and to predict gain from selection (Allard. 1999). Quantitative Trait Loci (QTL) for resistance to *C. zeae-maydis* has been identified but only a few estimates of the heritability of resistance have been calculated. While it is useful to have an estimate of the total genetic effect on a particular trait, such as broad-sense heritability, narrow-sense heritability provides a better estimate of the breeding value (Allard, 1999). Parent offspring regression is an effective means of estimating breeding value (Foolad and Jones, 1992).

The heritability of plant height in maize is high, with an h^2 value of about 0.8 (Schön *et al.*, 1993; Daniel and Bajtay, 1975). In diallel trials, it showed significant genotypic variance and strong positive heterosis. Bajtay and Daniel (1976) found positive heterosis even when crossing sweetcorn inbreds. The reason for this great heterosis in plant height could be overdominance, the accumulation of dominant alleles in different loci or epistasis (Hallauer and Miranda, 1981).

Nawar *et al.* (1991) estimated the genotypic and phenotypic variances and co-variances for plant height, and there were many authors who found significant variance for this trait (Malvar *et al.*, 1996; Hansen and Baggett, 1977; Subandi and Compton, 1974). The significant variance existing in plant and ear heights is due to the multiplication of alleles and to overdominance, according to many research findings. Either dominance (Guo *et a*l., 1986) or additive effects (Russell, 1976) are considered more important in the expression of plant and ear heights, though some authors assign the differences in plant and ear heights to extra chromosomal effects (Baynes and Brawn, 1973).

2.7 Correlation of traits in maize

Plant height is strongly associated with flowering date, both morphologically and ontogenetically, because internodes formation stops at floral initiation, which means that earlier flowering maize is usually shorter (Troyer and Larkins, 1985). Earliness and high yield were considered to be in reciprocal ratio to each other. In Hungary, Fleischmann (1974) proposed first the necessity of breaking this negative correlation. Modern varieties produce high yields despite flowering early. There is also a correlation between earliness and ear height. The higher the ear is, the later the plant matures (Surányi and Mándy, 1955), but earliness and lower ear height have no absolute reciprocality.

There are correlations between many other traits and plant height. The number of leaves (Allen *et al*., 1973) and the grain yield (McKee *et al*., 1974) are significantly correlated with plant height. In sweet corn, the grain yields (Tan and Yap, 1973) and ear length (Hansen, 1976) showed significant positive correlations with plant and ear height. In popcorn, the grain yield had a positive correlation and the popping expansion a negative correlation with both characters (Verma and Singh, 1979). Obilana and Hallauer (1974) found a significant correlation between plant and ear heights in unselected inbred.

Several workers have attempted to determine linkage between the characters on which the selection for high grain yield can be made. Annapurna *et al*, (1998) found that seed yield was significantly positively correlated with plant height, ear diameter, number of seeds per row and number of rows per cob. You *et al*. (1998) reported significant correlations between yield and number of rows per cob, number of grains per row and 1000-grain weight and also number

of grains per row and number of rows per cob. Khatun *et al*. (1999) observed that grain yield per plant was positively and significantly correlated with 1000-grain weight, number of kernels per cob, ear weight and ear insertion height. Orlyan *et al*. (1999) found that the most important traits influencing grain yield are number of grains per row and number of grains per cob. Characters like number of grains per row, 1000-grain weight, and cob diameter and plant height are useful in improving grain yield in hybrids. Maximum correlation of grain yield was obtained with number of kernels per row followed by plant height and cob length (Gautam *et al*., 1999). Cantarero (2000) found that late sowing reduced number of ears per plant, number of grains per ear and grain yield.

CHAPTER THREE 3.0

MATERIALS AND METHODS

3.1 Maize varieties used.

Two sources of open pollinated maize varieties (OPV's), *Okomasa* (Plate 1) and *Obatanpa* (Plate 2), both released by the CSIR-Crops Research Institute in 1988 and 1992, respectively, were used in the study.

Obatanpa 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. It has also been released formally in other African countries under various names including '*Faaba*' in Benin, '*Debunyuman*' in Togo and Mali, and '*Susuma*' in Mozambique. 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 5.4 ton/ha and is widely grown by Ghanaian farmers. *Okomasa* is a full season maize variety that is widely adapted throughout all the agro ecologies of Ghana. It has its source from CIMMYT Population 43 and is also a white dent material with a yield potential of 6.5ton/ha.

3.2 Experimental Location.

The study was carried out at the breeding nursery of the CSIR-Crops Research Institute at Fumesua, Kumasi which is located on Lat. 6^{o} 43'N and 1^{o} 36'W., and falls within the Forest zone of Ghana. The location experiences two rainy seasons, the major season which stretches

from April through July and the minor season from August to November. The soils at Fumesua are classified as Ferric acrisols and belong to the Asuansi series with about 5cm thick top layer of dark gritty clay loam

3.3 Field planting and hybridization

In the field, three major activities were carried out and these included:

(1) Development of the F_1 material,

(2) Advancing the F_1 material to the F_2, and

(3) Recovery of the recurrent parent through two backcrosses.

