

**STUDIES ON COMBINING ABILITY AND HETEROSIS FOR  
AGRONOMIC CHARACTERS AND MALTING QUALITY TRAITS OF  
GRAIN SORGHUM  
[*Sorghum bicolor* (L.) Moench]**

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School of Agriculture and Agricultural Technology, Federal University of  
Technology, Yola, in fulfillment of the requirements, for the award of the  
degree of Doctor of Philosophy (Ph. D) in Crop Breeding.**

**August, 2010**

## **DECLARATION**

I Danlami BELLO of the Department of Crop Production and Horticulture, Federal University of Technology Yola, Nigeria, declare that this work was carried out in its original form by me.

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**Date**

## **APPROVAL PAGE**

This thesis entitled ‘Studies on Combining Ability and Heterosis for Agronomic Characters and Malting Quality traits of grain sorghum [*Sorghum bicolor* (L.) Moench]’ by Danlami BELLO has met the regulations governing the

award of the degree of Doctor of philosophy (Ph. D) in Crop Breeding of the Federal University of Technology, Yola and is approved for its contribution to Knowledge and literary presentation.

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## DEDICATION

This work is dedicated to my wife, Dinger Bello and children, Caleb, Joshua and Joan.

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## ABSTRACT

The utilization of sorghum for brewing technology in Nigeria has not been fully realized due to shortage of good quality sorghum for industrial malting purpose, since not all sorghum varieties are suitable for malting. This study was carried out to estimate combining ability and heterosis of parents and their respective hybrids in sorghum, with

a view to identifying promising individuals or crosses with good breeding values for high yield and good malting qualities. Four males (testers) and eight females (lines) were inter-mated using line x tester mating design. In all, there were twelve parents and thirty two generated hybrids. The forty four entries were evaluated in a Randomized Complete Block Design with three replications in three locations. Results of the general analysis of variance indicated significant mean squares among the parents and crosses for almost all agronomic and malting qualities studied, in both the individual and combined location analyses, suggesting that the sorghum population under study is highly variable and therefore would respond to selection. Combining ability analysis of variance, however, showed that GCA (Line), GCA (tester) and SCA (line x tester) were significant for most characters, indicating the importance of additive and non-additive gene actions in the expression of these characters. The non-additive gene effect was higher than the additive gene effect as revealed by the  $\delta^2_{gca}/\delta^2_{sca}$  ratios which were less than unity, revealing the predominance of non additive genes. High general combining ability were observed from Samsorg 40, Samsorg 41 and Samsorg 17 for the male parents, while Ex Gerio, Ex Guyuk, Ex Girei, Ex Mbamba, Ex Gulak and Ex Wagga were among the highest general combiners from the female parents; suggesting that genotypes resulting from the crosses of these parents would lead to improvement in agronomic and malting qualities. Although significant level of high-parent heterosis was observed, this differs with locations, perhaps due to differences in environmental effect on the genotypes for most of the agronomic and malting traits studied. This equally implied that great potential exists for increased production of sorghum with good agronomic and malting qualities. Economic heterosis for important agronomic characters such as yield and important malting quality traits such as germination energy, germination capacity, beta and alpha amylases, were recorded for the hybrids Ex Girei x Samsorg 41, Ex Mbamba x Samsorg 40, Ex Gulak x Samsorg 17 and Ex Gulak x Samsorg 40 which equally recorded higher mean performances for these characters. These genotypes with further improvement can be produced on commercial scale as hybrids for the malting industries in Nigeria and beyond. Correlations between agronomic character such as yield per plant and germination capacity and between yield per hectare and both germination capacity and energy were positive and can consequently lead to simultaneous improvement of genotypes with excellent agronomic and malting traits in this sorghum population. From this study, characters such as inter-node length and panicle width which are negatively correlated with days to anthesis and days to maturity will enhance earliness to maturity when selected for in a breeding program. Similarly, selecting for high water sensitivity will reduce plant height as depicted from the findings of this study.

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## LISTS OF SYMBOLS

|            |          |
|------------|----------|
| $\alpha$   | Alpha    |
| $\beta$    | Beta     |
| $^{\circ}$ | Degrees  |
| %          | Per cent |
| $\delta^2$ | Variance |

## ABBREVIATIONS

1000sd weight (g) = 1000 seed weight (g)

|              |   |
|--------------|---|
| AD           | After Death   |
| ANOVA        | Analysis of Variance  |
| BC           | Before Christ   |
| °L           | Degrees Lintner   |
| $\delta^2 e$ | Error variance  |
| EBC          | European Brewing Convention   |
| EMS          | Expected Mean Squares   |
| Ex           | Naming system adopted by plant Breeders for cultivars not yet developed |
| FAO          | Food and Agriculture Organization                                       |
| GCA          | General Combining Ability   |
| HCN          | Hydrocyanic acid  |
| HPH          | High Parent Heterosis   |
| IAR          | Institute for Agricultural Research, Samaru                             |

|   |  |
|---|--|
| ICRISAT   | International Crops Research Institute for Semi-Arid Tropics             |
| ICSV  | ICRISAT Sorghum variety developed in collaboration with IAR Samaru Zaria |
| IoB   | Institute of Brewing   |
| KSV   | Kano short Sorghum developed at IAR Samaru Zaria                         |
| Leaf Length                                       | Flag leaf length   |
| Leaf width  | Flag leaf width  |
| L° /kg  | Litre degrees per kilogram   |
| MS  | Mean Squares   |
| Number leaves/ plant = Number of leaves per plant |  |
| Rep   | Replication  |
| SABS  | South African Bureau of Standards  |
| SAMSORG   | Samaru Sorghum (variety developed at IAR Samaru)                         |
| SAS   | Statistical Analysis System  |
| SCA   | Specific Combining Ability   |
| SDUg <sup>-1</sup> malt                           | Sorghum Diastatic Unit per gram of malt                                  |
| SK  | Short Kaura  |
| TFMSA Trihalogenatedmethylsulfonamides            |  |
| $\delta^2_{GCA}$                                  | Variance of GCA  |
| $\delta^2_{SCA}$                                  | Variance of SCA  |
| °Wk   | Windisch-kolbach unit  |

## CHAPTER ONE

### 1.0

### INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench], with  $2n=20$  diploid number of chromosomes, is a cultivated tropical cereal crop. It is generally, although not universally, considered to have been domesticated in Northeastern tropical Africa, possibly the Ethiopian regions from as early as 1000BC (Balole and Legwaila, 2006). Today sorghum is cultivated across the world particularly in the warm climate areas. FAO (2003) reported that out of the 700 million hectares planted with cereals, sorghum occupies about 45 million hectares with Asia as the leading continent (19.6 million hectares) followed by Africa (15.7 million hectares). It is quantitatively the world's fifth largest most important cereal grain, after wheat, maize, rice and barley (FAO, 2003). World annual production of sorghum was 69 million metric tons in 2001 out of which Africa produced about 20 million metric tons, which makes the crop quantitatively the second most important cereal grain in Africa after maize. Nigeria, Sudan, Ethiopia and Burkina Faso accounted for 7.08m tons, 4.47m tons, 1.54m tons and 1.37m tons respectively in 2002, which is nearly 70% of Africa's production (FAO, 2003). The cereal is an important food crop in sub-Saharan Africa on account of its drought tolerance and its ability to withstand periods of water-logging. The area under cultivation has steadily increased over a period of time from 18.5 million hectares in 1989 to 20.2 million hectares in 2001 (FAO, 2003), but the average yield trend has been downwards. By contrast, in the Indian sub-continent during the same period, sorghum production area declined by 37% but yield increased by 80%, with about 880kg/ha (FAO, 2003). Further, FAO (2003) pointed out that hybrid variety utilization and increase in genetic research have been the major factors for the increase in yield recorded.



In Africa, Taylor and Dewar (2001) observed that, over the past 25 years sorghum production has increased steadily from 11.6 million tons in 1976 to 20.3 million tons in 2001. The increase in production has been as a result of increase in the land area under cultivation, although there has been no overall improvement in yield per hectare. Average yield range remains 500kg/ha - 900kg/ha on the farmers' fields, which in turn means that there is often no surplus sorghum, without which processing industries cannot be created. Further, Taylor and Dewar (2001), stated that the reason for the stagnation of yield is related to the narrow genetic base of the more recently bred high yielding sorghum varieties, which are grown under the traditionally low-input farming system adopted in most regions. However, where intensive agriculture is practiced with improved varieties or hybrids, Taylor and Dewar (2001) noted that yields are higher and comparable with other major cereals. For example, in South Africa, they reported that average commercial yield in 2001 was 2.34 tons/hectare compared to 2.49 tons/hectare for maize. Continued increase in cultivated area is environmentally damaging and in the long run unsustainable, so effort must be intensified to improve sorghum yield in Africa. Higher yields are essential not for rural food security alone but also for increased commercialization and raw material for industrial uses.

Sorghum is processed into a very wide variety of attractive and nutritious traditional foods, such as semi leavened bread, porridges, steam cooked and non-fermented beverages (Onwueme and Sinha, 1991). They also reported that sorghum plants are used as fodder, forage or silage, but caution has to be taken as it is dangerous to feed the leaves and stems of poor and drought stricken plants to young stock as they may contain poisonous glucoside. They further contended that tillers produced after harvesting (ratoons), particularly their leaves are very poisonous. The poison is present from germination and disappears gradually with age. Purseglove (1972) pointed out that on hydrolysis by enzyme action, the cyanogenic glucoside durum yields hydrocyanic acid (HCN) and as little as 0.5g HCN can kill a cow. Further, the use

of sorghum for the manufacture of wax, starch, edible oil, production of syrup in the pharmaceutical industries and adhesive has been reported (ICRISAT, 1993). Sorghum however, remains the grain of choice for brewing traditional African beers (Novellie, 1982). New products such as instant soft porridge and malt extracts are also great successes in a competitive environment of multinational enterprises such as Nigeria (Okafor and Aniche, 1980).

Sorghum has been proved (Okafor and Aniche, 1980; Obilana, 1985; Palmer, 1992) to be the best alternative to barley for large scale lager beer brewing, particularly in Nigerian breweries, since the ban on the importation of barley (which is very expensive to import) by the Federal Government in 1988. However, the potential utilization of sorghum for brewing technology has not been fully realized due to acute shortage of good sorghum crop for industrial malting purpose, since not all sorghum varieties or cultivars are suitable for malting or brewing. The red sorghum has been suggested to be unsuitable because of its tannin content while the white and yellow cultivars have been reported to have good brewing properties, (Agu *et al.*, 1995). SAMSORG-41 (ICSV400) to date has the best malting quality but has low yield due to its average panicle size and so, it is not popular with the local farmers whose primary concern is economic returns (Alhassan, 2005). There is therefore, an urgent need to increase productivity through breeding for higher yield per hectare and good malting quality which hitherto has lagged behind. Good traditional malting (but poor yielding) sorghum varieties have quickly been replaced by high yielding varieties that are poor malters. Improved diastatic (alpha and beta amylases) activities ensure better fermentable malt extracts. The primary quality criterion is the sorghum potential to produce malt with high diastatic power (Palmer, 1992).

