

EFFECT OF GENOTYPES AND NITROGEN ON GRAIN QUALITY OF SORGHUM

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## Abstract

Sorghum (*Sorghum bicolor* L. Moench) is cultivated as an important food grain in the semi-arid regions of Africa. Processed grain sorghum is traditionally consumed as porridge, couscous, traditional tô or beer. The quality of such foods is highly dependent upon grain characteristics. Sorghum grain quality traits mainly include kernel hardness, kernel weight, kernel size, protein content and kernel color. Grain quality traits are often influenced by environment, genotypes, fertilizer management and their interaction. The objective of this study was to determine the impact of different levels of nitrogen application (0, 45, and 90 kg ha<sup>-1</sup>) on grain quality of selected sorghum genotypes.

The field experiment was conducted at three locations in 2010 (Manhattan, Ottawa, and Hays) and at two locations in 2011 (Manhattan and Ottawa). The experiment was laid in split plot randomized complete bloc design and replicated four times. The main plots were assigned to three N regimes: control (0 kg N ha<sup>-1</sup>), half recommended rate (45 kg N ha<sup>-1</sup>) and recommended rate (90 kg N ha<sup>-1</sup>). The subplots were assigned to twelve genotypes (six hybrids and six inbred lines). Plot size was 6.1 m x 3.0 m with a row spacing of 0.75 m. After harvest, grain quality traits (hardness, weight, diameter and protein content) were evaluated using standard procedures and the data subjected to statistical design using SAS. There were significant effects of genotype for most grain quality traits across both locations in Manhattan. Inbred lines SC35 and SC599 had maximum hardness at all locations while hybrid 95207, had the lowest hardness for all locations. Also, Inbred lines SC35 and Tx340 had maximum protein content at all the locations. While hybrids 95207, 26056, 23012 had the lowest protein content.

Genotypes Tx430, SC35, had higher hardness and with higher protein content were classified as high quality. We conclude that application of N (45 or 90 kg ha<sup>-1</sup>) significantly

improved grain protein, but not other quality traits. There are opportunities to improve grain protein through fertilizer management and plant breeding.

*Key Words:* Genotypic variability, hardness, weight, diameter, protein content, N level.

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## **Dedication**

I would like to dedicate this work to my family. To mom and Dad, thank for your unending love and support. The patience and prayers support from my wife Mme Diallo Houa Dembele and my son Mamadou Diallo for their patience and prayers.

# Chapter 1 - Literature review

## Importance of Sorghum

Sorghum (*Sorghum bicolor* L. Moench) along with pearl millet constitutes the staple cereal of millions of people living in the hot, drought-prone tropical regions in Africa and India (Maunder, 2002). Sorghum outperforms other cereals under various environmental stresses and is thus generally more economical to produce. More than 35% of world sorghum is grown directly for human consumption. The rest is used primarily for animal feed and as industrial raw material. The U.S is the largest producer and exporter of sorghum, accounting for 20% of world production and almost 80% of world sorghum exports in 2001–2002 followed by India and Nigeria (USDA-FAS, 2003). It is usually cultivated as a food and fodder crop by subsistence farmers in rainfed condition. In many part of the world, sorghum is traditionally consumed as staple food and in the production of a various food items such as; flat bread, porridge, couscous, alcohol, edible oil and syrup. In the United States, South America, and Australia, sorghum grain is used primarily for livestock feed and in a growing number of ethanol plants. In the livestock market, sorghum is used in the poultry, beef and pork industries. Stems and foliage are used for green chop, hay, silage, and pasture. A significant amount of U.S. sorghum is also exported to international markets where it is used for animal feed and ethanol fermentation. Sorghum has recently appeared in food products in the U.S because of increas in gluten-free food products. Sorghum is an excellent substitute for wheat for those who cannot tolerate gluten. Sorghum is used to make both leavened and unleavened breads (National Sorghum Producers, Sorghum 101, 2010). Good-quality sorghum has the feeding value that is equivalent to that of maize (*Zea mays* L.).

The United States is the number one producer and exporter of grain sorghum in the world. In 2010, it produced 8,773,000 million metric tons, while in 2011 the figure dropped to 6,246,000 million metric tons. The total area planted to grain sorghum in 2010 was 13,510,000 ha<sup>-1</sup>, while it was 13,667,500 ha<sup>-1</sup> in 2011 (USDA–NASS, 2011). Kansas is the largest producer of grain sorghum in the US. Average yields in Kansas ranged from 2700 – 5080 kg ha<sup>-1</sup> within the last five years (USDA–NASS, 2011). This implies yields vary from year to year. Grain sorghum is well suited to the dry arid climate. Grain sorghum is a very important crop both in the economy and cropping system in the U.S. especially in Kansas (USDA–NASS, 2011). Sorghum is the most drought tolerant summer crops grown in the central great plain regions. It has been estimated that about 1.2 million hectare of sorghum are currently cultivated each year by farmers in Kansas. In Kansas it has been reported that, majority of grain sorghum cultivated is grown under dry land conditions. In the U.S, 90% of grain sorghum is primarily used as feed in the livestock industry (USDA-NASS, 2010). In recent years, sorghum is also used in the bio–fuel industry, industrial manufacturing and as alternative food sources.

Sorghum is one of the most drought tolerant cereal crops currently under cultivation making it an excellent choice for arid and dry areas. It offers farmers the ability to reduce costs on irrigation and other farm expenses. The International Water Management Institute (IWMI) warns that by the year 2025, about 25 percent of the world's population will experience severe water scarcity. However, water productivity in both irrigated and rain-fed acres can be increased through the use of more water-use efficient crops, like sorghum. The production of sorghum is increasing due to the introduction of improved varieties and hybrids around the world. In the world several improved sorghum varieties adapted to semi-arid and tropic environments are released every year by sorghum breeders. In Africa especially in Mali, most of the sorghum

breeding programs have been focused on agronomic performance to improve production of sorghum. However, in African traditional agricultural systems, grain quality is an essential requirement. Many grain quality criteria can be identified because of the wide range of sorghum culinary dishes made by different African ethnic groups. Most of the varieties of sorghum cultivated in West Africa have therefore adequate grain quality characteristics with adaptation to low soil fertility, abiotic (drought and temperature), and biotic stress.

The influence of different physical and biochemical sorghum grain characteristics on the quality of traditional food has been established (Bello et al., 1990; Fliedel, 1995; Taylor et al., 1997). Endosperm texture, i.e. the relative proportion of corneous to floury endosperm, has been described as being one of the most important characteristics affecting sorghum food quality (Rooney and Murty 1982; Rooney et al., 1986). Bello et al. (1990) reported that corneous endosperm sorghum generally produced good quality *tö* (a West African traditional thick porridge) with a firm texture, and softer endosperm sorghum produced poor quality of *tö* with a softer texture. However, Fliedel (1995), who developed a laboratory test to screen advanced breeding material for *tö* quality, found no correlation between *tö* firmness and grain vitreousness; instead, it was observed that varieties with high amylose content, high starch solubility and good dehulling properties gave a good quality *tö*. The dehulling of sorghum grains depends on grain hardness or vitreousness (Reichert et al., 1981; Fliedel et al., 1989). Hard and corneous grains give a higher dehulling yield and produce flours with lower lipid, ash and fiber contents and thus better quality *tö* (Bello et al., 1990; Fliedel, 1995).

Sorghum competes with rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), pearl millet (*Pennisetum glaucum* L.), and maize (*Zea mays* L.) as a food crop in developing countries particularly in Africa. The processing and food-making properties of sorghum grain are affected

by many physical and chemical properties. The consumption of sorghum, as opposed to rice, wheat, and maize, as a staple food in arid region of Africa and Asia is so diverse that no single criterion of quality can be identified. This has hindered progress of plant breeders in selecting agronomically improved sorghum hybrids with acceptable grain quality (Cagampang and Kirleis, 1982).

### **Factors Limiting Sorghum Production**

Despite its importance, the production of sorghum as food and feed grain is limited by many constraints. Most biotic and abiotic stresses (disease, insect, weeds, temperature, drought, and salinity) faced by crop plants are related to environmental conditions (Olson et al., 1990; Simpson and Daft, 1990; Kocsy et al., 2004; Luna et al., 2005; Li et al., 2004, 2006; Garrett et al., 2006). These stresses limit yields of sorghum throughout the world especially in Africa as there are little resources to mitigate the effects of these stresses. Because of those factors the majority of smallholder farmers, especially in the semi-arid tropical regions of Africa, are not able to produce enough sorghum to meet family needs in most years.

#### ***Biotic Stresses***

Like all crops, grain sorghum is subject to infectious diseases which can sometimes limit production. Fungal diseases cause significant losses in both yield and quality, particularly in areas where improved cultivars have been adopted. Specific diseases may include anthracnose caused by *Colletotrichum spp.*, sorghum ergot is a disease caused by a fungus (*Claviceps africana*) and Charcoal rot caused by *Macrophomina phaseolina* are among the major diseases in sorghum-growing regions. Insect pests constrain production in many areas. Stem borers (*Oberea myops*) are endemic in many areas; head bugs (*Eurystylus immaculatus*) and midge (*Stenodiplosis sorghicola*) are most important in Western Africa; and shoot fly (*Atherigona*

*soccata*) causes substantial losses in late and off-season sowings in both Asia and Africa. In some areas production is constrained by birds, which attack the crop particularly during the grain-filling stage. Another major constraint to sorghum production is *Striga*, a parasitic weed that attaches itself to the sorghum roots from where it draws its moisture and nutrient requirements, inhibiting plant growth, reducing yields and in severe cases, causing plant death. In Africa, especially in Mali, *Striga* cause 90% of damage to sorghum in some region. Some *Striga*-resistant sorghum varieties have been developed by breeding program, but these varieties generally mature earlier than local varieties (but *Striga*- susceptible), often before the end of the rainy season. This results in increased susceptibility to grain moulds, greatly limiting the adoption of these varieties by farmers.

### ***Abiotic Stresses***

Nutrient-poor, degraded, acidic soil, drought and heat stress limit sorghum productivity worldwide. Both drought and heat stress affect plant growth, development, yield and quality of sorghum. In Africa the agricultural system depend largely upon rain-fall. As a result, it is highly vulnerable to changes in climate variability, seasonal shifts, and precipitation patterns. Any amount of temperature increases will result in increased water stress. Roughly 70 % of the population is dependent on agriculture 40 % of all exports are agricultural products (WRI, 1996). Limited quantities of fertilizer are used for the cultivation of sorghum, due to high cost and poor economic conditions of the farmers. The use of N fertilizer varies among the developed and developing countries. FAO (2010) reported that in 2009/2010 Africa used 1,566,000 tons of N fertilizer and 1,776,000 tons in 2010/2011. However, US during the same period used 7,833,000 tons and 7,461,000 tons in 2010/2011. In Mali Nitrogen deficiency is prevalent for smallholder and the average rate of fertilizer application is 8 kg of nutrients per hectare in comparison with



100 kg per hectare in the U.S and India and 220 kg per hectare in China (UN Millennium Project Task Force on Hunger, 2005). Nitrogen fertilizers are expensive inputs costing agriculture more than US\$45 billion per year (Ladha et al., 2000). As a result of high fertilizer costs, application rates in Africa especially in Mali are the lowest in the world and continue to decline even though soils in Mali are considered as poor as those in Latin America and Asia (Kidane et al., 2006). In Mali, the price has more than doubled in price in the last five years and therefore has become, and will continue to be, too expensive to produce any economic benefit for the vast majority of Mali's subsistence farmers. The increase in the value of the harvest of maize, sorghum, and millet that is produced by chemical fertilizer is less than the cost of the fertilizer. Mengel and Kirkby (2001) mentioned that corn and sorghum yield would have dropped by 41% and 19%, respectively, without nitrogen fertilizer application. Rising labor costs have also affected most farm operations, from land preparation, weeding and bird scaring to harvesting and grain processing. Another factor, important in Africa, is changing food preferences.

Climate change is also slightly becoming a limiting factor of sorghum production. Whether or not climate change will cause an increase or decrease in overall rainfall in the various parts of Africa, it is quite clear it has already dramatically increased the irregularity and unpredictability of rains. This has already reduced yields dramatically in most of sub-humid and semi-arid Africa, and it is also damaging to soil fertility because irregular rains dramatically reduce the biomass.

In order to increase their incomes, many farmers are moving from sorghum production to rice, wheat, cotton, cowpea, and maize. Inadequate government policy support also limits the expansion of sorghum production in many countries. For example, in Africa, as government production support measures for sorghum are relatively small compared to rice, and maize. In a

number of developing countries price support policies for sorghum has been drastically reduced or eliminated, mainly as a result of market deregulation.

In U.S sorghum industry is comprised of a variety of diverse markets, including international exports, biofuels, livestock feed, food aid and seed reserves, which make up the majority of the sorghum market distribution. U.S. sorghum is also purchased by the food and baking, pet food, bird seed and aquaculture industries, to name a few. In Africa especially in Mali sorghum market is slightly increasing because of the news uses developed by IER. In recent years IER has developed some varieties to improve the quality of the local beer called Dolo.

## **Growth and Development of Sorghum**

Grain sorghum goes through three distinct stages of development after emergence – seedling development, panicle initiation and reproduction. The growth of sorghum plant is defined from stage 0 (emergence) to stage nine (physiological maturity). The time required for the plant to go through each stage is dependent upon genotype and environmental condition during the growing season (Vanderlip, 1993).

### ***Stage 0: Emergence***

Emergence occur when the coleoptiles is visible at the soil surface and occur 3 to 10 days after planting. This stage depends upon soil temperature, moisture condition, depth of planting, and seed vigor. Cool, wet condition during this period favors disease organisms that may damage stand.

### ***Stage 1: Three Leaf Stage***

During this stage the growing point is still under the soil surface. It occurs when the collars of three leaves can be seen without dissecting the plant. This stage occurs usually 10 days after emergence depending largely to the temperature.

### ***Stage 2: Five Leaf Stage***

The five leaf stage occurs approximately 3 week after emergence. The root system also develops rapidly at this stage. At this stage the, stresses from weed competition, nutrients, water, or insects can significantly reduce yield if not correct.

### ***Stage 3: Growing Point Differentiation***

This stage will occur 30 days after emergence. The growing point differentiation changes from vegetative stage (leaf producing) to reproductive (panicle producing) stage. The total number of leaves has been determined and potential head size will be determined. At this stage nutrient uptake is rapid and constant supplies of nutrient and water are necessary to ensure maximum growth. About one third of the total leaf area has fully developed 7 to 10 leaves and the lower 1 to 3 leaves may have been lost.

### ***Stage 4: Final Leaf Visible***

At this stage the flag leaf (final leaf) is visible. All except the 3 to 4 leaves are fully expended representing about 80% of the total leaf area potential. The lower 2 to 5 leaves of the plants have been lost. Light interception is approaching maximum, growth and nutrient uptake continue at a rapid rate. Any reference to leaf number from now on should be from the top, counting the flag leaf as leaf number one.

### ***Stage 5: Boot Leaf Stage***

At this stage all leaves are fully expended, providing maximum leaf area and light interception. The head is full size and is enclosed in the flag leaf sheath. Peduncle elongation is beginning and will result in exertion of the head from the flag leaf sheath.

### ***Stage 6: Half Bloom***

Half bloom is usually defined as when one half of the plant in a field or are in some stage of bloom. However, because an individual sorghum head flowers from the tip downward over 4 to 9 days. At this stage approximately one half of the total dry weight of the plant has been produced. Nutrient uptake has reached almost 70, 60, and 80 percent of total for N, P, and K respectively. Time required from planting to half bloom depend largely on the maturity of the hybrid and environmental condition.

### ***Stage 7: Soft Dough***

Between half bloom and soft dough grain fills rapidly and grain is formed. The culm loses weight. Lower leaves continue to senesce with 8 to 12 functional leaves remaining at this stage.

### ***Stage 8: Hard Dough***

By hard –dough stage about three-fourths of the grain dry weight has accumulated. Nutrient uptake is essentially complete. Severe moisture stress of an untimely freeze before the grain reaches physiological maturity will result in a light, chaffy grain. Additional leaves may have been lost.

### ***Stage 9: Physiological Maturity***

Maximum total dry weight of the plant has occurred. Physiological maturity can be determined by the dark spot on the opposite side of the kernel from the embryo. Grain moisture and physiological maturity varies with hybrid and environmental conditions. Grain moisture content at physiological maturity depends on the hybrid and growing condition also. It usually is between 25 and 35 percent moisture. After physiological maturity, the remaining functional leaves may stay green or die and brown rapidly.

## **Effect of Nitrogen on Sorghum Physiology, Growth and Yield Traits**

Nitrogen fertilizer is an expensive but essential input for optimum production of non-leguminous crops on rotation. Nitrogen fertilizer use for grain crops can be reduced by using leguminous crops. Despite nitrogen being one of the most abundant elements on earth, nitrogen deficiency is certainly the most common nutritional problem affecting plants worldwide. In general very little quantity of fertilizer is used for the cultivation of sorghum in West Africa, probably because of high cost and poor economic conditions of the farmers. The application of fertilizer has been known to increase yield of sorghum (Bathcal et al., 1971; Turkhede and Prasad, 1978; and Pawar et al., 1980).

