

Pearl Millet Nutritional Quality and Fertilization of Sweet Corn in Senegal.

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ABSTRACT

Agricultural production is the main source of income and major employer in many countries in Africa, including Senegal. Commercial sweet corn (*Zea mays L. ssp. saccharata*) production in Senegal is increasing in response to global marketing opportunities and offers producers the ability to increase income and diversify the cropping base. Production of optimum sweet corn yield and quality depends on adequate nutrient supply, particularly, nitrogen (N). Current N recommendations are based on recommendations specific to corn for grain. This study aimed to identify tools to estimate sweet corn N status and determine the most appropriate fertilizer dosage for sweet corn. Non-destructive remote sensing tools and ion exchange resin membranes (IEMs) were used to evaluate the effect of fertilizer dose. No differences in ear yield or yield components, normalized difference vegetation index (NDVI) values, biomass, N uptake or leaf N concentration due to fertilizer dose were detected at Ndiol. However, significant relationships existed between NDVI values and yield, biomass, and N uptake at the V9 growth stage. Only yield was affected by fertilizer dose at Sangalkam, and no consistent relationships were found between chlorophyll meter readings and others measured parameters. Treatment differences due to fertilizer dose for available NH_4^+ at V9 (Ndiol), and NO_3^- at V5 (Sangalkam) were found, however further research is needed to fully evaluate the usefulness of IEMs to measure available soil N. Based on these studies, sweet

corn fertilizer rates should likely be based on 75% of the dose applied to field corn, however more work is needed to confirm this finding.

Pearl millet (*Pennisetum glaucum* (L) R. Br.) is the most widely grown staple crop in Senegal. Introduction of drought tolerant millet genotypes has helped mitigate the effect of increased water shortage in the region, but little is known about the nutritional composition of these genotypes. Our objective was to compare millet grain nutritional composition among and between putative drought tolerant and drought sensitive pearl millet lines under drought stress and well-watered conditions. One field experiment was conducted in 2014 at the National Center for Agronomic Research (CNRA) of Bambey, Senegal (16°30' and 16° 28' N; 15°44' and 15°42' W). The experiment utilized a split-plot design with four replications. Water regime was the main plot experimental factor while genotype, a total of 20 was the sub-plot. Pearl millet genotypes were divided into three contrasting groups based on drought tolerance for comparisons. Water stress did not affect 100-grain weight, test weight, protein, soluble protein, starch, sugars, amino acids or vitamin B2 content of grains among VPD-groups. Accumulation of these constituents of pearl millet grain appear to be genetically controlled and are probably not affected by late drought stress. However, differences were noted among genotypes as the sensitive VPD-group accumulated greater soluble protein, starch and soluble sugars (except sucrose) than the tolerant and medium VPD-groups. The tolerant VPD-group, however, accumulated greater protein and vitamin B2 content. Arginine, proline and serine content was greater in the sensitive VPD-group, while lysine, aspartic acid, and glutamic acid were greater in the tolerant VPD-group. Glycine, histidine, threonine, alanine, tyrosine, valine, methionine, leucine, isoleucine, and phenylalanine were relatively equal in tolerant and sensitive VPD-

groups. Calcium and Na levels were affected by water stress in the sensitive VPD-group, but differently. Calcium content was greatest for the sensitive group under drought stress, while sodium was the lowest. Iron accumulation in sensitive VPD-group increased under water stress. Potassium decreased for all VPD-groups under stress, while across water regime, K levels in the drought-sensitive group were lower. Selection for drought appears to effect many of the nutritional constituents of pearl millet grain, however many of these differences appear to be directly related to parameters known to effect plant water relations.

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GENERAL AUDIENCE ABSTRACT

The research reported in this thesis explores challenges and solutions in two different agriculture crops in Senegal. Commercial sweet corn (*Zea mays L. ssp. saccharata*) production can offer added diversity and income for smallholder farmers while changes in the nutritional composition of pearl millet (*Pennisetum glaucum* (L) R. Br.) due to selection for drought tolerance can directly impact household nutritional security.

The specific objectives for the sweet corn study were to: (1) determine if differences in sweet corn nutritional status can be detected using non-destructive remote sensing (RS) tools such as the GreenSeeker® and SPAD meter; and (2) whether anion-and cation-specific resin membranes can be used to monitor soil N dynamics and relate this to sweet corn RS readings, plant biomass, plant N uptake, yield and quality when produced under different nutrient levels in Senegal. Overall, readings generated using the GreenSeeker® were correlated with sweet corn yield, leaf mass and N uptake during mid-vegetative growth.

The objective of the pearl millet study was to compare grain nutritional composition among and between putative drought tolerant and drought sensitive pearl millet cultivars and lines. Drought sensitive genotypes produced more sugars, soluble protein, starch and some individual amino acids in grains, while drought tolerant varieties accumulated more protein, vitamin B2, and some individual amino acids. Grain nutrient content of pearl millet genotypes selected and identified as drought tolerant and drought sensitive are nutritionally

different, thus selection for drought tolerance affect pearl millet grains nutrient concentration level.

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Chapter 1: Introduction

1. Presentation of Senegal

Senegal, the western-most country of continental Africa, covers an area of 196,722 km² and is located between latitudes 12° and 17° 30'N and longitudes 11°30 'and 17°30'W. It is bordered in the north by Mauritania, to the east by Mali, to the southeast by Guinea and south by Guinea Bissau. Senegal's population was estimated to about 14.6 million with an annual growth rate of 2.89 % in 2014 (World Bank, 2016).

Senegal is located in the sudano-sahelian zone and has a semi-arid tropical climate that is characterized by a dry and rainy season from November to May and from June to October, respectively. The climate is divided into three zones based on rainfall. The Sahelian region is characterized by an annual rainfall less than 300 mm up to 500 mm; the Sudanese domain receives between 500 and 1200 mm of rain whereas the sub-Guinean region can receive more than 1200 mm of rainfall (ANSD, 2013).

Senegalese soils are generally sandy in the north of the country, ferruginous in the central regions, and lateritic in the south (Fall, 2008). The Senegalese soil types are grouped based on their formation factors (parent material, topography) (Tappan, et al., 2004) from which six agro-eco-geographical homogeneous zones were identified (FAO, 2007). The Delta and the Senegal River Valley (22,472 km²) are part of the Sahelian zone and the dominant soils are hydromorphic soils, and vertic soils. The main cultivated crops in this area are sorghum (*Sorghum bicolor (L.) Moench ssp*), maize (*Zea mays*) and rice (*Oryza sativa*). The Pastoral Region of Ferlo (36,289 km²), located in the Sahelian climatic zone, is under an ongoing desertification process due to excessive utilization of woody species

by shepherds. The dominant soils are ferruginous tropical sandy soils, slightly leached. The Northern part of the “Peanut Basin” (14,783 km²) which is in the Sudanese domain is composed of ferruginous tropical sandy soils, slightly leached. The southern and southwest regions of the “Peanut Basin” (23,945 km²) have ferruginous tropical and ferralitic soils; loamy sands over fine sandy loam at depth on which peanuts (*Arachis hypogaea*), sorghum (*Sorghum bicolor* (L.) Moench ssp), maize (*Zea mays*) and cassava (*Manihot esculenta*) are cultivated. The Niayes or Long Coast (8883 km²) has a vocation of vegetable and fruit tree production and the predominant soils in this region are ferruginous tropical sandy soils, and poorly developed coastal sands. The Lower and Middle Casamance (16 632 km²) soils are predominantly ferralitic tropical, ferruginous tropical sandy to clayey soils over laterite in the east and hydromorphic in the valleys. It is the area of dense forest and the traditional rice production zone. The Eastern Senegal and Haute Casamance regions (73 718 km²) have shallow loamy and gravelly soils over laterite on plateaus; deep, sandy to loamy, leached tropical ferruginous soils in the valleys and terraces and provide woody fuel used in the cities.

Arable land in Senegal represents 15.1 % of the land area in 2014 (The World Bank, 2016). Most of the land is cultivated only during the rainy season despite the presence of significant groundwater resources. Senegal has significant freshwater and underground resources but these are unevenly distributed spatially. Currently, irrigated agriculture represents only 2% of arable land in Senegal. The major constraints to Senegalese agriculture are soil salinization and acidification, deforestation, water erosion of plateau and valley slopes, wind erosion, reduction of biodiversity and plant biomass, drought

(République du Sénégal, 2016), but also inadequate government agriculture development policies, and rapid population growth (Cisse et al., 2004).

2. Millet and Maize cultivation in Senegal

Senegal is in the sahelian region where agriculture occupies the primary production activity (Noba, 2002) and contributes to improving food security through the provision of food and raw materials (Mbaye et al., 2014). The cultivable land is estimated to be around 3.8 million hectares for which around 2.3 million are actually cultivated (Dieye and Gueye, 2002). The principal cultivated crops are peanut, cotton, millet, sorghum, rice, maize, cowpea, and cassava. Cereals are reported to provide approximately 65% of calories and 61% of proteins consumed in Senegal (Ministere de l'Agriculture et de l'Elevage, 2001). Millet is the major staple food and cereal crop grown on approximately 973,000 ha (Dieye and Gueye, 2002). Millet is cultivated across the country and farmers yields often range from 0.5 and 0.6 t ha⁻¹ (Corniaux and Fall, 2005) while the potential achievable yield is more than 3 t ha⁻¹ with good management (Khairwal et al., 2007). In fact, the overall produced millet in Senegal does not exceed 600 000 tons per year mainly because of limiting factors like low soil fertility, weeds and diseases, recurrent drought, but also due to institutional and economic constraints (Mbaye, et al., 2014). Pearl millet is traditionally cultivated by many farmers, ie, without any applied fertilizer or no more than 60 kg ha⁻¹ of fertilizer (Rai et al., 2008), and with little or no use of manures, similarly to many countries in the Sahel (Khairairwal et al., 2007). Millet is cultivated in Senegal using family labor either in pure continuous cropping in farmers' field near houses,

and in rotation with peanuts or in association with cowpea in scrubland (Ministere de l'Agriculture et de l'Elevage, 2001).

Generally, in the sahelian region agriculture is dominantly rain fed, and over 90% of Senegalese agriculture is rain dependent (Dankelman, 2008). Rainfall affects agricultural activities mainly because of the uncertainty of the rains and risk of drought which is a major constraint on rain fed agriculture. The rains are fluctuating across the country and from year to year, and have declined 35% since 1996 (Dankelman, 2008).

In west Africa pearl millet varieties are classified as either Sanio (globular softer endosperm) characterized by a long season or Souna (obovate, hexagonal and lanceolate, harder endosperm) characterized by a short season (McDonough et al., 2000; Corniaux and Fall, 2005). Improvement of these traditional pearl millet varieties began in 1931, and a large array of new pearl millet hybrids have been created since (e.g., Souna-2 in 1965, then Souna-3 in 1969, and GAM-73, IBMV-8402, SOSAT-C88, etc.) (Corniaux and Fall, 2005). These improvements have demonstrated the possibility to increase the productivity of pearl millet. Some of these hybrids were vulgarized in rural areas, and others are still under the testing phase (Ministere de l'Agriculture et de l'Elevage, 2001). The objectives of these selections were mainly focused on productivity and yield stabilization, and consumer acceptability by targeting panicle size, tolerance to diseases and pests, to weeds (e.g. Striga), and to drought (Bezançon et al., 1997). Due to the importance of pearl millet in Senegalese agriculture and for human nutrition, these hybrids should be investigated in terms of their nutrient composition as affected by the selection to stresses such as drought. Thus we evaluated the nutritional characteristics of putative drought tolerant and drought

sensitive pearl millet genotypes to understand the effect of selection for drought tolerance on grain composition.

Maize is the second most cultivated cereal in Senegal and represent 22% of total cereal production. Maize is also cultivated across the country precisely in Oriental-Senegal, Sine-Saloum, Casamance and in the River valley (Ndiaye and Niang, 2010) and relatively with the same cultural practices as millet. Maize cultivated under the rainy season is also limited by drought, low soil fertility, lack of varieties adapted to environmental condition and lack of appropriate technical cultural practices (Cornaux and Fall, 2005). However, contrary to millet, maize is cultivated under irrigation, mainly in the Senegal River valley. This is mainly the result of the government's efforts to intensify maize production, but also dry season cultivation favoring horticultural production development (Cornaux and Fall, 2005; FAO, 2007). In fact, from 1960 Senegal adopted political decisions to develop irrigated agriculture (Dieye and Gueye, 2002; FAO, 2007). The irrigated agricultural systems in Senegal still occupies less than 10% of production (USAID, 2010). However, a total of 600 000 persons in the north of the country, 3068 farmers' organizations were identified as practicing irrigated agriculture for a total of 75000 ha (FAO, 2007). Sweet corn, a recently introduced subspecies of maize for grain, is abundantly cultivated in these irrigated lands. The sweet corn produced is for exportation purposes, and is generally produced from November to May by multinational companies. The main producers are Grands Domaines du Senegal (GDS) created in 2003, Société de Cultures légumières (SCL) created in 2006, and others such as Van Oers begun in 2007 and producing sweet corn since 2012, and SEMAF-SARL initiated in 2003. The total exported sweet corn reached around 3 730 990 kg in 2011 for a return value of about \$2 Million USD (Diouf,

2013). Because of the important export value, the Senegalese government is making efforts to develop sweet corn production for increasing the role of Senegal in the export market of horticultural products, but also to extend sweet corn cultivation to small producers. A further objective is to reduce the importation of processed sweet corn in Senegal via the development of locally processed sweet corn. For corn production, research demonstrated that optimum productivity can be obtained on small farms when the farmers have access to good production technology and production inputs (mineral, and organic fertilization and cultivation techniques) (Cornaux and Fall, 2005). This is not yet the case in Senegal in terms of accessibility of technology and cultivation techniques for sweet corn.

Pearl millet and sweet corn were included in the research and development strategies in Senegal supported by USAID/ERA through the Feed the Future program. The experiments described were part of studies conducted under the USAID/ERA project to 1) characterize the agronomic potential and fertilizer need of several sweet corn varieties and the applicability of remote sensing tools for in-season nitrogen (N) monitoring; and 2) to analyze and compare the physical and biochemical characteristics of putative drought tolerant and drought sensitive pearl millet varieties intended for further selection of drought tolerance.