Although over the years, mass selection and recurrent selection within the traditional tropical sorghum have led to some yield increase, these increases according to Obilana (1985) and Tadesse *et al.* (2008) have been largely insignificant (700-800kg/ha) at farmers' level and only a few of these varieties are good malters. Moreover there are extensive local germplasm of

sorghum available in Adamawa State (Sajo and Kadams, 1999) and in spite of its providing the basis for adaptation being genetically more diverse, it remains unutilized in the development of hybrids and good malting sorghum. One remedial option, among others, is to increase research and development effort towards the development of hybrids and other varieties with good malting quality. The potential for sorghum to be a driver of economic development in Nigeria is enormous but largely depends on the success of research and development particularly in hybrid development programs. Improvement of yield potential and of malting qualities in sorghum requires selection of parents capable of transmitting these desirable traits. A rational approach to such breeding programs for superior plant types as suggested by several workers (Simmonds, 1979; Simmonds, 1996 and Kadams, 2000) is the use of combining ability analysis based on progeny test data, for evaluating parents and crosses for wide range of quantitative characters. This is because the choice of selection and breeding procedure adopted for the genetic improvement of any crop is largely influenced by the magnitude of genetic variability, relationships between economically important characters and the nature of gene action governing the inheritance of these desirable characters.

Plant breeders (Monpare and Sanghy, 1982, Nwasike *et al.*, 1992, Mushonga *et al.*, 1994 and Kadams, 2000) have shown that combining ability and heterosis studies involving local germplasm is essential for the selection of suitable parents for hybridization and identification of promising F<sub>1</sub> hybrids for further exploitation in breeding programs. This study will therefore bring about improvement of the local landrace accessions of sorghum with good yield and good malting quality for industrial purpose, so as to make use of new economic opportunities in the malting and weaning food industries, which are offered today to sorghum growers throughout Nigeria. The general objective of the study was to investigate the combining abilities and heterosis for agronomic and malting quality traits in grain sorghum. The specific objectives were to:

1. estimate variances due to General Combining Ability (GCA) and Specific Combining Ability (SCA), that will indicate the type of gene action governing inheritance of characters in sorghum.
2. estimate the General Combining Ability effects among the genetically diverse parents in order to identify superior genotypes with good agronomic and malting quality traits.
3. estimate the Specific Combining Ability (SCA) among the hybrids in order to identify superior cross combinations.
4. estimate the level of heterosis among the F<sub>1</sub> hybrids, and
5. estimate the correlation coefficients among the agronomic characters and among the malting quality traits.

## **CHAPTER TWO**

### **LITERATURE REVIEW**

## **2.0**

### **2.1 Botany of sorghum**

Sorghum with  $2n=20$  diploid number of chromosomes is from the Poaceae (*Graminae*) family, tribe *Andropogoneae*. It is an erect annual plant, main stem can attain a height of 1.5-6m while the panicle varies between 25-75cm (Balole and Legwaila, 2006). Studies by Doggett (1988) and Onwueme and Sinha (1991), showed that tillers come out in some cultivars when they are grown as a ratoon crop. First a single main root is produced from a large number of much branched lateral roots which are formed from the lowest nodes of the stem. The stem is erect, dry or juicy, sweet, grooved and nearly oval. The peduncle (top internode) is not grooved. The leaves are alternate in two ranks; the leaf sheaths are 15-55cm long and encircle the stem, the midrib is prominent.

According to Leland (1985) and Murty, *et al.* (1994), flowering (anthesis) of sorghum marks the end of vegetative growth due to merismatic activity. Sorghum usually flowers in 55 to 70 days in warm climates but may range from 30 to more than 100 days. The Sorghum head begins to flower at its tip and graduates successively downward over 4 to 5 days period. At the time of flowering the glume opens and the "three" anthers fall free while the two feathery stigmas from the single ovary protrude on the stiff styles. Flowering of a panicle may be spread over a period of 4 to 9 days depending upon cultivar, panicle size, temperature and relative humidity. In cooler climates flowering periods are usually extended. As a glume opens, the stigma and anthers emerged outwards. Usually the stigmas and anthers protrude just before the glume open and frequently anthers protrude first. The time from commencement of glume opening to completion of closing is about 1-2 hours and varies with cultivar. The glume closes shortly after pollination, though empty anthers and stigmas still protrude (except in the long

glume types). The florets of some of the very long glume type do not open for fertilization a phenomenon known as *Cleistogamy*. Sorghum is primarily self-pollinated, that means pollen from a head fertilizes most of the ovules on the same head. Sorghum also has an out crossing tendency estimated to be 5-15% for cultivated varieties and 30% for the wild varieties. The amount of cross-pollination is normally higher in the top quarter of the panicle, possibly because the stigmas emerging in this region have relatively less access to pollen from the same panicle. It is generally considered to be a wind pollinated crop. Pollen is viable for 3-6 hours as long as it is contained in the anthers even after dehiscence. However, during field pollination pollen is collected in paper bags and its viability deteriorates rapidly and can survive for less than 20 minutes. Pollen can be collected from flowering panicles for a few hours after dehiscence. Pollen is usually available from 4-10 days since not all the panicles on the field flower at the same time. The stigmas remain receptive up to a week (7 days); however, they are most receptive during the first 3 days after their emergence.

## **2.2 Line x Tester Mating Design**

Line x tester mating design as suggested by Kempthorne (1957) has been extensively used to estimate General Combining Ability (GCA) effects of parents and Specific Combining ability effects of crosses. GCA and SCA variance estimates using line x tester mating design have been useful in devising breeding strategies in sorghum and other crops (Mushonga *et al.*, 1994, Ahmed *et al.*, 2003, Mehmet *et al.*, 2003). Further, Kempthorne (1957) pointed out that in line x tester mating design, the lines are the females which are emasculated while the testers are the males whose pollen are used to pollinate the lines. This mating design involves the mating of n-lines to n-testers; he also stated that this mating design has an advantage of being able to accommodate the crossing of large number of lines to genetically broad-based testers. More so, large number of crosses obtained using other mating designs such as diallel mating design is

reduced to manageable size (number) using line x tester mating design. Kempthorne (1957) further pointed out that, with the line x tester mating design the combining ability variances of parameters and combining ability effects of more genotypes can be obtained than with most other designs.

### **2.3 Combining ability**

The term combining ability expresses the relative performance of a line in hybrid combination and could be expressed as either general or specific combining ability. Sprague and Tatum (1942) defined General Combining Ability (GCA) as the average performance of lines in hybrid combinations and Specific Combining Ability (SCA) as those instances in which certain hybrid combinations are either better or poorer than should be expected on the average performance of the parent inbred lines included. Falconer (1981), also pointed out that, General Combining Ability (GCA) is directly related to additive gene action while Specific Combining Ability SCA, on the other hand is commonly associated with all effects which cannot be accounted for by schemes such as dominance, epistasis and genotype and environment interaction (Rosenow, 1972, Nwasike *et al.*, 1992, and Kadams, 2000). Information regarding the different types of gene action and relative magnitude of genetic variance helps in the determination of the relative roles of additive and non-additive gene effects and also helps in determining the appropriate breeding procedures to be utilize in improvement programs. *Per se* performance or mean performance of varieties or cultivars in a large number of yield trials may give an idea of their relative superiority, but this would not necessarily reflect their ability to produce better cross combinations with a number of similar lines (Kadams, 2000). In his contribution, Matzinger (1962), observed that estimates of GCA and SCA effects from series of crosses, estimate positive values for genetic effects of the base population from which parental varieties are sampled. Kenga (2000) pointed out that, GCA is relatively more important than the SCA in previously unselected materials while SCA assume greater importance in materials which have been

previously selected for GCA. He also observed that, knowledge of combining ability effect is essential for selection of suitable parents for hybridization and identification of promising hybrids for further exploitation in a breeding program.

## **2.4 Choice of parents**

Information on combining ability effects of genotypes is essential for improvement of any crop. This important information when available in sorghum serves as essential tool to the sorghum breeder in the choice of better parental combination for hybridization and identification of promising hybrids for further improvement in selection programme (Muhammed and Sultan, 2003). Combining ability studies basically serve the following purposes: (i) it helps in the selection of suitable parents for cross combination by estimating the general and specific combining ability effects of parents and crosses respectively. (ii) it also helps in early detection of promising progenies from non-promising ones.

A thorough knowledge of the parental materials helps to assure the success of a breeding programme (Ghani, 1980). Whitehouse *et al.* (1958) pointed out that breeders of self-pollinated annual crops are faced with the problems of choosing the best parents for hybridization. According to Quinsenberry (1967), procedures for selecting parents may range from being relatively simple to rather complex in nature depending on the breeding objectives. Pederson (1974) suggested that in choosing superior parents, it is necessary to carry out trials with replications over time and space and to rank the genotypes according to their mean values for the characters of interest.

Paroda and Joshi (1970), Dhonuskshe and Rao (1979) and Muhammed and Sultan (2003), pointed out that, the study of combining ability is a useful tool in classifying parental lines in terms of their hybrid performances. As such, it is a great aid in selecting parents which when crossed will give rise to more desirable segregates. However, Chaudhary and Singh (1978)



argued that, the *per se* performance based on realized means could be adequate for selecting the best cross combinations when exploiting heterosis in hybrid seed production. This would eliminate the complicated calculations of specific combining ability estimates, which may be biased as it is based on certain assumptions. Lupton (1961) described the use of the diallel mating design in the planning of a breeding program to help solve the problem of choice of parents. He suggested comparing estimates of yield and variances resulting from trials with  $F_3$  and  $F_4$  obtained from randomly selected  $F_2$  plant progenies from each of the component crosses. The use of the vector method for choosing parents was suggested by Grafius (1964) to avoid excessive data taking. According to him, with this method, it was possible to construct populations which closely approach an ideal one for a large number of traits and also at the same time have a low variance for many of these traits, so that an individual selection for one trait might be expected to be in focus for the other traits as well. According to Bailey and Comstock (1976) and Busch *et al.* (1974), the first step in any plant breeding program is parental selection. From their theoretical and empirical results, they concluded that the probability of recovering a superior progeny genotype is greater if both parents are similar in performance as opposed to one parent being inferior in one or more traits.

## **2.5 Genetic control of sorghum parameters**

### **2.5.1 Plant height**

Patil and Thombre (1986), Rafiq *et al.* (2002) all reported the preponderance of non-additive gene action in the control of plant height. Earlier, Lukhele (1981) and Monpare and Sanghy (1982), reported significant mean squares for SCA for plant height in sorghum, suggesting the importance of non-additive genetic effect in the control of this character.

### **2.5.2 Number of leaves per plant**

In a study to evaluate genetic analysis of grain yield and its components in sorghum, Patil and Thombre (1986), reported the effect of non-additive gene action in the control of number of leaves per plant. Yang (1991), Jianming and Mitchel (2001) and Rafiq *et al.* (2002), also obtained similar results in their various studies on sorghum.

### **2.5.3 Panicle length**

Patil and Thombre (1986) and Pillai *et al.* (1995) reported non-additive gene action for the control of panicle length in sorghum.

### **2.5.4 Panicle width**

Several authors (Patil and Thombre, 1986, Pillai *et al.* 1995 and Rafiq *et al.* 2002) indicated higher SCA than GCA for panicle width in sorghum, indicating that non-additive gene action is predominantly responsible for its control.

### **2.5.5 Leaf length and 2.5.6 Leaf width**

Patil and Thombre (1986) and Pillai *et al.* (1995), showed that GCA variance was more important than SCA variance for leaf length and leaf width in their study using Line x Tester in sorghum.