Plate1: Cobs of Okomasa (Normal OPV- Recurrent parent)

Plate 2: Cobs of *Obatanpa* (QPM donor parent)

3.3.1 Development of F_1's

During the minor season of 2007, the development of F_1's was carried out. Sixty rows of *Okomasa*, a normal open-pollinated variety (OPV) were planted as the female alongside twenty rows of *Obatanpa*, a Quality Protein Maize (QPM) composite in a crossing block. Before the appearance of the silk, developing ears were protected with a transparent plastic bag to ensure that emerging silks are not contaminated with unwanted pollen. At anthesis, pollen was collected using brown paper bags from agronomically good plants in the *Obatanpa* population, bulked and used to pollinate equally good plants in the *Okomasa* block.

At harvest forty-five clean cobs devoid of rot and of good husk cover were selected. These were dried and shelled separately into envelopes for the next breeding program in the next season.

3.3.2 Production of F_2 seed

The forty-five F_1 families were planted ear to row during the 2008 major season. Row length was 5m long with spacing of 45cm between hills and 75cm between rows. Twelve hills per row with three seeds per hill was obtained and thinned to one plant per hill at establishment. At flowering, three to four good plants in each row or family were selfed to generate the F_2 progeny. At harvest 96 F_2 families were selected, dried and shelled separately into envelopes. Each family was screened on the light box and the modified kernels i.e. the kernels carrying the heterozygote (O_2O_2) as well as the homozygote dominant O_2O_2 and homozygote recessives o_2o_2 were counted and their numbers recorded.

3.3.3 Backcrossing

During the 2009 major season, the 96 F_2 families that were subjected to light box screening, and the selected modified kernel with a modification score of between 25% and 50% were planted ear-to-row. Also, ten rows each of the donor (*Obatanpa*), the recurrent parent (*Okomasa*), and the F_1 progeny were planted alongside the F_2 Families in a crossing block. At flowering pollen was collected from healthy plants in the recurrent (*Okomasa*) population and used to pollinate three to four good plants per family in the F_2 Families to generate backcross one (BC_1). The following crosses were also made: F_1 x Recurrent parent (*Okomasa*)

1. F_1 x Donor parent (*Obatanpa*)
2. F_1 x F_1……F_2

At harvest two hundred and seventy-six BC_1 families were selected. These were shelled earto-row. During the 2009 minor season the 296 BC1 families were planted. A random sample of one hundred families was sent to IITA-Ibadan, Nigeria for biochemical analysis to determine

their lysine and tryptophan levels. At flowering, three good plants from each family were selfed to generate BC_1S_1. After harvest the BC_1S_1 families were subjected to light box screening to select those families carrying the opaque-2 modifiers. These will be advanced to BC_2 in 2010 and later possibly recombined to form an experimental variety.

Ten rows each of the two parents and the crosses that were generated between the F_1 and the two parents were also planted and agronomic data including days to 50% silking, plant height, and ear height were taken. Other parameters taken after harvest included cob length, cob diameter, and number of rows per cob.

3.3.4 Data collection

The following quantitative traits were measured in the field during the pre-harvest stage:

1. **Plant height**: The height of twenty plants in centimeters were randomly selected and measured with a graduated measuring stick from the ground level to the node bearing the flag leaf
2. **Ear height**: The height of the ear from ground level to the node bearing the uppermost ear from the same plants from which plant heights were recorded were also measured.

The following post harvest data were also recorded:

1. **Cob length**: The lengths of 20 randomly selected cobs were measured in centimeters using a caliper.
2. **Cob diameter**: The diameter of the 20 cobs of which the lengths were measured was also measured using a caliper.

3. **Rows per cob**: The numbers of rows per cob of the 20 cobs were counted and the average recorded.

Other investigations undertaken were in the laboratory, and these included the following:

1. The 276 F_2 material generated were screened under light box and the ratios of Homozygote dominant O_2O_2, heterozygote O_2o_2, and homozygote recessives o_2o_2 counted and recorded. (Plate 3)
2. Proximate analysis of bulk samples of the parents and the crosses generated to determine the percentages of crude protein (CP), crude fiber (CF), and ash was undertaken at the QPM laboratory at Crops Research Institute.

3.3.5 Data analysis

1. The chi-square test was employed to determine if the ratios of segregations observed at F_2 s fitted expected genetic ratios.

3. Genetic analysis using GenStat statistical package was employed to generate means and then compute the variances.

- Environmental variance (Ve)

This was arrived at using the formular:

Ve = Vp1+Vp2+VF_1/3 where Vp1= variance of parent 1 (*Okomasa*), Vp2= variance Of parent 2 (*Obatanpa*), and VF_1 = variance of the Cross between the two parents.

- Additive genetic variance (Va)
  This is also computed using:

$2VF_2 – (VBC_1 + VBC_2) = 1/2a$, where VF_2 = variance of the F_2, VBC_1= variance of BC_1, and VBC_2 = variance of BC_2.

- Dominance genetic variance (Vd) = $4(VF_2) – 2a - 4e$

- Broad sense and Narrow sense heritability are computed as follows:

1) Broad sense heritability (H^2b) = Vg/Vp, where Vg = genetic variance and Vp = phenotypic variance.
2) Narrow sense heritability (h^2n) = Va/Vp, where Va = additive genetic variance and Vp = phenotypic variance.

CHAPTER FOUR RESULTS

4.1 Genetic analysis of kernel modification in the F_2 generation.

Kernel modification at the F2 generation and BC1S1 were studied. The results are presented in table 2 below.

Table 2.Genetic analysis of kernel modification in the F2 generation.