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Chapter 2: In-Season Tools for Determination of Critical Nutrient Levels for Sweet Corn (*Zea mays* ssp. *Saccharata*. L.) Hybrid in Senegal

ABSTRACT

Sweet corn (*Zea mays* L. ssp. *saccharata*) production has increased considerably worldwide in recent decades and this is also true in Senegal. Though the industry is growing, there are currently no published fertilizer recommendations for sweet corn production in Senegal. Current recommendations are mainly based on the recommended dose of nitrogen (RDN) for corn for grain. This study aimed to identify tools to estimate sweet corn nitrogen (N) status and determine the most appropriate fertilizer dosage for sweet corn. Anxious about the undesirable effects of both inadequate and excess N application, the GreenSeeker®, chlorophyll meter, and ion exchange resin membranes (IEMs) were tested for their applicability for in-season N assessment at the V5 and V9 growth stages at Ndiol and Sangalkam, Senegal. The doses of fertilizer were 75%, 100% and 125% RDN applied as complete fertilizer (15-15-15; approximately 25% of total N need) at planting with the remainder as urea, in-season. No differences in ear yield or yield components, NDVI values, biomass, N uptake or leaf N concentration due to fertilizer dose were detected at Ndiol. However, significant relationships existed between NDVI values and yield, biomass, and N uptake at the V9 growth stage at this site. Only yield was affected by fertilizer dose at Sangalkam, and no consistent relationships were found between SPAD readings and other measured parameters. Treatment differences due to fertilizer dose for available NH_4^+ at V9 (Ndiol), and NO_3^- at V5 (Sangalkam) growth stages were found

however, these findings are not sufficient to affirm the applicability of IEMs to measure soil available N. The proposed recommended sweet corn fertilizer rate based on the study findings would be 75 % RDN (99 kg N ha⁻¹) in the study areas of Senegal.

1. INTRODUCTION

Maize (*Zea mays L.*) is the third most important cereal crop worldwide following rice and wheat and provides a large diversity of uses and broad possibilities for exploitation (Spandana Bhatt et al., 2012; Sunitha and Reddy, 2012). Sweet corn (*Zea mays L. ssp. saccharata*) is a hybridized type of maize that differs from corn for grain due to gene mutation resulting in decreased starch synthesis and increased proportion of polysaccharides (sugar) in seed endosperm (OGTR, 2008; Boyhan, 2011; Kumawat et al., 2014). Sweet corn sugar content typically ranges from 13 to 15%, but may contain up to 25% sugar in the immature grain (Spandana Bhatt et al., 2012; Singh et al., 2014) depending on genetics and environment. Sweet corn is produced both for fresh consumption and as a raw material for the canned food industry (Coskun et al., 2006). The desirable flavor and nutritional quality of sweet corn are responsible for increased global demand for sweet corn production (Szymanek et al., 2005). While sweet corn production and consumption is highest in the USA, Canada, and Australia, demand is notably increasing in India and other Asian countries (Najeeb et al., 2011). Moreover, sweet corn has been grown in some regions in Africa with different levels of production. In western Africa, especially in Senegal, sweet corn is cultivated only in small quantities and is mainly exported to European countries (Diouf, 2013). Sweet corn was introduced in the Senegalese market in 2004 with the intent to diversify the national market of exported fruit and vegetables, while increasing profitable outcomes of gardening (Diouf, 2013). In order for Senegal to intensify sweet corn production, increased knowledge of agronomic factors influencing or inhibiting this crop's growth and maturation are necessary.

Cultivating good quality sweet corn relies on the integrated management of the crop itself and soil which it will be grown. One of the crucial variables that need to be managed is crop

nutritional status that occurs through fertilizer additions. Cruz et al. (2015) stated that, besides improving productivity, appropriate fertility supports the development of well-shaped sweet corn kernel and ears for processing. McCall (1983), Gardner et al. (2000) and Farhadi-Afshara et al. (2009) collectively reported that sweet corn yields depend upon variety or hybrid (genetic capacities), soil type, climatic conditions, and cultural practices. Thus, crop responses to fertilization may not always be accurately predicted. Therefore, successful crop production will require research in each of Senegal's specific agronomic zones to increase crop yield while striving for high grain quality (De Grazia et al., 2003). Successful sweet corn fertilization requires relatively high proportions of N for vegetative and generative development (Salardini et al., 1992; Cordea et al., 2011). It can be challenging to determine the exact dose of mineral fertilizer needed to support crop yield because of various biotic and abiotic factors, but also because of conditions such as mineral fertilizer application rate, timing, placement, or the nature of fertilizer used, all impacting production sustainability (Phillips, 2015). However, efficient N application rates for sweet corn growth have been determined worldwide where corn is cultivated intensively. This efficiency is in part linked to the use of crop sensors (GreenSeeker® and SPAD meter), important tools developed as nutrient management strategies for improved N use efficiency (NUE) compared to traditional N rate determinations (Phillips, 2015).

Ion exchange resin membranes (IEMs) have also been useful in predicting soil N availability. Adoption of these technologies in Africa could facilitate the elaboration of N fertilizer recommendations for farmers (Teboh et al., 2012). In fact, Mashingaidze et al. (2013) stated that the low agronomic N use efficiency (ANUE) observed in the semi-arid region of sub-saharan Africa is mainly the result of poor nutrient management, especially N timing, source, and rate. Remote sensing technologies being used for several years as decision support tools with the ability

to adjust for soil N and in situ (field) variability when determining the adequate rate optimizing crop NUE (Martin et al., 2007; Mashingaidze et al., 2013) can similarly be an alternative for crop production improvement in Africa.

Therefore, with the overall goal of improving sweet corn production in Senegal under both irrigated and rain fed conditions, this study was conducted to test whether remote sensing tools and IEMs can be used to determine optimum N fertilizer rates for sweet corn cultivation in Senegal.

2. LITERATURE REVIEW

Sweet corn is a corn subspecies that has been historically documented since the 18th century and is classified as a vegetable plant (Szymanek et al., 2005). Sweet corn's distinction from other maize types comes from the higher kernel sugar content compared to corn for grain. Sweet corn varieties or hybrids are differentiated further into categories of: "normal sugary" (su); "sugary enhanced" (se) and (se+); and "shrunk" (sh2) (also called "super sweet"). These varieties differ from one another in their sugar content, taste and softness, as well as the rates at which starches are transformed to sugar (Diver et al., 2008; Singh et al., 2014). Sweet corn varieties can also be differentiated base on the vegetative growth period length. Typically varieties maturing 70 to 80 days after sowing are known as early maturity, those reaching maturity at 85 to 90 days, as medium maturity and late varieties maturing 95 to 110 days after sowing (Szymanek et al., 2005).

In sweet corn production, quality is the main determinant of market suitability. Besides high sugar content, sweet corn has high moisture (73%), and the solid component (22%) is a good provider of carbohydrates (81%), protein (13%) and lipids (3.5%) (Kumawat et al., 2014). Sweet corn is gluten free (Szymanek et al., 2005). In order to achieve the highest crop quality rating, intensive management practices are required from planting to harvest (Kwabiah, 2004). Sweet corn

is typically cultivated using irrigated conditions and water is typically the most limiting factor in sweet corn production (y Garcia, 2009). The lowest water needs for sweet corn development are in the initial period (seedling) of vegetative growth, while maximum water requirements coincide with tasseling, silking and grain filling. Inadequate water at reproductive growth stages can drastically reduce yield and quality (Rogers, 2007; Szymanek et al., 2005). Additionally, it's been reported that adequate irrigation is required for an optimum response to N fertilization (Gardner et al., 2000).

2.1 Agronomy of sweet corn

Sweet corn can be grown similarly to maize (corn) grain; however, sweet corn production in the early growing season may be more challenging due to reduced seedling vigor. Reduced early vigor results from its low starch content (OGTR, 2008). Maize growth requires warm daytime temperatures greater than 10 °C and fertile, deep and well drained soils with soil pH ranging between 5.5 and 7.0 (OGTR, 2008; Serafin and Carrigan, 2014). Sweet corn performs poorly in waterlogged, cold, clay, dry sandy, and piedmont and mountain soils (Szymanek et al., 2005). Consideration of soil profile is very important for sweet corn production because roots can reach a depth of 1.2m (Beckingham, 2007); however, most plant roots are located in the first few centimeters (30 cm) of the root zone from the soil surface (Rogers, 2007; Serafin and Carrigan, 2014). Water needs for the whole growing season of sweet corn can approach 400 mm, and it can tolerate temporary drought, especially during vegetative growth (Szymanek et al., 2005). Minimum soil temperature during sowing should be higher than 12 °C, and ideally between 14 and 16 °C (Beckingham, 2007; Najeeb et al., 2011). At silking temperatures over 30 °C can limit the success of pollination and can reduce yield and quality (Szymanek et al., 2005).

Corn is an annual grass which can reach a height of 1 to 4 meters, and depending on field condition plant density can range from 35,000 to 70,000 plants per hectare (OGTR, 2008). Sweet corn sowing depth should be 3 to 5 cm; row spacing should not be closer than 75 to 90 cm (Diver et al., 2008); and distance between plants in a row not less than 20 to 30 cm (Szymanek et al., 2005). Sweet corn harvest occurs when the silks are dried and the outer leaves still green. The kernels should be in a milky or very early dough stage (OGTR, 2008; Boyhan, 2011) with a moisture content ranging between 70 and 75% (Szymanek et al., 2005). Depending on the hybrid, moisture, and temperature, harvest is usually from 75 to 105 days after planting (corresponding to 21 to 28 days after silk appearance) (OGTR, 2008). Najeeb et al. (2011) reported that hybrids of 95 days reach maturity at 120 to 125 days in the temperate region of Kashmir in India. Sweet corn can produce from 1 to 3 spikes plant⁻¹ with spike length between 10 and 20 cm (Szymanek et al., 2005).

Sweet corn is affected by insects such as corn earworm (*Helicoverpa zea*), European corn borer (*Ostrinia nubilalis*), corn rootworm (*Diabrotica*), cutworm, and also by some fungal diseases (smut, rust, downy mildews), bacterial diseases (Stewart's bacterial wilt, wallaby ear), and viral diseases (maize dwarf mosaic) (Beckingham, 2007; Diver et al., 2008). In Kansas (USA) for example, an average of 5 to 15% of yield loss due to diseases is documented each year (Jardine, 2007). Pesticides, fungicides, resistant varieties, crop rotation or early sowing are all suggested management strategies to help alleviate yield and quality losses due to diseases and pest attacks (Diver et al., 2008). Weeds compete with sweet corn plants for resources and that competition can reduce plant growth and yield. For every 500 g gained in weed dry matter in a field a corresponding 500 g loss of corn plant dry mass has been documented (Regehr et al., 2007). Control of weeds can be done using various methods, such as use of herbicides, cultivation, and crop rotation

(Regehr et al., 2007; Najeeb et al., 2011). Birds and animals like monkeys can also damage sweet corn ears and reduce yield.

2.2 Plant fertilizer demand

Adequate nutrient availability in soil is vital to crop establishment. Providing sufficient nutrients by adding fertilizer helps assure of crop yield and quality. Appropriate fertilization is known to facilitate early growth, good rooting establishment, and resistance to diseases (Szymanek et al., 2005). The nutrients most needed by corn are N, potassium, phosphorus calcium, magnesium, and zinc (Szymanek et al., 2005; Beckingham, 2007; Leikam and Mengel, 2007). Potassium and N removal from the soil by sweet corn roots is greater compared to phosphorus, calcium, magnesium and zinc (Szymanek et al., 2005; Leikam and Mengel, 2007). However, Wu et al. (1993) reported from their study using different fertilizer combinations that N and phosphorus contributed more to sweet corn yield than potassium. Other essential plant nutrients are needed but in smaller quantities (Beckingham, 2007). Table 2.1 contains general sufficiency ranges of sweet corn for N, P, and K in plant leaves as identified by Bryson et al. (2014).

In general, one ton of corn grain requires 22 to 27 kg of N, of which 12 to 16 kg is accumulated in the grain, and 5 kg phosphorus where 3 kg is found in the grain (Birch et al., 2003). Potassium requirement is 10 kg t⁻¹, of which 3 to 4 kg is contained in the grain (Serafin and Carrigan, 2014). Beckingham (2007) reported that 28 t ha⁻¹ sweet corn take up a total of 310 kg N ha⁻¹, 40 kg P ha⁻¹ and 210 kg K ha⁻¹. From these totals, 110 kg ha⁻¹ of N, 16 kg ha⁻¹ of phosphorus, and 60 kg ha⁻¹ of potassium are accumulated in the cob and grain portion. N is critical in plant growth and development, and is often a main limiting factor for corn production in both irrigated and non-irrigated production systems (Birch et al., 2003). N is an essential component of many

processes including proteins, enzymes, DNA, RNA, ATP, chlorophyll, auxin and cytokinins synthesis in plants (green cobs, fodder, and grains) (Raven et al., 2004; Hawkesford et al., 2012; Andrews et al., 2013; Kumawat et al., 2014). In fact, proteins generally contain 80 to 85% of the total N in plants (Li et al., 2013).

According to Meena et al. (2013) an optimum N supply encourages photosynthesis which improves dry matter accumulation for the duration of the growing season. N fertilizer can be applied before or during sowing and during the growing season as a sidedress application (Leikam and Mengel, 2007). N removal from soil by a corn plant is slow from emergence to 25 days; only 10% of the total N required is used (Basden et al., 2006). Uptake rate increases more rapidly beginning about 25 days after emergence, and by silking, 60% of the total season N accumulation has been taken up by the plant (Szymanek et al., 2005; Leikam and Mengel, 2007). Wu et al. (1993) reported that the greatest rate of N uptake occurred during the tasseling stage of the sweet corn plant. Timing of N application is thus very important since the plants have periods of high nutrient requirement. Providing the majority of N supply to corn in its period of low uptake can lead to N loss through various mechanisms (Basden et al., 2006). Okumura et al. (2014) found that application of N to plants at stage V4 compared to V8 promoted a greater plant height and leaf area index. He argued that N availability at early stages resulted to better foliar and root development as well as a greater nutrient accumulation by the plant for the rest of its cycle. Sweet corn plants typically absorb N from soil as the main inorganic forms of N usable by plants, NH_4^+ -N and NO_3^- -N (Li et al., 2013). Mills and McElhannon (1982) reported that NO_3^- uptake was higher during tasseling to silking, while NH_4^+ absorption was greater during grain development. Many plants can also use the organic form of N (Andrews et al., 2013; Wang et al., 2014).