### **2.5.7 Panicle exertion**

Tadesse *et al.* (2008), reported the contribution of additive genetic effect in the determination of this trait in sorghum.

### **2.5.8 Inter-node length**

Rafiq *et al.* (2002) reported the preponderance of higher GCA variance than SCA variance for the control of this character in sorghum

### **2.5.9 Days to booting or anthesis**

Rafiq *et al.* (2002), in their study on combining ability in Post-rainy season sorghum reported the preponderance of additive gene action over non-additive gene action in the control of this character. Senthil and Palanisamy (1994), previously, obtained similar results, indicating that

time to anthesis is governed by additive gene effect in sorghum. Additive gene action was also found important in the control of time to anthesis by Hugar *et al.* (1986).

#### **2.5.10 Days to maturity**

In studies on GCA and SCA variances in sorghum, Govil and Murty (1973) and Rafiq *et al.* (2002) reported high magnitude of additive gene over non-additive gene actions for the control of days to maturity. In a similar study, Monpare and Sanghy (1982), reported preponderance of additive genetic variance for this same character. A similar report was also made by Tadesse *et al.* (2008) in their study of combining ability for major morpho-agronomic traits on sorghum parental lines.

#### **2.5.11 1000 grain weight**

Hicks *et al.* (2002) in their study noted that both male and female parents were highly variable and expressed high levels of genetic variation in general combining ability for 1000- grain weight. Significant SCA for this character was not observed. This provided information that greater amount of genetic variability in this population was due to GCA effect which indicates additive type of gene action being involved for this character. Therefore, these parents might be exploited for varietal improvement in different cross combinations for yield improvement. According to Singh *et al.* (1982), best performing cross may actually be produced by crossing two parents having the highest GCA. Hugar *et al.* (1986) also reported additive gene action in the control of this character.

#### **2.5.12 Grain yield**

Toure *et al.* (1996) reported significant general combining ability accounting for 81% variability for grain yield among crosses signifying the importance of additive gene effect over non additive gene effect in determining grain yield character among crosses. Grain yields have also been reported to be under the influence of non-additive gene action (Monyo *et al.*, 1988, Kinbeng and

Nwasike, 1994). However, studies by Rao (1970) and Nagur and Murty (1970) indicated highly significant GCA and SCA mean squares for grain yield and other yield components studied. The result shows that additive as well as non additive gene actions control the inheritance of grain yield and its components. In studies conducted by Defranca (2000) and Harer and Bapat (2000), GCA and SCA mean squares were significant for grain yield with the GCA values greater than the SCA. They concluded that both additive and non additive gene effects were important in grain yield control but with a slight preponderance of additive gene effects. In situation like this where GCA for other traits predominates over SCA and SCA predominates over GCA for other traits, simultaneous exploitation of additive and non-additive components have been recommended under the use of recurrent selection for population improvement (Nwasike and Oyejola, 1989).

## **2.6 Malting qualities in grain sorghum**

Malting is the process whereby cereal grains are germinated under controlled condition of moisture, aeration and temperature (Owuama, 1999). During this process the growing grains develop hydrolytic enzymes which degrade the starchy endosperm to sugars, amino acids and other compounds which react to impart a malting taste and aroma after kilning (drying) process (Taylor and Dewar, 2001). They also pointed out that sorghum malting process involves three distinctive operations: Steeping, Germination and Drying. Further, they explained that, Steeping involves immersing the grains in water to imbibe sufficient water to initiate the metabolic process of germination, dirt, chaff and broken kernels are also removed by washing and floating. It also inactivates the tanning in tanning varieties, if not inactivated the tanning binds the malt amylase enzyme resulting in reduced sugar production. According to Aniche and Palmer (1990), malt is a germinated cereal grain, usually barley or sorghum that has been germinated for a limited period of time and then kilned (dried). Aniche and Palmer (1990) further reported that, malting process that generates the fermentable mono and disaccharides is dependent upon the

Alpa ( $\alpha$ ) and beta ( $\beta$ ) amylases and the maltose splitting enzyme - maltase, that develop in sorghum during germination. Quite a number of parameters have been reported to influence malting quality of cereal grain. These include: number of broken kernels and foreign materials, germination energy, germination capacity, moisture content of grains before malting, biochemical test such as diastatic activities, cold and hot water extracts, starch determination and protein (Aisien, 1989). Taylor and Dewar (2001) pointed out that not all sorghum yields suitable malts for the brewer, and selection is based on many criteria such as: rapid and synchronous germination of sorghum grains, even enzymatic degradation of the endosperm, an adequate complement of enzymes even after kilning and low level of fibrous materials, while some of the desirable agricultural properties include good yield, earliness, uniformity in grain ripening and disease resistance.

Haruna (1991) observed that low moisture content resulted in reduction in diastatic power while an increase of 20-25 (SDUg<sup>-1</sup> malt) was recorded for genotypes with high moisture content. He also reported reduction in diastatic power and other malting characteristics of sorghum genotypes when germinating sorghum was dried between 40 and 60°C. Whereas Novellie (1982) pointed out that marked losses resulted when drying temperature was raised to 70°C. Malting loss according to Haruna (1991) represents the combined losses during steeping, respiratory losses as carbon dioxide and water, and weight losses of shoot and roots. He opined that genotypes with high malting loss are undesirable for malting and attributed these losses to excessive growth during germination. The high malting loss of sorghum (20-30%) compared to barley (9-12%) has been attributed to variations in solubility patterns of their amylases (Malomo, 1989) as well as the high temperature employed during malting. Burgner and Labergue (1985) in their comparison study between sorghum and barley malt, showed that the optimum germination time at a temperature of 30°C, to produce malt grain of high diastatic power and good malt extract was 4-5 days for barley. Syed *et al.* (2000), reported significant variation in the

composition of protein, starch, fat ash and moisture in various sorghum genotypes they studied. This variation they added, was due to genetic variability among the cultivars.

#### **2.6.1 Germination energy (4mL. test) %**

Germination involves seedling growth. This process helps in mobilizing the sugar, while drying helps to reduce the moisture (to about 10%) which further ensures a shelf-stable product.

Germination energy assesses the rate at which 100 grains will germinate in a 9cm Petri dish containing two rings of filter paper in 4ml of water at 18°C after 72hrs. In any sample a proportion of the grains will not germinate because they are dead. Of those remaining some will not germinate when tested, although alive, such grains are immature and dormant (Ogu *et al.*, 2006).

#### **2.6.2 Germination capacity (viability) %**

Germination capacity (viability) assesses the percentage of corns which will germinate in 200ml of 0.75% hydrogen peroxide at 18°C after 72hrs. For malting, a grain should have a viability or germination capacity of at least 86-96% (Haruna, 1991). The difference between germination energy and germination capacity is a measure of the dormancy (%) of the grain sample. In mature grains, germination energy is close to germination capacity (Ogu *et al.*, 2006).

#### **2.6.3 Water sensitivity (%)**

This assesses the rate which 100 corns will germinate in a 9cm Petri dish, containing two rings of filter paper and 8ml of water at 18°C after 72hrs. Water sensitive grains require higher internal oxygen than does mature grains before they germinate (Ogu *et al.*, 2006).

#### **2.6.4 Hot water extracts (HWE)**

This is an analytical measure of the quantity of dissolved solids in sweet wort prepared from malt by small scale mashing process. Hot water (25 °C - 30 °C) extracts of malted sorghum had higher and sustainable amount of free amino acids than commercial barley malt. The hot water extract of sorghum malt yields about 66%-77% glucose to malt ratio than barley (Ogu *et al.*, 2006).

### 2.6.5 Diastatic Power (Dp)

Diastatic Power also called Diastatic activity or enzymatic power is the grains ability to break down starches into sugars. It is generally referred to only in malted grains which have begun to germinate. This process of germination is achieved by simply soaking the grains in water at a controlled temperature (mashing). Diastatic activity can also be enhanced by the inclusion of separately-prepared brewing enzymes. Diastatic power for a grain is measured in degrees Lintner (°L or °Lintner) or in Europe by Windisch-kolbach unit (°WK). The two measures are related by

$$^{\circ}\text{L} = \frac{^{\circ}\text{WK} + 16}{3.5} \quad \text{or} \quad ^{\circ}\text{WK} = (3.5 \times ^{\circ}\text{L}) - 16 \quad (\text{EBC, 2007}).$$

Generally, sorghum malt diastatic power ranges between 35°L to 150°L. However, diastatic power of between 38°L to 78°L is recommended for sorghum grain to be considered for commercial or industrial malting. The Diastatic activity development in some sorghum cultivars is very limited compared to that of barley malt. However, some sorghum cultivars can develop significant levels of this important maltose producing enzyme. Mushonga *et al.* (1994) reported that the variance due to GCA of lines was only significant in one season while SCA variance was significant in both seasons, which suggest that, non-additive type of gene action is primarily involved in determining diastatic activity. Mushonga *et al.* (1994) further observed a ratio of less than one, between GCA and SCA, which is also indicating that non-additive gene action was more important than additive gene action in governing diastatic activity.

### 2.6.6 Moisture content

Mushonga *et al.* (1994) reported significant differences among hybrids for all traits measured except grain moisture content. Similarly, they also observed significant SCA x Environment interaction for this trait but did not indicate the type of gene action controlling the trait.

### 2.6.7 Cold water extract

Cold water extract is a soluble product of enzyme hydrolysis from malting process, and this includes readily available sugar and amino acids within the endosperm of malt (Agu, 2005). The type of gene action controlling this trait has not been reported.

#### **2.6.8 $\alpha$ -amylase**

Agu (2005) reported that alpha-amylase catalyses random hydrolysis of starch chains at alpha (1,4) glucosidic linkages distant from the ends of the chains and from alpha (1,6) linked branches in the chain. During malting, significant quantity of alpha-amylase is produced from the embryos of sorghum while  $\beta$ -amylase is activated from latent forms in starchy endosperm. The formation of alpha amylase requires adequate oxygen and can be hindered in the presence of excess carbon dioxide. Amylase activity usually measured in SDU (Sorghum Diastatic Unit); an SDU (/g malt) of 25-1873 SDU is accepted in sorghum depending on the variety. Also the type of gene action controlling this trait has not been reported.

### **2.7 Selection methods**

Traits governed by additive gene action can easily be fixed in selection programs. For this reason Reddy (1976) and Chaudhary and Singh (1978) concluded that simple selection techniques would be effective for the improvement of characters under the control of additive gene action. Naidu *et al.* (1984) indicated that characters under the influence of additive x additive genetic components of variation are mostly improved through simple selection methods such as the pedigree and single seed descent which isolate desirable plant types from segregating generations.