Acc. No.	OO >50%	Oo 25-50%	oo <25%	Total	---Observed--- QPM	Non QPM.	Chi-squared on expectation of 1Qpm: 3 non Qpm.
1	45	104	277	426	45	381	0.372
2	86	78	375	539	86	453	0.147
3	68	70	322	460	68	392	0.185
4	65	210	275	550	65	535	1.366
5	92	84	345	521	92	429	0.094 6
78	88	319	485	78	407	0.139	
7	69	122	347	538	69	469	0.264 8
47	70	279	411	47	349	0.313 9	
21	104	389	514	21	493	0.775 10	
18	60	290	368	18	350	0.717	
11	35	50	283	368	35	333	0.425 12
19	117	223	358	19	340	0.691	

Chi-square calculated less than corresponding tabular value (5% 1.d.f= 3.841) indicates that the Observed value shows a goodness of fit to the genetic ratios expected

Table 3. Genetic analysis of kernel modification in the BC1S1 generation.

Acc. No.	OO >50%	Oo 25-50%	oo <25%	Total	--Observed--- QPM	Non QPM.	Chi-squared on Expectation 1Qpm: 3 Non Qpm
1-2-1	78	298	22	398	100	298	0.048

1-2-2	**101**	**321**	**21**	**443**	**111**	**332**	**0.111**
3-1-1	**30**	**10**	**40**	**80**	**20**	**60**	**0.278**
4-2	**50**	**270**	**5**	**325**	**81**	**244**	**0.166**
6-3	**99**	**308**	**10**	**417**	**104**	**313**	**0.002**
6-5-1	**65**	**228**	**15**	**308**	**77**	**231**	**0.026**
6-5-2	**11**	**320**	**8**	**384**	**96**	**288**	**0.828**
8-1-1	**67**	**309**	**15**	**391**	**98**	**293**	**0.111**
8-1-3	**105**	**290**	**4**	**399**	**100**	**299**	**0.002**
8-2-1	**58**	**270**	**11**	**339**	**85**	**254**	**0.112**
8-2-2	**71**	**230**	**15**	**316**	**79**	**237**	**0.001**
8-4-1	**49**	**106**	**79**	**234**	**59**	**176**	**0.032**
9-1-1	**85**	**320**	**25**	**430**	**108**	**323**	**0.050**
10-2	**120**	**300**	**4**	**424**	**106**	**318**	**0.837**
10-3-1	**90**	**320**	**32**	**442**	**111**	**332**	**0.040**
16-1-2	**83**	**169**	**105**	**357**	**89**	**268**	**0.005**

Chi-square calculated less than corresponding tabular value (5% 1.d.f= 3.841) indicates that the observed value shows a goodness of fit to the genetic ratios expected

Kernels were observed to have segregated into three levels; above 50% or completely opaque, 25%-50% opaque, and below 25% opaque. The frequencies of kernels above the 50% or fully Opaque (homozygote dominant) were observed to be lower than the heterozygote (between 25-50%) as well as the homozygote recessives (below 25% opaque).

The observed ratios of QPM to Non QPM ranged from 107:423 (1:4) to 6:470 (1:10). The expected ratios of QPM to Non QPM recorded values between 90:269 kernels and 138:413. The calculated chi-square values range between 0.041 and 1.366, which are less than the

corresponding tabulated chi-square value of 3.841 at 5% 1 df, indicating that the observed ratios of QPM to Non QPM showed a goodness of fit to the expected genetic ratios.

The heterozygotes in the F_2 materials were backcrossed to the recurrent parent and further selfed the following season to obtain BC1S1. These were also subjected to light box screening and the results are presented in Table 3. Contrary to the observations in the F_2 generation, the frequencies of the homozygote dominant and recessives were lower than the heterozygote, but recorded similar trend in the ratio of QPM to Non QPM as in the F_2 generation. Observed ratios ranged, between 20:60 and 111:332. Calculated chi-squared

values ranged between 0.001 and 0.837, which were below the corresponding tabulated chisquared value of 3.841($P<0.05$) 1 df; indicative of a goodness of fit to the expected.

4.2. Components of variation and related parameters.

Components of variation studied included environmental variance, additive genetic variance, dominance genetic variance and phenotypic variance of the measured parameters including plant height, ear height, cob length, cob diameter, and rows per cob. Broad sense (H^2b) and narrow sense (h^2n) heritability were estimated for. The results are shown in Table 4 below.

Table 4. Environmental Variance (Ve), Genetic Variance (Vg), Phenotypic Variance (Vp) and Heritability Estimates of Measured Parameters.

Ve	Va	Vd	Vp	H^2 b	h^2n

Plant height	8.76	38.54	114.46	161.46	0.95	0.24
Ear height	8.87	16.46	22.60	47.93	0.81	0.34
Cob length	0.13	0.02	0.36	0.51	0.75	0.04
Cob diameter	0.0	0.0	0.0	0.0	0.0	0.0
Rows per cob	0.10	-0.18	0.2	2.6	0.5	-0.9

Ve = Environmental variance. Va = Additive genetic variance. Vd = Dominance genetic variance. Vp = Phenotypic variance. H^2b =Broad sense heritability. h^2n = Narrow sense heritability.

For environmental variance, plant height recorded 8.76 whilst ear height and cob length recorded 8.87 and 0.13 respectively.

Cob diameter and rows per cob recorded 0.0 and 0.10, respectively. Additive genetic variance was -0.18 for rows per cob, 0.0 for cob diameter and 0.02 for cob length. Plant and ear heights recorded 38.54 and 16.46, respectively.