Studies on the effect of fertilizer rate on sweet corn growth, yield and yield component, as well as protein content reported that these parameters typically increased with increasing N fertilizer application (Bravo-Ureta et al., 1995; Oktem et al., 2010; Kumawat et al., 2014). Meena et al. (2012) and Sunitha and Reddy (2012) found that the highest yield and the best quality of sweet corn was obtained with increasing N uptake and increasing fertilizer rate. Oktem (2005) reported that an increase in N rate from 150 to 300 kg ha⁻¹ led to a 42% increase fresh ear yield; however a 3.5% decrease in fresh ear yield was noted when the rate of applied N reached 350 kg ha⁻¹. Total soluble sugar content was found to decrease with increasing N (Chamberland, 1978), whereas lysine content followed the opposite trend (Wu et al., 1993). Chlorophyll content in leaves similarly increased with increased N supply (Amer, 1991; Wu et al., 1993; El-Yazied et al., 2007), and a positive relationship was found between yield and chlorophyll concentration in the ear leaf (Wu et al., 1993). Differences in chlorophyll concentrations due to varieties were noted by Amer (1991) as well as nitrate level in the kernels (Cordea et al., 2010). Comparatively to the previous authors, Mullins et al. (1999) reported very little variation in the measured variables between fertilization treatment and varieties. A similar response was observed by Sharma and Sood (1974) with no significant difference for fertilizer rate between 60 and 120, and between 120 and 180 kg N ha⁻¹. Significant difference was noted between 0 and 60 kg N ha⁻¹ which was the recommended rate for economic yield.

Nitrogen is known to be a very dynamic nutrient in soils, and plant N uptake efficiency is dependent upon factors such as: environmental conditions (climate, and soil type), plant varieties, their density, fertilizer type, application timing, and fertilizer rate. Different optimum N fertilizer application rates for sweet corn are reported in the literature. Cruz et al. (2015) have reported, based on a linear equation, a maximum productivity of total and marketable ears with 300 kg N

ha⁻¹ in the state of São Paulo, Brazil. Okumura et al. (2014) that in the state of Paraná in southern Brazil, an optimum marketable yield occurred when sidedressing 110 kg N ha⁻¹ regardless the variety, time of application, and year of growing. The optimum preplant application rate of 20 kg N ha⁻¹ was similar in all treatments. In Oktem et al. (2010) application of 320 kg N ha⁻¹ gave the highest nitrogen-use efficiency (NUE), which is reported as the ratio of yield per kilogram to applied N. This rate also corresponded to the optimum marketable yield performance for ‘Vega’ sweet corn hybrid used in the study in Sanliurfa, Turkey. Mullins et al. (1999) recommended 134 kg N ha⁻¹ for production of sweet corn in Tennessee. Studies conducted to identify the optimum N rate for sweet cultivation (row spacing of 75 x 25 cm) in Nigeria report optimum yield when 120 kg N ha⁻¹ was applied (Akintoye and Kintomo, 2011).

It is important to note that the optimum N rates usually reported in the literature are relative to specific plant density and environmental conditions. Plant population density is crucial since it plays a significant role in the level of competition between plant for nutrient, water and light. An amalgamation of various studies have reported that under yield limitations, plants at lower density more effectively exploit natural resources, whereas plants at highest plant density endure harsh competition (Spandana Bhatt, 2012). This stress can induce a great decrease in plant leaf area, grain yield, and kernel number per plant (Oktem, 2005). Kumar (2009) reported that the highest green cob and fodder yields resulted application of 120 kg N ha⁻¹ when paired with a plant density of 83,333 plants ha⁻¹ whereas a population of 111,111 plants ha⁻¹ resulted in lighter ears, fewer kernel cob⁻¹ and lower quality. Oktem et al. (2005) in studies in the semi-arid region in Turkey recommended a rate of 300 kg N ha⁻¹ with plant density of 64,930 to achieve high yield (approximately 16 t ha⁻¹) of fresh ears. Spandana Bhatt (2012) proposed application of 240 kg N ha⁻¹ with a density of 80,000 plants ha⁻¹ on a clay loam soil with low available N in India. A study

in Iran, conducted under warm conditions, reported that a density of 89,000 plants per ha⁻¹ resulted in the highest yield (8.8 t ha⁻¹) and provided efficient weed control (Farhadi-Afshar et al., 2008).

Many authors who have investigated the optimum N rate for sweet corn cultivation have reported that very high N rates can cause a decrease in yield and quality, and increase the cost of production (Chamberland, 1978; Amer, 1991; Oktem et al., 2005). Several authors like Straw et al. (1993) and Bravo-Ureta et al. (1995) stated that excessive N fertilization can cause environmental issues such as surface water and ground water pollution, whereas low N application can result to loss of yield (Amer, 1991; De Grazia et al., 2003). John et al. (2003) pointed out that high rates of N may increase nitrogen concentration in vegetables, and eventually cause health problems. In fact, Cordea et al. (2010) stated that high nitrate content in the kernels can be alarming if improper ear storage conditions occur because nitrite can be transformed into nitrite, which can be dangerous for humans. They also reported that at 8 mg kg⁻¹ body weight, nitrate can be lethal to humans. These consequences (low yield and quality, pollution of the environment or toxicity) deriving from poor N management during crop production highlight the importance of improved N management for sweet corn.

2.3 Crop sensors and in season N monitoring

Nitrogen is often the most deficient nutrient in many soils used for agronomic crops, and is also usually the main limiting factor to plant growth (Andrews et al., 2013). To date, there is not a single generalized management strategy to assure high N use efficiency (NUE) for field crops (Raun et al., 2005). The critical consideration with N is how to assure nitrogen availability to meet plant needs throughout the growing cycle while possibly avoiding N losses (Birch et al., 2003). Traditional methods for monitoring N in-situ often fail to elucidate the dynamics of labile nutrients.

Plant tissue analysis generally requires the use of destructive sampling and analytical methods (Buzas et al., 2006). These destructive methods for assessing plant N status are expensive (Pinkard et al., 2006), and may not be practical when evaluation of N status is needed in real time (Yao et al., 2007). Advanced approaches track N more effectively in soils and in plant tissues during the entire growing season. Remote sensing tools provide non-destructive approaches to estimate chlorophyll and N content in plants using leaf optical properties (i.e., reflectance or transmittance) of radiant energy (Schepers et al., 1996). To evaluate plants N status, crop sensors measure crop reflectance or transmittance, since absorption of photosynthetic wavelengths is related to the concentration of plant pigments (chlorophyll a and b) and N content (Tucker, 2010). The reflected, transmitted, or absorbed radiation by leaves delivers information that can be used to interpret plant physiological response to growth conditions (Hong et al., 2007). These measures may also enable the assessment of crop plant nutrient needs and fertilizer recommendation for specific stages of development (Yao et al., 2010; Gianquinto et al., 2011).

The handheld GreenSeeker[®] is an active sensor that generates its own red (660 ± 25 nm) and near infrared (NIR) (780 ± 25 nm) light, and as the sensor passes over the plant canopy, it measures incident and reflected light and calculates a normalized difference vegetation index (NDVI) (Yang et al., 2011; Teboh et al., 2012; Jones, 2013; Tadesse et al., 2015).

The equation for calculating NDVI is as follows:

$$\text{NDVI} = (\text{NIR}_{(\text{reflected})} - \text{Red}_{(\text{reflected})}) / (\text{NIR}_{(\text{reflected})} + \text{Red}_{(\text{reflected})}).$$

The values can range from 0.00 to 0.99, and generally healthier plants produce higher NDVI values (Tadesse et al., 2015). Healthy plant biomass absorbs more red lights and reflects more near-infrared light, indicating high chlorophyll content and N status (Lan et al., 2009; Teboh et al., 2012). The GreenSeeker[®] system has been used to evaluate plant health and vigor and to

determine optimum in-season N rates for maize (Tadesse et al., 2015; Teboh et al., 2012). Relationships of NDVI to N in plant and fertilizer rate, chlorophyll, crop yield, and biomass have been studied for various crop species (Amer, 1991; Yao et al., 2012). Tadesse et al. (2015) found that NDVI values increased with increasing rates of applied N ($r^2 = 0.93$ and 0.98), and with advancing plant growth stage (V4 to V6). This same study reported a stronger correlation between NDVI and maize grain yield as N rate and growth stage increased. Osborne (2007) evaluated the ability of the GreenSeeker® to predict canola yield, biomass, leaf N and N uptake. These studies concluded that NDVI readings correlated well with these measured variables. It is often reported that at early crop growth stages, NDVI is less effective for predicting crop N status due to low vegetation cover and soil background interference (Titolo, 2012). Jones (2013) observed that soil color and moisture affect NDVI readings. He explained that NDVI values were higher in wet soil than in dry soils due to the pronounced darker background caused by moisture. Additionally, sensor orientation relative to the target (nadir, parallel, 15 cm mask, 45° off nadir orientation) varied by soil series. Torino et al. (2014) observed that GreenSeeker® readings taken over multiple years were more effective at differentiating corn N status at V8 and V10, compared with V6. Titolo (2012) found that NDVI collected at V8 was better correlated with final yield than readings collected at V6. Similar findings were reported by Thomason et al. (2007) who found that NDVI collected after cumulative growing degree-days (GDD) ranging from 166 and 485, corresponding to the V5 and V9 growth stage, was more predictive than readings taken earlier. These findings underscore the importance of NDVI measurement timing for N management. Compared to the soil plant analysis development (SPAD) meter, the GreenSeeker® was typically unable to detect N deficiencies at early stages and predicted lower corn yields at early stages (Kitchen et al., 2010; Titolo, 2012). Kipp et al. (2012) reported that for row crops such as maize, the leaf levels from

which the reflectance signals are captured by the active sensors are difficult to determine and can influence NDVI readings depending on plant architecture and the growth stage. Winterhalter et al. (2013) compared passive sensors and active sensors and found that reflectance measures from active sensor were more confined in the upper part of the plant canopy. Kitchen et al. (2010) further explained that canopy reflectance sensors mostly measure leaves that are out of the whorl, near the top of the plant, while the chlorophyll meter is typically used to measure leaves that are under the whorl leaves, and that since N is a mobile nutrient deficiencies appear first on lower leaves.

The SPAD chlorophyll meter provide a measure of leaf greenness which is directly correlated with leaf chlorophyll and N content (Mulla, 2013). It is a suitable analytical portable tool for measuring N status in leaves during the growth of the crop (Byju and Anand, 2009). The nitrogen sufficiency index (NSI) was defined as the ratio of SPAD meter greenness readings for crops in a given field relative to SPAD readings for the same crop in a well-fertilized reference strip with no N deficiencies (Mulla, 2013). This NSI minimizes factors (diseases, water status, nutrients, and genotype) other than N that can affect leaf greenness and chlorophyll meter readings (Piekielek et al., 1997). A NSI less than 95% indicates N deficiency, and the relative level of sufficiency can be used to recommend the appropriate N rate to apply to correct the deficiency (Peterson et al., 1993). The SPAD meter measures light transmittance at wavelengths of 650 nm (red) and 950 nm (infrared) (Hoel, 1998; Byju and Anand, 2009) but allows correction for differences due to leaf thickness, water content, and others (Rori et al., 2011). The indexed chlorophyll content readings range from 0-50 with greater numbers indicating a higher chlorophyll status. Chlorophyll meters have been used to diagnose N status in corn (Wood et al., 1992; Waskom et al., 1996; Zhang et al., 2009; Kitchen et al., 2010; Yang et al., 2012; Jones, 2013; Torino et al., 2014.), wheat (*Triticum aestivum* L.) (Hoel, 1998), coffee (*Coffea canephora* L.) and

pear (*Pyrus communis* L.) trees (Netto et al., 2005; Netto et al., 2011), rice (*Oryza sativa* L.) (Cao et al., 2013), cassava (*Manihot esculenta* L.) (Byju and Anand, 2009), and cotton (*Gossypium hirsutum* L.) (Muharam et al., 2014). For most of these studies, SPAD meter values and chlorophyll content were strongly correlated. Rostami et al. (2008) reported that SPAD readings measured from corn leaves increased with increasing fertilizer rate and that the SPAD measures were low before the V7 growth stage, increased to the R1 stage and declined afterward. Another interesting finding from Rostami et al. (2008) was that SPAD reading related to N status of plants was correlated to grain yield. Using a regression analysis, Rostami et al. (2008) determined a linear correlation coefficient of $r^2 = 0.80$ between SPAD values and corn leaf N. Prior to silking leaf chlorophyll meter measurements are generally taken from the most recent fully matured corn leaf and after silking, from the ear leaf (Zhang et al., 2009). However, Zhang et al. (2009) stated that in a field study it can be challenging to see treatment effects on SPAD measurements before silking. They reported that differences in SPAD values were noticed when plants reached 50 cm height until about 150 cm. This height corresponds to measurements of leaves near the ear leaf. Nonetheless, compared to the GreenSeeker®, that SPAD meter is generally able to detect N deficiencies at earlier growth stages, and provide more accurate prediction of corn yields at earlier stages (Titolo, 2012). Jones (2013) reported that neither the GreenSeeker® nor the SPAD meter good indicators of corn plant N concentration prior to the V4 growth stage. In several studies, variations in SPAD readings were found related to the measured leaf, and the position at which the measurement was taken on the leaf. In Zhang et al. (2009) the uppermost fully developed corn leaf was used to determine corn N status, and because of the variation in N on the same leaf, four readings were averaged (on both edges, measurement was taken alongside the edge and the tip). Hoel (1998) reported that with winter wheat the middle of the upper most leaf was adequate to

measure leaf N. Overall, experience have shown that GreenSeeker® and SPAD meter are in many cases useful tools for in-season N assessment and recommendation. However, it should be noted that factors other than N can affect leaf greenness and photosynthetic activity in plant. Therefore, it may improve accuracy of recommendations to combine plant N monitoring with soil N status determination during the crop season. Combined, the result could be reduced yield loss and the environmental impact of excess N.