Singh *et al.* (1983) concluded from their studies that when characters are mainly governed by non-additive genetic variance it is necessary to maintain heterozygosity in the population. They suggested that this type of genetic variability could be handled by non-fixable breeding methods such as the triple cross technique followed by recurrent selection, which is likely to hasten the rate of genetic improvement. Mushonga *et al.* (1994) working on malting characteristics in sorghum suggested that, since diastatic activity is primarily non-additive, recurrent selection program to improve primarily specific combining ability is an appropriate method by which to breed for parents with good malting quality for hybrid development. Ahmad *et al.* (1979), working on wheat, and Yang (1991), working on sorghum, reported that for characters under non-additive type of gene action, bi-parental mating followed by recurrent selection is likely to result in substantial improvement of the character under selection. Similar recommendations have been made by Gill *et al.* (1972) and Byrne and Rasmusson (1974) in wheat, Doggett and Eberhart (1968) in sorghum and other self-pollinated crops. Yang (1991), however, cautioned that an important problem to consider in self-pollinated crops such as sorghum is that a recurrent selection scheme will only be feasible if commercially exploitable male sterility, a pre-requisite in heterosis breeding, is available.

Widner and Lebsack (1975), Jatasra and Paroda (1979) and Singh *et al.* (1983) suggested that in situations where both additive and non-additive variances are important in governing a character, breeding a homozygous stable line by the pedigree method would mean only a partial exploitation of the two genetic systems. They therefore suggested a breeding system that will exploit both additive and non-additive genetic effects simultaneously. The systems they advocated included bi-parental mating as well as mating of selected plants in early segregating generations. Nwasike and Oyejola (1989), however, suggested the use of recurrent selection to improve such population.

## **2.8 Genotype x environment (G x E) interaction**

Genotype x environment interaction is of major importance in a plant breeding program. Many potential genotypes are usually evaluated in different environments before selecting desirable ones. However, the performance of different genotypes often varies from one environment to another.

The interaction between various environments and genotypes usually estimated in the analysis of variance, although useful, fails to give adequate account of the dynamic response of varieties to different environments (Finlay and Wilkinson, 1963, Brancout and Lecomte, 2003). This method of analysis only indicates the extent of the influence of environment on the genotypes, but fails to determine superior lines or varieties, because the relative rankings of these genotypes differ. According to Eberhart and Russel (1966) and Brancout and Lecomte (2003), this causes difficulties in demonstrating the significant superiority of any genotype over the others. It is for this reason therefore that better methods of analysis that can identify superior genotypes, across environments have been adopted.

The Biadditive Factorial Regression Analysis (BIAREG) and Multiplicative Interaction Model (AMMI) Brancout and Lecomte (2003), are few examples. In a trial to explain genotype x environment interaction using BIAREG and AMMI methods for comparison, 13 lines of sorghum in 14 environments for 2 years, Brancout and Lecomte (2003), reported that the interactive pattern of genotypes and environment was similar for both models for heading date and grain yield. Other alternative analyses used apart from those mentioned above include Principal Component Analysis (PCA), Cluster analysis and Geometrical methods in which each genotype or environment is represented by a point in Multi-dimensional Euclidean space and analysis of Stochastic dominance.

## **2.9 Heterosis and hybrid sorghum seed production**

### **2.9.1 Heterosis**

Heterosis or hybrid vigor is the difference between the hybrid mean and the mean of the two parents (Falconer and Mackay, 1996). This is called mid parent Heterosis. However, higher parent heterosis known as heterobeltiosis (that is the performance of the hybrid over the better parent) is very important if the goal is to have hybrids with economic heterosis. Duvick (1999), however, observed heterosis as the increase in size or rate of growth of off-spring over its parent, for example, hybrid vigor in crop plants can be observed as increase in yield of grain or reduction in number of days to flower. Panwar *et al.* (1983), in their earlier contribution, pointed out that, the exploitation of hybrid vigor appears to be an alternative for making further increase in yield. They stated that over the years, heterosis has been extensively explored and utilized for increase in a number of economically important crop species such as sorghum, millet, maize, potato and cotton. The basic information, which would seem valuable from heterosis breeding point of view, would be the estimation of the amount of heterosis and the way to exploit it for commercial cultivation. Kadams (2000) reported heterosis of between 13.2-20.5% as opposed to 7.2- 19.7% reported by Barker and Varughese (1992) in *Triticale*. This level of heterosis if realized under commercial planting rate, could be exploited for hybrid seed production.

The key to obtaining hybrid vigor according to Barker and Varughese (1992) is genetic diversity. They reported that, when crosses involve genetically and geographically diverse parents, high yielding hybrids could be produced. Although high heterotic effect or response does not always mean high SCA according to Singh *et al.* (1982), Kadams (2000) reported, that high *per se* performance of crosses indicates high SCA and in this case since high SCA denotes high heterosis, it then follows that *per se* performance of crosses could be used to predict crosses with high heterotic response. Toure *et al.* (1996) reported 35 to 72.95% of heterosis for grain yield of F<sub>1</sub> over mid parent mean in sorghum. In practice, however, the selection of productive hybrids may not be appreciated by the expression of heterosis of F<sub>1</sub> above mid parent mean, but rather by the performance of F<sub>1</sub> mean in relation to that of the better parent (heterobeltiosis) or

other hybrid and parental lines. Jianming and Mitchel (2001) while studying seedling growth under cold temperature stress in grain sorghum, reported significant high parent heterosis (HPH) values for number of leaves, vigor, and dry weight in many crosses, meaning that hybrids were superior to the better parents involved in each cross. Duvick (1999) reported 6-47% heterosis over mid parent in sorghum while Presterl and Weltzien (2003) reported mid parent heterosis for grain yield of individual population crosses ranging between -14% and 30% when averaged across the first location while the range across the second location was between -9 and 17% for the same trait and crosses. Kaul *et al.* (2003), in their study on heterobeltiosis and combining ability in sorghum using line x tester, reported significant mean performance between two crosses with a corresponding significant positive heterobeltiosis for grain yield per plant (101.4 %). Mushonga *et al.* (1994), reported heterosis for diastatic activity from -63.7 to 54.0% and heterobeltiosis from -69.3 to 39.1% in the first season and from -67.6 to 100.8% and heterobeltiosis from -74.8 to 78.4% in the second season. This display of differences they stated was due to co-adapted genes at many loci, interacting in an epistatic manner. When such populations are crossed, the F<sub>1</sub>s are adapted to neither of the different environment.

In tropical maize lines, Betran *et al.* (2003), opined that, heterosis can be affected by environment. In a study using 17 tropical maize lines, heterosis was greater under drought stress and smaller under low N-conditions than under nonstress conditions. This shows that abiotic conditions affect the expression of heterosis. In millet Ouendeba, *et al.* (1993), reported a mid parent heterosis of up to 81% in grain yield. Gravois (1994) while working on rice, indicated absence of dominance on grain yield as the average value for mid parent heterosis was not significant.

### **2.9.2 Hybrid sorghum seed production using male sterility**

Male sterility in plants is caused by an interaction of sterility inducing factors in the cytoplasm with genetic factors in the nucleus; this is called cytoplasmic genetic male sterility. In sorghum

such system was discovered in 1954 by Stephens and Holland, who noticed from crosses of Milo x Kafir that cytoplasmic male-sterility (CMS) existed, resulting from an interaction between Milo cytoplasm ( $A_1$ ) and Kafir nuclear genome. The  $F_2$  generation of this cross exhibited some male sterile plants, whereas the progenies of the reciprocal cross, Kafir x Milo were all fertile. Male sterility was expressed only when Milo was used as female parent thereby indicating the importance of Milo cytoplasm.

Three types of parental lines are used for hybrid seed production, these are: the R-line (pollen parent, homozygous for nuclear restorer genes with normal or male fertile cytoplasm), the A-line (female of seed parent homozygous for nuclear non-restorer genes with male-sterile cytoplasm) and the B-line (maintainer of A-line, homozygous for nuclear non-restorer genes with normal cytoplasm).

Today, in the entire sorghum seed industry and the sorghum hybrid grown by farmers are based on Milo ( $A_1$ ) type of cytoplasm; only in a few instances are other cytoplasm used to induce male sterility. Different cytoplasmic male sterility systems have been developed. The  $A_2$  cytoplasm was first reported by Schertz and Pring (1981). The source of  $A_2$  cytoplasm is 1S66c in the coudatum group (Guinea race) from Ethiopia. The source of nuclear gene and the maintainer is 1S322c, which is in the Roxburghii group (Guinea race) from India. The designation  $A_3$  was given to the male sterility inducing cytoplasm in 1S1112c (SC193) by Quimby (1980), who proposed that plants with  $A_3$  cytoplasm are “Ultra female” which reduces the probability of finding restorers.

### **2.9.3 Hybrid sorghum seed production using chemical gamatocides**

The only feasible way to obtain commercial hybrid in sorghum or any self pollinated crop is through the inducement of male sterility. Male sterility is artificially induced by spraying chemical *Gametocides* to cause stamen sterility without harming the pistil. The plant whose

stamen is made sterile by the use of *Gametocide* (chemical) can be used as female parent for hybrid seed production. Two or more selected lines are planted in alternate strips and one is utilized as female (chemically sterilized) and is pollinated by the other line for producing hybrid seed. *Trihalogenatedmethylsulfonamides* that is, *trihalomethylsulfonamide* (TFMSA) is a new class of male *Gametocide* that induces male sterility, specifically, without detectable effects on other plant functions. TFMSA is currently being used to induce male sterility in the cereal crops such as sorghum, maize and wheat, with great success (Dale, 2004). Further, Dale (2004) pointed out that florets from TFMSA-treated plants were not capable of converting C-glutamate to C-proline and anther transport capacity of C-proline in TFMSA treated plants was significantly reduced. It is therefore inferred that TFMSA induces male sterility by interfering with the transport of proline from the site of synthesis to the site of accumulation, ultimately starving the developing anther of proline. 100-200µg of TFMSA has been used in green house study in sorghum to obtain 100 percent male sterility while in maize 200µg plant<sup>-1</sup> of TFMSA produced 100 percent male sterility (Dale, 2004). Other chemical hybridizing agent such as Ethrel, have been used with great success in hybrid seed production. Earlier, Borghi *et al.* (1973) reported the application of 4000-8000 ppm of Ethrel as the most effective concentration for inducing sterility at early boot stage in wheat.

## **2.10 Correlation of characters in sorghum**

Correlation, is the degree of association between characters, and can be strong or weak, positive or negative (Eckebil *et al.*, 1977 and Kadams, 2000). The cause of correlation may either be genetic or environmental. Genetic correlation, which is of great value to breeders, usually occurs through pleiotropy or linkage and it is the cause of transient correlation particularly in populations derived from crosses between divergent strains (Falconer, 1981). Correlated responses are of interest to breeders because they provide information on the type of change that

selection for one trait could cause in another (unselected) trait. This information is useful in the construction of selection indices for simultaneous improvement of two or more traits. Pleiotropy is simply a characteristic of gene whereby it affects two or more characters. The degree of correlation arising from pleiotropy expresses the extent to which two characters are influenced by the same gene. Correlation resulting from pleiotropy is the overall or net effect of all segregating genes that affect both characters, while others may increase or reduce the characters; the former tends to cause positive correlation and the latter negative correlation. When they vary together in the same magnitude, the coefficient is usually negative.

Success in breeding programs requires evaluation in environments that are representative of the target population of environments (Falconer and Mackay, 1996). However, the problem with testing in marginally productive environments when breeding for good performance in these environments is that the ratio of genetic to non genetic variance frequently is less than in highly productive environments (Falconer and Mackay, 1996). The environmental correlation is the correlation of environmental deviation together with non-additive variance (Falconer, 1981). The “genetic” and “environmental” correlations correspond to the partitioning of covariance into additive genetic components versus all the rest.