Dominance genetic variance were higher with plant height recording a value of 114.46 and 22.60 for ear height. Cob length and cob diameter had values of 0.36 and 0.0, with rows per cob recording 0.20. Broad sense heritability for plant height, ear height and cob length are highly significant: 0.95, 0.81 and 0.75, respectively.

Narrow sense heritability, which is of utmost importance to the breeder, recorded very low values of 0.24, 0.34, and 0.04 for plant height, ear height, and cob length, respectively. The low values indicated that the environmental influence is minimal to the phenotype the variety under study. This means that plant height, ear height, and cob length be improved through the manipulation of genes.

Table 5. Variation of plant and ear height of maize generations

Parameter	Generation	Mean	Variance	CV%
Plant height	*Okomasa* (P1)	216.8	9.07	1.38
	Obatanpa (P2)	202.6	4.31	1.03

	F_1	201.3	12.89	1.78
	F_2	253.9	56.57	2.95
	BC_1	209.4	14.09	1.81
	BC_2	264.4	22.70	1.82
Ear height	*Okomasa* (P1)	133.3	11.91	2.59
	Obatanpa (P2)	108.3	4.30	1.91
	F_1	146.1	10.41	2.21
	F_2	102.2	22.75	4.67
	BC_1	110.7	18.73	3.94
	BC_2	107.1	7.24	2.51

Table 6. Variation in cob characteristics of maize generations.

Parameter	Generation	Mean	Variance	CV%
Cob length	*Okomasa* (P1)	20.1	0.15	1.90
	Obatanpa (P2)	19.2	0.18	2.20
	F_1	21.5	0.07	1.21
	F_2	19.7	0.23	0.24

		BC_1	20.3		0.07	0.13		
		BC_2	21.4		4.4	0.02		
Cob diameter	*Okomasa* (P1)		4.7		0.0		0.0	
Obatanpa (P2)	4.6		0.0		0.0			
		F_1	4.8		0.0	0.0		
		F_2	4.8		0.0	0.0		
		BC_1	4.7		0.0	0.0		
		BC_2	4.9		0.0	0.0		
Rows per cob	*Okomasa* (P2)	15.0		0.18		2.80		
	Obatanpa (P2)	16.4		0.10		1.95		
		F_1	15.3		0.01	0.65		
		F_2		14.5		0.08		1.93
BC_1	14.7		0.0		0.0			
		BC_2	15.3		0.11	2.16		

Mean plant height value for BC_2 recorded the highest of 264.4 cm, followed by the F_2 generation (253.9 cm), which are far above the mean of the parents P1 and P2 (Table 5 above). The F_1 recorded the lowest value of 201.3 cm. Mean F_1 and BC_1 values were within parental limits.

Mean ear height followed a different trend. The F1 generation recorded the highest value of 146.1cm, followed by P1 (133.3 cm), BC_1 (110.7 cm), P2 (108.3 cm), BC_2 (107.1 cm) and F_2 recording the lowest value of 102.2 cm. The F_2 recorded the highest variance of 56.57 for

plant height, followed by BC_2 (22.70), with P2 recording the least of 4.31. For ear height F_2 again recorded the highest variance of 22.75%, followed by BC_1 (18.73%).

P2 again had the lowest variance value of 4.30. For plant height, the F_2 recorded the highest coefficient of variation of 2.95% with P2 recording a value of 1.03%. Ear height of the F_2 generation recorded the highest CV of 4.67%, and P2 ranking lowest (1.91%). The values recorded for plant and ear heights in the parents and their crosses are an indication of the predominance of minor genes controlling the two traits. This is confirmed by the low narrow sense heritability of 0.24 and 0.34 (Table 4).

Mean cob length recorded 21.5 cm in the F_1 generation as the longest, followed by BC_2 (21.4 cm), BC_1 (20.3 cm), P1 (20.3 cm) with the F_2 generation recording the shortest of 19.7 cm (Table 7). BC_2 obtained the highest value for variance, followed by F_2, P2, P1, with BC_1 and F_2 recording 0.07 each. Coefficients of variation were generally very low with P2 recording 2.20% being the highest, followed by P1 (1.90%), with BC_2 recording the lowest CV value of 0.02%. Values for cob diameter ranged between 4.6 cm and 4.9 cm. These values were credited to P2 and BC_2 respectively. BC_1 and P1 recorded 4.7 each while F1 and F2 recorded 4.8 each, indicating that the sizes of the cobs were very uniform. Variances and coefficient of variation were therefore 0.0 and 0.0% respectively. Number of rows per cob ranged between 14.5 in the F_2 generation and 16.4 in the P2. P1 recorded the highest coefficient of variation value of 2.80% followed by BC_2 (2.16%), P2 (2.16%), F_2 (1.93%) and F_1 (0.65%) in that order. BC_1 recorded less variation since variance and coefficient of variation were both zero.

4.3 Association among traits in various generations.

In P1, all the traits or parameters measured showed positive correlation ranging from 0.15 for plant height and cob length, to 0.82 for plant height and ear height (Table 7). Plant height and ear height, as well as number of rows per cob and cob diameter were highly significant (0.82 and 0.68) respectively (Table 7). Similar trends were observed in P2 (Table 8) where all the traits showed positive correlation, with plant height and ear height being significant (0.77). Ear height and number of rows per cob recorded the lowest correlation value of 0.05 (Table8). In the F_1, 50% of traits recorded negative correlations, but again plant height and ear height recorded positive and significant value of 0.73(Table 9). F_2, BC_1, and BC_2 recorded low to medium values, with between 20% and 50% of the traits being negatively correlated, (Table 10-12).