### ***Physiological Traits and Growth Traits***

Sorghum a C<sub>4</sub> crop uses nitrogen (N), CO<sub>2</sub>, solar radiation and water more efficiently than most crops C<sub>3</sub> (Anten et al., 1995; Young and Long, 2000). Nitrogen is one of major factors limiting photosynthesis and crops yield. High nitrogen result in an increase rate of carbon assimilation also of C<sub>4</sub> plants like sorghum which can be attributed to high investment of nitrogen into the photosynthetic mechanism. Nitrogen deficiency significantly reduced leaf area, leaf chlorophyll content and leaf photosynthesis rate resulting in lower biomass production. The reduction of leaf chlorophyll content affect directly leaf photosynthesis rate. Zhao et al. (2003) reported that plant components of dry weights, leaf dry weight had the greatest and root dry weight had the smallest decrease under N deficiency. They concluded that leaf N and chlorophyll concentrations were linearly correlated. Leaf area and leaf photosynthetic rates are directly associated with plant dry matter production. Sorghum grain yield is closely related to green leaf area (Borrell and Douglas, 1997) and leaf photosynthetic rate (Locke and Hons, 1988; Peng et

al., 1991). Leaf N and chlorophyll concentrations are important physiological parameters of detecting crop plant N status. Fertilizer N recommendation is usually depending on soil N status.

***Yield Traits (seed-set, seed numbers, seed size, grain yield, and harvest index)***

Nitrogen is one of the main inputs of the sorghum but it constitutes also the major factors limiting crop yield. The adequate application of nitrogen fertilizer is fundamental to maximize crop yield. (Mengel and Kirkby, 2001) mentioned that sorghum yield would have dropped from 41% to 19%, respectively without nitrogen fertilizer application. (Mahum et al., 2003) reported that application of nitrogen fertilizer increased crude protein, fodder, and dry matter yield in forage sorghum. Rashid et al. (2007) indicated an increase in the grain yields with an increase in N levels. They reported that grain yield increased from 2.92 to 5.61 t ha<sup>-1</sup> in the plots that were treated with 90 kg N ha<sup>-1</sup> compared with the control plots with 0 kg N ha<sup>-1</sup>. The increase in grain yield with nitrogen levels up to 90 kg N ha<sup>-1</sup> was attributed to the gradual increase in grain number and weight of grain per panicle with nitrogen level up to 90 kg N ha<sup>-1</sup>.

According to Jaynes et al. (2001) adequate supply of N to crops essential to optimize crop yields, mismanagement of N, such as excessive N application, can cause contamination of groundwater. Therefore, efficient monitoring of plant N status and appropriate N fertilizer management are essential to balance the factors of increasing cost of N fertilizer, the demand by the crop, and the need to minimize environmental perturbations, especially water quality (Jaynes et al., 2001).

***Sorghum Grain Quality (nutritional and seed size),***

Definition of grain quality depends on the grain type and its end use. It includes a range of properties that can be defined in terms of physical, sanitary, and intrinsic characteristics. Physical characters include moisture content, kernel weight, kernel size, total damaged kernels,

and broken kernels. Grain quality is also related to fungi count, insects and insect fragments, rodent excrements, foreign material, toxic seeds, pesticide residue, odor and dust (Wenwen Xiang, 2007). Oil content, protein content, hardness, density, and starch content are classified as intrinsic characteristics (Henry and Ketlewell, 2007). The quality properties of a grain are affected by its genetic traits, the growing period, timing of harvest, grain harvesting and handling equipment, drying system, storage management practices, and transportation procedures (Mazur et al., 1999).

Grain hardness is an important attribute in the processing of cereal grains and in the end products such as breads and snack foods (Bettge and Morris, 2000). Textural quality of cooked sorghum grain determines acceptability to consumers (Cagampang, Griffith, and Kirleis, 1982). For example, sorghum porridges that are too soft and sticky adhere to the teeth and palate during consumption. Grain sorghum cultivars that consistently produce relatively firm and nonsticky porridges are preferred by consumers. In sorghum, grain hardness is the most important and consistent characteristic that affects porridges (Rooney, Kirleis, & Murty, 1986). Aboubacar and Hamaker (1999) reported that hard sorghum grain produced flours containing a high proportion of coarse particles with low ash and high damaged starch content and yielded a higher proportion of desirable sorghum couscous granules. Kernel hardness (endosperm texture) affects the processing properties of the grain and the resulting products. Grains with a high proportion of corneous endosperm tend to be more resistant to breakage during decortications (dehulling) and milling than grain with a high proportion of floury endosperm. During milling hard grains tend to yield proportionally cleaner endosperm of large particle size than soft grains. This is because the corneous endosperm is easily separated from intact starchy endosperm giving a higher yield. In the field, hard grains are also more resistant to insect and mould damage than soft grains.

Endosperm texture affects storage quality of the grain. Insects more easily attack soft floury endosperm sorghum than hard corneous sorghum. Sorghum kernel hardness (endosperm texture) is the proportion of corneous (vitreous) fraction of the endosperm with respect to the floury or soft endosperm fraction. The proportions determine endosperm texture. The relative proportions of the corneous and floury endosperm vary among sorghum types. This variation is mainly influenced by genetic factors. But it is also influenced by the environment. Kernel hardness can also be influenced by other factors such as moisture. It also plays a role in plant defense against molds (Jambunathan et al., 1992), weathering, and insect attack (Waniska, 2000). For sorghum, hardness is reported to be significantly related to cooking quality parameters such as adhesion, cooked grain texture, alkali gel stiffness (Cagampang et al., 1984), porridge quality (Akingbala and Rooney, 1987), and production of high-quality couscous granules (Aboubacar and Hamaker, 1999). Milling quality of sorghum grain has been related to grain hardness as well (Rooney and Waniska, 2000). Commonly, large sorghum kernels are harder than small ones and related to higher quality grain (Lee et al., 2002). The milling quality of sorghum is determined by the kernel shape, density, hardness and structure (Rooney, 2003).

Sorghum kernel weight is determined by kernel growth rate and total duration of grain filling, also related to grow position within the sorghum panicle (Gabriel et al., 2005; Buffo et al., 1998). Sorghum kernel weight contributes highly to yield determination. The weights of the kernels increase by over 10% within a panicle (Heiniger, Vanderlip, and Kofoid, 1993). The kernel moisture content and kernel density are two components majors of weight. These two components are correlated with milling value (Munck et al (1981). Sorghum kernel color varies from dull white, yellow, and brown to red, which is also an important component for sorghum grain quality. Because usually the seed with a red coat has a good chance of high tannin content which is not good for food and feed use, light color are more preferred. Lighter flour is more



favorable in markets. Chemical quality parameters such as protein, starch and mineral contents are certainly big grain quality components and play an important role in sorghum nutritional value.

The grain compositions of sorghum vary due to many factors including the nature of the hybrid or genetic, soil and environmental conditions. Protein content of sorghum germplasm accessions varied from 4.4% to 21.1% with a mean value of 11.4% (Subramanian and Jambunathan, 1984). Crude protein determinations by Worker and Ruckman (1968) on six cultivars and 35 hybrids grown in the southwestern desert, indicated that significant increase in protein content were obtained when mean temperature were cooler than 26.5 C during anthesis and 20 days thereafter. Rooney (1971) reported significant protein differences, due to location, from three sorghum varieties grown at 21 locations in the U.S. He also reported significant protein difference attributed to location from seven varieties grown at 10 Texas locations. Nutrient accumulation and distribution studies (Lane and Walker, 1961) have shown changes in nutrient accumulation of sorghum at distinct stages but decrease at the inception of flowering. In the early stage of grain formation it begins again. Vanderlip reported that 50% of the total of plant nitrogen was contained in the grain sorghum at physiological maturity. Protein content of sorghum grain has been reported to increase with increase in the level of nitrogen applied (Waggle et al., 1967; Reddy and Hussan, 1968). The nitrogen application not only affects sorghum forage production but also improve its quality from view point of protein contents (Patel et al., 1994).

Grain diameter is also an important parameter. Large sorghum kernels with corneous endosperm are usually preferred for human consumption (FAO, 1995). Variation in kernel size occurs not only between cultivars but within a cultivar obtained from a different location or

season (Wills & Ali, 1983). Relatively little is known about the effect of sorghum kernel size on food quality independent of genotype and environmental effects. Wills and Ali (1983) reported the effect of kernel size on the decorticating characteristics of 28 Australian sorghum cultivars and suggested cultivars with kernels of non-uniform size should be separated into different sizes and each size grade dehulled for different times for optimal yields during dehulling. Ungraded and sized grain (<4.00 mm; <3.35 and <2.80 mm diameter, respectively) of 28 sorghum cultivars was dehulled in a pearler (Kett Husk Pearler) for 60 s. The decorticating recovery was higher for kernels <2.80 mm than for kernels <3.35 mm, and kernels <4.00 mm gave the lowest recovery.

### ***Response of Nitrogen Fertilization on Nitrogen Use Efficiency in sorghum Fertilizer***

Sorghum genotypes are known to vary in their response to nitrogen. Little information is available on the response of grain sorghum genotypes differing in nitrogen (N) use efficiency (NUE). Nitrogen fertilizer is one of the most essential and extensively applied nutrients. But leaching losses of N fertilizer are an economic problem for farmers and pose environmental concerns for the public. Therefore it is critical to have crop plants that will use fertilizer and soil N more efficiently for grain and forage production. Genotypic differences in N uptake, partitioning, and N use efficiency (unit DM per unit N in DM) have been reported for others crops including maize (Bruetsch and Estes, 1976) and grain sorghum (Maranville et al., 1980). Anderson et al. (1985) observed that the ability of a maize genotype to increase grain yield with high N rates was not necessarily associated with greater NUE values. Gardner et al. (1994) found that sorghum cultivars with greater NUE had reduced grain yield. Wheat genotypes have been found to differ in total plant N and N harvest index (NHI), with genotypes exhibiting the greatest N accumulation at harvest producing the greatest yields of grain and protein (Desai and Bhatia, 1978). Higher rates of N fertilizer have been found to increase grain N content and grain yield in

grain sorghum (Muchow, 1988). The physiological processes of carbohydrate partitioning and N metabolism are associated. Thus, genotypes with differences in grain yield potential may have differences in N accumulation and NUE (Sinclair and de Wit, 1975).

Landrace cultivars that have adapted to low N environments may possess different stress coping mechanism than do domesticated cultivars developed in contemporary breeding programs (Pearson, 1985). Physiological processes, which are related to N stress tolerance, frequently relate to leaf area and performance in term of gas exchange rates and stomata conductance from a given supply of leaf N (Pavlik, 1983; Field, 1983). Leaf morphological and anatomical features can also influence these physiological processes and contribute to NUE (Pavlikl, 1983; Longstreth and Nobel, 1983). Leaf size (Bhagsari and Brown, 1986), leaf thickness (Alagrswamy et al., 1988) and internal leaf anatomy (Nobel et al., 1975) have all been associated with photosynthetic N efficiency.

### **Nitrogen Management of Sorghum**

Nitrogen is essential for plant growth (Mosier et al., 2004) and it is still one of the major factors limiting crop yield (Zhao et al., 2005). Nitrogen deficiency effects on plant growth, leaf photosynthesis and hyper spectral reflectance properties of sorghum. Nitrogen is the most limiting nutrient for crop production in many of the world's agricultural areas and its effective use is important for the economic sustainability of cropping system (Fageria and Baligar, 2005), They reported that low N recovery of N is not only responsible for high cost of crop production, but also for environmental pollution. Nitrogen management is necessary to optimizing its utilization while decreasing pollution risk and operational cost. To achieve economically viable returns, like nitrogen, is necessary to maximize yields in all seasons. There is a need to use the minimum amount of nitrogen required any time during growing season (Sheehy et al., 1998).

Although adequate supply of nitrogen to crops is fundamental to optimize crop yields, mismanagement of nitrogen, such as excessive nitrogen application, can result in contamination of groundwater (Jaynes et al., 2001). Crops response and nitrogen use efficiency (NUE, defined as the ratio of yield to mineral N supply, regardless of source) are important for evaluating N requirement of sorghum and reaching maximum and economic yield.

### ***Time and Rate of Application***

Timing and placement of N fertilizer have a major influence on the efficiency of N management system. Nitrogen should be applied to a crop at a time that avoid stress period of using sensitive at critical stages and provide adequate N when needed by the crop. Placement of N fertilizer should aim at maximizing availability of N while minimizing potential losses. It is important to find the optimum N level to reduce the expense of the farmers. There is a need to use the minimum amount of nitrogen required for the maximum growth rate at any time during the growing season (Sheehy et al., 1998). The nitrogen requirement for crop production has traditionally been determined from field experimentation involving different rates of application of nitrogen fertilizer (Muchow et al., 1998). Variable responses to the application of nitrogen fertilizer have been observed in maize and in sorghum (Muchow et al., 1990). Studies with grain sorghum have shown that fertilizer knifed-in at planting has increased yields relative to broadcast application (Lamond, 1987; Sweeney, 1989; Khosla et al., 2000). According to Nimje and Gandhi (1993) the application of nitrogen fertilizer at the rate of 80 kg ha<sup>-1</sup> significantly improves germination, seedling vigor, grain and straw yield as well as protein content. Tripathi and Bhan (1995) found that 60 kg N ha<sup>-1</sup> as two split (one portion at planting furrow 2-3 cm below the seed and the remaining portion side dressed about 35 days after planting) significantly increase the sorghum yield and its attributes. Rashid et al. (2007) studied impact of nitrogen

levels in grain sorghum. They reported a positive relationship between nitrogen levels and crude protein content of sorghum grain. They found that the crude protein content of the grain showed a linear increase with N application, attaining the maximum at 120 kg N ha<sup>-1</sup>. The effect of different levels of nitrogen in the form of urea and the partial replacement of urea by farm yard manure (FYM) and groundnut cake on sorghum grain yield, protein content, and different protein fractions were studied by Patel et al. (1983). They reported that the crude protein content of the grains increased progressively and significantly as the dose of urea, urea + FYM and urea + GNC increased. Campbell and Pickett (1968) reported nitrogen fertilizer affected the protein production significantly but variation among lines was much greater. Khosla, et al. (1992) indicate that production of sorghum on soil testing high in mineral N (50 kg N ha<sup>-1</sup> in the surface 0.3 m) at planting should not receive any starter-band N in conjunction with sidedress N application of 130 kg N ha<sup>-1</sup> for optimum economic return to N fertilization. For soils testing low in mineral N, 40 kg N ha<sup>-1</sup> starter-band in conjunction with 130 kg N ha<sup>-1</sup> sidedress N should optimize the sorghum yields in most situations. Time of nitrogen application have lead to the general conclusion that should be applied nearest to the time of crop needs.

### ***Source of Nitrogen***

Ammonium nitrate, anhydrous ammonia, urea and urea-ammonium nitrate (UAN) solution are the four main sources of N fertilizers. These forms of nitrogen have two sources: nitrogen containing mineral and the vast quantity of nitrogen in the atmosphere. The nitrogen in soil mineral is released as the mineral decomposes. This process is usually slow and contributes slightly to nitrogen nutrition on most soil. Atmospheric nitrogen is generally a major source of nitrogen in soils. In the atmosphere nitrogen exists in the very inactive form N<sub>2</sub> and to be useful in the soil it must be converted. Plants, animals and microorganisms can die of nitrogen

deficiency, surrounded by  $N_2$  they cannot use. This conversion is accomplished two ways. Some  $N_2$  are oxidized to  $NO_3$ . The  $NO_3$  dissolves in raindrops and falls into the soil. The quantity of nitrogen added to the soil in this form is directly related to thunderstorm.

The supply of available nitrogen in soils is often supplemented by nitrogen released from soil organic matter or organic materials added to soils (manure, residues of forage legumes, etc.). Some microorganisms can transform atmospheric  $N_2$  to manufacture nitrogenous compounds for use in their own cells. This process, called biological nitrogen fixation, requires energy; therefore, free-living organisms that perform the reaction, such as *Azotobacter*, generally fix little nitrogen each year, because food energy is usually scarce. Most of this fixed nitrogen is released for use by other organisms upon death of the microorganism. Some plants (legume) like cowpeas, soybeans and peanuts have bacteria such as *Rhizobia* that infect (nodulate) the roots. These legumes may fix up to 113.39 kg of nitrogen per acre and are not usually fertilized. When the quantity of nitrogen fixed by *Rhizobia* exceeds that needed by the microbes themselves, it is released for use by the host legume plant. This is why well-nodulated legumes do not often respond to additions of nitrogen fertilizer. They are already receiving enough from the bacteria.

### ***Loss of Nitrogen in Field***

Nitrogen can go through many transformations in the soil. All these transformations are often grouped into a system called the "nitrogen cycle". The nitrogen cycle contains several routes by which plant-available nitrogen can be lost. Fertilizer N can be lost from crops and soil in many ways including: gaseous plant emission; soil denitrification; surface runoff; volatilization; and leaching (Raun and Johnson, 1999). Nitrate-nitrogen  $NO_3^-$  is usually more subject to loss than is ammonium nitrogen  $NH_4$ . The mechanism of nitrogen loss includes leaching, denitrification, volatilization, and crop removal. The nitrate form of nitrogen is so

soluble that it leaches easily when excess water percolates through the soil. This can be a major loss mechanism in coarse-textured soils where water percolates freely, but is less in finer-textured, where soil is more impermeable, and percolation is very slow. In the denitrification process  $\text{NO}_3^-$  is converted to gaseous oxides of nitrogen or to  $\text{N}_2$  gas, both unavailable to plants. Denitrification can cause major losses of nitrogen when soils are warm and remain saturated for more than a few days. Losses of  $\text{NH}_4^+$  nitrogen are less common and occur mainly by volatilization. Ammonium ions are essentially anhydrous ammonia ( $\text{NH}_3$ ) molecules with extra hydrogen ( $\text{H}^+$ ) attached. When this extra  $\text{H}^+$  is removed from the  $\text{NH}_4$  ion by another ion such as hydroxyl ( $\text{OH}^-$ ), the resulting  $\text{NH}_3$  molecule can evaporate, or volatilize from the soil. This mechanism is most important in high pH soils that contain large quantities of  $\text{OH}^-$  ions. Crop removal represents a loss because nitrogen in the harvested portions of the crop plant is removed from the field completely. Because many agricultural systems favor the accumulation of plant residues at the soil surface, the nitrogen in crop residues is recycled back into the system and is better immobilized rather than removed. A quantity of nitrogen is eventually mineralized and may be reutilized by a crop. Many study showed that even under the best management, 30-50% of the applied N is lost through different routes (Stevenson, 1985), and hence more fertilizer has to be applied than that actually needed by the crop to offset for the lost. The lost of N cause a negative impact on the environment (Kessel et al., 1993; Gosh and Bhat, 1998). High quantity of chemical fertilizer causes soil degradation and environmental pollution (William, 1992). In no-tillage system nitrogen loss is much higher than tillage system. No-tillage system, often characterized by an accumulation of crop residues on the soil surface, result in greater C, n, and water content in the upper 5-10 cm of soil compared with conventional tillage (Blevings et al., 1977; Doran, 1980) consequently, facultative anaerobes and denitrifying bacteria are more

numerous in no-tillage soil (Doran, 1980) and therefore, higher denitrification losses have been reported in no-tillage soils than plowed soils (Rice and Smith, 1982; Linn and Doran, 1984).