Phenotypic correlation or correlation of phenotypic value is the association between two characters that can be directly observed. This is estimated from measurement of the two characters in a number of individuals of the population (genotypic and environmental factors). Relationships among traits of economic importance are valuable in making sound decisions in breeding programs especially for selection purposes. Generally, sequence of selection of traits in breeding program depends on their relative importance, their ease of selection and their inter relationship with one another. Selection in the above sequence using good parental materials enhances the production of superior genotypes. Totok (1997) reported a genetic correlation of 0.75 and 0.89 for grain yield per plant with number of productive panicles and panicle weight,

respectively. In a study on genetic association among sorghum genotypes, Bello *et al.* (2001), reported a significant positive association between plant height and grain yield (0.84) and also between panicle length and grain yield. They suggested that these characters were most reliable for yield improvement in this population, owing to their significant high positive relationship with yield. In another trial to estimate the correlation of economically important traits in sorghum varieties, Sadia *et al.* (2001), observed that a highly significant positive genetic correlation existed between all the traits measured namely: plant height, panicle length, 1000 seed weight, number of grains per panicle, days to 50% flowering, days to 95% maturity, grain yield, stover yield and total dry matter. These traits also had significant positive genetic correlation with yield. Aba and Zaria (2000) reported a highly significant positive genetic correlation between length of first leaf and second leaf sheaths (0.69), while a negative significant genetic correlation (-0.84) was found between length of first leaf sheath and width of spikelet in sorghum.

In a study to determine the relationship between enzyme development, malt extract yield and sugar profile in malted sorghum, Agu (2005), reported a significant positive relationship between diastatic activity and extract yield. Agu (2005) further, observed that, significant positive correlation also exist between diastatic activity and level of glucose produced than maltose. However, he observed that when  $\beta$ -amylase is higher than  $\alpha$ -amylase more maltose than glucose is produced in the sorghum worts. Finally, he pointed out that, these observations would play an important role in the choice of parents for hybrid sorghum malting quality.



## **CHAPTER THREE**

### **MATERIALS AND METHODS**

#### **3.0**

##### **3.1 Genetic materials**

Four Sorghum varieties designated as Samsorg 14, Samsorg 41, Samsorg 17 and Samsorg 40 improved for malting at the Institute for Agricultural Research (IAR) Samaru, Zaria, were collected in 2004 from the Institute. These were used as male parents (Testers), while eight local sorghum cultivars with good malting qualities (from personal communication with users) designated as Ex-Gulak, Ex-Gerio, Ex-Wagga, Ex-Guyuk, Ex-Pella, Ex-Girei, Ex-Mbamba and Ex-Numan, collected within Adamawa State, Nigeria in the same year (2004) were used as female parents (Lines); in all, there were 12 parents. General descriptions of the 12 parental genotypes are given in Table 1.

##### **3.2 Preliminary evaluation of genetic materials used in the experiment**

The twelve parents collected in 2004 were sown on 3 (three) 5m long ridges each, in the rainy season of 2004, as described by Murty *et al.* (1994). This was to determine the number of days to booting for each genotype, so as to provide a guide for ‘nicking’ time of these genotypes during crossing. The preliminary trial was carried out at the Lake Gerio farm project in Jimeta (Lat. 9° 18'N and Long. 12° 15'E).

##### **3.4 Crossing nursery and crossing procedures**

###### **3.3.1 Crossing nursery**

The crossing nursery (Figure 1) was sited at the Lake Gerio farm project in Jimeta (Lat. 9° 18'N and Long. 12° 15'E). It has an altitude of 200m above sea level, within the Northern Guinea Savannah Zone of Nigeria. The soil type is clay loam. The site was previously cropped with maize and free from any nearby sorghum field.

**Table 1: Genotype designation, pedigree, source and description of materials used in the study**

| <b>S/No</b> | <b>Genotype designation</b> | <b>Pedigree</b>   | <b>Source</b>            | <b>Brief description</b>  |
|-------------|-----------------------------|---|--------------------------|---|
| 1.          | SAMSORG-14 (KSV-8)          | Developed at IAR Samaru, Modified by bulk method from pure line selection, among materials from Uganda. | IAR Samaru Zaria         | Tall variety, semi compact panicle with white seed; average malting quality. High yielding. Medium maturing variety (140-150 days).             |
| 2.          | SAMSORG-41 (ICSV-400)       | 1512611x5c108-3   | IAR Samaru Zaria         | Medium tall variety, semi compact panicle with cream coloured seed; very good malting characteristics, early maturing variety (105 - 115 days). |
| 3.          | SAMSORG-17 (SK-5912)        | Selection from local collection of Kaura, through mutation Breeding (i.e. mutant from short Kaura)      | IAR Samaru Zaria         | Medium tall variety with compact panicle, it has yellow seeds. Very Good for brewing. Late maturity (160-180 days).                             |
| 4.          | SAMSORG-40 (ICSV-111)       | SPV-35xE -35-1  | IAR Samaru Zaria         | Medium tall variety, semi compact panicle and white grains. Good for food and average malting quality. Early maturing variety (100-110 days).   |
| 5.          | Ex-Gulak                    | Cultivated local variety  | Gulak<br>Madagali LGA    | Medium tall variety, compact panicle with reddish seed; used for local brewing. It is an early variety (110 - 112 days).                        |
| 6.          | Ex-Gerio                    | Cultivated local variety  | Jimeta<br>Yola North LGA | Medium tall cultivar, compact panicle with reddish grains, good for food and local brewing, late maturing (160-162 days).                       |
| 7.          | Ex-Wagga                    | Cultivated local variety  | Wagga<br>Madagali LGA    | Medium tall cultivar, long compact panicle with white grains. Good for food and local brewing. Late maturing (170-172 days).                    |
| 8.          | Ex-Guyuk                    | Cultivated local variety  | Guyuk<br>Guyuk LGA       | Medium tall cultivar, long compact panicle with yellow grains, good for food and local brewing. Late maturing (170-172 days).                   |
| 9.          | Ex-Pella                    | Cultivated local variety  | Pella<br>Hong LGA        | Tall cultivar with long compact panicle with white grains. Good for food and local brewing. Late maturing (160-162 days).                       |
| 10.         | Ex-Girei                    | Cultivated local variety  | Girei<br>Girei LGA       | Medium tall cultivar with loose panicle it has reddish seeds. Good for food and brewing. Extra early maturing (80 - 85 days).                   |
| 11.         | Ex-Mbamba                   | Cultivated local variety  | Mbamba<br>Yola South LGA | Medium tall cultivar with long compact panicle with reddish seeds. Extra early maturing (80 - 85days). Good for food and local brewing          |
| 12.         | Ex-Numan                    | Cultivated local variety  | Numan<br>Numan LGA       | Medium tall cultivar with long compact panicle. It has reddish seeds and Good for food and local brewing. Middium maturing (140 - 145 days).    |

| BLOCK I |        | BLOCK II |        | BLOCK III |        | BLOCK IV |        |
|---------|--------|----------|--------|-----------|--------|----------|--------|
| LINES   | TESTER | LINES    | TESTER | LINES     | TESTER | LINES    | TESTER |
| 5       | 1      | 5        | 2      | 5         | 3      | 5        | 4      |
| 6       | 1      | 6        | 2      | 6         | 3      | 6        | 4      |
| 7       | 1      | 7        | 2      | 7         | 3      | 7        | 4      |
| 8       | 1      | 8        | 2      | 8         | 3      | 8        | 4      |
| 9       | 1      | 9        | 2      | 9         | 3      | 9        | 4      |
| 10      | 1      | 10       | 2      | 10        | 3      | 10       | 4      |
| 11      | 1      | 11       | 2      | 11        | 3      | 11       | 4      |
| 12      | 1      | 12       | 2      | 12        | 3      | 12       | 4      |

**Fig. 1: Out-line of the Crossing Nursery for the F<sub>1</sub> Seed Production.**

**Key:**

1= SAMSORG 14  
 2=SAMSORG 41  
 3= SAMSORG 17  
 4= SAMSORG 40  
 5= Ex-Gulak  
 6= Ex-Gerio  
 7= Ex-Wagga  
 8= Ex-Guyuk  
 9= Ex-Pella  
 10= Ex-Girei  
 11= Ex-Mbamba  
 12= Ex-Numan

There are 4 blocks, each with 8 plots of the Lines 5,6,7,8,9,10,11 and 12 and 8 plots of the Testers 1,2,3 and 4 opposite respective lines in an isolated field.

### 3.3.2 Crossing procedures

Seeds of the 12 genotypes were “stagger-planted” at intervals (Figure 1), so as to “nick” the parents due to variation in number of days to anthesis (booting). Irrigation scheduled was twice weekly until harvest while weeding was carried out once every two weeks until harvest, no fertilizer was used in the crossing nursery. Crosses were performed for two years (2005/2006 and 2006/2007 dry seasons); this was in order to generate enough F<sub>1</sub> seeds for field trial over three locations in 2007 cropping season. Crosses were carried out following the line x tester mating design (Table 2). The crosses were performed using hand emasculation technique as described by Leland (1985) and Murty *et al.* (1994). A total of 32 crosses were generated, which gave rise to a total of 44 entries, including the 12 parents.

The procedures began with trimming of portion of the head that have anthesised, each head was trimmed so that individual florets remain uniformly spaced such that panicle florets occur in cluster of two or three. Emasculation was done by grasping the floret between the thumb and the fore-finger; the emasculation needle was then inserted between the glume below the mid-portion of the floret and then slowly moved across the inner surfaces of the glume so as to break the filaments. The needle was then slowly lifted out and upwards, pulling the three detached anthers. Emasculated panicles were then covered with large envelope (bag) made from brown paper, the date of emasculation was then written on the bag with indelible ink.

Crosses were carried out within 3 days, during morning hours, as follows; fresh pollen was collected from designated male parent (using the brown envelope) and carried immediately to the receptive female parent. The bag covering the female parent was removed and the bag with the collected pollen was inserted on the female head, then held tightly around the peduncle and shaken vigorously filling the air in the bag with pollen. After pollination, the bag is secured properly using clips over the head. The date of crossing and the parents crossed were clearly written on the envelope.