Table 7. Correlations among phenotypic traits of *Okomasa* (P_1)

	COD	COL	EHT	PHT	ROC
COD					
COL	0.36				
EHT	0.39	0.23			
PHT	0.23	0.15	0.82*		
ROC	0.68*	0.15	0.42	0.20	

*Significant at 5% level

COD= Cod diameter, COL= Cob length, EHT= Ear height, PHT= Plant height, ROC=Rows/cob.

Table 8. Correlations among phenotypic traits of *Obatanpa* (P_2)

	COD	COL	EHT	PHT	ROC
COD					
COL	0.25				
EHT	0.09	0.18			
PHT	0.06	0.22	0.77*		
ROC	0.06	0.19	0.05	0.08	

*Significant at 5% level

COD= Cod diameter, COL= Cob length, EHT= Ear height, PHT= Plant height, ROC=Rows/cob

Table 9. Correlations among phenotypic traits of the F_1 generation

	COD	COL	EHT	PHT	ROC
COD					
COL	0.07				
EHT	0.00	0.19			
PHT	0.00	0.00	0.73*		
ROC	0.00	0.02	0.00	0.04	

*Significant at 5% level

COD= Cod diameter, COL= Cob length, EHT= Ear height, PHT= Plant height, ROC=Rows/cob

Table 10. Correlations among phenotypic traits of the F_2 generation

	COD	COL	EHT	PHT	ROC
COD					
COL	0.52				
EHT	0.01	0.04			
PHT	0.47	0.20	0.39		
ROC	0.00	0.02	0.12	0.00	

*Significant at 5% level

COD= Cod diameter, COL= Cob length, EHT= Ear height, PHT= Plant height, ROC=Rows/cob

Table 11. Correlations among phenotypic traits of BC_1 generation

	COD	COL	EHT	PHT	ROC
COD					
COL	0.20				
EHT	0.00	0.00			
PHT	0.00	0.23	0.42		
ROC	0.00	0.00	0.00	0.00	

COD= Cod diameter, COL= Cob length, EHT= Ear height, PHT= Plant height, ROC=Rows/cob

Table12. Correlations among phenotypic traits of BC_2 generation

	COD	COL	EHT	PHT	ROC
COD					
COL	0.02				
EHT	0.14	0.23			
PHT	0.00	0.26	0.47		
ROC	0.20	0.09	0 00	0.09	

COD= Cod diameter, COL= Cob length, EHT= Ear height, PHT= Plant height, ROC=Rows/cob

CHAPTER FIVE DICUSSION

The study had the objective of enhancing the nutritional qualities of a high yielding open pollinated maize variety and conducting studies into the genetics of backcross breeding that could enhance selection gain.

Very little progress can be achieved in plant breeding without information on the mode of inheritance of traits of economic importance such as the opaque-2 gene.

The study is very relevant in the area of genetics and plant breeding especially with backcross breeding which involves the transfer of desirable traits from one genotype (donor) to another (recurrent parent) in order to improve the quality of the recurrent parent which lacks that trait being transferred while trying to maintain the traits of the recurrent parent.

The introgression of the opaque-2 gene which is in the homozygous recessive state into maize cultivars which are deficient in high lysine and tryptophan such as *Okomasa* is long

overdue because the variety under improvement, (*Okomasa*), possesses a yield potential that competes favourably with any of the quality protein maize hybrids in the national maize programme.

5.1 Genetic analysis of kernel modification in the segregating generations

Kernel modification of the F2 and BC1S1 generation recorded lower frequencies of QPM to Non-QPM., that is, the number of kernels that were fully opaque, otherwise known as the homozygote dominant kernels and the heterozygote were lower than the homozygote recessives (Plate 4).

Plate 3. Ears of F_2 segregated material. Bleached white kernels are the completely opaque material. (Right of plate 4 below).