Estimation of soil N available for plants is difficult mainly because of the dynamic nature of N in soils. In fact, the bioavailability of soil nutrients depends on soil chemical, physical and mineralogical properties which also influence ion-exchange in the soil (Sherrod et al., 2002). In soil, ion exchange resin membranes (IEMs) are used for soil testing and are an alternative to chemical extraction methods for determining nutrient (bio) availability (Qian et al., 1992; Sato and Comerford, 2006; Barghouthi et al., 2012). In fact, in vitro soil extraction with potassium chloride provides accurate estimates of nitrate (NO_3^-) pools and exchangeable ammonium (NH_4^+), but little information on N flux (Bowatte et al., 2008). Ion exchange resin membranes are most useful when used as a measure of soil nutrient availability over a defined time period, rather than as a measure of absolute plant nutrient availability (Sherrod et al., 2003). Other positive characters of IEMs compared to other techniques comprise the quantifiable surface area, good soil contact, reduced soil disturbance during incorporation, reduced time compared to soil sampling and grinding prior to analysis, and reduced possibility of losing part of the exchange surface area (Meason and Idol, 2008). They have been reported to be reliable for soil nutrient ion testing (such as NO_3^- , NH_4^+ , PO_4^{3-} , and K^+) in agricultural and nonagricultural systems, since they have a noticeable sensitivity to plant environmental conditions, and mimic nutrient uptake by roots (Pare et al., 1995; Barghouthi et al., 2012). In fact, studies have shown that IEMs are sensitive to soil moisture

content, mineralization and immobilization, and less sensitive to temperature (Duarte, 2003; Szilerry et al., 2005; Johnson et al., 2005). Nutrient supply rate is the relative amount of nutrient ions from the soil absorbed on resin membranes over a time period. The nutrient supply rate is typically expressed as μg (or μmol) per cm^2 (Harrison and Maynard, 2013). Comparative studies have concluded that nutrient supply rates determined from resin membranes have a good relationship with ions concentrations (μg) per gram of soil (Qian et al., 1992; Pare et al., 1995; Harrison and Maynard, 2013, Mallarino and Atia, 2005) in crop fields and forests, however, some did not find correlation for specific ions (Szilerry et al., 2006). Ion exchange membranes have been used to estimate N supplying power of different soils (Qian and Schoenau, 2005) and to assess soil N mineralization rates (Harrison and Maynard, 2013). Nyiraneza et al. (2011) conducted a study in a corn field to investigate soil type, time of anionic exchange membrane (AEM) removal from soil and placement distance effect on AEM-adsorbed N and P. Qian and Schoenau (2000) reported r values ranging from 0.79 to 0.96 between canola plant N uptake and available N (sum of NO_3^- and NH_4^+) measured by IEMs in the soil under different N supply. Noulas et al. (2013) observed that phosphorus supply rate determined with AEMs was more highly correlated to P uptake by plants ($r^2 = 0.96$) than to other yield-related parameters. Potassium availability also measured with IEMs was highly correlated to traditional extraction methods ($r^2 \approx 0.70$) in a range of soil types (Qian et al., 1996). In a study investigating rotation effect on nutrient availability, N recovered from resin membrane closely reflected N uptake by wheat plant in different rotations (Salisbury and Christensen, 2000). Investigating the ability of ion exchange membrane to evaluate NO_3^- availability in a forage and corn field, Ziadi et al. (2006) not only found not only that NO_3^- adsorbed on resin membrane increased significantly with increasing N fertilizer rates, but also sorbed NO_3^- had a significant correlation with crop N uptake. These results suggest that IEMs can be used to

evaluate soil N availability in corn fields. However, some disadvantages of IEMs are noted. When used in situ it is often difficult to convert IEMs data to units for field use, such as kg ha^{-1} of available nutrient and to determine the amount of soil solids in contact with the IEMs (Pare et al., 1995). Additionally, when left in soil for a long period of time (more than two weeks) IEMs function as sink can no longer be guaranteed because it may behave as a dynamic exchanger (Qian and Schoenau, 2002). Few to no-published work are available on the use of ground based crop sensors and IEMs for investigation of crop nutritional status and crop fertilizer recommendations in Senegal or the broader Sahel and Sudan-Sahelian regions of west Africa.

3. Specific objectives

The specific objectives of this study were to: (1) determine if differences in sweet corn nutritional status can be detected using non-destructive remote sensing (RS) tools such as the GreenSeeker® and SPAD meter; and (2) whether anion-and cation- specific resin membranes can be used to monitor soil N dynamics and relate this to sweet corn RS readings, plant biomass, plant N uptake, yield and quality when produced under different nutrient levels in Senegal.

4. Hypothesis

Our hypotheses are that (1) fertilizer application will affect sweet corn yield and quality and will influence the market value of the product and farmer profitability. (2) Improper fertilization will lead to reduced yield and quality. (3) Non-destructive RS tools can help provide feedback to farmers on optimum in-season N rates. (4) Anion- and cation- specific resin membranes will provide a simple, useful, economical tool to track soil N dynamics under field conditions prevalent in Senegal.

5. MATERIALS AND METHODS

5.1 Experimental site

Experiments were conducted at two sites during the growing seasons of 2015 from April to August in Senegal. One site was located at the ISRA experimental site of Sangalkam in the Dakar region near latitude 14°46' 52" N and longitude 17°13' 40" W. The climate of the area is a Coastal Sahelo-Sudanian (Southern Canarian) characterized by mean annual rainfall of 400 to 600 mm (Camara et al., 2014). The climate data during the growing period are presented in Figure 2.1. Situated in the Niayes or Long Coast, the soil type of Sangalkam is moderately hydromorphic gley (FAO, 2007). The second site is in the Saint-Louis region at the ISRA experimental site of Ndiol (16°08'N, 16°19'W, 7 m). The soil type is predominantly red-brown sandy soil. The climate is a Continental Sahelian characterized by a mean annual rainfall of 200 to 300 mm (Diop et al., 2008). Climate data during the growing period for this site are presented in Figure 2.2.

5.2 Experimental design

The experiment was conducted in a randomized complete block design with four replications in each of our two locations. The factors studied were three fertilizer rates applied as NPK fertilizer (15-15-15) and urea, providing a total of 99, 131 and 165 kg N ha⁻¹ (Table 2.2). Our reference rate in this experiment is the 100% recommended dose of nitrogen (RDN) fertilizer for cereal (grain) corn per the horticultural development center (CDH) of Senegal affiliated to the Senegalese institute of agricultural research (ISRA). Five sweet corn hybrids (Table 2.3) purchased from Société de Culture Légumière (SCL) in Senegal were used in this study. A total of 60 plots were planted at each site. Additional location and production management information are

included in Table 2.3. At Ndiol, each experimental plot consisted of five rows of plants with 75 cm between rows and 25 cm between plants. Plots were 5 m in length and 3 m wide (total area of 59 m x 26 m). At Sangalkam, each plot had four rows of plants with plots 3 m in length and 2.25 m wide (total area of 48 m x 36 m). Plots were separated by a 1 m between adjacent plots and 2 m alleys (1.5 m in Sangalkam). Planting density was approximately 55,500 plants ha⁻¹ at both sites.

5.3 Agronomic practices

Before sowing, soil was tilled at a depth of 25 cm. At the time of sowing two sweet corn seeds were planted in each hole at a depth of 3 cm. The fertilization treatment consisted of a broadcast application with a mild incorporation using a garden rake of the total amount of 15-15-15 fertilizer per plot one day before planting to achieve the three fertilization rates. A split application was done for sidedress, 40% of the total urea (46% N) was applied at the V6 growth stage; 60% was applied at the V10 growth stage. Urea was band applied at the base of each individual plant. At both experimental sites, water was supplied to plants daily using a sprinkler system delivering 18 L water hour⁻¹ for a period of four hours. Twenty-one days after emergence, seedlings were thinned to one plant per hill to obtain the desired plant density. Weeding was performed manually with hoes. The fungicide Mocap (ethoprop) was used to treat potential diseases, 50 kg ha⁻¹ was applied the same day as preplant fertilizer in broadcast and incorporated using a garden rake. During harvest at Ndiol ears were harvested from the three center rows and yield was determined from an area of 6 m². At Sangalkam, the two center rows were harvested and yield was calculated on an area of 2.25 m². Of the total ears harvested in each plot, those deemed to be of marketable quality (healthy ears, ie free of insect damages) were weighed intact and used to calculate yield. Healthy ears were those free from insect and disease damage and with fully

developed kernels. In addition, a representative six-ear subsample was collected and were measured for length and diameter.

5.4 Soil samples

Soil samples, 12 individual cores at depths of 0-15 and 15-30cm, were collected from each experimental area prior to planting and composited. Samples were analyzed for nitrate (NO_3^-) and ammonium (NH_4^+), and other routine soil analyses (Table 2.4). Soil samples were air dried, sieved through a 2 mm screen, and extracted with 2 M KCL (Bremner and Keeney, 1966) prior to analysis for $\text{NH}_3\text{-N}$ and $\text{NO}_3\text{-N}$. Ammonia and $\text{NO}_3\text{-N}$ concentrations were determined using automated injection flow analysis (Lachat Instruments, Milwaukee, WI) with QuickChem sodium salicylate method 12-107-06-2-A (Hofer, 2001) and QuickChem 12-107-04-1-B using Cd reduction (Knepel, 2003), respectively. Other nutrients were extracted with Mehlich-1 using a 1:5 soil:extractant ratio and analyzed with inductively coupled plasma-atomic emission spectroscopy (Maguire and Heckendorn, 2011). The pH was determined using a 1:1 soil:water mixture, and cation exchange capacity (CEC) was measured following extraction with Mehlich 1 buffer containing 0.05N HCl in 0.025N H_2SO_4 (Maguire and Heckendorn, 2011). The C:N ratio was determined by dry combustion using vario MAX CNS Element Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany).

5.5 Ion exchange membranes

The Ion Exchange Membranes (IEMs) were purchased as sheets from Membranes International Inc., and cut into 2.5 x 15 cm strips. The polymer structure of the IEMs was gel polystyrene cross linked with divinylbenzene. The CMI-7000 Cation Exchange Membrane (CEM)

ionic form was sodium, the cation exchange capacity (CEC) was 1.6 ± 1 (meq/g), and was stable in a pH range of 1-10. The AMI-7001 Anion Exchange Membrane (AEM) ionic form was chloride; the anion exchange capacity (AEC) was 1.3 ± 1 (meq/g) and was stable in a pH range of 1-10. Two weeks after sowing, in each experimental plot three cation and three anion resin membranes, each stapled to a wooden plot marker were buried at 15 cm depth, approximately 30 cm from the plant row and equidistant to two adjacent plants in the row. Before use, IEMs were kept in a NaCl solution (468 g of NaCl dissolved in 2 L distilled water) for 24 hours for regeneration. One set of cation and anion membranes were removed from each plot at V5, V9 and at harvest. After removal, IEMs were rinsed with deionized water and kept moist in plastic bags until analysis. For NH_4^+ and NO_3^- extraction, each IEM was placed in a 500-ml centrifuge tube containing 50 ml of 1-M KCl, and shaken for 1 h. The NH_4^+ and NO_3^- concentration of the eluant was measured by Lachat QuikChem AE flow-injection autoanalyzer and ion chromatography. Because the resin was stapled to a stake on one side, the reactive surface area was considered to be 37.5 cm^2 . The amounts of adsorbed NH_4^+ and NO_3^- on IEM were expressed as $\mu\text{g N /cm}^2 \text{ IEM/ length of time the IEM strip was in the soil}$.

An adsorption study was conducted to determine the maximum adsorption capacity of the $2.5 \times 15 \text{ cm}$ IEMs strips. In a separate experiment, membranes were soaked in 1-M NaCl for 48 hours, and then kept in KNO_3 and NH_4Cl solutions for CEM and AEM strips, respectively for 7 days to assure maximum adsorption. Solution concentration ranges from 100 to 6000 ppm for KNO_3 , and 100 ppm to 3000 ppm for NH_4Cl was used. Each IEM had a duplicate for each concentration, and the reactive surface area considered was 75 cm^2 . After 48 hr, IEM's were extracted with 1M KCl and NH_4^+ and NO_3^- concentrations determined by the Lachat QuikChem AE flow-injection autoanalyzer and ion chromatography. The amounts of adsorbed NH_4^+ and NO_3^-

on IEM were expressed as $\mu\text{g N/cm}^2$ IEM. The maximum ions adsorption capacity of the AEM's was $473 \mu\text{g NO}_3^- \text{cm}^2$, and $581 \mu\text{g NH}_4^+ \text{cm}^2$ for CEM's.

5.6 GreenSeeker® and SPAD meter readings

GreenSeeker® (Trimble Navigation Limited, Sunnyvale, CA) handheld crop sensor readings were collected at V5 and V9 at a height of 60 cm from plant canopy from the two central rows from each experimental plot at Ndiol. At Sangalkam, SPAD-502 chlorophyll meter (Konica Minolta Sensing Inc., Osaka, Japan) readings were taken at V5 and V9 from three locations on the most recently collared leaf from six plants from the two middle rows of each plot and averaged to generate one value. GreenSeeker® and SPAD meter measurements were taken on the same day as resin membrane removal.

5.7 Biomass and plant tissues sampling

Biomass and plant tissue sampling was conducted only at Ndiol. At V9, all plants from one meter of row were cut at ground level (surface area 0.75 m^2). Plant biomass was sun dried to a constant mass, weighed and ground to pass a 2 mm sieve. Plants tissue were analyzed for total N were determined using dry combustion using a Vario MAX CNS macro elemental analyzer (Elementar, Hanau, Germany), while P and K were determined using ICP after digestion of the samples per Isaac and Johnson (1998). Plant N uptake at V9 was determined as the product of nutrient concentration and biomass.

5.8 Statistical analysis

Analysis of variance was performed using the GLIMMIX procedure available in SAS (SAS Institute, 2011). Although N was applied in a split application, analysis was conducted based upon

the total rates of applied N. The influence of fertilizer treatments on plant N, P, and K concentration, biomass, N uptake, ear yield, ear diameter, ear length, NDVI values, SPAD meter readings and available N in soil measured at two growth stage (V5 and V9) was separated using Tukey's HSD to determine significance between treatment means. Due to different measures at the two locations and due to interactions of site and fertilizer rate, data are analyzed and presented by location. Using the PROC REG procedure in SAS (SAS Institute, 2011), possible correlations were used to fit a quadratic model representing soil available N, NDVI, and SPAD meter values relationship to N uptake, yield and yield component.

6. RESULTS AND DISCUSSION

6.1 Sweet corn yield and yield components

Fertilizer dose did not affect ear yield, ear length, or ear diameter data collected at Ndiol, or ear diameter or ear length at Sangalkam (Table 2.5). At V9, leaf N, P, and K as well as leaf biomass and plant N uptake did not differ due to fertilizer dose at Ndiol. These results obtained for yield differ from Shaver et al. (2011) who reported yield variation due to fertilizer rate difference. They are also not in concordance with Mullins et al. (1999) who reported increased yield with increasing N rate (0-168 kg ha⁻¹) but with no significant differences detected in plant height or diameter. The absence of N uptake, yield and yield component differences may reveal that there was sufficient N available from all treatments at Ndiol. At Sangalkam, yield differences due to N treatment were detected (P<0.0056) (Table 2.5). The ear yield obtained from the 100% RDN (131 kg N ha⁻¹) and 75% RDN (99 kg N ha⁻¹) were not significantly different (10.3 and 9.8 t ha⁻¹, respectively), but 125% RDN (165 kg ha⁻¹) had the lowest ear yield with 5.3 t ha⁻¹ (Figure 2.3).