Nitrogen fertilizer management is a critical problem in high residue because of lesser N available (Rao and Dao, 1996). This occurs because of slower N mineralization (Phillips et al., 1980), greater N immobilization (Rice and Smith, 1984), denitrification (Rice and Smith, 1982), and  $\text{NH}_3$  volatilization (Terman, 1979). Gordon and Whitney, 1995 reported that below optimum soil temperatures in no-till environments cause lower nutrient availability in the early part of the growing season (Gordon and Whitney, 1995). In general the application method used in no-tillage systems is broadcast either solid ammonium nitrate or urea, or spraying urea ammonium nitrate (UAN) solutions on the soil surface immediately before or after planting (Mengel et al., 1982). However, surface application of N fertilizer can result in significant N losses through ammonia volatilization. (Eckert, 1987; Fox and Piekielek 1987; Mengel et al., 1982) have shown that similar N application rates of broadcast UAN produced lower yields than either injected or surface-banded UAN.

### **Methods to Minimize Loss of N**

The best management practice for timing of nitrogen (N) fertilizer applications is to apply fertilizer as close as possible to the period of rapid crop uptake. Managing N in this way will minimize losses of N from the field and will ensure adequate nitrogen availability to the crop during critical growth periods. The loss of N from rooting zone can be minimized by maintaining applied N in the ammonium form during period of excess rainfall prior to rapid N uptake by crops (Nelson and Huber, 1992). Schwab and Murdock (2005) state mentioned that depending on the soil conditions, some inhibitors can slow the conversion of ammonium nitrogen ( $\text{NH}_4\text{-N}$ ) to nitrogen ( $\text{NO}_3\text{-N}$ ) by a few weeks. The loss of nitrogen not only cause



trouble to the farmer but also cause hazardous impact on the environment (Kessel et al., 1993; Gosh and Bhat, 1998). It is necessary to find not only the optimum N level but also the appropriate way for the application of n to minimize the loss. Tripathi and Bahan (1995) found that 60 kg N ha<sup>-1</sup> as two portions (one portion at planting in furrow 2-3cm below the seed and the remaining portion side dressed about five weeks after planting) increase significantly the sorghum yield and its attributes.

The method of foliar application of nitrogen is effective especially in dry regions where the unavailability of moisture limit the uptake of nutrients by the plants. Using the method of foliar spray should be effective. Jamal (1991) conclude that the grain and straw yields increase with soil application, whereas the protein content of the grain was increasing with foliar spray of urea. In all crops, rapid uptake of N occurs during the maximum growth period. There is not much risk of N loss when fertilizer is applied at the beginning of the period of rapid growth. There are two main additives methods to reduce losses from N fertilizer, Agrotain and N-Serve. Both are effective at reducing the risk of N loss in certain N management systems. N-Serve is a nitrification inhibitor that is used mainly with anhydrous ammonia. Although it can be used with other N sources, its benefits are most proven with ammonia. Nitrification is the conversion of ammonia or ammonium to nitrate. This process happens naturally in all soils. Nitrate is the form of N that is susceptible to loss, so slowing fertilizer conversion to nitrate reduces the risk of loss. Using N-Serve with anhydrous ammonia to slow down conversion is a best management practice. Agrotain is a urease inhibitor that is used primarily with urea and secondarily with urea-ammonium nitrate solution. Use of Agrotain is a best management practice when urea is broadcast and not incorporated with tillage or irrigation. Urea left on the soil surface is susceptible to loss to the air, beginning on about the third or fourth day after application and

continuing until at least half an inch of rain occurs. Agrotain can be coated on urea granules and is effective at delaying N loss until rain occurs. There are two main additives available that help to reduce losses from N fertilizer, Agrotain and N-Serve. Both are effective at reducing the risk of N loss in certain N management systems. N-Serve is a nitrification inhibitor that is used mainly with anhydrous ammonia. Although it can be used with other N sources, its benefits are most proven with ammonia. Nitrification is the conversion of ammonia or ammonium to nitrate. This process happens naturally in all soils. Nitrate is the form of N that is susceptible to loss, so slowing fertilizer conversion to nitrate reduces the risk of loss. Using N-Serve with anhydrous ammonia to slow down conversion is a best management practice. Research shows that N loss from surface-applied urea can range from 0 to 50 percent, and 25 percent appear to be the average loss. The amount of loss depends on weather conditions; loss is greatest with warm, windy weather and a moist soil surface but is ended by rain that moves the urea into the soil. Agrotain often helps to reduce this loss and to improve yield (Plant Protection Program College of Agricultural Resource, 2006).

## **Hypothesis**

We hypothesized that nitrogen application increases sorghum grain quality traits (hardness, weight, diameter and protein content) of sorghum genotypes.

## **Objective**

The objective of this study is to determine the impact of different levels of nitrogen application (0, 40, and 90 kg ha<sup>-1</sup>) on grain quality of selected sorghum genotypes.



## Chapter 2 - Effect of Genotypes and Nitrogen on Grain Quality of Sorghum

### Introduction

Sorghum (*Sorghum bicolor* L.) is an important crop, usually cultivated as a feed and fodder crop by subsistence farmers in rainfed in Africa especially in Mali. In some parts of the world, it is consumed as staple food and is also used in the production of a variety of by-products like alcohol, edible oil, and sugar. In general, very little quantity of fertilizer is used for the cultivation of sorghum, probably due to high cost and poor economic condition of the farmers. Although fertilizer application increases crop production and it has universally been acknowledged that the more you pay to the crop the more you will gain", inappropriate practices that are followed during cultivation lead to low output of the applied fertilizer compared with the actual potential of fertilizer efficiency. In addition, even under the best management practices, 30%-50% of the applied N is lost through different routes (Stevenson, 1985), and hence more fertilizer has to be applied than that actually needed by the crop to compensate for the loss. The loss of N not only causes trouble to the farmer but also causes hazardous impact on the environment (Kessel *et al.*, 1993; Gosh and Bhat, 1998). High inputs of chemical fertilizer for sustainable crop production cause soil degradation and environmental pollution (William, 1992). Thus, it is necessary the optimum N level.

Nimje and Gandhi (1993) reported that the application of nitrogen fertilizer at the rate of 80 kg ha<sup>-1</sup> significantly improves germination, seedling vigor, grain and straw yields as well as protein content. Since nitrogen is critical nutrient for growth development of crops, low nitrogen supply, besides limiting yield may also have impact on general grain quality characteristics and

nutritional value of the crop. There the present study aimed at determining the impact of nitrogen fertilization on grain quality in selected sorghum genotypes.

## **Materials and methods**

In summer 2010 and 2011, a two-year study was initiated to determine the effect of nitrogen levels on grain quality of selected sorghum genotypes. Test locations in 2010 were Unit 1 (Irrigated) and Unit 7 (rainfed) sites at Ashland Bottoms Research farm near Manhattan KS and at the Western Kansas Research Station, Hays KS. The 2011 studies were conducted again at Unit 1 and Unit 7 sites at Manhattan and the East Central Experiment Station at Ottawa, KS. Soils at Manhattan were silt loam (Unit1) and reading silt loam (Unit7). The Hays and Ottawa soil were silt loam.

Average maximum and minimum temperatures, precipitation and relative humidity during the growing season (May to October) for the study areas are in Figures 1 through 3. The experiments were implemented on conventional tillage in three of the locations, Hays, Ashland bottom Unit 1 and Unit 7, and were no till in Ottawa. The previous crop in Unit 1 and Hays was sorghum while in Unit 7 and Ottawa it was soybean and maize, respectively for 2010. However, in 2011 the previous crop in Unit 1 was sorghum, soybean in Unit 7 and was corn in Ottawa.

Precipitation and temperature which are the two most important climatic factors that affect crop growth during the growing season varied among the locations and years of the study.

In Manhattan, the growing season mean maximum temperatures were 28.8°C and 29.3°C in 2010 and 2011, respectively. While the minimum temperatures were 15.8°C and 15.4°C in 2010 and 2011, respectively. The rainfall was 355.4 mm, and 457.1 mm in 2010 and 2011, respectively (Figure 1)

Besides the mean maximum temperatures for Ottawa were 28.8°C and 29.5°C in 2010 and 2011, respectively. The minimum temperatures were 16.4°C and 15.6°C in 2010 and 2011, respectively. The rainfall was 666.7 mm, and 351.7 mm in 2010 and 2011, respectively (Figure 2).

In Hays the growing season maximum temperature was not different from the other two locations with a value of 28.7°C. Rainfall amount were higher in 2010 than 2011 in Manhattan and Ottawa thus making 2011 a dry year as compared to 2010 (Figure 3).

Across all the locations in 2010, Hays had the least amount of precipitation with a total of 332 mm (Data from KSU Weather Library).

## **Experimental Details**

The randomized complete block experimental design in a split plot arrangement with four replications was used. The main plots were assigned to three N levels. Control (0 kg ha<sup>-1</sup>), half recommended rate (45 kg N ha<sup>-1</sup>) and the recommended rate (90 kg N ha<sup>-1</sup>). The sub plots were assigned to six hybrids (23012, 26056, Tx3042xTx2737, CSR1114xR45, 99480, and 95207) and six inbred lines (SC35, SC599, B35 Tx430, Tx2783, and Tx7000) of varying drought tolerance characteristics (pre-flowering and post-flowering drought tolerance) (Table 2). Each plots dimension was 6 m long and 4 rows unite whit row spacing of 0.75 m. The central two rows were harvested for yield estimated to eliminate any border effects, and the grain quality parameters included in this thesis were based on grain harvested from the central rows. Sorghum varieties were sown on a well-prepared seedbed. Before sowing, a composite soil sample from 0.15 and 0.60 cm soil depth was collected from the experimental plots and analyzed for physico-chemical properties.

## **Crop Management**

The general management operation at each location is presented in Table 3. The nitrogen fertilizer source was urea (46% N). The fertilizer was hand broadcast 10 d to 14 d after emergence along the rows of each plot. Planting was done in May and June across all the locations. Weeds were controlled with pre-emergence herbicides applied at labeled rates using a tractor mounted boom sprayer. At Manhattan (Unit 1), Callisto at 0.37 L ha<sup>-1</sup> and Bicep at 2.75 L ha<sup>-1</sup> was used. Similarly, at Manhattan (Unit 7), Lumax 2.84 at the rate of 2.9 L ha<sup>-1</sup> and Bicep at 3.3 L ha<sup>-1</sup> was sprayed. However, at Ottawa, Atrazine at the rate of 1.1 L ha<sup>-1</sup> and 2, 4-D at 1.1 L ha<sup>-1</sup> was applied. While at Hays, Atrazine and Parallel were used at the rate of 2.4 L ha<sup>-1</sup> and 1.8 L ha<sup>-1</sup> respectively.

Hand weeding was also used when necessary to remove late emerging weeds during the growing season. Maturity the central two rows were harvested and threshed using a two row plot combine. The grain samples were collected separately for each plot. The harvested grain samples were sent to the USDA laboratory for determining grain quality parameters including protein content.

## **Measurements**

Samples were cleaned before analysis by sieving over a screen with 2.0-mm triangular openings. Glumes, broken kernel, and foreign matter were removed by hand when necessary. Grain samples were subjected to a sequence of measurements performed by the SKCS 4100 includes weight (mg), hardness (%), and diameter (mm). Each of the measurements (weight, diameter, and hardness) were indirect and were calibrated against reference laboratory methods. Weight measurement is calibrated against mass determined using an analytical balance (AND HR-60) for single seeds with weights of 12–80 mg (U.S. method). Single characterization

diameter and hardness measurement were conducted using a SKCS 4100 (Perten Instruments North America Inc., Reno, Nevada, USA). Total nitrogen in sorghum was determined by the micro-Kjeldahl method (AOAC, 1975), and the crude protein content was calculated by multiplying 6.25 with N content of grain.

### **Statistical Analyses**

Analyses of variance were performed for the dependent variables, (kernel hardness, weight, diameter, and protein content) content using the SAS version 9.1 with GLM at an alpha level of 0.05. Data for the two years 2010 and 2011 experiments were analyzed separately due to contrasting climate conditions between the years during the growing season. For significant variables, means separation was accomplished using LSD test procedure. Whenever interactions were significant, main effects were ignored and interactions effects were discussed.



## Results

### Nitrogen Effects on Grain Quality Traits in 2010

#### *Manhattan Unit 1*

In 2010 at Manhattan Unit 1, there was significant effect of genotype on hardness, weight, diameter and protein content in sorghum grains ( $P < 0.0001$ ). However, effect of nitrogen rate was not evident for all these variables ( $P > 0.05$ ) except the diameter ( $P < 0.0001$ ). The effect of interaction between N rate x genotype was not significant for all the variables (Table 3).

#### *Genotypic differences in physical characteristics*

The mean values of the four characteristics studied for the genotypes used in 2010 at Manhattan (Unit 1) are showed in Table 7. There were significant differences among genotype ( $P < 0.0001$ ) on hardness, weight, diameter and grain protein content. For grain hardness, test values ranged from 23.21 to 72.61 percent. Genotypes SC35, and SC599, had higher grain hardness value compared to genotypes B35, and Tx340. Besides, genotypes 99480, Tx2783, CSR1114xR45 had higher hardness value when compared to genotypes Tx3042xTx2737, 23012, 26056, Tx7000. While genotypes 95207 had the lowest grain hardness value.

For kernel weight, grain weight varied from 23.69 to 30.23 mg. Averaged across nitrogen, genotypes Tx340 was generally superior in terms of kernel weight relative to genotypes SC35, CSR1114R45 and Tx7000. In addition, genotypes 99480, 26056, 95207, Tx3042Tx2737 were significantly ranked higher kernel weight when compared to genotypes SC599, 23012, and B35. While genotypes Tx2783 had the lowest kernel weight value. Grain diameter varied from 1.96 to 2.53 mm. Genotypic difference showed genotypes Tx340 was superior when compared to genotypes SC35, SC599, B35, Tx2783, CSR1114xR45, 26056, 23012, Tx3042xTx2737, 99480, and 95207. While genotype Tx2783 had the lowest grain diameter value. The protein content

varied from 8.58 to 12.53%. Genotypes Tx340 (12.53%), SC35 (12.34%), B35 (12.20%) were generally superior in terms of protein content value relative to Tx7000, SC599, Tx2783, 99480, 23012, Tx3042xTx2737, CSR1114xR45, 26056, 95207.

#### ***Grain physical property and protein content as affected by applied nitrogen rate***

There were no significant effects of N levels for all traits except grain diameter which was significant different ( $P < 0.05$ ). At 45 kg N ha<sup>-1</sup> (56.76%) kernel hardness was lower compared to 90 kg N ha<sup>-1</sup> (57.18%) and 0 kg ha<sup>-1</sup> (58.89%). At 45 kg N ha<sup>-1</sup> similar response was obtained for crude protein content. But crude protein values increased slightly with increasing N fertilizer levels from 45 to 90 kg N ha<sup>-1</sup>. On average, the highest protein content 10.62 % was produced at 90 kg ha<sup>-1</sup> of N. For grain kernel weight, weight values were similar at 45 kg N ha<sup>-1</sup> (25.80 mg) and 90 kg N ha<sup>-1</sup> (25.95mg) when compared to 0 kg N ha<sup>-1</sup> (26.26mg) mm). Similar response was obtained for grain diameter (Table 8).

#### ***Genotype by N interaction effect on grain quality***

The interaction genotypes by nitrogen was not significant for all traits

#### ***Manhattan Unit 7***

At Manhattan (Unit 7) in 2010 analysis of variance showed that there significant differences ( $P < 0.0001$ ) among genotypes for hardness, weight, diameter, and protein content of the sorghum grain. There were significant effect of N rate for hardness, diameter, and protein content. In the nitrogen test except the weight which was not significant ( $P > 0.05$ ) all the variables were significant ( $P > 0.0001$ ). The effect of the interaction was significant for sorghum grain hardness, and protein content but not for weight and diameter (Table 4).