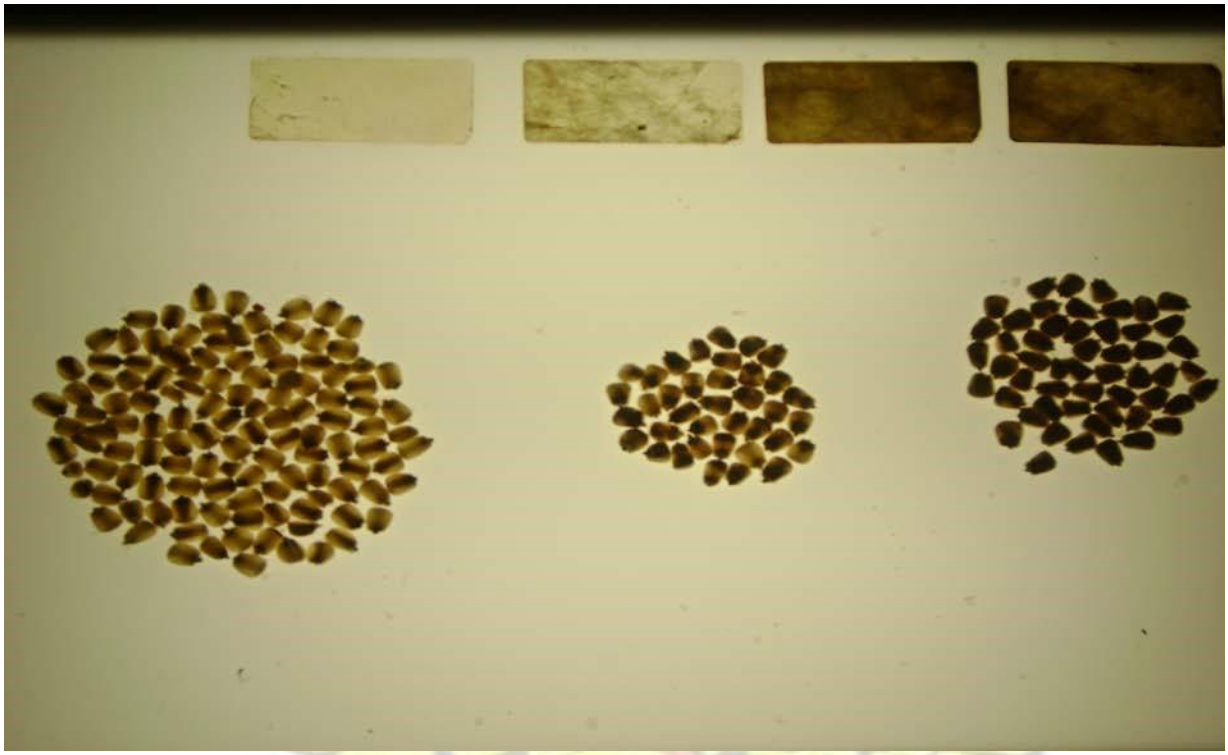

Plate 4. Kernel modifications as observed on a light box: (from left, <25% opaque, 25-50% opaque and completely or >50% modification)

Notwithstanding their frequencies, the ratio of QPM to Non-QPM recorded various values, but showed a goodness of fit to the expected 1:3 genetic ratios. Similar results were reported by several scientists. Ravindra *et al* (2004) in their work on combining high protein quality and hard endosperm traits through phenotypic marker selection in maize reported that kernels from F2 plants segregated for hardness and various levels of modification. They further reported that the frequency of the fully opaque and completely modified kernels are very low,

indicating that there could be several minor genes controlling kernel modification in quality protein maize.

Earlier studies by Lopes and Larkins (1995) revealed the existence of two additive modifier genes that significantly influenced kernel modification in maize.

5.2 Correlations among parameters measured

Correlation studies in plant breeding enables the breeder to, in a way predict the consequences in selecting for a particular trait. For example if two traits are positively correlated, it means that selecting for one trait assures the breeder that the other trait is also being selected for.

The study showed that in the both recurrent and donor parents all the measured traits recorded positive values, with plant and ear heights being highly positive. The other generations including the F_1, F_2, BC_1, and BC_2 recorded some levels of negative and very low values. This observation is contrary to those reported by several scientists, for example, Allen *et al* (1973), and Mckee *et al* (1974), reported that there were correlations between many traits and plant height especially number of leaves and grain yield. The negative correlation values observed for the F_1, F_2, BC_1 and BC_2 could be attributed to a moisture stress experienced during the reproductive phase of growth.

Several scientists have attempted to determine linkage between characters by which the selection for high yield can be made. Annapurna *et al* (1998) found that seed yield was significantly positively correlated with plant height, ear diameter, number of seeds per row

and number of rows per cob. You *et al* (1998) reported significant correlations between yield and number of rows per cob, number of grains per row and 1000 grain weight, and also number of grains per row and number of rows per cob. Khatun *et al* (1999) reported that the most important traits influencing grain yield were number of grains per row and number of grains per cob.

Characters like number of grains per row, 1000 grain weight, and cob diameter and plant height are useful in improving grain yield in maize hybrids. Highest correlation of grain yield was obtained with number of kernels per row, followed by plant height and cob length (Gautam *et al.*, 1999).

Cantarero (2000) found that late sowing reduced number of ears per plant, number of grains per ear and grain yield.

The negative correlation values recorded in the F_1, F_2, BC_1 and BC_2 could have been attributed a moisture stress experienced during the reproductive period of growth, and this could be averted by remedying the situation by ensuring adequate moisture supply.

Results from this study showed similar trend in some cases especially in *Okomasa* (P1) and *Obatanpa* (P2) where all the parameters measured, showed positive correlation, with plant height and ear height giving significant values. Plant height is known to correlate positively with many traits Allen *et al* (1973) and Mckee *et al* (1974). Those families that were successfully converted and showed opaqueness should be recombined and evaluated for yield

in preliminary and advance yield trials in multi-locations, to ascertain adaptability and for eventual release.

CHAPTER SIX SUMMARY AND CONCLUSION

The study on the conversion of normal maize to quality protein maize using the backcross approach favoured a recessive gene, the opaque-2 gene.

At the F_2 generation, segregation of kernels into QPM and Non-QPM were in the ratio of between 107:423 (1:4) and 6:470 (1:10). The expected ratios of QPM to Non-QPM recorded values between 90:268 kernels and 138:413. The Calculated Chi-square values ranged from between 0.041 and 1.366, which are less than the corresponding tabulated chi-square value of 3.841($P<0.05$) 1df, therefore indicating that the observed ratios of QPM to Non-QPM showed a goodness of fit to the expected genetic ratio of 1:3. Similar trends were observed at the BC1S1 generation. This therefore indicates that there was successful transfer of the opaque-2 gene from the donor parent to the recurrent parent thereby conferring the much desired lysine and tryptophan onto the recurrent parent.