**Table 2: Outline of Line x Tester mating design comprising 12 Parents and 32 crosses (44 entries)**

| <b>Parents</b>   | <b>Males ♂</b> |          |          |          |
|------------------|----------------|----------|----------|----------|
| <b>Females ♀</b> | <b>1</b>       | <b>2</b> | <b>3</b> | <b>4</b> |
| <b>5</b>         | 5x1            | 5x2      | 5x3      | 5x4      |
| <b>6</b>         | 6x1            | 6x2      | 6x3      | 6x4      |
| <b>7</b>         | 7x1            | 7x2      | 7x3      | 7x4      |
| <b>8</b>         | 8x1            | 8x2      | 8x3      | 8x4      |
| <b>9</b>         | 9x1            | 9x2      | 9x3      | 9x4      |
| <b>10</b>        | 10x1           | 10x2     | 10x3     | 10x4     |
| <b>11</b>        | 11x1           | 11x2     | 11x3     | 11x4     |
| <b>12</b>        | 12x1           | 12x2     | 12x3     | 12x4     |

**Key:**

|                |               |              |
|----------------|---------------|--------------|
| 1= SAMSORG 14  | 5x3=Ex-Gulak  | x SAMSORG 17 |
| 2=SAMSORG 41   | 6x3=Ex-Gerio  | x SAMSORG 17 |
| 3= SAMSORG 17  | 7x3=Ex-Wagga  | x SAMSORG 17 |
| 4= SAMSORG 40  | 8x3=Ex-Guyuk  | x SAMSORG 17 |
| 5= Ex-Gulak    | 9x3=Ex-Pella  | x SAMSORG 17 |
| 6= Ex-Gerio    | 10x3=Ex-Girei | x SAMSORG 17 |
| 7= Ex-Wagga    | 11x3=Ex-Mbamb | x SAMSORG 17 |
| 8= Ex-Guyuk    | 12x3=Ex-Numan | x SAMSORG 17 |
| 9= Ex-Pella    | 5x4=Ex-Gulak  | x SAMSORG 40 |
| 10= Ex-Girei   | 6x4=Ex-Gerio  | x SAMSORG 40 |
| 11= Ex-Mbamba  | 7x4=Ex-Wagga  | x SAMSORG 40 |
| 12= Ex-Numan   | 8x4=Ex-Guyuk  | x SAMSORG 40 |
| 5x1=Ex-Gulak   | 9x4=Ex-Pella  | x SAMSORG 40 |
| 6x1=Ex-Gerio   | 10x4=Ex-Girei | x SAMSORG 40 |
| 7x1=Ex-Wagga   | 11x4=Ex-Mbamb | x SAMSORG 40 |
| 8x1=Ex-Guyuk   | 12x4=Ex-Numan | x SAMSORG 40 |
| 9x1=Ex-Pella   |               |              |
| 10x1=Ex-Girei  |               |              |
| 11x1=Ex-Mbamba |               |              |
| 12x1=Ex-Numan  |               |              |
| 5x2=Ex-Gulak   |               |              |
| 6x2=Ex-Gerio   |               |              |
| 7x2=Ex-Wagga   |               |              |
| 8x2=Ex-Guyuk   |               |              |
| 9x2=Ex-Pella   |               |              |
| 10x2=Ex-Girei  |               |              |
| 11x2=Ex-Mbamba |               |              |
| 12x2=Ex-Numan  |               |              |

### **3.4 Experimental sites for progeny evaluation**

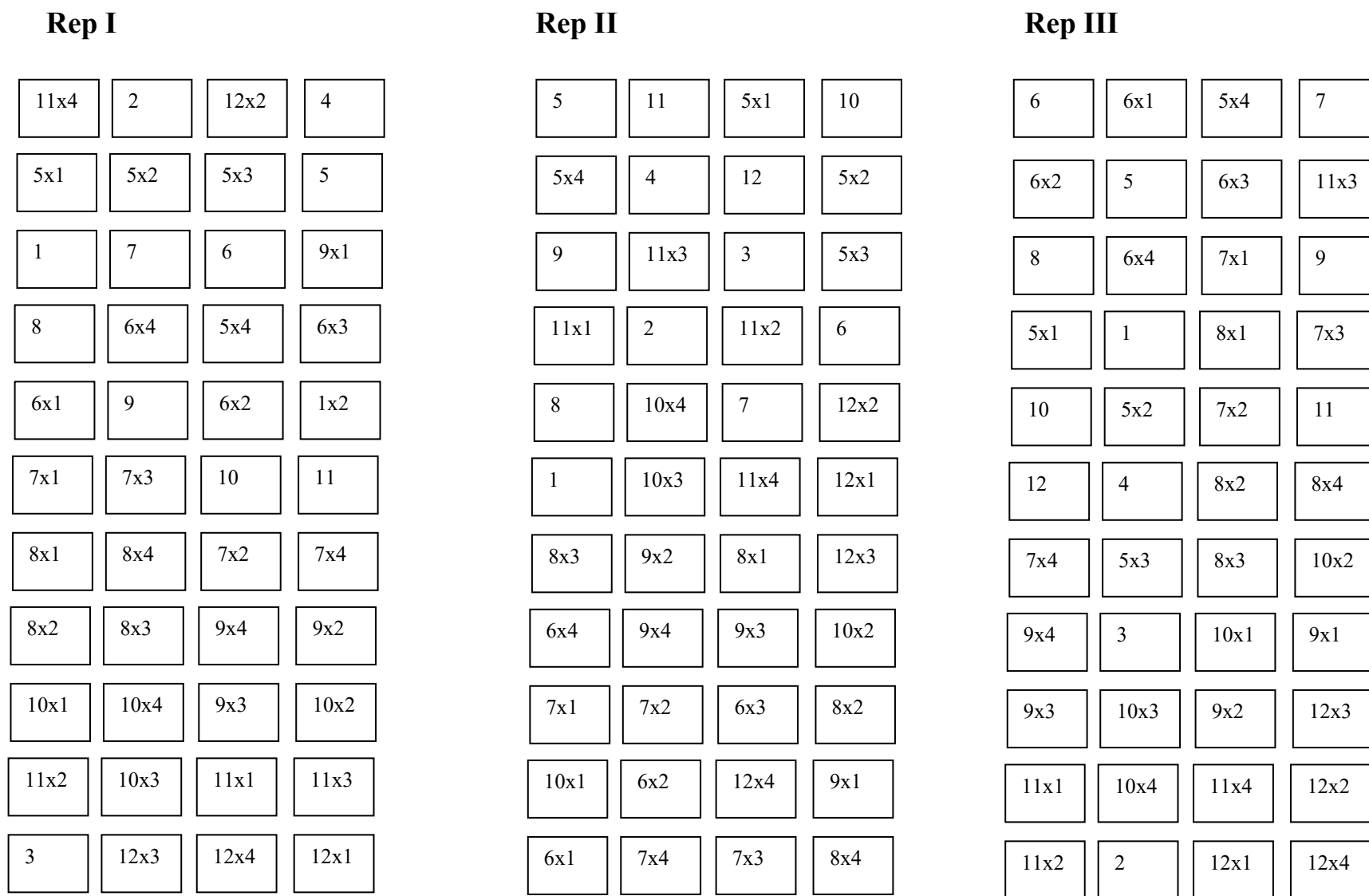
Evaluations of the Progenies were conducted over three locations in Adamawa State. The locations were as follows: College of Agriculture experimental farm site in Ganye (Lat. 09° 08'N and Long. 11° 05'E) within the northern guinea savanna zone of Nigeria, the soil type of the experimental site was sandy loam (Adebayo and Tukur, 1999); College of Agriculture Mubi, students' experimental farm site (lat. 10° 03'N and Long. 13° 07'E) also within the northern guinea savanna zone of Nigeria, Mubi has an altitude of 286m above sea level, the soil type of the site was clay loam and Teaching and Research Farm of the Department of Crop Production and Horticulture Federal University of Technology, Yola (Latitude 09° 18'N and Longitude 12° 15'E), within the northern guinea savanna zone of Nigeria, the soil type was clay loam.

### **3.5 Experimental design for progeny evaluation**

The 44 entries were laid in a “4 x 11” Randomized Complete Block Design (RCBD) with three replications (Figure 2), in all the three locations earlier described. Each entry was sowed on a two 5m ridged plot, spaced 0.75m apart (that is, a plot size of 5m x 1.5m = 7.5m<sup>2</sup>) at an intra row spacing of 0.30m apart. The total field area at each location was 67m x 11m (737m<sup>2</sup>). Two to three (2-3) seeds were sown per hill and later thinned to one plant per hill which gave rise to 16 plants per row with a total of 32 plants per plot (42,667 plants/ha).

### **3.6 Cultural practices**

Seeds were sown at each location (experiment site) following land preparation, on 6 /6/07 in Ganye, 1 /7/07 in Mubi and on 13 /7/07 in Yola, due to variations in rainfall establishments across these locations. Manual weeding using hoe was carried out once every two weeks throughout the experiments while N.P. K. (27:13:13) fertilizer was applied at the rate of 60kg N/ha, 30kg/ha of phosphorus (P<sub>2</sub> O<sub>5</sub>) and 30kg/ha potassium (K<sub>2</sub>O) in two split doses at 3 and 6 weeks after sowing. There was no noticeable pest or disease outbreak including *Striga*, throughout the experiments in the three locations.



**Fig. 2: Field layout in a “4 x 11” Randomized Complete Block Design (RCBD) with three replications.**

**Key:** (1) SAMSORG 14 (2) SAMSORG 41 (3) SAMSORG 17 (4) SAMSORG 40 (5) Ex-Gulak (6) Ex-Gerio (7) Ex-Wagga (8) Ex-Guyuk (9) Ex-Pella (10) Ex-Girei (11) Ex-Mbamba (12) Ex-Numan

### **3.7 Data collection on agronomic characters and malting quality traits**

#### **3.7.1 Agronomic characters**

At maturity 10 plants in the middle rows (with least border effects) were sampled at random for measurements on the following characters:

3.7.1.1 Plant height (cm): The length of the stem measured from ground level to the tip of the panicle.

3.7.1.2 Number of leaves per plant: This is the number of leaves on the plant.

3.7.1.3 Flag leaf length (cm): The length of the boot leaf measured from the base to the tip.

3.7.1.4 Flag leaf width (cm): The width of the boot leaf measured at the widest point.

3.7.1.5 Panicle exertion (cm): The length of the stem measured from the base of the boot leaf to the base of the panicle

3.7.1.6 Inter-node length (cm): The length of each stem divided by the number of nodes on the stem.

3.7.1.7 1000-grain weight: The weight of 1000 grains from the bulk grains per plot.

3.7.1.8 Panicle length (cm): The length of the panicle from the collar to the tip.

3.7.1.9 Panicle width (cm): The width of the panicle at the widest diameter of the panicle.

3.7.1.10 Days to anthesis: Number of days from sowing to the date when 50% of the plants on a plot flowered (anthesised).

3.7.1.11 Days to maturity: Number of days from sowing to when 95% of the plants on a plot have reached physiological maturity.

3.7.1.12 Yield per plant (g): Grain yield estimated in g/plant

3.7.1.13 Yield per hectare (kg/ha): Grain yield estimated in kg/ha

#### **3.7.2 Data collection on malting quality traits**

The malting traits considered were germination energy, germination capacity, moisture content, water sensitivity, cold water extract, hot water extract, diastatic power and alpha amylase. These parameters (for both parents and F<sub>1</sub>S) were analyzed following standard procedures, in the laboratory of the International Beer and Beverage Industries, IBBI Kaduna, Nigeria.

##### **3.7.2.1 Germination energy (%)**

The IOB (1986) method was used; two whatman no. 1 filter papers were placed at the bottom of a 9cm Petri-dish and 4ml. of distilled water was accurately transferred onto it. Then 100 grains were counted



and placed on the filter paper taking care to ensure that all grains were in good contact with the moist filter paper. The Petri dish was then incubated at 18°C in a growth cabinet for 24, 48 and 72 hours, the grains are examined and germinated grains removed on each occasion. Grains which have not germinated were counted after 72 hours, the percentage of sprouted grains were counted at the end of incubation (72 hours) and expressed as germination energy (GE) using the formula,

$$GE\% = 100 - n$$

Where GE (4ml.) % =Germination Energy in 4ml. water

n =Number of un-germinated grains

### 3.7.2.2 Germination capacity (viability) %

Grains showing no evidence of germination were in each case placed on fresh moist papers and incubated as above for a further 48hrs. Grains which had germinated were counted and added to those germinating after 72 hours; the new percentage was recorded as the germination capacity (EBC, 2007).