#### ***Genotypic differences in physical characteristics***

The effect of sorghum genotypes averaged across N rates on kernel hardness, kernel weight, kernel diameter, and protein content at Manhattan Unit 7 in 2010 is presented in Table 9. The data showed that there were significant differences ( $P < 0.05$ ) among genotype for all variables. The grain hardness ranges were 69.93 to 82.40 percent, 22.92 to 28.87 mg for weight, 1.88 to 2.47 cm for diameter, and finally 8.69 to 11.92 percent for protein content. Average across the nitrogen the genotype SC599, Tx3042Tx2737, 26056, Tx2783, CSR1114R45, 99480 had higher kernel hardness compared to 23012, B35, Tx340, SC35, and Tx7000. While genotype 95207 had the lowest hardness value. For sorghum grain protein content, genotype Tx340 (11.92 %) had the maximum grain protein content. Genotype SC35 (10.83 %), and SC599 (10.76 %) were ranged higher when compared to Tx3042Tx2737, Tx7000, B35, CSR1114R45. Besides, genotype Tx2783, had higher protein content when compared to 95207, 26056, and 23012. Also the lowest grain protein content was recorded in genotype 99480 (8.69%).

#### ***Grain physical property and protein content as affected by applied nitrogen rate***

There were significant ( $P < 0.05$ ) effects of N regimes on all the variables except gain weight. Sorghum grain hardness ranged from 74.69 to 78.78. Grain hardness values increased slightly with increasing N fertilizer levels from 0 to 90 kg ha<sup>-1</sup>. Crude protein also increases significantly from 0 to 90 kg ha<sup>-1</sup>. (Table 10).

#### ***Genotype by N interaction effect on grain quality***

Genotype by N interaction was significant ( $P < 0.05$ ) for crude protein content. Genotypes CSR1114R45, and Tx2783 had similar response for protein but was significantly higher at 45 kg ha<sup>-1</sup>. Whereas at 45 kg N or 90 kg N ha<sup>-1</sup>, genotypes 23012, SC599, and Tx430 had similar response. While genotypes SC35, SC599, and Tx340 had higher crude protein content at 45 kg N ha<sup>-1</sup> or 90 kg N ha<sup>-1</sup>. Genotypes 95207 and 99480 has similar response for crude protein content

at 45 kg N ha<sup>-1</sup>. Overall, when averaged across the genotypes, the lowest crude protein content was produced at 0 kg N ha<sup>-1</sup> for genotype 23012 and Tx3042Tx2737 (Figure 4).

For grain hardness, genotype by N interaction was significant (P<0.05). Grain hardness was apparent at all the N regimes among genotypes 23012, and Tx3042Tx2737 but was significantly higher at 90 kg N ha<sup>-1</sup>. The highest grain hardness value was obtained at 90 kg N ha<sup>-1</sup> for genotypes SC599 and Tx3042Tx2737. While averaged across the genotypes, the lowest hardness value was produced at 0 kg N ha<sup>-1</sup> for genotypes Tx7000 (Figure 4).

### ***Ottawa***

At Ottawa in 2010 analyze of variance revealed that there were differences among genotypes for hardness, weight, diameter, and protein content of the sorghum grain. However, the individual effect of nitrogen rate was not evident for any of the variables at P (>0.05). The result showed that there were significant interactions between sorghum varieties and nitrogen fertilizer rate for only protein content at P (>0.0001). Whereas, the effect of interaction between N rate x genotype was not significant for the others traits namely hardness, weight, and diameter (Table 5).

### ***Genotypic differences in physical characteristics***

The result showed in Table 11 represent the effect of sorghum genotypes averaged across N rates on kernel hardness, kernel weight, kernel diameter, and protein content at Manhattan Unit 7 in 2010. There were significant differences (P<0.0001) among genotype for all variables. The ranges were 60.87 to 75.91 percent for grain hardness, 25.73 to 29.40 mg for weight, 2.16 to 2.36 cm for diameter and 6.98 to 9.46 percent for protein content. For grain hardness genotypes 99480, SC35 and SC599 was significant higher grain hardness with 76.09, 75.91 and 75.24 % respectively when compared to CSR1114R45, 23012, Tx7000. Besides, genotypes

Tx3042Tx2737, Tx340, 26056, had significantly higher kernel hardness when compared to 95207, and Tx2783. Similar response was observed for kernel weight (Table 11). For grain diameter, genotypes Tx7000, and 95207 had significantly higher grain diameter when compared to genotypes 26056, Tx3042Tx2737, Tx340, SC35, and 23012. Besides, genotypes CSR1114R45, 99480, and Tx2783 had the lowest grain diameter. For crude protein content, genotype SC35 (9.46%), and Tx2783 (9.12%) had significant maximum crude protein content when compared to Tx7000, Tx340, and SC599. The lowest crude protein (6.98%) was obtained by genotype 26056. There is no much difference between hybrids and inbred lines in term of grain protein content.

#### ***Grain physical property and protein content as affected by applied nitrogen rate***

There were no significant ( $P>0.05$ ) effects of N regimes on all the variables except grain hardness which was highly significant ( $P<0.05$ ). Sorghum grain hardness ranged from 66.40 to 71.90%. Grain hardness values decreased slightly with increasing N fertilizer levels from 0 to 90 kg ha<sup>-1</sup>. Crude protein content also increases significantly from 45 to 90 kg ha<sup>-1</sup>. At 0 kg N or 45 kg N ha<sup>-1</sup>, crude protein decrease (Table 12).

#### ***Genotype by N interaction effect on grain quality***

The interaction between genotype and N was significant for crude protein content. For crude protein content, no evidence for difference was apparent between 45 kg N ha<sup>-1</sup> or 90 kg N ha<sup>-1</sup> for genotype 26056 and 95207. In addition, no evidence for difference was apparent at 0 kg N ha<sup>-1</sup> or 90 kg N ha<sup>-1</sup> for genotypes Tx430 and Tx7000. For crude protein content, the highest value was obtained at 45 kg n ha<sup>-1</sup> or 90 kg N ha<sup>-1</sup> for genotypes SC35 and Tx2783. Overall, when averaged across the genotypes, the lowest crude protein content value was obtained at 45

kg N ha<sup>-1</sup>. However, genotype 23012 showed no statistical difference at 0 kg N ha<sup>-1</sup>, 45 kg N ha<sup>-1</sup> and 90 kg N ha<sup>-1</sup> (Figure 6).

### ***Hays***

At Hays in 2010, the results in Table 6 indicated that, hardness, weight, diameter, and protein content, were not significantly affected by different nitrogen rates ( $P > 0.05$ ). There was significant effect of genotype on all variables of the sorghum grains  $P < 0.0001$ . Except the protein content in which the interaction between genotypes and nitrogen was significant ( $P < 0.0001$ ), there was no significant effect of N rate and genotypes interaction for hardness, weight and diameter.

### ***Genotypic differences in physical characteristics***

The mean values of the four characters studied for the genotypes used in 2010 at hays are shown in Table 13. There were significant differences among genotype for hardness, weight, diameter and grain protein content. The kernel hardness test ranges were 64.02 to 78.13 percent, 26.78 to 30.91 mg for weight, 2.07 to 2.44 cm for diameter, and 10.81 to 12.82 percent for protein content. Genotypic difference showed genotypes SC599 (79.54%), and 99480 (78.13%) had significantly higher value for grain hardness when compared to genotypes 23012, 26056, B35. While genotypes SC35, 95207, had significantly lower grain hardness value. For kernel weight, similar response had been recorded. For grain diameter, genotypes Tx7000 (2.44 mm), Tx340 (2.44 mm), and 95207 (2.33 mm) had significantly higher when compared to genotypes 26056, 23012, 99480. While genotypes Tx2783 (2.07 mm) had significantly lower grain diameter. The genotype B35, SC35 and Tx3042Tx2737 produced maximum kernel protein content with 12.82%, 12.17%, and 12.02% while genotype 23012 (10.81%) had significantly

lower crude protein content. All the hybrids and inbred lines had significantly greater total protein content.

#### ***Grain physical property and protein content as affected by applied nitrogen rate***

There were no significant ( $P>0.05$ ) effects of N regimes on all the variables except grain diameter which was highly significant ( $P<0.05$ ). Sorghum grain hardness ranged from 73.00 at 90 kg N ha<sup>-1</sup> to 73.56% at 0 kg N ha<sup>-1</sup>. Grain hardness values decreased slightly with increasing N fertilizer levels from 0 to 90 kg N ha<sup>-1</sup>. Kernel weight increases from 0 kg n ha<sup>-1</sup> to 90 kg N ha<sup>-1</sup>. Maximum kernel weight was obtained at 90 kg N ha<sup>-1</sup> (29.05 mg) when compared to 0 kg n ha<sup>-1</sup> or 45 kg N ha<sup>-1</sup> with (28.57 mg) and (28.84 mg) respectively. Similar responses were observed for grain diameter and grain protein content (Table 14).

#### ***Genotype by N interaction effect on grain quality***

Genotype by N interaction effect was evident on crude protein content. No significant difference was apparent at 0 kg N ha<sup>-1</sup> or 45 kg n ha<sup>-1</sup> among genotypes 23012, B35 and Tx7000. In addition the difference was highly significant at 45 kg N ha<sup>-1</sup> or 90 kg n ha<sup>-1</sup> for genotypes 26056 and B35. The highest crude protein content value was obtained at 45 kg N ha<sup>-1</sup> for genotype Tx340. Overall, the crude protein content is significantly high for all genotypes (Figure 7).

### **Nitrogen Effects on Grain Quality Traits in 2011**

#### ***Manhattan Unit 1***

In 2011 at Manhattan Unit 1, there was significant effect of genotype on hardness, weight, diameter and protein content of the sorghum grains  $P (<0.0001)$ . However, effect of nitrogen rate was not evident for all these variables  $P (>0.05)$  except the protein content  $P$

(<0.0001). Interaction effect of genotype and N rate was not significant for all the variables (Table 15).

### ***Genotypic differences in physical characteristics***

The mean values of the four characters studied for the genotypes used in 2011 at Manhattan are presented in Table 18. There was significant effect on all grain traits. The kernel hardness test ranges were 73.48 to 82.13%. Among the genotypes, SC599 and 99480, Tx3042Tx2737 had higher grain hardness when compared to B35, 26056, SC35, CSR1114R45, Tx340, 23012. While genotypes, Tx2783, 95207, and Tx7000 had lowest grain hardness. The kernel weight was ranged from 23.20 to 30.91 mg. Genotypes Tx340, Tx3042Tx2737, and 26056 had heavier kernel weight when compared to SC35, Tx7000, B35. While genotype SC599 had the lowest kernel weight. The grain diameter was ranged from 1.95 to 2.51 mm for diameter. The effect of genotypes on grain diameter showed that genotypes Tx340, Tx3042Tx2737, B35, CSR1114R45, 26056, 23012, SC35, Tx7000, had higher diameter compared to 99480, SC599, and Tx2783. Genotypic difference showed genotypes SC599 (79.54%), and 99480 (78.13%) had significantly higher value for grain hardness when compared to genotypes 23012, 26056, B35. For crude protein, the test range was from 8.87 to 12.24%. The effect of genotypes on crude protein content showed that genotypes Tx340, Tx7000, and CSR1114R45 had higher crude protein value when compared to SC599, B35, SC35, Tx2783, Tx3042Tx2737, and 23012. While genotypes 95207, 26056, Tx2783, 99480 had the lowest crude protein content.

### ***Grain physical property and protein content as affected by applied nitrogen rate***

There were no significant ( $P>0.05$ ) effects of N regimes on all the variables except grain protein which was highly significant ( $P<0.0001$ ). Sorghum grain hardness ranged from 77.06 at



0 kg N ha<sup>-1</sup> to 78.64% at 90 kg N ha<sup>-1</sup>. Grain hardness values increased slightly with increasing N fertilizer levels from 0 to 90 kg N ha<sup>-1</sup>. Maximum grain hardness was obtained at 90 kg n ha<sup>-1</sup> when compared to 0 kg N ha<sup>-1</sup> or 45 kg N ha<sup>-1</sup>. Similar responses were observed for grain protein content (Table 19).

### ***Genotype by N interaction effect on grain quality***

The interaction genotypes by nitrogen was not significant for all traits

### ***Manhattan Unit 7***

Analysis of variations, main effects and their interaction effects in Manhattan Unit 7 in 2011 are shown in Table 16. There was significant effect of genotype (P<0.0001) for all the variables. There were no effect (P>0.05) of nitrogen treatment for grain weight and diameter. However there were highly significant effect (P<0.0001) of nitrogen for hardness, and protein content. The interaction between genotypes and nitrogen was no significant for any of the variables.

### ***Genotypic differences in physical characteristics***

The mean comparison value (Table 20) showed that there was significant effects genotype on hardness, weight, diameter and grain protein content. The kernel hardness varied from 69.90 to 91.07. Genotypes 99480, B35, SC35 were superior to genotypes SC599, Tx340, Tx3042Tx2737 for hardness. In addition, genotypes 23012, 26056, CSR1114R45 had higher grain hardness when compared to genotypes 95207, Tx340. While genotypes Tx7000 had lowest grain hardness. For kernel weight, the result showed that genotypes Tx7000, was superior to genotypes CSR1114R45, 26056, Tx340, Tx3042Tx2737. Besides, genotypes 95207, 23012, B35, and 99480 were ranked higher when compared to genotypes Tx2783 and SC599. When averaged across grain diameter, grain diameter varied from 1.72 to 2.61 mm. Genotypes Tx7000

had significantly greater grain diameter when compared to genotypes Tx340, CSR1114R45, and SC35. In addition, genotypes 26056, Tx3042Tx2737, 95207, and 23012 were superior to genotypes B35, and 99480. While genotypes SC599, and Tx2783 had lowest grain diameter. For crude protein content, genotypes Tx7000, Tx340, and CSR1114R45 were ranked higher when compared to genotypes B35, 26056, Tx3042Tx2737, and Tx2783. Besides, genotypes 99480, 23012, had higher crude protein content when compared to 95207 which had lowest value.

#### ***Grain physical property and protein content as affected by applied nitrogen rate***

Effect of N regimes was significant ( $P < 0.05$ ) on all the variables except kernel weight. Grain hardness ranged from 80.08 at 0 kg N ha<sup>-1</sup> to 82.87% at 45 kg N ha<sup>-1</sup>. Kernel weight values decreased slightly with increasing N fertilizer levels from 0 to 90 kg N ha<sup>-1</sup>. Similar response was also obtained for grain diameter. Kernel weight increases from 0 kg N ha<sup>-1</sup> to 90 kg N ha<sup>-1</sup>. Crude protein content was ranged from 11.52% at 0 kg N ha<sup>-1</sup> to 12.29% at 90 kg N ha<sup>-1</sup>. Maximum protein content was obtained at 90 kg N ha<sup>-1</sup> (12.29%) (Table 21).

#### ***Genotype by N interaction effect on grain quality***

The interaction genotypes by nitrogen was not significant for all traits

#### ***Ottawa***

At Ottawa in 2011 analysis of variance showed that there were significant effects of genotype for all variables except the protein content. Effect of N regimes was not significant ( $P > 0.05$ ) for all the variables. The interaction between genotypes and nitrogen was significant for grain hardness. While there was no significant effect of N rate and genotypes interaction for grain weight, diameter, and protein content (Table 17).

#### ***Genotypic differences in physical characteristics***

The result showed in Table 22 represent the effect of sorghum genotypes averaged across N rates on kernel hardness, kernel weight, kernel diameter, and protein content at Ottawa in 2011. There were significant differences ( $P < 0.0001$ ) among genotype for any variables. The grain hardness was ranked from 69.35 to 79.10. The result showed that genotype 26056 was superior to genotypes 99480, 95207, Tx3042Tx2737, B35 for grain hardness. In addition, genotypes SC599, SC35, and Tx340, were ranged higher when compared to 23012, and Tx2783. While genotype Tx7000 had lowest value of grain hardness. For kernel weight, genotypes test range was from 24.22 to 27.39. Genotypes CSR1114R45, and 95207 had higher kernel weight when compared to genotypes Tx3042Tx2737, SC35, 26056, 99480, B35, 23012, and Tx340. While genotypes Tx7000, SC599, and Tx2783 had lowest kernel weight. For grain diameter, genotypes 95207, SC35, and CSR1114R45 had higher grain diameter when compared to B35, 26056, 23012, 99480, Tx3042Tx2737, SC599, Tx340 and Tx7000. While genotype Tx2783 had the minimum value of grain diameter. For grain protein content, genotype CSR1114R45, 23012, 26056, 99480, were superior to genotypes Tx2783, Tx340, B35, 95207, and SC599. While genotypes Tx3042Tx2737, and Tx7000 had lowest crude protein content.

#### ***Grain physical property and protein content as affected by applied nitrogen rate***

There were no significant ( $P > 0.05$ ) effects of N regimes on all the variables except gain hardness which was highly significant ( $P < 0.05$ ). Sorghum grain hardness ranged from 71.78% at 90 kg N ha<sup>-1</sup> to 76.75% at 45 kg N ha<sup>-1</sup>. Maximum grain hardness values were obtained at 45 kg N ha<sup>-1</sup>. Similar response was produced for crude protein content. Grain diameter decreased slightly with increasing N fertilizer levels from 0 to 90 kg ha<sup>-1</sup> (table 13).