Components of variation of parameters measured showed high broad sense heritability of 0.95, 0.81, and 0.75 for plant height, ear height, and cob length. Narrow sense heritability for plant height, ear height, and cob length as well as cob diameter and number of rows per cob recorded very low values. Additive genetic variance for all traits studied recorded low values, indicating that 5% of the phenotype is attributed to environmental variance while 95% of the phenotype is attributable to genetic variance.

Significant and positive correlation values were obtained between plant height and ear height in the two parents (P1 and P2) as well as the F_1. The F_1, BC_1, and BC_2 generations recorded negative correlations for most of the traits. The significant correlation between plant height and ear height can be relied upon by breeders to select parents in a breeding program. The pedigree or backcross method of breeding can be used to incorporate the opaque-2 gene into Non-QPM or normal maize varieties that have been released by the national maize programme to enhance their nutritional status. Since the trait of interest is controlled by a recessive gene, breeders must use a large F_2 population during selection since its frequency in the F_2 is of a smaller proportion. In conclusion, plant and ear heights can be relied on as a criterion in selecting parental lines in a breeding programme.

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APPENDICES

Appendix 1 Plant height, ear height, cob length, cob diameter and rows per cob showing means, standard deviation (SD), variance, coefficient of variation (CV) and Standard error (SE) of *Okomasa.*

ACC. No.	PHT	EHT	COL	COD	ROC
1	200.7	90.0	19.5	4.9	14
2	230.5	90.5	19.3	4.6	14
3	200.0	110.0	16.5	4.8	16
4	220.5	110.0	16.0	5.2	14
5	230.0	120.0	20.3	4.3	18
6	230.5	140.0	19.0	4.2	18
7	240.0	130.5	17.5	4.0	16
8	200.2	90.0	18.0	5.0	16
9	210.0	120.0	19.5	5.2	18
10	220.0	100.0	20.3	4.2	20
11	230.5	130.7	19.5	4.3	16
12	240.0	120.8	22.0	5.3	14
13	220.0	90.0	24.0	5.0	17
14	210.6	120.6	18.0	5.2	14
15	190.5	90.0	19.3	4.8	20
16	210.6	110.5	18.5	4.0	16
17	220.0	90.5	19.0	4.6	19
18	210.8	100.5	17.3	4.2	18
19	210.4	100.0	20.5	4.6	14
20	210.0	110.5	19.0	4.3	16
Mean	216.8	133.3	20.1	4.7	15
SD	3.01	3.45	0.39	0.0	0.42
S^2	9.07	11.91	0.15	0.0	0.18
CV %	1.38	2.59	1.90	0.0	2.80 SE
5.0	4.9	0.4	0.1	0.3	

Appendix 2 Plant height, ear height, cob length, cob diameter and rows per cob showing means, Standard deviation (SD), variance, coefficient of variation (CV) and Standard error (SE) of *Obatanpa*

ACC. No.	PHT	EHT	COL	COD	ROC
1	200.0	140.0	20.0	5.0	16
2	210.0	100.0	21.3	4.5	13
3	198.2	96.5	20.0	5.6	18
4	200.5	101.5	22.0	4.8	18
5	215.0	103.5	21.3	4.5	14
6	220.0	100.0	22.5	4.6	16
7	210.0	155.0	20.3	5.3	16
8	216.0	100.0	22.5	5.5	14
9	198.0	98.5	20.5	4.2	16
10	200.5	90.0	19.5	4.6	14
11	186.3	95.5	24.5	4.8	14
12	190.5	100.5	17.3	4.2	14
13	200.3	100.0	21.0	5.5	13
14	205.2	130.5	27.0	4.5	14
15	195.8	95.0	19.3	5.0	14
16	186.9	98.0	21.0	5.6	18 17
	200.5	105.6	22.0	4.5	16
18	200.3	115.5	22.3	5.6	18
19	216.0	119.0	24.2	5.0	16 20
	198.5	98.5	20.0	4.8	14
Mean	202.6	108.3	19.2	4.6	16.4 SD
2.08	2.07	0.42	0.0	0.32	
S^2	4.31	4.30	0.18	0.0	0.10
CV %	1.03	1.91	2.20	0.0	1.95
SE	3.1	15.8	1.9	0.1	0.5

Appendix 3 Plant height, ear height, cob length, cob diameter and rows per cob showing means, standard deviation (SD), variance, coefficient of variation (CV) and Standard error (SE) of F_1

ACC. No.	PHT	EHT	COL	COD	ROC
1	200.5	120.0	20.0	4.6	15
2	200.7	90.0	19.5	4.8	14
3	160.0	80.0	18.0	4.5	15
4	180.0	100.0	17.0	4.0	14