### 3.7.2.3 Moisture content determination (%)

The moisture was determined by the direct oven method of AOAC (1984). The samples were milled and 10g of each sample was weighed into Petri dish of known weight. The samples were then placed in an oven at 105°C for 3hrs. The dried samples were removed from the oven and placed in desiccators for 25 minutes to cool prior to weighing, then placed in an oven for drying and reweighing until a constant weight is achieved. The moisture content was obtained using the formula:

$$\text{Moisture Content (\%)} = \frac{W_1 - W_2}{W_3} \times 100$$

Where:

|       |   |   |
|-------|---|---|
| $W_1$ | = | Weight of sample + dish before drying             |
| $W_2$ | = | Weight of sample + dish after drying (on cooling) |
| $W_3$ | = | Weight of sample used                             |

#### 3.7.2.4 Water sensitivity (%)

The IOB (1986) method was also used; two whatman no. 1 filter papers were placed at the bottom of a 9cm Petri-dish and 8ml. of distilled water was added to it; 100 grains were counted and placed in good contact with the moist filter paper. The Petri dish was then incubated at 18<sup>0</sup>C, in a growth cabinet for 24, 48 and 72 hours. Germinated grains were counted at the end of 24, 48 and 72 hours as water sensitivity (%).

#### 3.7.3 Sorghum malt production

Based on methods of EBC (2007), sorghum malt was produced as follows: 1kg of grains from each sample was steep in water over-night (18hrs.) at room temperature (28<sup>0</sup>C). Subsequently water was changed at six hourly intervals until the moisture content of the sorghum was 30 %. Steeping lasted for 40hrs. for every sample with an air rest of 4hrs. 0.1% formaldehyde was added to the steep water to reduce microbial load on the sorghum grains and improve germination.

The grains from each sample were then allowed to germinate in a large beaker. The newly-germinated grains were spread at room temperature on a large glass sheet lined with moist blotting paper. Evaporation was prevented by use of a sheet of polythene. Grains were mixed and sprinkled with water at approximately six hourly intervals to ensure good enzyme and extract development. Successful modification of the grains was determined by chewing the grains which had then developed sweet taste. Germination lasted for three days. The germinated grains were kilned in a hot air oven at 55<sup>0</sup>C until the moisture content was 5.8 %. It was then raised to 65<sup>0</sup>C until the seeds moisture content was 4%; the temperature was then maintained at 65<sup>0</sup>C until the grains become friable. Rootlets were then removed. For analysis purpose, at the end of malting a kilogram sample was weighed and ground in an electric grinder and passed through number 30 sieve to obtain particle size of 0.5mm. The ground malt was used for the following analyses:

3.7.3.1 Cold water extract (CWE) %: 10g of the ground malt was digested with 200ml distilled water containing 12ml of 0.1M ammonia for 3hrs at 30<sup>0</sup>C, stirring every 30mins. Grains were allowed to settle.

It was then filtered off and the specific gravity at 20°C was determined. The cold water extract (CWE) was calculated by the equation:  $CWE = (\text{specific gravity} - 1000) \times 20 (\%)$

3.7.3.2 Diastatic power (DP) °L (Lintner method): 5ml of the supernatant from the above sample was shaken with 100ml of buffered 2% soluble starch at 21°C for 1hour. 30ml of 0.1N NaOH were used to stop further diastatic action after which the volume was made up to 200ml with distilled water at 21°C. The mixture was filtered against 5ml of mixed Fehling's solution using methylene blue as indicator. Diastatic power (DP) was calculated thus:

$$DP = \frac{2000}{xy} - \frac{20}{x} \text{ (°L)}$$

Where; x = volume (ml) of malt extract

y = volume of starch solution required for reduction of 5ml of Fehling's solution.

3.7.3.3 Hot water extract (HWE) L°/kg : 50g of ground malt was mashed at 60°C and following the procedures described for cold water. The hot water extract (HWE) was calculated by the equation;

$$HWE = (\text{specific gravity} - 1000) \times 4.8$$

3.7.3.4 α-Amylase (Dextrin units) DU: The crude substrate was prepared by mixing 1% starch with excess β-amylase dissolved in 20mM sodium acetate buffer containing 10 mM CaCl<sub>2</sub> (pH=5.7). Five grams of milled malt of each sample was prepared by extracting in 80mL of extraction buffer (20mM sodium acetate buffer pH = 5.7). The mixture was shaken for 30mins. and centrifuged at 2,000 rpm for 10 mins. at 4°C. The supernatant was removed and diluted appropriately (x20), before 0.5 mL of the enzyme extract was used in duplicate for assay. Substrate (0.5mL) was mixed with 0.5mL of the diluted enzyme and the mixture was allowed to digest for 5min. at 25°C. Dilute iodine solution (10mL) was added to the digest to stop the enzyme reaction. The colour of the iodine-dextrin complex was determined using a spectrophotometer (Philips PU 8730 UV/VIS scanning spectrophotometer and Philips colour plotter) at 565nm. Digests containing no substrate or no enzyme were also examined at 565nm. The alpha-amylase in Dextrin Units was calculated using the formula:

$$DU = A_{565nm} (\text{absorbance}) \text{ units} \times 2 \times \text{Dilution}$$

Where;  $A_{565}(\text{absorbance}) \text{ units} = A_{565} \text{ substance control} - A_{565} \text{ Assay value}$

### 3.8 Statistical analyses

#### 3.8.1 Analysis of Variance (ANOVA)

Analyses of variances were conducted using individual plot means for each location and then combined across locations. Locations were considered as random effect while genotypes were considered as fixed effects. All statistical analyses of variance and linear genetic models used in this study were based on methods described by Singh and Chaudhary (1985) using the Generalized Linear Model (GLM) of SAS package (SAS Institute, 1999). Forms of the general and the specific combining ability analyses of variances for individual and combined locations are presented in Tables 3-6. The linear genetic model assumed for the general ANOVA for the individual location was as indicated below:

$$Y_{ij} = \mu + e_i + r_j + (er)_{ij} + e_{ij}. \quad (\text{Singh and Chaudhary, 1985})$$

**Where**

$Y_{ij}$  = effect of the  $i^{\text{th}}$  entry on the  $j^{\text{th}}$  replication

$\mu$  = General mean

$e_i$  = effect of the  $i^{\text{th}}$  entry ( $e=1\ldots 44$ )

$r_j$  = effect of the  $j^{\text{th}}$  replicate ( $r= 1,2,3$ )

$(er)_{ij}$  = entry x replication Interaction

$e_{ij}$  = error associated with each observation.

While for the combined analysis across locations; the following linear model was used:

$$Y_{ijk} = \mu + g_i + l_j + (gl)_{ij} + B_{ijk} + (gl)_{ij} + e_{ijk} \quad (\text{Singh and Chaudhary, 1985})$$

**Where:**

$Y_{ijk}$  = observation of any variable in the  $K^{\text{th}}$  replication in the  $j^{\text{th}}$  location on the  $i^{\text{th}}$  genotype.

$\mu$  = the general mean.

$g_i$  and  $l_j$  = effect of the  $i^{\text{th}}$  genotype in the  $j^{\text{th}}$  location.

$B_{ijk}$  = effect of the  $K^{\text{th}}$  replication in the  $j^{\text{th}}$  location on the  $i^{\text{th}}$  genotype

$(gl)_{ij}$  = genotype x location interaction effect

$e_{ijk}$  = random error associated with  $K^{\text{th}}$  replication in the  $j^{\text{th}}$  location on the  $i^{\text{th}}$  genotype.

**Where:**  $i = 1\ldots 44$ ,  $j = 1,2,3$ ,  $k = 1,2,3$ ;

**Table 3: Form of general analysis of variance and expectation of mean squares for individual****location**

| Source of variation | DF  | MS             | Expected mean squares          |
|---------------------|-----|----------------|--------------------------------|
| Replication (r-1)   | 2   |                |                                |
| Entries (e-1)       | 43  | M <sub>4</sub> | $\delta^2_{err} + r\delta^2_e$ |
| Parents (p-1)       | 11  | M <sub>3</sub> | $\delta^2_{err} + r\delta^2_p$ |
| Crosses (c-1)       | 31  | M <sub>2</sub> | $\delta^2_{err} + r\delta^2_c$ |
| Parents vs crosses  | 1   |                |                                |
| Error (r-1) (e-1)   | 86  | M <sub>1</sub> | $\delta^2_{err}$               |
| Total (re)-1        | 131 |                |                                |

Key: c= crosses, p= parents, loc= location, e= entries, e<sub>rr</sub>= error, r=replication and Ms<sub>1-4</sub>= mean squares

**Source:** Singh and Chaudhary (1985)

**Table 4: Form of general analysis of variance and expectation of mean squares across locations**

| Source of variation             | DF  | MS             | Expected mean squares   |
|---------------------------------|-----|----------------|---|
| Locations (loc-1)               | 2   |                |   |
| Rep/ loc. Loc(r-1)              | 6   |                |   |
| Entries (e-1)                   | 43  | M <sub>7</sub> | $\delta^2_{err} + r\delta^2_{rc\ loc.} \delta^2_p + p\ loc\ \delta^2_e$ |
| Parents (p-1)                   | 11  | M <sub>6</sub> | $\delta^2_{err} + r\delta^2_{rc\ loc.} \delta^2_p$                      |
| Crosses(c-1)                    | 31  | M <sub>5</sub> | $\delta^2_{err} + r\delta^2_{p\ loc.} \delta^2_c$                       |
| Parents vs crosses (p vs c)     | 1   |                |   |
| Loc. x Entries (Loc-1)(e-1)     | 86  | M <sub>4</sub> | $\delta^2_{err} + r\delta^2_{e(loc)}$                                   |
| Loc. x Crosses (Loc-1)(c-1)     | 62  | M <sub>3</sub> | $\delta^2_{err} + r\delta^2_{c(loc)}$                                   |
| Loc. x Parents (Loc-1)(p-1)     | 22  | M <sub>2</sub> | $\delta^2_{err} + r\delta^2_{p(loc)}$                                   |
| Loc. x (Parents vs Crosses)     | 2   |                |   |
| Error (Rep/Loc)(e-1)            | 258 | M <sub>1</sub> | $\delta^2_{err}$  |
| Total [(Rep/Loc) (r-1) (e-1)]-1 | 524 |                |   |

Key: c= crosses, p= parents, loc= location, e= entry, e<sub>rr</sub>= error, r=replication and M<sub>1-7</sub>= mean squares  
 $\delta^2$ = variance due to any component.