Yield differences at Sangalkam might be due in part to the difference in the number of healthy ears between N treatments. The lowest number of healthy ears was associated with 125% RDN (Figure 2.4). At this site, no differences in ear length, ear diameter or SPAD meter readings were noted (Table 2.5). Using regression to evaluate the relationship for the number of healthy ear and yield, 67% of yield variation was explained by the number of healthy ears at Sangalkam (Figure 2.5). This suggests that the various rates of applied N did not necessarily influence yield through variation in yield components but through quality and the number of marketable ears. Cruz et al. (2015) found no differences in ear length, ear diameter, or number or percent of marketable ears when N rates ranged from 0-300 kg ha⁻¹. However, at high N doses there was an increase in the number of diseased ears. These authors postulated that higher disease incidence resulted from a reduction in the production of phenolic compounds (such as lignin), that can reduce the damaging effects of fungi. Oktem et al. (2010) reported N treatment (0-360 kg ha⁻¹) differences for sweet corn yield, ear length and diameter; however, N rates differing by 40 kg ha⁻¹ or less did not show significant differences for ear length or diameter. In a study similar to ours, Meena et al. (2013) reported differences in sweet corn green cob yield and ear length for N rates between 100% RDN, 75% RDN and 125% RDN. Sweet corn growth rate and ear length increased up to 125% RDN, but green cob yield was greater for 100% RDN compared to 75% RDN. The results obtained our study in both locations, concerning yield and yield components, suggest that 99 kg N ha⁻¹ would be sufficient to obtain high yield at the lowest cost. Kumawat et al. (2014) reported a recommendation of 90 kg N + 40 kg P₂O₅ ha⁻¹ for maximum green cob yield (9.85 t ha⁻¹) production of sweet corn in southern Rajasthan.

Among sweet corn hybrids, JKMH-45 had the highest ear yield (12.4 t ha⁻¹), ear length, V9 leaf biomass, and V9 leaf N uptake at Ndiol, while Prime Plus had the smallest ear diameter

(Table 2.6). The higher yield of JKMH-45 compared to other hybrids likely reflects an overall improvement in growth as revealed from higher V9 leaf mass and N uptake. Also JKMH-45 hybrid performed better probably because of its provenance from tropical region (India) compared to the other varieties adapted to temperate environment. At Sangalkam, JKMH-45 had the largest ear diameter and ear length (Table 2.5). Ear yield of GSS and JKMH-45 was higher than for Prime Plus (4.9 t ha^{-1}) (Table 2.6). Mullins et al. (1999) and Kumawat et al. (2014) also reported performance (yield, ear length and diameter, leaf N concentration, N uptake) differences between hybrids used in their study. In the report by Kumawat et al. (2014), the hybrid Sugar 75 produced 13.34 t ha^{-1} of green cob.

6.2 GreenSeeker[®] and SPAD meter readings

At Ndiol, NDVI readings at V5 and V9 growth stages did not reveal N treatment differences (Table 2.5). This is not surprising since differences in plant N uptake and N concentration were also not detected among N treatments. These findings are contrary to those of Tadesse et al. (2015) where NDVI values reflected N treatment differences beginning at the V4 growth stage, and as crop demand increased with growth stage (V6) the differences became more distinct. However, Titolo (2012) did not find treatment differences detectable with NDVI prior to V6. This inability to differentiate treatment differences could be due to soil background effects on GreenSeeker[®] readings since at early growth stages (such as V5) the crop canopy does not provide full ground cover (Martin et al., 2007; Tadesse et al., 2015). It may also be that differences in total plant N uptake at these early stages are small and undetectable with this method.

At Ndiol there were no differences in NDVI measured among sweet corn hybrids at V5 (Table 2.5). However, at V9, JKMH-45 had the highest NDVI followed by SHY 1036 and GSS

(Table 2.6). Nitrogen uptake at V9 was also highest for JKMH-45 and while mean N uptake did not differ among the other hybrids, the trend was similar to that of NDVI (Table 2.6).

Overall, significant relationships between NDVI and plant N uptake ($R^2 = 0.68$) and between NDVI and plant biomass using a power function ($R^2 = 0.59$) at V9 were found (Figures 2.6 and 2.7, respectively). Findings on biomass and N uptake relationships to NDVI are similar to those of Freeman et al. (2007) that reported from a three year experiment that NDVI was strongly correlated with plant biomass and corn plant N uptake for data collected from V8 to V10 in Oklahoma, USA (Freeman et al., 2007). Based on the relationship between NDVI and yield at Ndiol, an equation estimating yield for sweet corn at the V9 growth stage with an R^2 value of 0.50 was developed (Figure 2.8). Thomason et al. (2007) also report that NDVI recorded at the V6 to V9 growth stages as could be used to reliably predict corn grain yield in Virginia, USA. Tucker (2010), working in Kansas, found a reasonably strong relationship ($R^2 > 0.50$) between NDVI and corn grain yield at the V8 and V9 growth stages under non-stressed conditions. Martin et al. (2007) reported a good relationship of NDVI with corn yield and biomass ($R^2 = 0.61$ and 0.64 , respectively) at V9, but no relationship was found at growth stages prior to V6. Tadesse et al. (2015) however found correlation between corn grain yield and NDVI value at V4 ($R^2 = 0.77$) through V6 ($R^2 = 0.79$) growth stages in Ethiopia. At the Ndiol study site, NDVI collected at V9 was a good predictor of plant leaf biomass and N uptake as well as final ear yield over N rates and hybrids tested (Table 2.5). It was also useful for detecting differences in N status among hybrids at V9 (Table 2.6).

At the Sangalkam experimental site, the SPAD meter was used to nondestructively assess corn N status at V5 and V9. There were no differences in SPAD meter readings among N treatments at either V5 or V9 growth stages (Table 2.5; Table 2.7). Waskom et al. (1996) also

reported that SPAD readings collected at V6 did not reliably detect N treatment differences in irrigated field corn. These findings are not in concordance with Jones (2013) who reported that SPAD meter readings effectively detected N treatments and estimated N status of corn plant at the V5 growth stage. Titolo (2012) also reported net differences in SPAD readings affected by N rates at the V9 plant growth. The lack of differences in SPAD readings likely reflect the same trend for not differences in leaf mass and N uptake at the time readings were collected.

No significant relationship between SPAD values and yield was found at Sangalkam for either development stages (V5, V9) (data not shown). These results differ from the findings of Titolo (2012) where strong relationships between SPAD readings and yield with R^2 values greater than 0.7 were found from V4 to V5, and also with the findings of Rostami et al. (2008) where SPAD values measured at V7 were correlated with corn grain yield. While ear yield at Sangalkam was affected by fertilizer dose, no other factors were influenced (Table 2.7). Differences between fertilizer doses that affected yield likely appeared after V9. Thus the inability of the SPAD to estimate this effect is likely due to measurement timing.

Tucker (2010) has reported that while currently available plant canopy reflectance sensors are helpful, they can fail to accurately predict N needs in situations such as when there is no currently detectable N deficiency or when measures are collected prior to the V8 growth stage. They also noted that N applied preplant or at planting may limit the ability of sensor readings to distinguish N needs, especially in early plant development prior to the V9 growth stage. Overall, in this study, neither the GreenSeeker® nor SPAD meter detected N treatment effects, however the GreenSeeker® (NDVI) readings at V9 had a good relationship with ear yield, N uptake and biomass.

6.3 Ion exchange membranes

Differences in fertilizer dose effects on NO_3^- and NH_4^+ adsorbed by IEMs were limited for both sites (Table 2.8). At Ndiol, differences due to fertilizer dose were only detected for available NH_4^+ at V9 ($P < 0.06$) with the level of NH_4^+ higher for 125% RDN compared to 75%. (4.4 and 3.1 $\mu\text{g N cm}^2$, respectively). At Sangalkam available NO_3^- at V5 ($P < 0.0832$) differed due to fertilizer dose (Table 2.8). In this instance, the NO_3^- levels measured from 75% RDN were significantly less than from 100% RDN (36.9 and 98.7 $\mu\text{g N cm}^2$, respectively).

Available NO_3^- and NH_4^+ measured using IEMs did not reveal any relationships with leaf N uptake, N concentration, ear yield, NDVI or SPAD meter values at any of the growth stages at either location. In a study designed to determine phosphorus and sulfur supply in wheat (*Triticum aestivum* L), Redman (2002) found no correlation between wheat P and S uptake and available P and S resulting from AEMs desorption. Ziadi et al. (2006) findings differ with the results of this study. Ion exchange membranes were used in forage and corn studies and they found strong relationships ($R^2 = 0.94$ and 0.52 , respectively) between available NO_3^- sorbed from AEMs and N uptake by forage and corn. Also, in their study, AEMs detected increasing NO_3^- availability with increasing applied N rate (Ziadi et al., 2006).

In our study the IEMs were inserted in soil two weeks after preplant fertilizer application, the applied N could have been lost through the soil profile before IEMs insertion due to the daily irrigation. These parameters and the sandy textured characteristic of the soil at Ndiol experimental site could explain why at V5 growth stage significant differences in available N were not detected. IEMs also remained in soil for at least four weeks and according to Bowatte et al. (2008) more than seven days residence time of IEMs in soil can result in an inability to detect N differences in soil because once there is ionic equilibrium between IEMs surface and the contact soil solution

further adsorption from IEMs may not occur. This would therefore affect the interpretation of available N in soil. Salisbury (1999) found that available N sorbed from IEM PRS probes provided the best estimation of wheat N uptake after burial for seven days compared to two, four and eight weeks burial. In another study, Meason and Idol (2008) in a tropical forest system reported that a burial of two weeks was optimum to avoid IEM saturation in soils rich in labile nutrients. Qian and Schoenau (2002) report that possible competition between plant and buried resin during the growing season may occur and should be taken into account. These reported phenomena could, in part, explain the lack of N treatment differences found by the AEM and CEM strips in this study. Results obtained in this study do not support the use of IEMs for assessment of in-season N availability for sweet corn in these systems.

7. CONCLUSION

Plant biomass, N uptake, N, P, and K concentration, ear yield, ear length and ear diameter were not significantly affected by fertilizer dose, except at Sangalkam where the highest fertilizer rate resulted in the lowest ear yield. Considering these findings, 75% RDN would be the proposed rate of fertilizer to apply for sweet corn production in the two locations in Senegal. Hybrids used in this experiment revealed differences in genetic potential. JKMH-45 produced greater yield, ear length and ear diameter, N uptake, biomass, and NDVI at Ndiol. At Sangalkam however, only Prime Plus produced lower yield than JKMH-45 but JKMH-45 had higher ear length and diameter.

GreenSeeker® and SPAD meter readings were generally similar for all fertilizer doses. However, NDVI values collected with the GreenSeeker® at V9 were able to predict ear yield, leaf biomass and N uptake. This positive relationship indicates that the GreenSeeker® could be used to estimate yield for sweet corn production in the northwest part of Senegal. No relationship between

SPAD readings and yield or yield components were found likely due to measurement timing. Ion exchange membranes used in-situ over four or more weeks did not identify differences in soil N levels among fertilizer doses. Measures of NO_3^- and NH_4^+ desorbed from the IEMs similarly did not show any relationship with NDVI values, SPAD meters readings, biomass, N uptake, N concentration, yield or yield components. Additional testing will be necessary to properly explore use and application of these sensors in sweet corn production prior to providing conclusive recommendations.

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Table 2.1. Sufficiency range of the major elements in sweet corn plant.

Essential elements	V6 growth stage	5 - 6 week old	End of silk
Major elements	----- %-----		
Nitrogen (N)	3 - 4	3.5 - 4.5	2.2 - 2.7
Phosphorus (P)	0.3 - 0.5	0.3 - 0.5	0.25 - 0.4
Potassium (K)	2.5 - 4	2.8 - 3.8	1.40 - 2.5
Calcium (Ca)	0.5 - 0.8	0.5 - 0.9	0.6 - 1.1
Magnesium (Mg)	0.25 - 0.5	0.2 - 0.5	0.2 - 0.5
Sulfur (S)	0.4 - 0.6	0.21 - 0.7	0.21 - 0.7

Adapted from Bryson et al. (2014)

Table 2.2. Rates of fertilizer applied at planting and as side-dress and resulting total N.

Treatment	Preplant fertilizer, N-P ₂ O ₅ -K ₂ O	Sidedress N	Total N
	-----kg ha ⁻¹ -----		
75% RDN [†]	23-23-23	76	99
100% RDN	30-30-30	101	131
125% RDN	38-38-38	127	165

[†] - Recommended dose of N

Table 2.3. Experimental locations, dates for significant agronomic practices, corn hybrid and source.

Location	Latitude	Longitude	Corn planting	V5 sampling	V9 sampling	Harvest
-----Date-----						
Ndiol	16°08'N	16°19'W	4/08/2015	5/22/2015	6/19/2015	7/08/2015
Sangalkam	14°46' N	17°13' W	6/25/2015	7/20/2015	7/30/2015	8/19/2015

Hybrid	Color	Origin
Prime plus	Yellow	Top Mountain
SHY 1036	Yellow	Syngenta seed-SCL
Columbus	Yellow	Syngenta seed-SCL
GSS	Yellow	Syngenta seed-SCL
JKMH-45	Yellow	Top Mountain

Table 2.4. Chemical properties of soil from experimental plots from 0 - 15 and 15 - 30 cm depth.

	Ndiol		Sangalkam	
	15 cm	30 cm	15 cm	30 cm
pH [†]	6.9	6.9	6.0	6.6
CN Ratio	9	8	11	13
CEC, meq 100g ⁻¹	2.0	1.9	11.8	11.0
	-----mg kg ⁻¹ -----			
Nitrate‡	0.1	0.2	0.8	0.4
Ammonium‡	1.2	0.6	0.7	0.5
P [§]	26	29	117	77
K	45	60	39	30
Ca	265	246	1585	1729
Mg	70	62	310	247
Zn	0.7	0.7	3.9	1.6
Mn	9.6	10.7	14.2	10.8
Cu	0.6	0.4	0.4	0.3
Fe	6.4	6.2	17.6	18.5
B	0.2	0.1	0.5	0.5

[†] 1:1 soil:water

[‡] 2M KCL

[§] Mehlich I

Table 2.5. Analysis of variance for effect of corn hybrid and fertilizer dose on plant height, V9 plant leaf mass, V9 leaf N uptake, V5 NDVI, V9 NDVI, ear yield, ear length, and ear diameter, Ndiol and Sangalkam.

Location	Source	Plant height	V9 Leaf N				V9 NDVI	Ear yield	Ear length	Ear diameter
			V9 Leaf mass	uptake	V5 NDVI					
-----Pr>F-----										
Ndiol	Hybrid	ns	***	***	ns	***	***	***	***	***
	Fertilizer dose	**	ns	ns	ns	ns	ns	ns	ns	ns
	Hybrid * dose	**	ns	ns	ns	ns	ns	ns	ns	ns
-----Pr>F-----										
	Source	Plant height	V9 Leaf N				V9 SPAD	Ear yield	Ear length	Ear diameter
		V9 Leaf mass	uptake	V5 SPAD						
-----Pr>F-----										
Sangalkam	Hybrid	**	na	na	ns	ns	***	***	***	***
	Fertilizer dose	ns	na	na	ns	ns	**	ns	ns	ns
	Hybrid * dose	**	na	na	ns	ns	ns	ns	ns	ns

* Significant at the 0.01 probability level.