5	200.0	90.8	20.3	5.0	14
6	200.5	110.0	21.5	5.3	15
7	220.5	130.0	20.0	5.0	15
8	200.5	85.0	19.0	4.5	15
9	200.5	100.5	18.5	4.0	14
10	180.7	120.0	21.0	4.0	15
11	190.5	100.0	20.5	4.0	14
12	200.5	110.5	19.5	4.5	15
13	220.5	120.0	20.0	5.5	13
14	210.0	80.0	21.0	4.9	14
15	190.0	80.0	21.5	5.0	14
16	220.0	95.5	20.6	5.3	15
17	230.0	110.7	19.5	5.0	14
18	200.0	110.5	19.0	4.8	14
19	200.5	110.0	18.0	4.0	15
20	210.0	100.5	20.0	4.8	15
Mean	201.3	146.1	21.5	4.8	15.3
SD	3.59	3.23	0.26	0.0	0.10
S^2	12.89	10.41	0.07	0.0	0.01
CV %	1.78	2.21	1.21	0.0	0.65 SE
4.9	2.8	0.5	0.1	0.4 Appendix 4 Plant	

height, ear height, cob length, cob diameter and rows per cob showing means, standard deviation (SD), variance, coefficient of variation (CV) and Standard error (SE) of F_2

ACC. No.	PHT	EHT	COL	COD	ROC
1					
220.5	130.5	22.5	5.0	14	
2	230.0	140.2	18.0	5.5	15
3	240.5	140.5	21.0	5.0	14
4	220.5	130.0	20.4	4.3	14
5	240.0	135.5	17.6	4.1	13
6	190.6	100.5	18.9	4.0	14
7	200.5	120.0	21.3	4.6	14
8	250.5	160.0	20.5	4.5	15
9	260.0	150.0	23.0	5.0	16
10	250.0	140.0	22.6	4.8	13
11	230.0	130.0	20.5	4.3	14
12	210.0	110.0	19.9	5.5	15
13	220.8	160.0	22.3	5.5	18
14	240.5	160.7	20.2	5.0	17
15	250.6	160.8	19.6	5.3	16

16	200.7	85.0	20.5	4.3	14
17	250.0	140.6	21.0	5.6	18
18	230.8	130.0	18.5	5.0	17
19	250.5	140.0	16.5	4.0	14
20	180.5	95.0	18.2	4.6	15
Mean	253.9	102.2	19.7	4.8	14.5
SD	7.50	4.77	0.48	0.0	0.28 S^2
56.57	22.75	0.23	0.0	0.08	
CV %	2.95	4.67	0.24	0.0	1.93 SE
3.7	3.3	0.3	0.1	0.1	Appendix 5 Plant

height, ear height, cob length, cob diameter and rows per cob showing means, standard deviation (SD), variance, coefficient of variation (CV) and Standard error (SE) of BC_1

ACC. No.	PHT	EHT	COL	COD	ROC
1	195.0	90.0	21.0	5.0	14
2	210.0	102.0	20.0	4.8	15
3	200.5	95.5	19.5	5.2	15
4	198.0	85.0	20.0	4.0	14
5	201.0	120.5	18.5	4.9	14
6	220.0	100.0	19.0	4.5	15
7	210.0	120.5	22.0	5.0	15
8	198.5	95.0	22.5	5.3	16
9	200.0	130.5	20.5	4.9	15
10	212.0	120.0	19.5	3.6	14
11	223.0	115.5	20.0	4.2	15
12	215.5	160.0	17.3	5.3	14
13	198.5	100.5	20.0	5.0	15
14	197.8	99.0	20.3	4.5	15
15	215.0	150.5	19.3	4.0	14
16	196.0	96.0	21.0	4.6	15
17	178.0	90.5	20.0	5.0	15
18	250.0	123.0	22.3	4.9	14
19	213.8	108.5	21.0	4.6	14
20	245.5	110.5	22.0	5.0	16
Mean	209.4	110.7	20.3	4.7	14.7
SD	3.80	4.33	0.23	0.0	0.0
S^2	14.09	18.73	0.07	0.0	0.0

CV %	1.81	3.94	0.13	0.0	0.0 SE
3.8	4.4	0.3	0.1	0.2 Appendix 6 Plant	

height, ear height, cob length, cob diameter and rows per cob showing means, standard deviation (SD), variance, coefficient of variation (CV) and Standard error (SE) of BC_2

ACC. No.	PHT	EHT	COL	COD	ROC
1	200.0	140.0	20.0	5.0	16
2	210.0	100.0	21.3	4.5	13
3	198.2	96.5	20.0	5.6	18
4	200.5	101.5	22.0	4.8	18
5	215.0	103.5	21.3	4.5	14
6	220.0	100.0	22.5	4.6	16
7	210.0	155.0	20.3	5.3	16
8	216.0	100.0	22.5	5.5	14
9	198.0	98.5	20.5	4.2	16
10	200.5	90.0	19.5	4.6	14
11	186.3	95.5	24.5	4.8	14
12	190.5	100.5	17.3	4.2	14
13	200.3	100.0	21.0	5.5	13
14	205.2	130.5	27.0	4.5	14
15	195.8	95.0	19.3	5.0	14
16	186.9	98.0	21.0	5.6	18
17	200.0	105.6	22.0	4.5	16
18	203.5	115.5	22.3	5.6	18
19	216.0	119.0	24.2	5.0	16
20	198.5	98.5	20.0	4.8	14
Mean	264.4	107.1	21.4	4.9	15.3 SD
4.80	2.69	0.20	0.0	0.33	
S^2	22.70	7.24	4.4	0.0	0.11
CV %	1.82	2.51	0.02	0.0	2.16
SE	2.1	3.8	0.5	0.1	0.4