**Source:** (Singh and Chaudhary, 1985)

### 3.8.2 Genetic analyses

#### 3.8.2.1 Combining ability analysis

In the analysis of combining ability, crosses were partitioned into male (tester) effects, female (line) effects and male x female effects. Combining ability analysis for individual location was based on the following statistical model:

$$Y_{ijk} = \mu + M_i + F_j + (M \times F)_{ij} + e_{ijk} \quad (\text{Singh and Chaudhary, 1985})$$

**Where;**

|                     |   |   |
|---------------------|---|---|
| i                   | = | 1,2 ...4 (testers)  |
| j                   | = | 1,2...8 (lines)   |
| k                   | = | 1,2,3(replications)   |
| $Y_{ijk}$           | = | $K^{\text{th}}$ observation on $i^{\text{th}}$ male and $j^{\text{th}}$ female. |
| $\mu$               | = | general mean.   |
| $M_i$               | = | effect $i^{\text{th}}$ male (tester).   |
| $F_j$               | = | effect of the $j^{\text{th}}$ female (Line)                                     |
| $(M \times F)_{ij}$ | = | Interaction effect and  |
| $e_{ijk}$           | = | error associated with each observation.   |

Combining ability analysis of variance across locations was based on the following linear model:

$$Y_{ijkl} = \mu + g_i + g_j + S_{ij} + L + (gl)_{il} + (gl)_{jl} + (SL)_{ijl} + e_{ijkl} \quad (\text{Singh and Chaudhary, 1985})$$

**Where**

|              |   |  |
|--------------|---|--|
| i            | = | 1, 2...4 (testers)   |
| j            | = | 1,2...8 (lines)  |
| l            | = | 1,2,3 (locations)  |
| k            | = | 1,2,3(replications)  |
| $Y_{ijkl}$   | = | observation on the hybrid between the $i^{\text{th}}$ male and the $j^{\text{th}}$ female in the $k^{\text{th}}$ replication and $l^{\text{th}}$ location. |
| $\mu$        | = | population mean common to all crosses in all replications within locations.  |
| $g_i$        | = | effect common to progeny of the $i^{\text{th}}$ tester.  |
| $g_j$        | = | effect common to progeny of the $j^{\text{th}}$ Line   |
| $s_{ij}$     | = | effect specific to the progeny of mating the $i^{\text{th}}$ tester and the $j^{\text{th}}$ Line.  |
| L            | = | the average effect of all the $l^{\text{th}}$ locations.   |
| $(gl)_{il}$  | = | genotype x location interaction effect on the $i^{\text{th}}$ tester in location   |
| $(gl)_{jl}$  | = | genotype x location interaction effect on the $j^{\text{th}}$ Line in location.  |
| $(sl)_{ijl}$ | = | effect specific to the progeny of mating the $i^{\text{th}}$ tester to the $j^{\text{th}}$ Line in location.   |
| $e_{ijkl}$   | = | Random error.  |

**Table 5: Form of combining ability analysis of variance and expectation of mean squares for individual location**

| Source of variation       | DF | MS               | Expected mean squares                            |
|---------------------------|----|------------------|--|
| Rep (r-1)                 | 2  |                  |  |
| Lines (female) (l-1)      | 7  | $M_l$            | $\delta^2 + r\delta^2 t \times l + rt\delta^2 l$ |
| Testers (male) (t-1)      | 3  | $M_t$            | $\delta^2 + r\delta^2 t \times l + rl\delta^2 t$ |
| Lines x Testers (t-1*l-1) | 21 | $M_{t \times l}$ | $\delta^2 + r\delta^2 t \times l$                |
| Error (mf-1) (r-1)        | 62 | $M_{err}$        | $\delta^2 e$                                     |
| Total (rfm)-1             | 95 |                  |  |

Key: l= line, t= tester, e or err= error, M = mean squares due to any component, r=replication and  $\delta^2$ = variance due to any component.

**Source:** Singh and Chaudhary (1985)

**Table 6: Form of combining ability analysis of variance and expectation of mean squares across locations**

| Source of variation                          | DF  | MS    | Expected mean squares  |
|--|-----|-------|--|
| Location(loc-1)                              | 2   |       |  |
| Rep/ loc. Loc(r-1)                           | 6   |       |  |
| Lines(l-1)                                   | 3   | $M_7$ | $\delta^2 + r\delta^2 \text{ loc} \times t \times l + r \text{ loc} \delta^2 tl + rf \delta^2 l$ |
| Testers(t-1)                                 | 7   | $M_6$ | $\delta^2 + r\delta^2 \text{ loc} \times t \times l + r \text{ loc} \delta^2 tl + rt \delta^2 t$ |
| Tester x line (t-1) (l-1)                    | 21  | $M_5$ | $\delta^2 + r\delta^2 \text{ loc} \times t \times l + r \text{ loc} \delta^2 t \times l$         |
| Loction x tester (loc-1)(t-1)                | 6   | $M_4$ | $\delta^2 + r\delta^2 \text{ loc} \times t \times l + rt \delta^2 \text{ loc} \times t$          |
| Location x line                              | 14  | $M_3$ | $\delta^2 + r\delta^2 \text{ loc} \times t \times l + rf \delta^2 \text{ loc} \times l$          |
| Location x tester x line (loc-1) (t-1) (l-1) | 42  | $M_2$ | $\delta^2 + r\delta^2 \text{ loc} \times t \times l$   |
| Pooled error                                 | 186 | $M_1$ | $\delta^2 e$   |
| Total [(r.f.m.loc.) -1]                      | 287 |       |  |

Key: L= line, t= tester, Loc= location, e= error,  $M_{1-7}$  = mean squares, r=replication and  $\delta^2$ = variance due to any component.

**Source:** Singh and Chaudhary (1985)

### 3.8.3 Estimation of Genetic components

The following genetic components of variance were calculated from the expectation of mean squares according to Singh and Chaudhary (1985).

$$\text{Testers} = \frac{M_{St} - M_{Stxl}}{rl}$$

$$\text{Lines} = \frac{M_{Sl} - M_{Stxl}}{rt}$$

$$\delta^2_{gca} = \frac{M_{Sl} - M_{Stxl} + M_{St} - M_{Stxl}}{r(l+t)}$$

$$\delta^2_{sca} = \frac{M_{Slxt} - M_{se}}{r}$$

To determine the gene action, the variance ratio was calculated thus:

$$\frac{\delta^2_{gca}}{\delta^2_{sca}}$$

**Where:**

|            |   |   |
|------------|---|---|
| $M_{St}$   | = | mean squares for testers.                 |
| $M_{Sl}$   | = | mean squares for lines                    |
| $M_{Stxl}$ | = | testers x lines interaction mean squares. |
| $M_{se}$   | = | error mean square.                        |
| $r$        | = | number of replications = 3                |
| $l$        | = | number of lines = 8                       |
| $t$        | = | number of testers = 4                     |

### 3.8.4 Estimation of GCA and SCA effects

The values of GCA (males), GCA (females) and SCA effects were estimated based on the procedures of Singh and Chaudhary (1985). Combining ability were estimated as the mean of each cross expressed as a percentage of the mean of all crosses and the general effect for each male and female, and the specific effect for each was calculated as follows:

$$gca(\text{testers}) = g_t = \frac{x_{.j.}}{lr} - \frac{x_{...}}{ltr}$$

$$gca(\text{lines}) = g_i = \frac{x_{i..}}{tr} - \frac{x_{...}}{ltr}$$

$$sca(\text{testers x lines}) = s_{ij} = \frac{x_{ij.}}{r} - \frac{x_{i..}}{lr} - \frac{x_{.j.}}{tr} + \frac{x_{...}}{tlr}$$



**Where:**

$x_i$  = mean performance of crosses within a given tester averaged over all replications within environments and lines.

$x_j$  = mean performance of crosses within a given line averaged over all replications within environments and testers.

$x$  = experimental mean

$t$  = testers

$l$  = lines

$r$  = number of replications.

$x_{i..}$  = number of lines

$x_{...}$  = grand total

$x_{.j.}$  = number of testers

$x_{ij}$  = number of crosses

The standard error of General and Specific Combining Ability effects were computed using the formula of Cox and Frey (1984) and Monyo *et al.* (1988).

i)  $S.E. (gca \text{ tester}) = \sqrt{M_e / r \times l}$  and  $S.E. (gca \text{ line}) = \sqrt{M_e / r \times t}$

ii) Similarly, standard error of SCA effect was computed as follows:

$$S.E. (SCA \text{ crosses}) = \sqrt{M_e / r}$$

**Where:**

$M_e$  = error mean square.

$r$  = Number of replications = 3

$l$  = Number of lines = 8

$t$  = Number of testers = 4

$S E (GCA \text{ tester or line})$  = Standard error for gca tester or line

$S E (SCA)$  = Standard error for sca

### 3.8.5 Proportional contribution of line (females), testers (Males) and their interaction (l x t) to total variance.

(a) Contribution of Lines (l) =  $\frac{SS(l) \times 100}{SS \text{ (crosses)}}$

(b) Contribution of Testers (t) =  $\frac{SS(t) \times 100}{SS \text{ (crosses)}}$

(c) Contribution of (l x t) =  $\frac{SS(l \times t) \times 100}{SS \text{ (crosses)}}$

**Where:** SS=sum of squares of either line (l), tester (t) or crosses (l x t) respectively.

### 3.9 Heterosis

Heterotic effect as an increase of F<sub>1</sub> mean over the higher-parent was expressed as a percentage and calculated as follows using the formulae of Liang *et al.* (1972).

Heterosis of F<sub>1</sub> mean over the high parent (Higher-Parent Heterosis) that is, *Heterobeltiosis*.

$$HPH = \frac{F_1 - H_p}{H_p} \times 100$$

**Where:**

F<sub>1</sub> = Mean of F<sub>1</sub> hybrid performance.

HP = Mean of better parent.

HPH = Higher parent heterosis

### 3.10 Correlations

#### 3.10.1 Phenotypic (ph), Genotypic (g) and Environmental (e) Correlation coefficients

The variance and covariance matrices necessary for calculating genotypic and phenotypic correlation coefficients were obtained from the mean squares and mean cross products of genotypes, phenotypes and error for the different traits (characters) measured in the replicated trials pooled over the three environments (Table 7). The genotypic and phenotypic correlations between pairs of traits (characters) were calculated according to Singh and Chaudhary (1985). The genotypic correlation coefficient (r<sub>g</sub>) was computed as follows:

$$r_g = \frac{M_{gx \cdot gy} - M_{ex} \cdot e_y}{\sqrt{(M_{gx} - M_{ex})(M_{gy} - M_{ey})}}$$

Phenotypic correlation coefficient (r<sub>ph</sub>)

$$r_{ph} = \frac{M_{gx \cdot gy}}{\sqrt{M_{gx} - M_{gy}}}$$

and environmental correlation (r<sub>e</sub>)

$$r_e = \frac{M_{ex \cdot ey}}{\sqrt{M_{ex} - M_{ey}}}$$

**Table 7: Form of Anova and Ancova for pairs of characters across Locations**

| Sources of variations | d.f. | Trait x  | Trait y  | Mean product      |
|-----------------------|------|----------|----------|-------------------|
| Replications (r-1)    | 2    | -        | -        | -                 |
| Entries(e-1)          | 43   | $M_{gx}$ | $M_{gy}$ | $M_{gx \cdot gy}$ |
| Error (r-1)(e-1)      | 86   | $M_{ex}$ | $M_{ey}$ | $M_{ex \cdot ey}$ |
| Total (re)-1          | 131  |          |          |                   |

**Source:** Singh and Chaudhary (1985)

**Where:**

$M_{gx \cdot gy}$  = mean product of trait x and y

$M_{gx \cdot}$  and  $M_{gy}$  = mean squares of trait x and y

$M_{ex \cdot ey}$  = error mean product of trait x and y

$M_{ex}$  and  $M_{ey}$  = error mean product of trait x and y respectively