** Significant at the 0.05 probability level.

*** Significant at the <0.0001 probability level.

ns Nonsignificant at the 0.1 probability level.

Table 2.6. Leaf mass, N uptake, NDVI, ear yield and yield attributes as influenced by sweet corn hybrid, Ndiol.

Location	Hybrid	V9 Leaf biomass		V9 Leaf N uptake		V9 NDVI	Ear yield		Ear length		Ear diameter		
		-----kg ha ⁻¹ -----					t ha ⁻¹		-----cm-----				
Ndiol	Columbus	692	b	11.6	b	0.39	dc	2.0	b	15.2	b	2.5	a
	GSS	823	b	12.1	b	0.43	c	2.4	b	15.0	b	2.7	a
	Prime Plus	708	b	10.3	b	0.37	dc	1.8	b	14.5	b	1.8	b
	SHY 1036	1009	b	17.5	b	0.56	b	2.2	b	14.4	b	2.9	a
	JKMH-45	2000	a	30.00	a	0.72	a	12.4	a	19.4	a	2.9	a

Within a column, means followed by the same letters are not significantly different (P< 0.1).

Table 2.7. Ear yield and yield attributes as influenced by sweet corn hybrid.

Location	Hybrid	Ear yield		Ear length		Ear diameter	
		t ha ⁻¹		-----cm-----			
Sangalkam	Columbus	6.0	ab	10.7	b	2.9	c
	GSS	10.7	a	13.3	b	3.0	bc
	Prime Plus	4.9	b	14.1	b	3.2	bc
	SHY 1036	9.7	ab	12.9	b	3.1	bc
	<u>JKMH-45</u>	<u>11.0</u>	<u>a</u>	<u>18.1</u>	<u>a</u>	<u>4.1</u>	<u>a</u>

Within a column, means followed by the same letters are not significantly different (P< 0.1).

Table 2.8. Analysis of variance and mean values for adsorption of NO₃⁻ and NH₄⁺ by ion exchange membranes at V5 and V9 growth stages, by fertilizer dose.

Location	Source	V5 NO ₃ ⁻	V5 NH ₄ ⁺	V9 NO ₃ ⁻	V9 N H ₄ ⁺	Harvest NO ₃ ⁻	Harvest NH ₄ ⁺
-----Pr>F-----							
Ndiol	Hybrid	ns	ns	ns	ns	ns	ns
	Fertilizer dose	ns	ns	ns	*	ns	ns
	Hybrid * dose	ns	ns	ns	*	ns	ns
Sangalkam	Hybrid	ns	ns	ns	ns	ns	ns
	Fertilizer dose	*	ns	ns	ns	ns	ns
	Hybrid * dose	ns	*	ns	ns	ns	ns
-----ug cm ⁻² -----							
Ndiol	Fertilizer dose	V5 NO ₃ ⁻	V5 NH ₄ ⁺	V9 NO ₃ ⁻	V9 N H ₄ ⁺	Harvest NO ₃ ⁻	Harvest NH ₄ ⁺
	75% RND†	10.8 a	9.9 a	21.3 a	3.1 b	15.7 a	3.2 a
	100% RND	6.5 a	10.3 a	15.6 a	3.3 ab	14.7 a	3.1 a
125% RND	10.4 a	9.5 a	24.9 a	4.4 a	11.1 a	3.3 a	
Sangalkam	75% RND	36.9 b	7.1 a	94.6 a	5.6 a	na	na
	100% RND	98.7 a	6.1 a	65 a	4.7 a	na	na
	125% RND	78.4 ab	8.8 a	103.7 a	4.9 a	na	na

* Significant at the 0.01 probability level.

** Significant at the 0.05 probability level.

*** Significant at the <0.0001 probability level.

ns Nonsignificant at the 0.1 probability level.

† - Recommended nitrogen dose

Within a column, means followed by the same letters are not significantly different (P< 0.1).

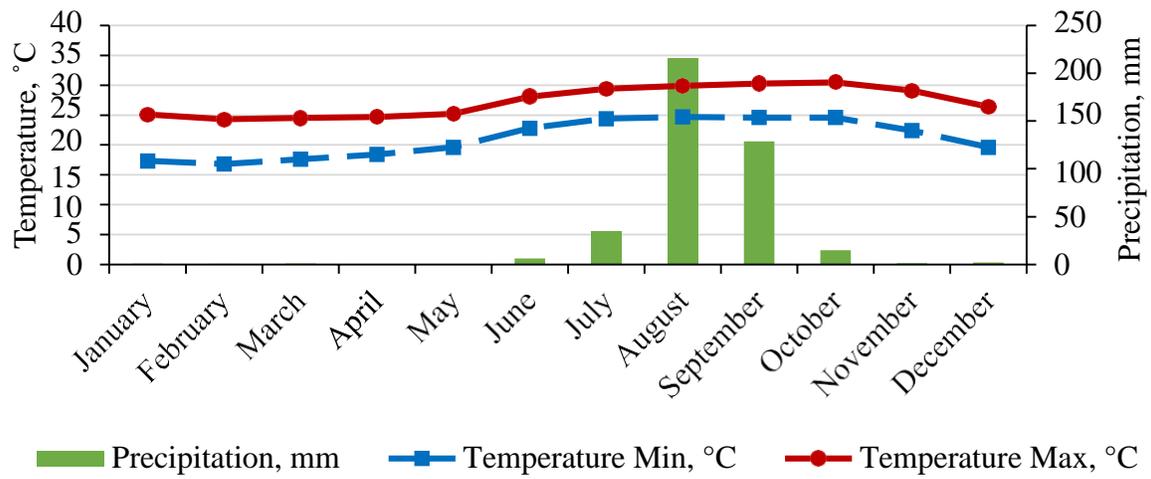


Figure 2.1. Monthly minimum, maximum temperature and total precipitation values during the growth period (May-August) of sweet corn in 2015, Sangalkam.

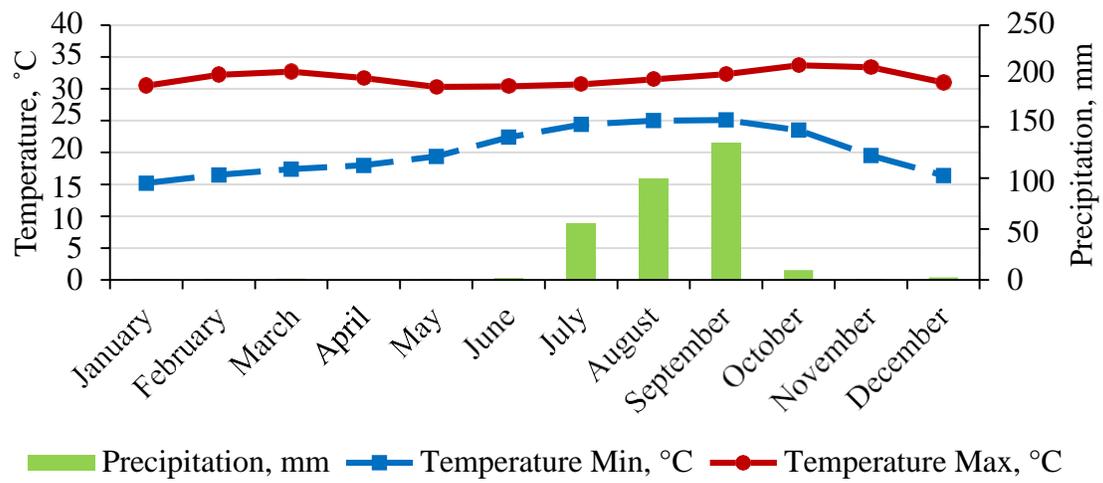


Figure 2.2. Monthly minimum, maximum temperature and total precipitation values during the growth period (April-July) of sweet corn in 2015, Ndiol.

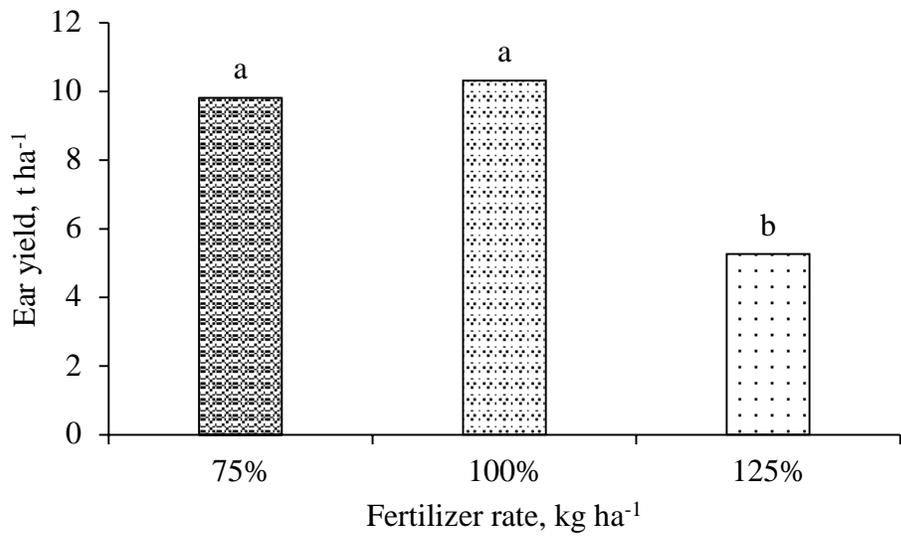


Figure 2.3. Yield variation due to fertilizer rates at Sangalkam experimental site.

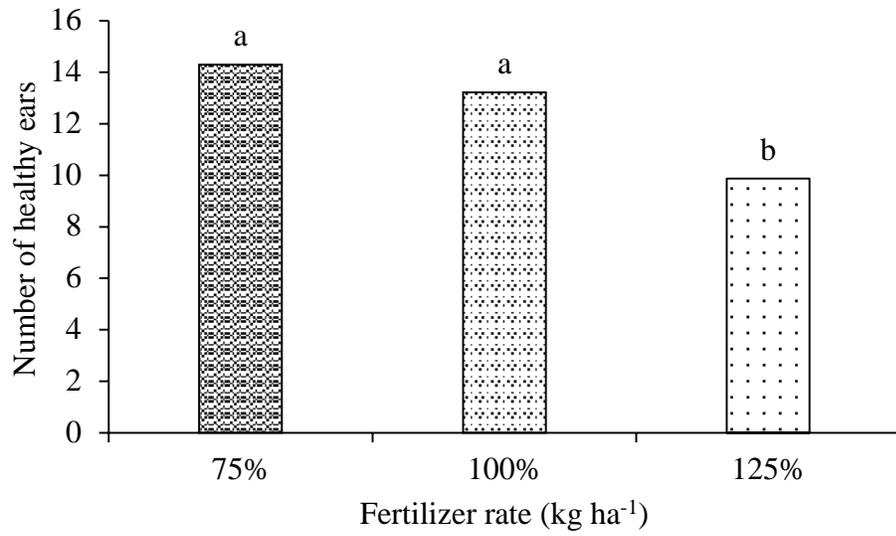


Figure 2.4. Number of healthy ears per plot as affected by fertilizer rate at Samgalkam experimental site.

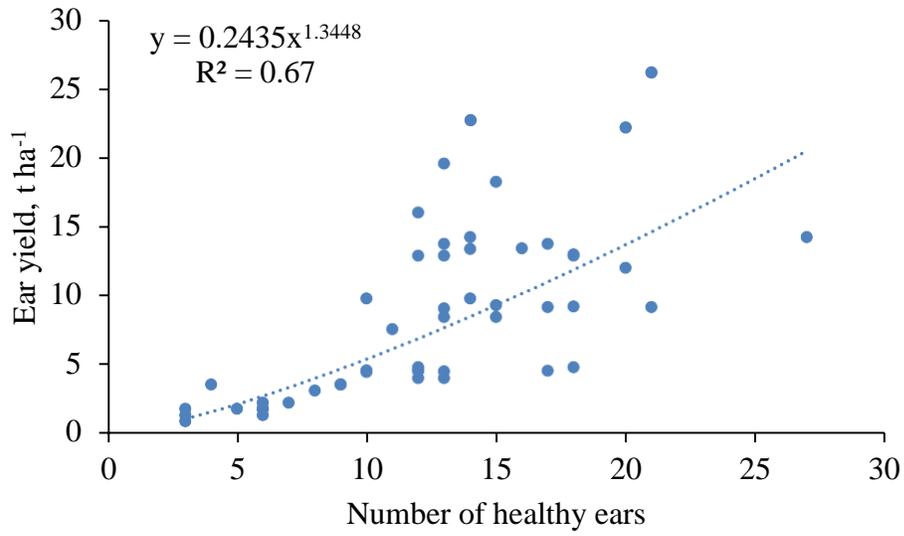


Figure 2.5. Relationship between the numbers of healthy ears per plot on total ear yield at Sangalkam experimental site.

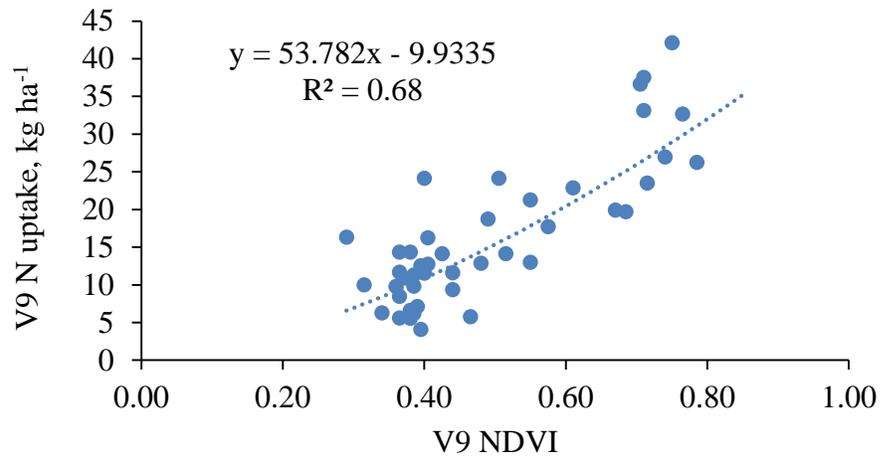


Figure 2.6. Relationship of NDVI measured with the GreenSeeker® sensor and leaf N uptake at the V9 growth stage, Ndiol.