#### ***Genotype by N interaction effect on grain quality***

The interaction genotypes by nitrogen was not significant for all traits

## **Principal Component Analysis (PCA)**

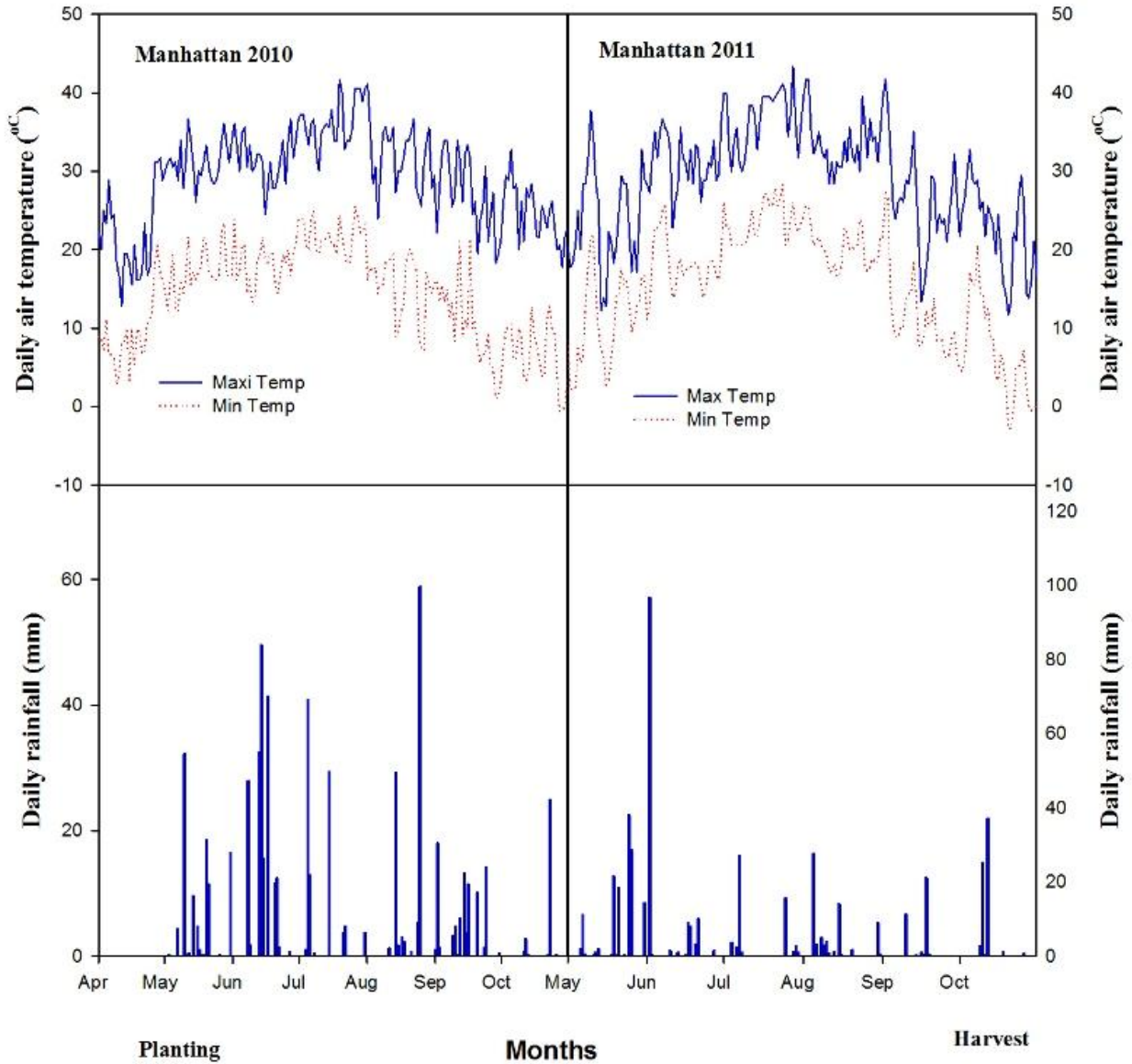
PCA is a multivariate technique for examining relationships among several quantitative variables and is especially a valuable analytical technique in exploratory data analysis. The PCA was carried out to identify the principal components of grain quality (hardness, weight, diameter, and protein content) that best described the genotypes with high and poor grain quality. Similarly, the response of genotypes for nitrogen level was done. The PCA identified the grain qualities that best separated the genotypes for their grain quality traits. However, the response of nitrogen on grain quality was not separated and all the levels of N are in on principal component vector. The first four principal component vectors (PC1, PC2, PC3 and PC4) accounted for 98.6 % of the total variability (Table 24). The PC1 eigenvector contrasted genotypes with high positive loadings for variables hardness, and protein. The PC2 eigenvector contrasted genotypes with high positive loadings for all the variables. The PC3 eigenvector contrasted genotypes with high positive loadings for variables diameter, and protein. The PC4 eigenvector contrasted genotypes with high positive loadings for variables hardness, and diameter (Table 24). On PC1, hardness had a loading of 0.99 and protein had 0.03. However in PC2, it had 0.09 and 0.11, respectively. The seed weight had the highest loading of 0.98 in PC2. Highest loading of protein content was observed in PC3 (0.993). Similarly, diameter loading was highest in PC4 (0.0998).

The biplot is a simply and specially scaled combination of PC scores and loadings (eigenvectors) that allow the approximate similarities and differences of the genotypes (the scores) to be displayed simultaneously and allow the different response variables (eigenvectors) to be associated with genotypes (Figure 8). A biplot of PC1 against PC2 revealed that there is considerable variation among genotypes in their response to nitrogen, with genotype score ranging from -9.5 to about 5.73 (Figure 8). The PCA separated the genotypes based on grain quality and the genotype. The genotypes with higher grain quality were placed on the right of the

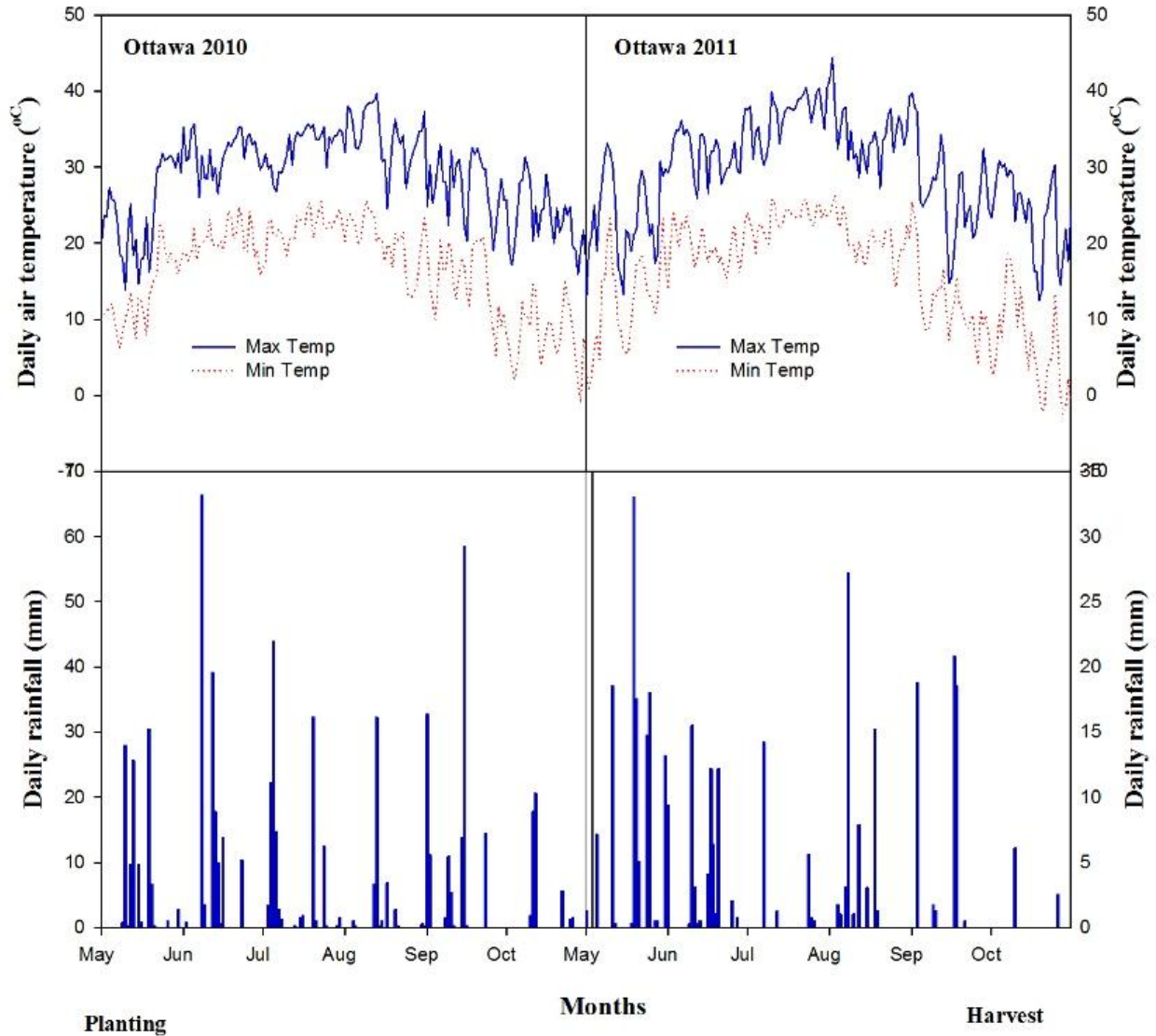
biplot while genotypes with low values were placed on the left of the biplot (Figure 8). The genotypes were divided into four groups based on the scores of the first two principal components (Figure 8): group 1 genotypes as high grain quality with positive scores for PC1 and PC2, group 2 as moderately high grain quality with positive PC1 and negative PC2 scores, group 3 as moderately low grain quality with negative PC1 and positive PC2 and finally group 4 as low grain quality with negative PC1 and PC2 scores (Table 25).

## Figures and Tables

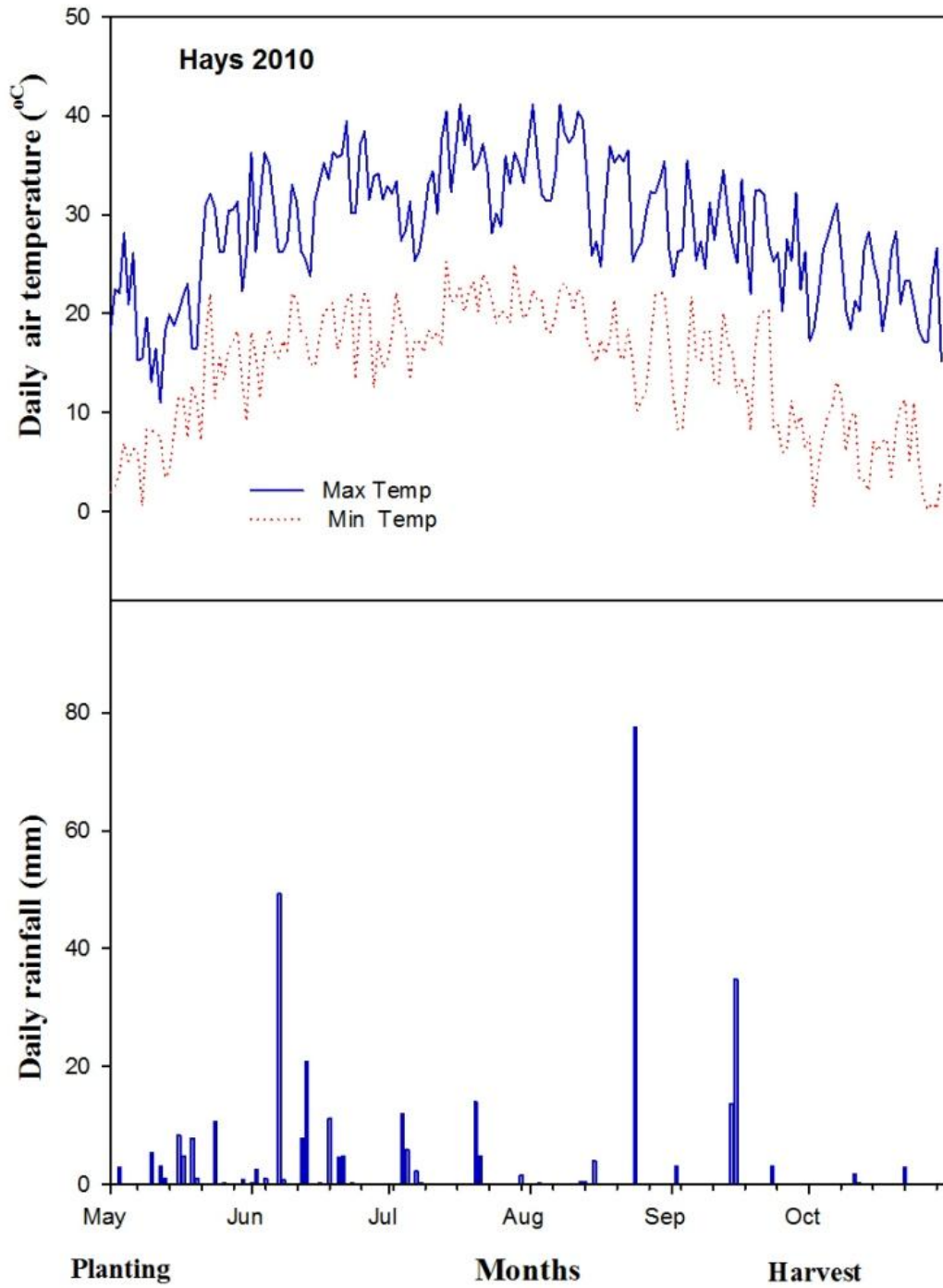
**Figure 1:** Daily maximum and Minimum Mean Air Temperatures and Rainfall from May to October 2010 and 2011 at Manhattan.



**Figure 2:** Daily Maximum and Minimum Mean Air Temperatures and Rainfall from May to October 2010 and 2011 at Ottawa.

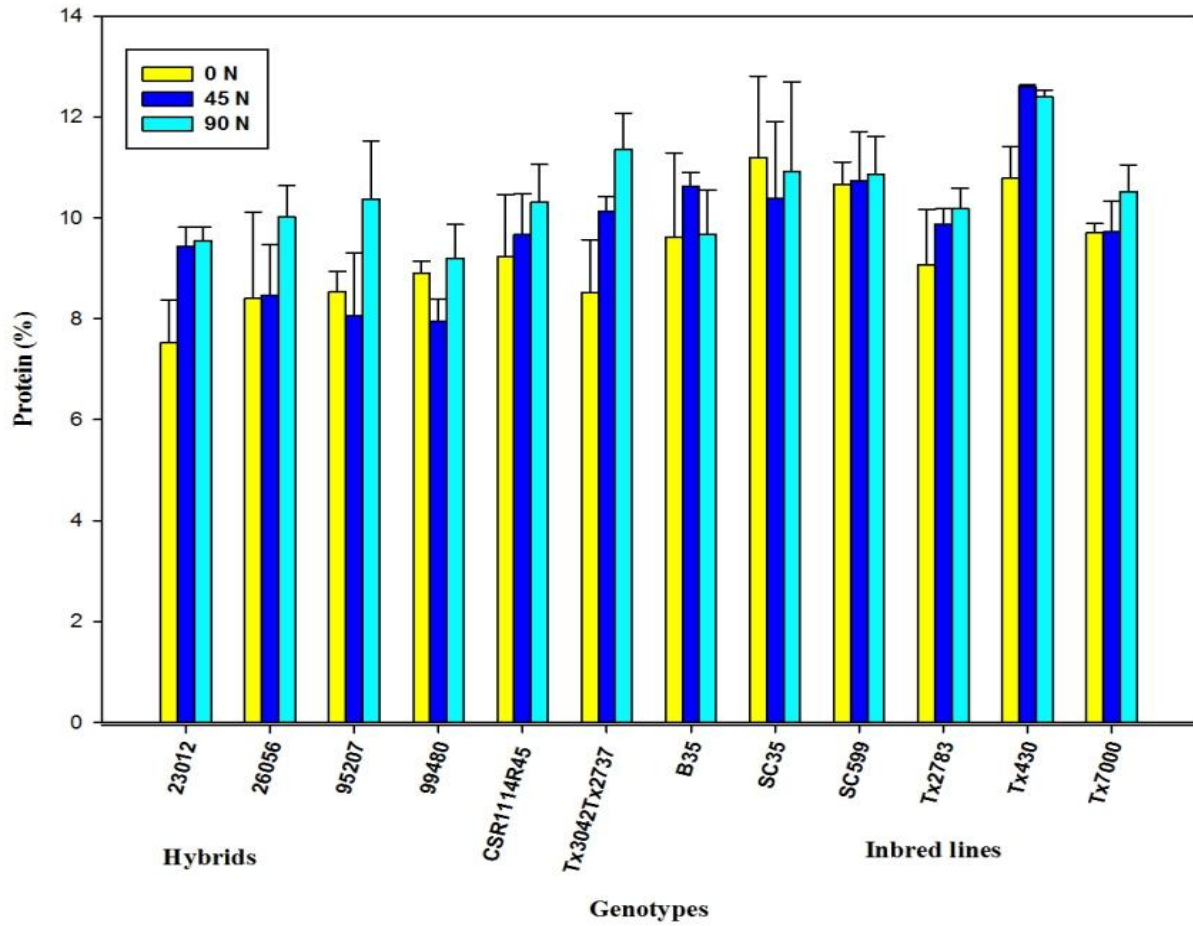


**Figure 3** Daily Maximum and Minimum Mean Air Temperatures and Rainfall from May to October 2010 at Hays.

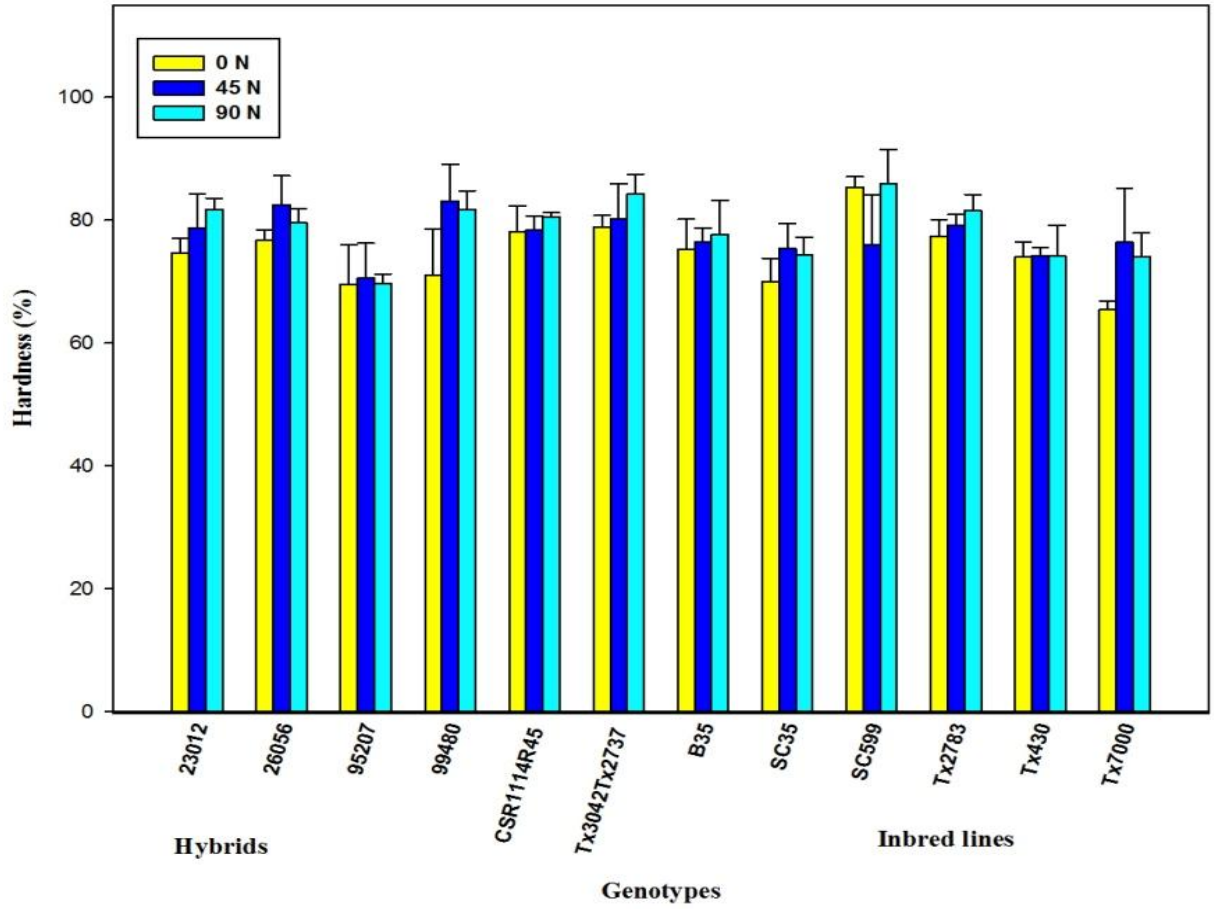




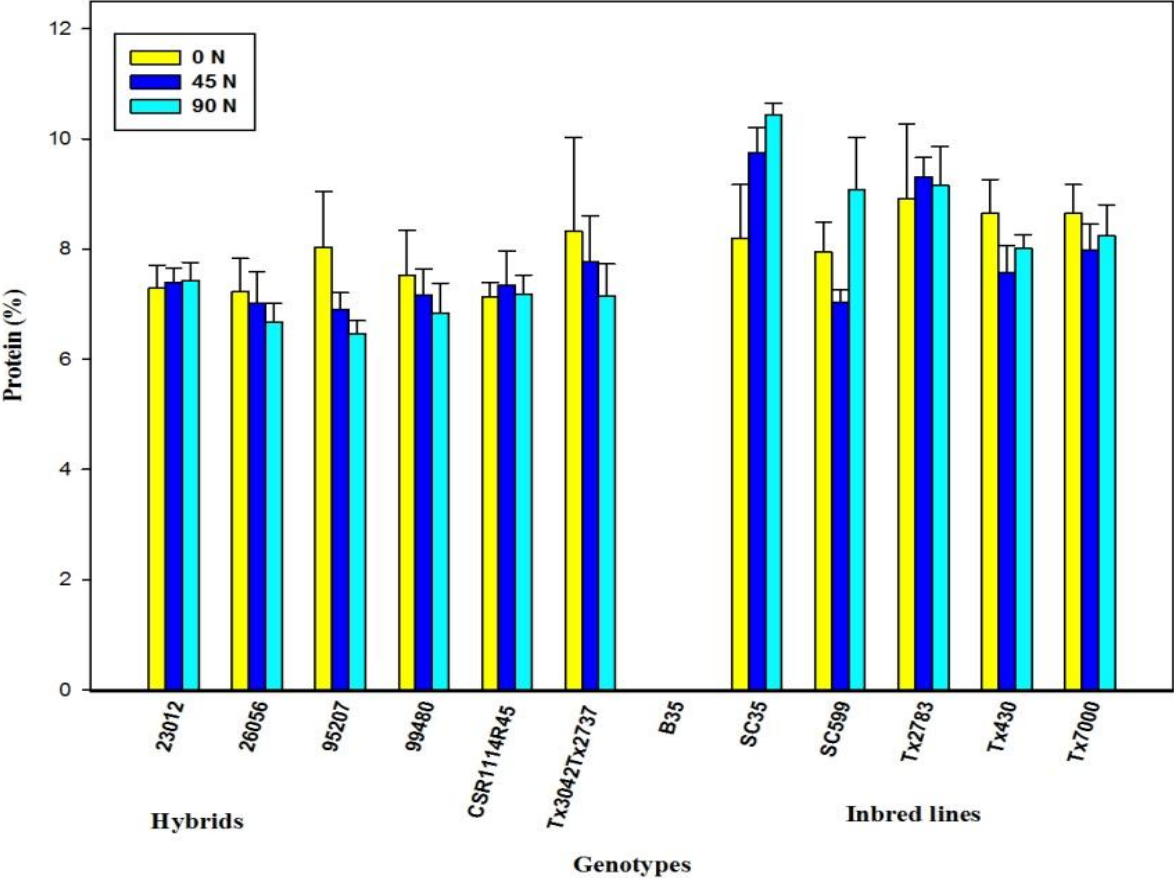
**Figure 4:** Interaction of genotypes and N rates on protein (%) at Manhattan (Unit 7) in 2010.



**Figure 5:** Interaction of Genotypes and N Rates on Hardness (%) at Manhattan (Unit 7) in 2010.



**Figure 6:** Interaction of Genotypes and N Rates on Protein (%) at Ottawa in 2010.



**Figure 7:** Interaction of Genotypes and N Rates on Protein (%) at Hays in 2010.

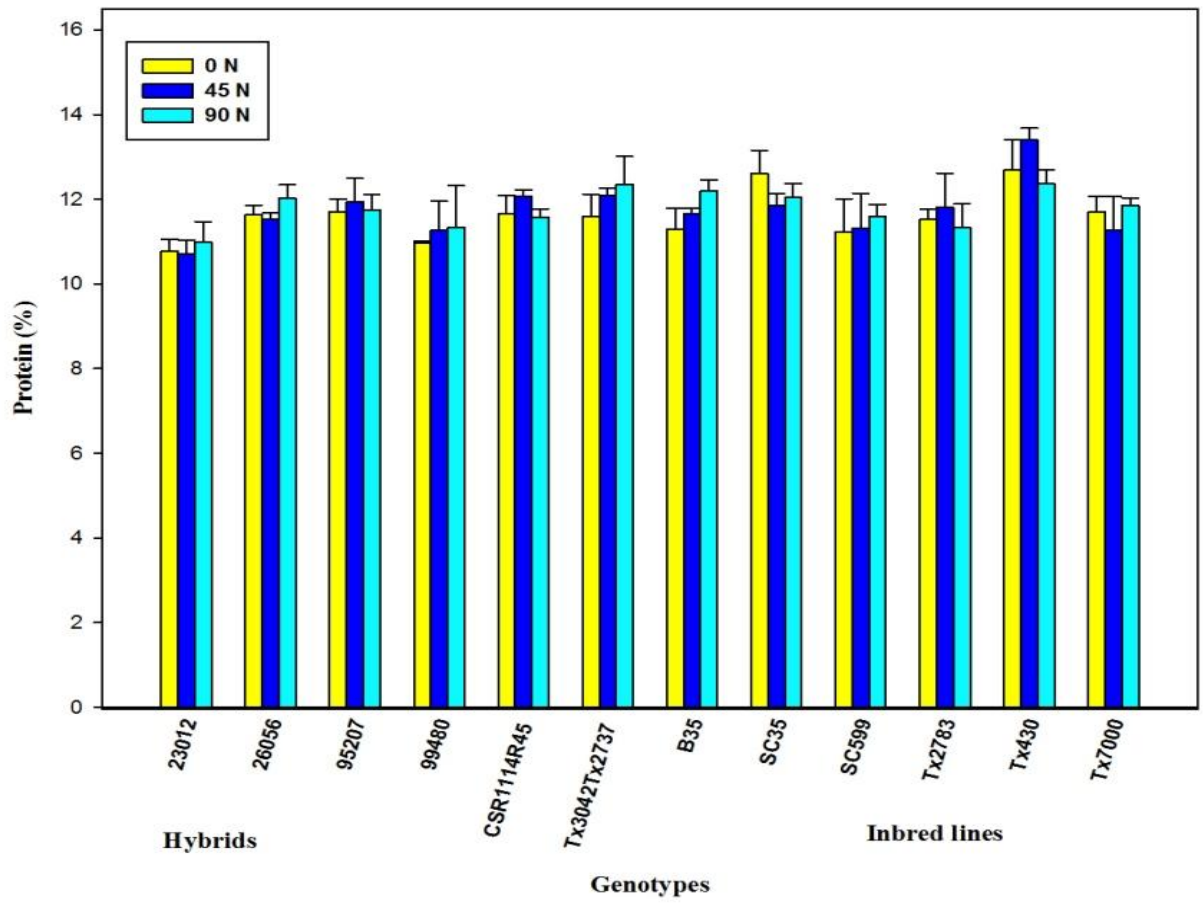


Figure 8: First and Second Principal Component Scores (PC1 and PC2) for the Identification of Sorghum Genotypes for Grain Quality Traits.

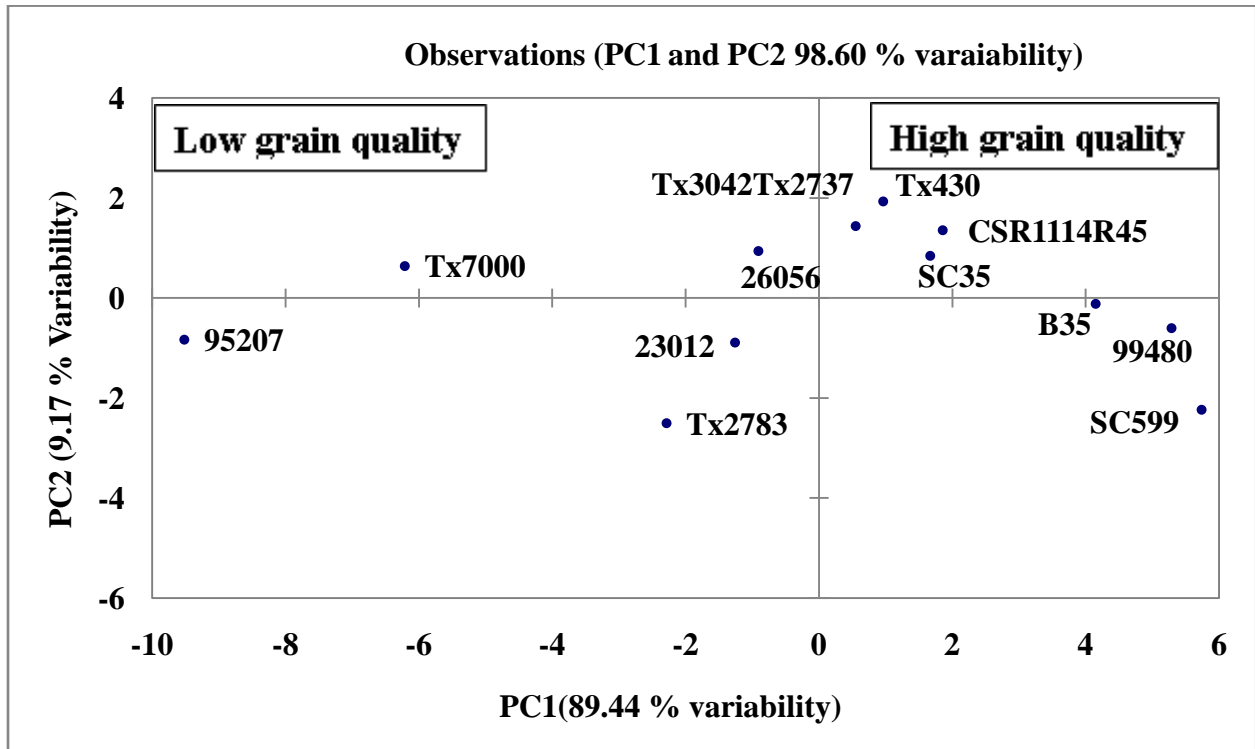


Table 1: Source and Characteristic of Genotypes Used in the Experiment during 2010 and 2011 Seasons.

Genotypes	Type	Characteristics	Source
23012	Hybrid	PreDFR, PostFDR	Crosbyton
26056	Hybrid	PreFDS, PostFDR	Crosbyton
Tx3042xTx2737	Hybrid	PostFDS	Experimental hybrid
CSR1114xR45	Hybrid	PostFDR	Experimental hybrid
99480	Hybrid	PreFDS, PostFDR	Crosbyton
95207	Hybrid	PreFDR, PostFDS	Crosbyton
SC35	Lines	Stay green (charcoal rot resistant)	Breeding material
SC599	Lines	Stay green (stalk rot resistant)	Breeding material
B35	Lines	Stay green (charcoal rot resistant)	Public inbred
Tx340	Lines	Non stay green	Public inbred
Tx2783	Lines	Non stay green	Public inbred
Tx7000	Lines	Non stay green (charcoal rot susceptible)	Public inbred

PreFDS: Pre-flowering drought susceptible.

PreFDR: Pre-flowering drought resistant.

PostFDR: Post-flowering drought resistant.

PostFDS: Post-flowering drought susceptible.

Table 2: Details of Various Cultural Practices Used in Conducting the Experiment in Kansas in 2010 and 2011.

Location	Planting Date	Nitrogen application	Harvesting	Herbicides application
_____2010_____				
Manhattan (Unit1)	May 25	June 9 (14)	Oct.10 (139)	June 25
Manhattan (unit7)	June 23	July 22 (42)	Nov.3 135)	June 24
Ottawa	May 28	June 13 (17)	Sep.29 (126)	May 29
Hays	June 11	June 18 (8)	Nov.11 (155)	June 6
_____2011_____				
Manhattan (Unit1)	June 6	June 22 (16)	Oct.27 (143)	June 6
Manhattan (unit7)	June 7	June 22 (15)	Oct 18 (134)	June 7
Ottawa	June14	July 8 (14)	Nov 11 (140)	May 5

Figures in parenthesis represent days after planting.

**Table 3:** Analysis of Variance (ANOVA) for Hardness, Weight, Diameter, and Protein Content of Grain Sorghum at Manhattan Unit 1 in 2010.

Source	Hardness (%)	Weight (mg)	Diameter (mm)	Protein content (%)
Genotype	<0.0001 <sup>***</sup>	<0.0001 <sup>***</sup>	<0.0001 <sup>***</sup>	<0.0001 <sup>****</sup>
N rate	0.2367 <sup>NS</sup>	0.2622 <sup>NS</sup>	0.0046 <sup>***</sup>	0.5403 <sup>NS</sup>
Genotype × N rate	0.6207 <sup>NS</sup>	0.5836 <sup>NS</sup>	0.4885 <sup>NS</sup>	0.5026 <sup>NS</sup>

\*, \*\*, \*\*\* Statistically difference at *P-value* 0.001, NS Non significant at *P-value* 0.05



**Table 4:** Analysis of Variance (ANOVA) for Hardness, Weight, Diameter, and Protein Content of Grain Sorghum at Manhattan Unit7 in 2010.

Source	Hardness (%)	Weight (mg)	Diameter (mm)	Protein content (%)
Genotype	<0.0001***	<0.0001***	<0.0001***	<0.0001***
N rate	<0.0001***	0.1422 <sup>NS</sup>	0.0375**	<0.0001***
Genotype × N rate	0.0448**	0.1529 <sup>NS</sup>	0.3132 <sup>NS</sup>	0.0087**

\*, \*\*, \*\*\* Statistically difference at *P-value* 0.001, NS Non significant at *P-value* 0.05

**Table 5:** Analysis of Variance (ANOVA) for Hardness, Weight, Diameter, and Protein Content of Grain Sorghum at Ottawa in 2010.

Source	Hardness (%)	Weight (mg)	Diameter (mm)	Protein content (%)
Genotype	0.0483*	0.0033**	0.0295*	<0.0001***
N rate	0.1134 <sup>NS</sup>	0.3971 <sup>NS</sup>	0.3608 <sup>NS</sup>	0.2581 <sup>NS</sup>
Genotype × N rate	0.3388 <sup>NS</sup>	0.2120 <sup>NS</sup>	0.1071 <sup>NS</sup>	<0.0001***

\*, \*\*, \*\*\* Statistically difference at *P-value* 0.001, NS Non significant at *P-value* 0.05

**Table 6:** Analysis of Variance (ANOVA) for Hardness, Weight, Diameter, and Protein Content of Grain Sorghum at Hays in 2010.