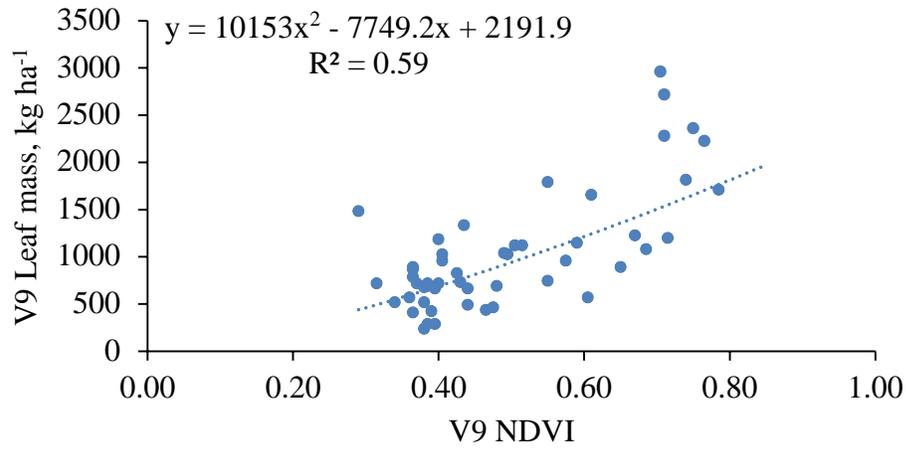


Figure 2.7. Relationship of NDVI measured with the GreenSeeker® sensor and leaf mass at the V9 growth stage, Ndiol.

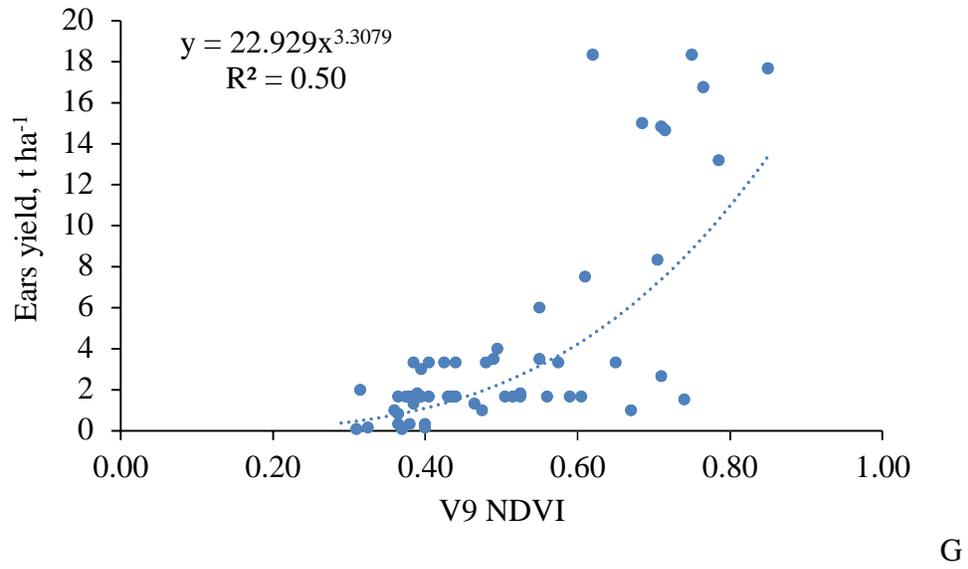


Figure 2.8. Relationship of NDVI measured with the GreenSeeker® sensor at the V9 growth stage and final ear yield, Ndiol.

Chapter 3: Effects of Selection for Drought Tolerance on Pearl Millet Seed Physical and Chemical Composition

ABSTRACT

Pearl millet (*Pennisetum glaucum* (L) R. Br.) is a cereal crop rich in essential nutrients which serves as an important food and feed resource in developing countries. It also has major socio-economic and nutritional importance. However, due to the recurrent and severe drought experienced in pearl millet growing areas in recent decades, the need for pearl millet cultivars with improved drought tolerance has increased. Often though, the main criteria associated with improved drought tolerance is only grain yield under stress. This aspect of selection for drought tolerance may result in unintended changes in other important crop attributes such as the nutritional profile. Experiments were conducted on a total of 20 pearl millet genotypes divided into three contrasting groups based on drought tolerance as determined by vapor pressure deficit (VPD) measured previously. The weight of 100 grains, grain test weight, protein, soluble protein, starch, minerals (Ca, Na, K, Fe, Mg, Mn), sugars, amino acids and vitamin B2 content of pearl millet grains were measured under two water regimes, well-watered and postanthesis water stressed, in a split plot design with four replicates in Senegal. Water stress did not affect 100-grain weight, test weight, protein, soluble protein, starch, sugars, amino acids or vitamin B2 content of grains among VPD-groups. Accumulation of these constituents of pearl millet grain appear to be genetically controlled and are probably not affected by late drought stress. Differences appeared between VPD-groups (genotypes) as the sensitive VPD-group accumulated more soluble protein, starch and soluble sugars (except sucrose) than the tolerant and medium VPD-groups. The tolerant VPD-group, however accumulated higher protein and vitamin B2 content. Arginine, proline and serine content

was greater in the sensitive VPD-group, while lysine, aspartic acid, and glutamic acid were greater in the tolerant VPD-group. Glycine, histidine, threonine, alanine, tyrosine, valine, methionine, leucine, isoleucine, and phenylalanine were relatively equal in tolerant and sensitive VPD-groups. Content of Ca, Fe and Na were affected by water stress in the sensitive VPD-group but differently. Calcium content was greatest for the sensitive group under drought stress, while sodium was the lowest. Potassium decreased for all VPD-groups under stress, while across water regime, K levels in the drought-sensitive group were lower. Selection for drought appears to effect many of the nutritional constituents of pearl millet grain, however many of these changes appear to be directly related to parameters known to effect plant water relations directly.

1. INTRODUCTION

In West Africa, agriculture is the main occupation and agricultural production is highly dependent on climate, including both precipitation amount and timing. Global climate change can accentuate the frequency and severity of rainfall-related stresses such as drought (Tuberosa, 2012). Climatic fluctuations interrupt all cultural operations from planting to harvesting (Redden et al., 2014) and make farming in the dry lands of West Africa particularly vulnerable to frequent droughts (Aune and Bationo, 2008). Indeed, worldwide, drought is the primary abiotic stress factor that limits crop productivity (Valliyodan and Nguyen, 2006; Ashraf, 2010). According to the Africa Human Development Report (2012), Sub-saharan Africa's agriculture is mainly based on cereal production and more than 75% of these cereals crops are produced in smallholder farming systems that are struggling to increase soil fertility.

Pearl millet (*Pennisetum glaucum* (L) R. Br.) is a cereal grain crop widely used and cultivated in drought-prone arid and semi-arid regions where it is well adapted to nutrient-poor soils and very hot and dry environments (Ibrahim et al., 1985; Yadav and Bhatnagar, 2001). It is the dominant cereal grown in Senegal and in most of the West African Sahel regions (Buerkert et al., 2001; CEDEAO-CSAO/OCDE, 2006). However, the recurrent variability of rainfall distribution in a single growing season results in varying intensity of drought stress for millet. This stress causes a tremendous loss in productivity and is especially harmful in low-yield farming systems that rely mainly on family labor. In Senegal more than 60% of the total active population participates in agriculture (Cisse et al, 2004) and 95% of the land where cash crops and subsistence crops are grown is subject to fluctuating rainfall (Lo and Tumusiime, 2013). This irregularity of rain results in a decrease in cereal production while the population of Senegal is increasing (Amouzou and Ndiaye, 2012, 2012). During the 2011-2012 growing seasons, millet production

was 481,000 T which is a 41% drop compared to the mean production from the previous five years because of the recorded rainfall disturbances (Amouzou and Ndiaye, 2012, 2012). Drought is typically the most limiting factor in rainfed crop production and can occur at any crop growth stage (Rai et al. 1999). Drought tolerance of currently available millet cultivars is inadequate to cope with extremes of irregular rainfall. Given the alarming situation resulting from global climate change, the importance of drought tolerant cultivars is greater than ever before. Obtaining more drought tolerant pearl-millet is a main focus for many breeding programs (Kole, 2006), but to achieve greater success, there needs to be further testing of high yielding cultivars and to evaluate and demonstrate the broad adaptation of new, drought tolerant pearl millet lines. However, it is important to know that yield performance and stability are not the only traits that are crucial to smallholder farmers in rural areas. All nutrients (such as minerals) contained in grains are sources of nutrients for human and animal diets, and noticeable deficiencies of these nutrients in grains tend to have an impact on consumers (Rose et al., 2015). In fact, a recent “smart nutrition” survey study conducted by the Ministry of health in Senegal in November 2015 revealed that the Global Acute Malnutrition rate (GAM) at national level is 9%. From this study, critically low GAM scores were noted in the north and northeastern regions, Louga (16.1%), Matam (16.5%), Saint Louis (14.7%) and Tambacounda (12.5%) (WFP Senegal, 2015). UNSCN (2013) revealed that malnutrition is more prevalent in rural areas, and 1/3 of registered child deaths in 2010 were due to under-nutrition. Therefore, there is a huge need in Senegal to increase millet production to fight malnutrition and hunger. To achieve this goal of greater food security for smallholder farmers, it is important to integrate the most effective and stable drought adaptation strategies with the best yielding cultivars and also to ensure that millet grain nutritional quality is maintained or improved (Bashir et al., 2014).

2. LITERATURE REVIEW

Pearl millet (*Pennisetum glaucum*, synonyms: *P. americanum*, *P. typhoides*) is an annual tillering diploid ($2n = 14$) highly cross-pollinated (C4) cereal crop, belonging to the family Poaceae and originating in tropical western Africa (Andrews and Kumar, 1992; Taylor and Duodu, 2010; Ul Hassan et al., 2014). It has a huge number of both wild and cultivated relatives and grows better than other common grain crops under arid and semi-arid conditions where it has evolved over thousands of years (McDonough et al., 2000; Khairwal et al., 2007). Pearl millet is nowadays the sixth-largest cereal crop cultivated in the world (Vietmeyer and Ruskin 1996, Shahidi and Chandrasekara, 2013). However, pearl millet is used as a food crop mostly in the developing countries of Asia and Africa. In developed countries such as the United States, pearl millet is grown as an annual forage crop during the summer, and also as seed for birds and for wildlife (Bezançon et al., 1997; McDonough et al., 2000). It is of increasing importance in the Middle East and Central Asia due to its tolerance to salinity (Rai et al., 2008). According to Khairwal et al. (2007) more than 93% of pearl millet grain is for food, and the remaining 7% is for animal and poultry feed. Beside food and feed uses, pearl millet crop residues are utilized for fodder, material for building and fuel for cooking in arid and semi-arid regions (Devos et al., 2006).

For all millets cultivated in the world, about 50% of the total area is pearl millet, with production of approximately 14 M ha in Africa and 12 M ha in Asia (Khairwal et al., 2007). Pearl millet cultivars are generally characterized by traits such as: maturity (early maturity, about 80 days; medium maturity, 100 days or so; late maturity, 180 days or more), height, amount of tillering, stem thickness and branching, leaf size and hairiness; seedhead size, shape and "tightness", number, length, rigidity, brittleness, and hairiness of bristles; size, shape and color of grain and the degree to which the glume adheres to the grain (Vietmeyer and Ruskin, 1996).

2.1 Pearl millet growth conditions and limitation

Pearl millet is known to grow under conditions where other grain crops will barely develop: low soil fertility, low moisture, and high temperature (McDonough et al. 2000; Loumerem, 2004). The optimum temperature for pearl millet growth is 30 - 34°C (Khairwal et al., 2007; Mangat et al., 1999), and during sowing the temperature of the soil should not be < 23°C (Khairwal et al., 2007).

Pearl millet can be cultivated in various soils; however, deep and well-drained soils are more conducive while waterlogged soils are not suitable for growing pearl millet (Khairwal et al. 2007, Lee et al., 2012, Mangat et al., 1999). In Africa and India, pearl millet is grown on soil with pH of 6.2 - 7 (Mangat et al., 1999). However, pearl millet can develop under low soil pH (4-5), and soils high in aluminum (SADAFF, 2011). Pearl millet yields respond well to fertilization and irrigation (Burton et al., 1972). Optimum rainfall is 600-800 mm, however pearl millet can develop in areas with rainfall < 350 mm (Khairwal et al., 2007), as low as 150 mm (Bezançon et al., 1999; Loumerem, 2004). In very low rainfall areas, short growing cycle pearl millet accessions are more suitable (SADAFF, 2011).

Pearl millet development can be divided in three phases according to Maiti and Bintinger (1981), and Khairwal et al. (2007). Phase one includes the following stages: seedling, roots formation, leaf and tiller growth, and tiller initiation. Phase two corresponds to establishment of leaves, tillers, panicles, flowering, and stigmas. The last phase begins with floret pollination and ends with physiological maturity when millet grains show a dark coloration at the base. Rapid and uniform germination of pearl millet requires a fine seedbed and planting depth no deeper than 3 cm (Christiansen, 2008). Pearl millet seed germination occur 2 to 3 days after sowing when there is adequate moisture. Adventitious roots responsible for water and nutrient uptake form at 6 to 7

days after emergence (Mangat et al., 1999), and can reach a maximum depth of two meters (Khairwal et al., 2007, Loumerem, 2004). In sandy soils, roots can spread up to three meters in circumference (Mangrat et al., 1999). Fully developed pearl millet plants can reach a height of 2 to 4 meters, depending on genotype (Mangrat et al., 1999). Flowering can occur at 40 to 55 days after sowing for early varieties (Lee et al., 2012; SADAFF, 2011) to 50-80 days after flowering for late varieties (Maiti and Bidinger, 1981). For optimum storage, grain moisture content needs to be 10-12% (SADAFF, 2011). Pearl millet seeds may be pearly white, pale yellow, brown, gray, slate blue or purple (Khairwal et al. 2007; SADAFF, 2011). With adequate management practices, pearl millet can yield up to 3680 kg ha⁻¹ (Lee et al., 2012).