Source	Hardness (%)	Weight (mg)	Diameter (mm)	Protein content (%)
Genotype	<0.0001 <sup>***</sup>	<0.0001 <sup>***</sup>	<0.0001 <sup>***</sup>	<0.0001 <sup>***</sup>
N rate	0.7912 <sup>NS</sup>	0.2390 <sup>NS</sup>	0.0709 <sup>NS</sup>	0.1661 <sup>NS</sup>
Genotype × N rate	0.9926 <sup>NS</sup>	0.7672 <sup>NS</sup>	0.3362 <sup>NS</sup>	0.0119 <sup>**</sup>

\*, \*\*, \*\*\* Statistically difference at *P-value* 0.001, NS Non significant at *P-value* 0.05

**Table 7:** Effect of Genotype and Nitrogen Levels on Hardness, Weight, Diameter, and Protein Content of Different Genotypes at Manhattan Unit 1 in 2010.

<b>Genotypes</b>	<b>Hardness (%)</b>	<b>Weight (mg)</b>	<b>Diameter (mm)</b>	<b>Protein content (%)</b>
<b><i>Hybrids</i></b>				
23012	47.81e	24.68dfe	2.14fe	9.51fe
26056	45.36fe	25.75dc	2.09fg	8.94fg
Tx3042xTx2737	50.10e	25.2de	2.07g	9.50fe
CSR1114xR45	62.89d	27.65b	2.28c	9.47fe
99480	67.81bdc	25.79dc	2.19de	9.58e
95207	23.21g	25.59dce	2.16e	8.58g
<b><i>Inbred lines</i></b>				
SC35	74.03a	27.74b	2.36b	12.34a
SC599	72.61b	24.97de	2.26c	11.33dc
B35	70.38bc	24.52fe	2.26c	12.20ba
Tx340	69.64bc	30.23a	2.53a	12.53a
Tx2783	65.30dc	23.69f	1.96h	10.92d
Tx7000	42.27f	26.31c	2.23dc	11.63bc
<b>LSD(0.05)</b>	5.18	1.13	0.06	0.61

**Table 8:** Means Comparisons of Hardness, Weight, Diameter, and Protein Content as Affected by Nitrogen Rate at Manhattan Unit 1 in 2010.

<b>N levels (N)</b>	<b>Hardness</b>	<b>Weight</b>	<b>Diameter</b>	<b>Protein content</b>
	(%)	(mg)	(mm)	(%)
0	58.89	26.26	2.24a	10.57
45	56.78	25.80	2.19b	10.45
90	57.18	25.95	2.20b	10.62
<b>LSD (0.05)</b>	NS	NS	0.03	NS

**Table 9:** Effect of Genotype and Nitrogen Levels on Hardness, Weight, Diameter, and Protein Content of Different Genotypes at Manhattan Unit 7 in 2010.

<b>Genotypes</b>	Hardness (%)	Weight (mg)	Diameter (mm)	Protein content (%)
<i>Hybrids</i>				
23012	78.39bc	24.06ih	2.12e	8.83e
26056	79.59bac	27.41ed	2.16e	8.97de
Tx3042xTx2737	81.10ba	28.11cd	2.23d	10.00c
CSR1114xR45	79.03bac	30.16b	2.37b	9.74c
99480	78.62bac	25.35gf	2.03f	8.69e
95207	69.93f	28.75cd	2.33cb	8.99de
<i>Inbred lines</i>				
SC35	73.25edf	28.87cb	2.29cd	10.83b
SC599	82.40a	22.92i	2.04f	10.76b
B35	76.44dc	26.27f	2.24d	9.97c
Tx340	74.14ed	31.53a	2.47a	11.92a
Tx2783	79.38bac	24.84gh	1.88g	9.71dc
Tx7000	71.97ef	25.93gf	2.15e	9.98c
<b>LSD (0.05)</b>	0.78	1.36	0.07	0.74

Table 10: Means Comparisons of Hardness, Weight, Diameter, and Protein Content as Affected by Nitrogen Rate at Manhattan Unit 7 in 2010.

<b>N levels (N)</b>	<b>Hardness (%)</b>	<b>Weight (mg)</b>	<b>Diameter (mm)</b>	<b>Protein content (%)</b>
0	74.69b	26.62	2.17b	9.35c
45	77.60a	27.18	2.22a	9.80b
90	78.78a	27.24	2.19ba	10.44a
<b>LSD (0.05)</b>	1.89	NS	0.038	0.37

Table 11: Effect of Genotype and Nitrogen Levels on Hardness, Weight, Diameter, and Protein Content of Different Genotypes at Ottawa in 2010.

<b>Genotypes</b>	<b>Hardness (%)</b>	<b>Weight (mg)</b>	<b>Diameter (mm)</b>	<b>Protein content (%)</b>
<i>Hybrids</i>				
23012	70.73ba	27.48bdac	2.32bac	7.37ed
26056	67.72ba	29.40a	2.36ba	6.98e
Tx3042Tx2737	68.30ba	29.32a	2.35ba	7.74cd
CSR1114R45	71.30ba	26.67dc	2.26bdac	7.22ed
99480	76.09a	26.93bdc	2.22bdc	7.18e
95207	61.18b	28.87ba	2.38a	7.14e
<i>Inbred lines</i>				
SC35	75.91a	27.94bac	2.31bac	9.46a
SC599	75.24a	25.84dc	2.21dc	8.02cb
Tx340	68.87ba	27.55bdac	2.33ba	8.08cb
Tx2783	60.87b	25.73d	2.16d	9.12a
Tx7000	70.44ba	28.91ba	2.37a	8.29b
<b>LSD (0.05)</b>	10.48	2.17	0.14	0.54



**Table 12:** Means Comparisons of Hardness, Weight, Diameter, and Protein Content as Affected by Nitrogen Rate at Ottawa in 2010.

<b>N levels (N)</b>	<b>Hardness</b>	<b>Weight</b>	<b>Diameter</b>	<b>Protein content</b>
	(%)	(mg)	(mm)	(%)
0	71.90	27.78	2.32	7.99
45	70.79	27.27	2.27	7.75
90	66.40	28.04	2.30	7.88
<b>LSD (0.05)</b>	NS	NS	NS	NS

**Table 13:** Effect of Genotype and Nitrogen Levels on Hardness, Weight, Diameter, and Protein Content of Different Genotypes at Hays 2010.

<b>Genotypes</b>	<b>Hardness (%)</b>	<b>Weight (mg)</b>	<b>Diameter (mm)</b>	<b>Protein content (%)</b>
<i>Hybrids</i>				
23012	75.84bdc	27.23fe	2.29bc	10.81g
26056	73.76fedc	29.44bc	2.34bc	11.72cde
Tx3042Tx2737	76.59bac	30.48ba	2.37ba	12.02cb
CSR1114R45	77.21bac	29.97ba	2.37ba	11.77cd
99480	78.13ba	28.54dc	2.29bc	11.19f
95207	65.76g	29.68b	2.42a	11.79cd
<i>Inbred lines</i>				
SC35	64.02g	28.02de	2.33bc	12.17b
SC599	79.54a	26.78f	2.20d	11.38fe
B35	75.10bedc	27.17fe	2.28dc	11.72cde
Tx340	72.51fed	30.91a	2.44a	12.82a
Tx2783	70.21f	27.24fe	2.07e	11.56de
Tx7000	71.73fe	30.39ba	2.44a	11.60de
<b>LSD (0.05)</b>	3.64	1.11	0.07	0.36

Table 14: Means Comparisons of Hardness, Weight, Diameter, and Protein Content as Affected by Nitrogen Rate at Hays in 2010.

<b>N levels (N)</b>	<b>Hardness (%)</b>	<b>Weight (mg)</b>	<b>Diameter (mm)</b>	<b>Protein content (%)</b>
0	73.56	28.57	2.29	11.61
45	73.53	28.84	2.33b	11.74
90	73.00	29.05	2.33	11.78
<b>LSD (0.05)</b>	NS	NS	NS	NS

Table 15: Analysis of Variance (ANOVA) for Hardness, Weight, Diameter, and Protein Content of Grain Sorghum at Manhattan Unit1 in 2011.

Source	Hardness (%)	Weight (mg)	Diameter (mm)	Protein content (%)
Genotype	0.0234 <sup>**</sup>	<0.0001 <sup>***</sup>	<0.0001 <sup>***</sup>	<0.0001 <sup>***</sup>
N rate	0.4365 <sup>NS</sup>	0.9350 <sup>NS</sup>	0.8834 <sup>NS</sup>	<0.0001 <sup>***</sup>
Genotype × N rate	0.7221 <sup>NS</sup>	0.9779 <sup>NS</sup>	0.9014 <sup>NS</sup>	0.7655 <sup>NS</sup>

<sup>\*</sup>, <sup>\*\*</sup>, <sup>\*\*\*</sup> Statistically difference at *P-value* 0.001, NS Non significant at *P-value* 0.05

**Table 16:** Analysis of Variance (ANOVA) for Hardness, Weight, Diameter, and Protein Content of Grain Sorghum at Manhattan Unit7 in 2011.

Source	Hardness (%)	Weight (mg)	Diameter (mm)	Protein content (%)
Genotype	<0.0001 <sup>***</sup>	<0.0001 <sup>***</sup>	<0.0001 <sup>***</sup>	<0.0001 <sup>***</sup>
N rate	0.0037 <sup>***</sup>	0.3785 <sup>NS</sup>	0.1189 <sup>NS</sup>	<0.0001 <sup>***</sup>
Genotype × N rate	0.2651 <sup>NS</sup>	0.9589 <sup>NS</sup>	0.6888 <sup>NS</sup>	0.1201 <sup>NS</sup>

\*, \*\*, \*\*\* Statistically difference at *P-value* 0.001, NS Non significant at *P-value* 0.05

**Table 17:** Analysis of Variance (ANOVA) for Hardness, Weight, Diameter, and Protein Content of Grain Sorghum at Ottawa in 2011.

Source	Hardness (%)	Weight (mg)	Diameter (mm)	Protein content (%)
Genotype	0.1368 <sup>NS</sup>	0.3544 <sup>NS</sup>	0.4627 <sup>NS</sup>	0.0444 <sup>*</sup>
N rate	0.0113 <sup>**</sup>	0.9963 <sup>NS</sup>	0.9369 <sup>NS</sup>	0.3268 <sup>NS</sup>
Genotype ×N rate	0.0022 <sup>***</sup>	0.7969 <sup>NS</sup>	0.4506 <sup>NS</sup>	0.0714 <sup>NS</sup>

\*, \*\*, \*\*\* Statistically difference at *P-value* 0.001, NS Non significant at *P-value* 0.05

**Table 18:** Effect of Genotype and Nitrogen Levels on Hardness, Weight, Diameter, and Protein Content of Different Genotypes at Manhattan Unit 1 in 2011.

<b>Genotypes</b>	<b>Hardness (%)</b>	<b>Weight (mg)</b>	<b>Diameter (mm)</b>	<b>Protein content (%)</b>
<i>Hybrids</i>				
23012	76.13ebdc	27.81bc	2.39a	10.03gfe
26056	79.77bac	31.04a	2.41a	9.42gh
Tx3042Tx2737	80.71ba	31.22a	2.45a	10.33dfe
CSR1114R45	78.63ebdac	30.58ba	2.40a	11.26bc
99480	80.44ba	25.01dc	2.04b	8.87h
95207	74.29ed	28.58ba	2.39a	9.67gf
<i>Inbred lines</i>				
SC35	78.63ebdac	29.93ba	2.39a	10.60dce
SC599	82.13a	23.20d	2.08b	10.92d
B35	79.39bdac	29.2ba	2.43a	10.75dce
Tx340	77.91ebdac	30.91a	2.51a	12.24a
Tx2783	74.90edc	24.22d	1.95b	10.38dfe
Tx7000	73.48e	29.24ba	2.37a	11.82ba
<b>LSD (0.05)</b>	5.30	2.86	0.17	0.74

Table 19: Means Comparisons of Hardness, Weight, Diameter, and Protein Content as Affected by Nitrogen Rate at Manhattan Unit 1 in 2011.

<b>N levels (N)</b>	<b>Hardness (%)</b>	<b>Weight (mg)</b>	<b>Diameter (mm)</b>	<b>Protein content (%)</b>
0	77.06	28.39	2.32	10.01c
45	78.39	28.31	2.30	10.52b
90	78.64	28.57	2.32	11.04a
<b>LSD (0.05)</b>	NS	NS	NS	1.98



Table 20: Means Comparisons of Hardness, Weight, Diameter, and Protein Content of Different Genotypes at Manhattan Unit 7 in 2011.

<b>Genotypes</b>	<b>Hardness (%)</b>	<b>Weight (mg)</b>	<b>Diameter (mm)</b>	<b>Protein content (%)</b>
<i>Hybrids</i>				
23012	82.16ced	26.59ed	2.30d	11.09fe
26056	81.37ed	30.31cb	2.37cbd	11.75dc
Tx3042Tx2737	84.07cebd	29.84cb	2.37cbd	11.64dec
CSR1114R45	80.99e	30.97b	2.41cb	12.80a
99480	91.07a	25.06e	2.06e	11.19dfe
95207	74.71f	26.84d	2.34cd	10.82f
<i>Inbred lines</i>				
SC35	86.05a	29.09c	2.39cb	12.47ba
SC599	84.70cb	20.56f	1.91f	12.02bc
B35	85.95a	26.42ed	2.29d	11.83c
Tx340	84.54cbd	29.89cb	2.44b	12.82a
Tx2783	72.32gf	22.09f	1.72g	11.55dce
Tx7000	69.90g	33.97a	2.61a	13.11a
<b>LSD (0.05)</b>	3.21	1.62	0.08	0.63

**Table 21:** Means Comparisons of Hardness, Weight, Diameter, and Protein Content as Affected by Nitrogen Rate at Manhattan Unit 7 in 2011.

<b>N levels (N)</b>	<b>Hardness</b> (%)	<b>Weight</b> (mg)	<b>Diameter</b> (mm)	<b>Protein content</b> (%)
0	80.08b	27.90	2.29a	11.52c
45	82.87a	27.47	2.26ba	11.96b
90	81.51ba	27.35	2.25b	12.29a
<b>LSD (0.05)</b>	1.60	NS	NS	0.31

**Table 22:** Means Comparisons of Hardness, Weight, Diameter, and Protein Content of Different Genotypes at Ottawa in 2011.

<b>Genotypes</b>	Hardness (%)	Weight (mg)	Diameter (mm)	Protein content (%)
<i>Hybrids</i>				
23012	71.83bc	26.47	2.19	10.17a
26056	79.10a	26.94	2.19	10.04a
Tx3042Tx2737	76.22ba	26.97	2.14	8.62b
CSR1114R45	75.94ba	27.39	2.20	10.41a
99480	76.55ba	26.54	2.16	10.17a
95207	76.33ba	27.09	2.27	9.59ba
<i>Inbred lines</i>				
SC35	72.79bac	26.93	2.24	10.07a
SC599	73.82bac	24.69	2.14	9.43ba
B35	76.21ba	26.46	2.19	9.87a
Tx340	72.32bc	26.01	2.13	9.87a
Tx2783	71.33bc	24.22	2.01	9.95a
Tx7000	69.35c	24.65	2.13	8.48b
<b>LSD (0.05)</b>	6.44	NS	NS	1.22

Table 23: Means Comparisons of Hardness, Weight, Diameter, and Protein Content as Affected by Nitrogen Rate at Ottawa in 2011.

<b>N levels (N)</b>	<b>Hardness</b>	<b>Weight</b>	<b>Diameter</b>	<b>Protein content</b>
	(%)	(mg)	(mm)	(%)
0	74.42ba	26.23	2.17	9.80
45	76.75a	26.17	2.17	9.91
90	71.78b	26.18	2.16	9.46
<b>LSD (0.05)</b>	3.22	1.43	0.09	0.61

Table 24: Eigenvectors of PC1, PC2 PC3 and PC4 of 12 Sorghum Genotypes for Grain Quality as Principal Component Analysis.

Parameter	Principle Component eigenvectors			
	PC1	PC2	PC3	PC4
Hardness	0.994	0.098	-0.045	0.000
Weight	-0.103	0.987	-0.113	-0.059
Diameter	-0.006	0.061	0.015	0.998
Protein	0.033	0.116	0.993	-0.022

Table 25: Classification of 12 Sorghum Genotypes Based on the Scores of First Two Principal Components (PC1 and PC2).

High grain quality (+PCA1, +PCA2)	Moderately high grain quality (+PCA1, -PCA2)	Moderately low grain quality (-PCA1, +PCA2)	Low grain quality (-PCA1, -PCA2)
Tx430 (0.96, 1.93)	B35 ( 4.14, -0.11)	Tx7000 (-6.21, 0.64)	95207 (-9.52, -0.82)
SC35 (1.66, 0.84)	99480 (5.28, -0.59)	26056 (-0.91, 0.94)	23012 ( -1.25, -0.88)
Tx3042xTx2737(0.54, 1.44) CSR1114/R45 (1.85, 1.36)	SC599 (5.73, -2.23)		Tx2783 (-2.28, -2.50)

Table 26: Effect of genotype and nitrogen levels on yield and components of yield traits of grain sorghum grown at Manhattan (Unit 7), Kansas, 2010.

<b>Treatment</b>	<b>Grain yield</b> Kg ha <sup>-1</sup>	<b>Harvest index</b> (ratio)	<b>200 kernel wt</b> (g)	<b>Kernel number</b> m <sup>-2</sup>
<b>Genotypes (G)</b>				
<i>Hybrids</i>				
23012	6563a	0.50ab	4.65f	27961ab
26056	6779a	0.50ab	5.27c	25745ab
Tx3042Tx2737	5239d	0.44cd	5.38c	19137bcde
CSR1114R45	6608a	0.50ab	4.93de	27353a
99480	6645a	0.46bc	5.73b	23642bc
95207	5968b	0.49abc	5.27c	21233bcd
<i>Inbred lines</i>				
SC35	2749g	0.27f	5.14cd	10204de
SC599	1774h	0.20g	5.65b	7382e
B35	4183e	0.35e	4.24g	18847bcde
Tx340	6148b	0.49abc	4.71ef	25791ab
Tx2783	3689g	0.39de	6.23a	12728cde
Tx7000	5545c	0.51a	4.90de	21998bcd
<b>N levels</b>				
0	5155b	0.42	5.08b	20535
45	5259a	0.43	5.20a	20184
90	5241a	0.42	5.24a	23953
F test probability	.....Pr>F	.....Pr>F	.....Pr>F	.....Pr>F
Genotypes	***	**	***	***
N levels	*	NS	*	NS
G x N	***	NS	*	NS

\*, \*\*, \*\*\* Significantly different at P = 0.05, 0.01, 0.001 respectively. NS = not significant.

Table 27: Effect of genotype and nitrogen levels on yield and components of yield traits of grain sorghum grown at Manhattan (Unit 1), Kansas, 2011.

<b>Treatment</b>	Grain yield Kg ha <sup>-1</sup>	Harvest index (ratio)	200 kernel wt (g)	Kernel number m <sup>-2</sup>
<b>Genotypes (G)</b>				
<i>Hybrids</i>				
23012	2934cd	0.35ab	5.81bc	9513de
26056	3798b	0.31bc	5.95ab	12194bc
Tx3042Tx2737	3568b	0.37a	5.96ab	12648b
CSR1114R45	4749a	0.36ab	5.35d	17812a
99480	1671f	0.28cd	6.26a	5539f
95207	3115c	0.35ab	4.6e	10099cde
<i>Inbred lines</i>				
SC35	987g	0.23d	5.25d	3764fg
SC599	1391fg	0.23d	5.62bcd	4483fg
B35	2156e	0.26cd	5.39cd	8692e
Tx340	2585de	0.37a	6.09ab	10925bcd
Tx2783	947g	0.29c	6.26a	3145g
Tx7000	1407fg	0.31bc	6.31a	4749fg
<b>N levels</b>				
0	2289b	0.32	5.60b	8249c
45	2422ab	0.31	5.90a	8414b
90	2617a	0.30	5.73ab	9226a
F test probability	.....Pr>F	.....Pr>F	.....	.....
Genotypes	***	***	**	***
N levels	*	NS	**	*
G x N	***	NS	**	NS

\*, \*\*, \*\*\* Significantly different at P = 0.05, 0.01, 0.001 respectively. NS = not significant.



Table 28: Nitrogen uptake at physiological maturity at Manhattan (Unit 7), Kansas, 2010.

<b>Treatment</b>	Grain yield Kg ha <sup>-1</sup>	Harvest index (ratio)	200 kernel wt (g)	Kernel number m <sup>-2</sup>
<b>Genotypes (G)</b>				
<i>Hybrids</i>				
23012	31.0bcd	15.6c	91.2cb	137cde
26056	37.1abc	21.8a	108b	166b
Tx3042Tx2737	39.0ab	21.5a	93.6cb	154bc
CSR1114R45	29.7bed	18.8c	107b	155bc
99480	44.1a	20.2a	135a	199a
95207	23.4de	14.8c	102b	141cde
<i>Inbred lines</i>				
SC35	20.5e	13.0c	94.4bc	128def
SC599	31.1bcd	18.8c	53.7e	
B35	36.5abc	15.4c	65.7ed	118ef
Tx340	37.4abc	27.3a	104f	137cde
Tx2783	22.2ed	18.5c	84.6bcd	125ef
Tx7000	29.0cde	16.2c	107b	153bcd
<b>N levels</b>				
0	28.5b	14.8b	72.7b	116c
45	36.3a	23.0a	104a	163a
90	30.5b	17.7b	101a	149b
F test probability	.....Pr>F	.....Pr>F	.....	
Genotypes	**	*	**	**
N levels	**	**	**	*
G x N	*	*	NS	*
Linear on N	NS	NS	NS	NS
Quadratic on N	NS	**	NS	NS

\*, \*\*, \*\*\* Significantly different at P = 0.05, 0.01, 0.001 respectively. NS = not significant.

## **Chapter 3 - Discussion**

### **Climate Condition**

The rainfall amount and distribution during the growing season (May to October) for 2010 and 2011 were not uniform in all locations. The large differences among the parameters observed during the study were mostly attributed to variations in seasonal rainfall and high temperature. In 2010, the rainfall was higher especially in the month of June through to September (Figure 1-3). However, in 2011 drought and high temperature stress was severe during the growing period which affected significantly crop growth. Drought is one of the most common environmental stresses that affected growth and development of grain sorghum in Manhattan, Ottawa and Hays. Yadav et al. (1999) reported that drought after flowering of sorghum decreased seed yield through reduction of number of panicles per unit area, seed per head and seed weight. Seed weight decline can be through decreased seed growth rate as well as seed filling period (Naseri et al., 2010). Similarly high temperature stress ( $>38^{\circ}\text{C}$ ) decreases sorghum grain yield (Prasad et al., 2006). Short periods of high temperature stress also decreased seed-set and seed numbers (Prasad et al., 2008).

### **Genotypic Response to Nitrogen Fertilizer for Quality Traits**

Genetic factors play a major part in determining grain composition. Environmental factors also have a role. Grain sorghum genotypes vary in their response to nitrogen fertilizer. In our study, there were significant differences among genotype for hardness, weight, diameter and grain protein content across all locations. One of the major components of sorghum grains is protein. Both genetic and environmental factors affect the protein content of sorghum. In sorghum the variability is large, probably because the crop is grown under diverse agroclimatic conditions which affect the grain composition. Wide variability has been observed in the

essential amino acid composition of sorghum protein Hulse et al. (1980); Jambunathan et al. (1984). There is variation among hybrid in terms of protein content inside and between locations. This finding is in agreement with finding of Miller et al. (1964) that grain from single hybrid varied in protein from 7 to 10% because of differences in climate and soil from eight locations.

Genetic factor also affect grain hardness of sorghum. There is variability among genotype for hardness in all location. Kotarski et al. (1992) demonstrated a higher *in vitro* rate of starch disappearance in a sorghum line with floury endosperm when compared to a sorghum line with vitreous endosperm. Pedersen et al. (1996) demonstrated considerable variation in percent vitreous endosperm among 16 sorghum conversion lines grown in a single year with percent vitreous endosperm ranging form 53 to 93%. Philippeau et al. (1999) reported a much wider range of crude protein (87–135 g kg<sup>-1</sup>), starch (601–720 g kg<sup>-1</sup>), and hardness (38.5– 79.1% vitreousness) among their 14 corn hybrids

Mahama (2012) reported that nitrogen regimes increase yield and yield component across the locations, genotypes and years. There were instances when half recommended rate was comparative to optimum N regimes. Thus, in such situation, it will be prudent to use the half recommended rate to reduce production cost. Variable response to the application on N fertilizer have been observed in sorghum (Muchow, 1990) owing to climatic, soil and genotypic factors across seasons and locations. Part of this yield variation is associated with difference in the capacity of the soil to supply N and in the efficiency of recovery of applied N fertilizer. The other component contributing to variable yield response to fertilizer N is the N requirement for yield determination. The N requirement is dependent on yield expectation in a given environment as determined by climatic management and cultivar.

## **Effect of Nitrogen Levels on Grain Quality Traits**

Most of the studies have long recognized the close association between nitrogen fertilizer and sorghum grain yield, and protein content but not often with hardness, diameter, and weight. The study shows variability among genotypes (hybrid and inbred lines) and the types in all the traits that were measured. Sorghum genotypes responded positively to N fertilizer application. This study did not show a significant effect of nitrogen on grain hardness, kernel weight, and kernel diameter. Many study reported increasing of nitrogen in cultivars of remarkably caused to increase in kernels weight (Khaliq et al., 2008). They found that increasing nitrogen fertilization rates led to a significant increase in grain weight and grain fertilizer as compared with control treatment. Similarly conclusion was reached by Said et al. (1996).

In our study the effect of nitrogen on kernel weight was generally not significant in 2010 and 2011 averaged across all environments. The variability of the seed weight observed in this current study might be due to decrease seed filling duration as result of the high temperatures (> 32°C) during the growing season especially in 2011. Despite the fact that environmental conditions were favorable at the time of flowering, but stress occurring 10 – 15 d before flowering, has the tendency to reduce seed weight. This condition prevailed in 2011. Thus, seed weight may be reduced if drought stress occurs immediately after seed set because of reduction of seed filling. As indicated in the results, 2011 was a dry year and this resulted in a reduction of seed weight for most of the genotypes.

Kernel hardness (endosperm texture) affects the processing properties of the grain and the resulting products. An increased N supply has been associated with increased kernel hardness (Kaye et al., 2007). Irrigation has been shown to result in softer kernels (Taylor et al., 1997). In general, dry milling and alkaline cooking for human food products is better with hard kernels (Johnson, 2005; Shandera et al., 1997), while wet millers and brewers prefer softer kernels with

lower protein concentrations (Fox et al., 1992). The determination of grain yield and hardness of food-grade sorghum hybrids grown in different production environments would assist grain merchandisers, farmers, and food processors in targeting environments and hybrids for value-added end-use markets. In our study, in 2011 drought and high temperature stress was severe during the growing period had the highest hardness compared to 2010. Research with sorghum and maize has shown that kernel density is greater under dryland conditions than irrigated conditions (Kaye et al., 2007; Kniep and Mason, 1989; Bauer and Carter, 1986; Duarte et al., 2005). Johnson (2005) found harder sorghum kernels produced under drier Texas growing conditions than in Kansas and Nebraska.

Protein content is one of the major components determining the quality of fodder crops and was influenced significantly by application of nitrogen. The result indicated that all the levels of nitrogen significantly affected the grain protein contents during 2010 and 2011. Crude protein contents showed linear increase with an increase in nitrogen level because a large proportion of the N in grain is remobilized from leaves and stems after anthesis rather than being taken up from the soil. Ercoli et al. (2008) found that dry matter and nitrogen increased up to maturity when fertilizer was not applied. They concluded that nitrogen in the grain was derived primarily by translocation from leaves and stems rather than by uptake from the soil during the period of grain formation. Knowles and Watkins (1993) found that most of the N that was taken up by wheat plants was translocated to the grain either directly or by mobilization from other plant parts. Other studies have shown that the relationship between grain protein concentration and N translocation or N-translocation efficiency is not consistent (Dordas, 2009; Asseng and Milroy, 2006). Conversely, Gooding et al. (2005) and Robert et al. (2001) have reported that protein concentration in grain might be improved by selecting genotypes that translocate a higher percentage of N from the vegetative organs to the grain. Positive correlations have been observed

in wheat between grain protein concentration and nitrogen harvest index (Saint Pierre et al., 2008; Paccaud et al., 1985). Sorghum grain protein concentration is increased by increasing N supply (Kaye et al., 2007; Kamoshita et al., 1998). A progressive increase of grain protein content with increase of N level may be also due to the reason that fertilizer enhanced the amino acid formation. Nimji and Gandhi (1993) and Hussain et al. (1999) reported that the application of nitrogen fertilizer at the rate of 80 kg ha<sup>-1</sup> significantly improves germination, seedling vigor, grain and straw yields as well as grain protein content. The increase in protein content with N application have also been reported by others researchers such as Path et al. (1984), Choudhary and Kaswasra (1984), Patel et al. (1994) and Matowo et al. (1997). Increasing nitrogen rates produced increasing protein content of sorghum grain on nine locations for a two years period (Robertson et al., 1969). The protein content was higher at Hays in 2010 compared to all locations which can be explained by the high residual N. The result also showed that mostly inbred lines performed better than hybrid in terms of grain protein content and hardness. Genotypes Tx430, SC35, SC599, and B35 had the highest protein content. Under low N stay green lines like SC35 and SC 599 seem to keep N in the grain. But when enough N is available these lines send more N to the grain. The potential exhibited by these genotypes can be exploit as good combiners in future breeding programs.

### **Principal Component Analysis**

The PCA is perhaps the most useful statistical tool for screening multivariate data with significantly high correlations (Johnson, 1998). The cluster analysis applied to the principal components divided the genotypes into four distinct groups (Figure 8; Table 25). The PC1 eigenvectors for variables hardness and protein content have high positive loadings, while variables weight and diameter have high negative loadings. The PC1 vectors indicated that

genotypes with high weight and optimum diameter do not necessarily have high grain hardness or high protein content. But, good grain quality will result only from high hardness and high protein content. Based on the biplot PC1 vs PC2 (Figure 8) genotypes Tx430, SC35, Tx3042xTx2737 and CSR1114/445 had higher hardness and with higher protein content were classified as high quality, and genotypes 95207, 23012, and Tx2783 were classified as low grain quality.

### **Nitrogen Use Efficiency (NUE) and Yield Traits**

In our study we found that there is no significant effect of nitrogen on grain quality. Inversely, nitrogen affects significantly grain yield and nitrogen use efficiency (NUE) (Tables 26-28). Sorghum genotypes varied for grain quality, NUE and yield. Many studies have reported variation for NUE and components of NUE at high and low N inputs (Gilteson et al. 1998, Sinebo et al., 2004) as well as significant effect of genotype and N fertilization (Le Gouis et al., 2000; Chardon et al., 2010). NUE and components of NUE were significant, influenced environments where soil test results show low residual N. The results indicated that there was genotypic difference in N uptake in plant parts (leaves, stems and grain). In both years, higher amount on N was translocated from the soil to the leaves relative to the stem at flowering stage but more N was mobilized from the leaves and stems to the grain at maturity. Among the genotypes the hybrids tended to take up more N than the inbred lines. This agrees with findings of Nakamura et al. (2002) that N absorption was regulated by root activities and was higher in hybrids than in local varieties or inbred lines in low-N conditions among grain sorghum genotypes. Greater N accumulation in the grain was associated with higher grain yields and NUE. Average across the genotypes and sites, grain N accumulation was greater than in the leaves and stems. During the grain filling stage, it is the N accumulated in leaves and stems

before flowering that is in large part remobilized to the grain and that contributes to grain N protein deposition (Mae, 1997). This could explain the higher N in the grain than the other plant parts that have been observed in our study. Nutrient uptake by sorghum is influenced by several factors including nutrient availability, soil water availability, soil organic matter, soil chemical and physical properties, type of previous crop, plant population and the genotype (Wortmann, 2007). This could explain the genotypic difference in the N uptake that has been observed in this current study (Adapted from Mahama; (2012)

Mahama (2012) also demonstrated grain yield and components of yield was generally better in 2010 than in 2011 averaged across all environments. For site year, the hybrids generally performed better than the inbred lines. Yield of the hybrids were 31% greater than the inbred lines. The better yield of the hybrids was manifested in both seed numbers and seed size averaged across locations and years. This is obvious because the hybrids have already gone through some improvement for higher yields as compare to the lines, which are purposely use for breeding programs. Contrast to low yields of the inbred lines, genotypes Tx2783 and Tx7000 both non–stay green lines were comparative to hybrids for grain yield especially in environment of high residual N. Efficiency of grain production in crop plants is frequently expressed as HI. Sinclair (1998) and Hay (1995) have reported that HI is an important trait associated with the dramatic increased in crop yield that have occurred in the twentieth century. Higher NUE has also been observed in rice varieties with high HI (Bufogle et al., 1997). The variation found for HI dynamics could be largely explained by difference in assimilation during grain filling and remobilization of pre–anthesis assimilates. Genetic variation for this trait has been reported in different crop types (Slafer et al. (1994); Kumudini et al., 2002. The potential exhibited by these genotypes can be exploit as good combiners in future breeding programs (Adapted from Mahama; 2012).



## **Conclusion and Future Activities**

In summary, grain sorghum genotypes vary in their response to nitrogen fertilizer. Sorghum genotypes responded positively to N fertilizer application. There was a significant effect of genotypes on grain quality traits. Increasing nitrogen fertilization rates led to a significant increase in grain protein content as compared with control. Grain quality traits of inbred lines were comparable with hybrids. Besides application of N significantly improved grain protein, but not other quality traits. There was a significant difference between sorghum hybrid and inbred lines in term of grain protein content. The study showed that mostly inbred lines performed better than hybrid in terms of grain crude protein content. The maximum grain protein content was obtained at the optimum N regime, followed by the half recommended rate and the least was the control. Overall, grain hardness (%) ranged from 23.21 to 84.70, kernel weight ranged from 20.56 to 33.97, grain diameter ranged from 1.72 to 2.53 and finally crude protein content ranged from 7.14 to 13.11.

Based on the result of this study there were no significant different for the entire trait except crude protein content which is easily comprehensive because of the richness of the soil in high residual N. In contrast the same study will be very useful for farmers in Africa especially in Mali where most of the soil has been used for long time without a substantial contribution of nitrogen and other nutrient such as phosphorus, potassium. There are opportunities to improve grain protein through fertilizer management and plant breeding. For grain hardness and crude protein content genotypes Tx430, SC35, SC599, and B35 were superior. These genotypes can be used in breeding program.

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