Pearl millet growth is often limited by abiotic (poor soil fertility and structure, high temperature and erratic rainfall), and biotic (weeds, diseases, pests) constraints. These constraints affect plant development and consequently grain yield. In fact, seedling emergence failure can occur as a consequence of sand blasting, high temperature and poor soil moisture, as well as pests (Harinarayana et al., 1999). In dry regions of Asia and Africa, surface soil temperature can reach 60°C leading to seedling failure or reduced plant vigor (Rai et al., 2008). Pearl millet is also sensitive to low soil moisture at flowering and grain filling stages (Mangat et al., 1999). Pearl millet is more sensitive to weed competition during the first month after planting. Weeds compete with pearl millet for nutrients, water and light (Khairwal et al., 2007). That makes early weed control important for adequate plant development (SADAFF, 2011). Pearl millet is also susceptible to diseases such as downy mildew (*Sclerospora graminicola*), ergot (*Claviceps fusiformis*), smut (*Tolyposporium penicillariae*) and rust (*Puccinia penniseti*) that infest panicles and grains (Khairwal et al., 2007; SADAFF, 2011; Harinarayana et al., 1999). Reducing losses due to weeds and diseases may require the use of herbicides, fungicides, cultural practices (e.g. crop rotation),

and management practices (e.g. early planting or early harvest), and also use of improved cultivars and hybrids (SADAFF, 2011).

2.2 Pearl millet nutritional value

Food grain quality is an important determinant of consumer acceptability (Nkama and Ikwelle, 1983), and more importantly it is critical for human well-being (Saleh et al., 2013). Physical attributes of pearl millet grain quality include: kernel weight, which ranges from 2.5 to 14.7 grams per 1000 grains (Singh and Nainawatee, 1999; McDonough et al., 2000); hardness and grain structure, chemical composition including carbohydrates, protein, lipid, ash, vitamins, amino acids content; and malting, cooking and processing qualities (Nkama and Ikwelle, 1983). Pearl millet is rich in energy, protein, and vitamins and minerals such as iron and zinc that are beneficial to people in areas where it is cultivated (Vadez et al., 2012). However, nutrient content often varies among cultivars. Carbohydrates are dominant in pearl millet grains (Singh and Nainawatee, 1999). Pearl millet typically contains between 62 to 71% starch, 22 to 29% amylose, 0.1 to 0.3% reducing sugars, and 1 to 3% soluble sugars (Singh and Nainawatee, 1999). Sucrose and raffinose account for 63 and 29 percent, respectively, of the soluble carbohydrates (McDonough et al., 2000). The additional soluble sugars found in pearl millet include stachyose (2.4 - 4.7%), glucose and fructose (3.2 – 6.3%) (Subramanian et al., 1981), and maltose (0.08 %) (Malleshi et al., 1986). Pearl millet protein content is generally 9 - 13%, but it can range from 6 to 21% among genotypes and environments (Andrews and Kumar, 1992; Khairwal et al., 2007). Pearl millet often has higher total amino acid content than sorghum and maize, but is equal to rice and wheat (Singh and Nainawatee, 1999). Typically, the most limiting amino acid in pearl millet is lysine (Burton et al., 1972; McDonough et al., 2000). Potassium, phosphorous, and magnesium are the predominant minerals in pearl millet (Singh and Nainawatee, 1999), but it is also a source of calcium, iron, zinc,

manganese (Arya et al., 2008; Bekoye, 2011), riboflavin, thiamine, niacin, lysine and tryptophan, and fat-soluble vitamins (Khairwal et al., 2007; Singh and Nainawatee, 1999). In general, millets have higher mineral and fiber content than rice or wheat, and contain beta-carotene, which rice does not (Millet Network of India, 2014). In comparison to maize, phosphorus (average 339 mg) is half again as much, iron (average 9.8 mg) is more than three times, and calcium (average 37 mg) is more than five times as much (Loumerem, 2004). Some studies have shown that high-iron/high-zinc pearl millet cultivars can significantly contribute to reducing malnutrition of young women and children (Cercamondi et al., 2013; Kodkany et al., 2013, Bashir et al., 2014). In fact, pearl millet could replace 25% of the rice in a child's diet without reducing the level of nitrogen, calcium, or phosphorus in the body (Vietmeyer and Ruskin, 1996). Thus, pearl millet diets can maintain a positive balance with respect to N and Ca. Compared to wheat, rice, or maize, pearl millet has higher ash content (McDonough et al., 2000); it also contains more calories, and almost twice in oil content (Vietmeyer and Ruskin, 1996). Pearl millet is gluten-free, and according to Leder (2004) and Bhardwaj et al. (2014), it is the only grain that conserves its alkaline properties after cooking, which make it optimum for people with gluten allergies. Antinutritional factors (polyphenols, goitrogen, phytic acid, trypsin inhibitors) are also found in pearl millet (Singh and Nainawatee, 1999). Taylor and Duodu, (2010) stated that phenolics in pearl millet grain and their antioxidants have protective or preventive disease effects. However, high levels of antinutritional components can cause diseases such as goiter, pancreas enlargement, and reduced nutrient availability (Singh and Nainawatee, 1999). Nonetheless, processes such as malting and decortication can reduce the concentration of antinutritional factors in pearl millet grain (McDonough et al., 2000, Lestienne et al., 2007). A malting procedure allowing germination for 48 hours reduced polyphenols and phytic acid in pearl millet grain up to 40%. Malting also

increased the content of some vitamins (A, B1, B2, C) (Rai et al., 2008). In a comparative study conducted by Adeola and Orban (1995), pearl millet grain fed to pigs had higher crude protein, gross energy and mineral content as well as a more preferable amino acid compared to corn. The digestibility of fat and amino acids was also higher for pearl millet, as well as N intake and absorption. Pearl millet forage has been reported to have greater crude protein compared to sorghum or maize, and animals fed pearl millet had more rapid growth compared to those fed sorghum (McDonough et al., 2000).

2.3 Effect of drought on grain quality

Water stress occurs when there is inadequate moisture for a growing plant to develop appropriately (Pandey et al., 2014). Water is typically a main determinant of millet yield, and the most critical stages for water stress in pearl millet are tillering, flowering and seed development (Khairwal et al., 2007). Hence, water stress at any of these periods affects the processes (biochemical and physiological modifications) of grain filling and nutrient accumulation in the growing grain, which in turn affects crop yield and quality (Kandpal and Appaji Rao, 1985; Yang and Zhang, 2006). Nutrient accumulation in developing grain is dependent on crop species, but also on the flux of nutrients from the maternal plant into the developing grains. This remobilization is influenced by the source-sink strength relationships during that period (Rose et al., 2015). The sink strength is referred to its size and to its activity (i.e. volume of grain endospermic cells, and the level of enzymes and factors that intervene in carbohydrate utilization and storage) (Ho, 1988; Saeedipour and Moradi, 2011). During water stress, the relationship between source-sink is affected due to the changes in growth priority and also because of photosynthesis alteration (Roitsch, 1999). In a tomato plant, from the flowering period to the fruit maturity, the potential

sink strength order was as following: fruit > young leaves > flowers > roots (Ho, 1984; 1988). Plants must provide enough nutrients to seeds to develop good seedlings with good germination (Rose et al., 2015) and assure continuation of the genetic line (Barnabas et al., 2008).

In a study conducted with finger millet (*Eleusine coracana* L.) by Kandpal and Appaji Rao (1985), a decrease in grain soluble protein content was observed when plants were water-stressed. These authors attributed this to a decline in protein synthesis resulting from the deprivation of water. However, in another study Mahalakshmi et al. (1985) noticed an increase in pearl millet grain protein and considered this to be a consequence of a decline in carbohydrate accumulation during grain filling under water stress. Anatala et al. (2015) found that the drought tolerant cultivar used in their study had higher protein content when stressed compared to well-watered conditions. Aparna et al. (2014) found that grain weight for tolerant varieties declined more compared to sensitive genotypes under pronounced water stress. Keshavarz et al. (2013) studied the effect of drought on physio-morphological traits and forage yield of millet (*Pennisetum americanum* L.) and found the highest protein and ash content in forage millet were obtained when soil moisture was maintained at field capacity. These values decreased when water stress was more severe. In wheat (*Triticum aestivum* L.), Eivazi and Habibi (2012) compared grain quality between two wheat varieties, one susceptible and the other tolerant to drought, and found that the susceptible genotype had higher grain protein than the tolerant genotype, while grain yield of the tolerant genotype was higher. Xie et al. (2003) also reported drought decreased wheat starch content and increased protein accumulation under water deprivation. Therefore, the degree of crop adaptation or tolerance to water stress, either naturally or through breeding, might affect nutrient content composition, either directly or indirectly. Desirable levels of grain fiber, starch, protein, lipids, vitamins, sugars,

minerals and amino acid profiles must be combined with a complex array of other agronomic traits required in a viable millet cultivar.

2.4 Pearl millet and selection for drought tolerance

Drought occurs due to a decrease or absence of rainfall and results in available water in soil that is insufficient for normal plant growth and development (Jaleel et al., 2009). One third of cultivable land in the world is subject to drought (Kholová, 2010a). In perennially dry areas where crop production is dependent on rainfall, crop production requires development and adoption of crops and cultivars that are adapted to drought. Resistance or tolerance to drought is a critical phenomenon which affect plants phenotypically and physiologically (Yadav et al., 2003, Yadav et al., 2005). It involves processes allowing plants to fully develop and maintain an acceptable yield in severely dry soil (Ibrahim et al., 1986). In fact, mechanisms involving tolerance or resistance to water stress include drought escape, drought avoidance and adaptation, or drought acclimation also referred as drought tolerance (Witcombe et al., 2008; Kholová, 2010a). These complex strategies involve phenological, morphological, anatomical and/or biochemical ability to regulate and maintain water in plants cells (Barnabas et al., 2008; Kholová, 2010a). Plants are known to be susceptible to drought in different growing stages defined as intermittent (mid-season) and terminal drought (Kholová, 2010a). Pearl millet is more susceptible to terminal drought than to intermittent drought since terminal drought directly affects the plant grain filling stage. The impact of intermittent drought can be mitigated through development of secondary tillers. This is referred to as developmental plasticity (Mahalakshmi et al., 1987; Vadez et al., 2012; Aparna et al., 2014). This phenomenon makes terminal drought the first target in pearl millet breeding for drought stress (Vadez et al., 2012). The major aim for drought stress improvement is to maintain yield components under drought condition that would be common for the environment of interest

(Witcombe et al., 2008; Jaleel et al., 2009; Vadez et al., 2012). Breeding programs screening pearl millet genotypes for tolerance and sensitivity to drought use a number of physiological measures to assess plant performance under water stressed environment (Ibrahim et al., 1986; Yadav and Bhatnagar, 2001; Serraj et al., 2003;). In fact, multiple traits relative to plant performance under normal and stress condition are being measured on plant leaves, stems, and roots to identify genotypes tolerant to drought (Yadav et al., 2003).

Transpiration rate (Tr) is an important trait related to drought tolerance. Plants are exposed to low and high vapor pressure deficit (VPD), and the Tr measured. Genotypes tolerant to drought are those that maintain a low Tr under high VPD (Belko et al., 2013). Plants that are more sensitive to VPD are better able to survive long lasting drought (Chen et al., 2014). In fact, some authors found that tolerant genotypes have lower transpiration rate compared to sensitive genotypes (Beggi et al., 2015) during vegetative and reproductive stages in both optimal, and drought conditions (Kholová, et al., 2012, 2010bc); Vadez et al., 2012). Therefore, selection for drought tolerance requires the knowledge of traits controlling effective water use in plant under both optimum moisture and drought (Ibrahim et al., 1986; Kholová et al., 2010c). Abscisic acid (ABA) is a stress hormone produced by plants that is responsible for several drought response mechanisms. Under drought stress, ABA accumulates in plant tissues (often leaves) and causes a stomatal closure, root elongation, and synthesis of osmoprotectants (proteins, sugars, proline accumulation, etc.) (Mugo et al., 2000). An increase in proline and protein content has been observed for water stressed pearl millet seedlings (Kandpal and Rao, 1985). Kholová, et al. (2010b) found that drought tolerant pearl millet cultivars had higher leaf ABA in well-watered condition, which could mean that ABA is linked to the drought tolerant quantitative trait loci (QTL) in pearl millet. The panicle harvest index (PNHI) is also an important variable used to measure plant susceptibility to drought. Defined as

the ratio of grain weight to total panicle weight (Bidinger et al., 2000; Serraj et al., 2003; Vadez et al., 2012), the PNHI is one of the drought tolerant quantitative trait loci (QTL, i.e. the parts of a genome linked to the specific phenotype) being identified and used in marker assisted breeding to screen and create drought tolerant pearl millet varieties (Serraj et al., 2003). The higher the PNHI value, the greater the plant's ability to conserve water for grain filling (Ibrahim et al., 1986). The drought resistance index (DRI) is a valuable parameter used to screen for drought tolerance. Studies conducted by Bidinger et al. (1987), found that 50% of yield variation under stress was explained by genotype yield potential and by flowering time; and the DRI was developed independently from yield potential and time of flowering. The DRI is a residual that accounts for tolerance to drought that is highly correlated with yield (panicle and grain) under drought condition (Ibrahim et al., 1986; Yadav et al., 2003). Yadav et al. (2003) found a strong correlation between the DRI and yield under stress ($r = 0.84$, $P < 0.0001$) in contrast to a negative correlation of the DRI to yield under well-watered conditions ($r = - 0.01$) and with flowering time ($r = - 0.01$). Like the PNHI, the DRI is higher for tolerant varieties indicating that there will be higher grain and biomass yield (Ibrahim et al., 1986). More modern techniques such as molecular and genomic approaches, mapping genes expressed and responsible for drought tolerance are currently being used to assess drought tolerance in pearl millet (Choudhary et al., 2015). While these breeding efforts have resulted in cultivars with improved drought tolerance, very few studies have reported the effect of drought tolerance selection on pearl millet grain nutritional composition.

3. General Objective

The objective of this study was to compare millet grain nutritional composition among and between putative drought tolerant and drought sensitive pearl millet cultivars and lines.

4. Hypothesis

Our hypotheses were: 1) that significant variation in millet grain nutritional composition likely exists; 2) that selection for drought tolerance in millet cultivars may indirectly influence grain nutritional composition; and 3) that improved understanding of millet seed nutritional profiles may improve food security and human nutritional status, especially for smallholder farmers.

5. MATERIALS AND METHODS

5.1 Description of the experimental site and conditions

The experiment was conducted from March to June 2014, during the dry season at the National Center for Agronomic Research (CNRA) of Bambey (16°30' and 16° 28' N; 15°44' and 15°42' W) situated in the semi-arid zone in Senegal. The soil at the experimental site is sandy with 91-94 % sand and 3.5-5.6 % of clay (Diouf et al., 2004).

The experimental design was a split plot with four replications, and two water regimes were applied: well-watered (WW) and water-stressed (WS). The water-stressed regime was applied after flowering (terminal drought) by stopping irrigation approximately three weeks before harvesting the spikes. Irrigation was done by providing the plant with 20 mm of water twice a week (on Mondays and Fridays), therefore the WS environment received 120 mm of water less than the WW one. Cultivars of interest for this study were 20 genotypes of pearl millet selected in previous testing for variation in expected drought tolerance based on a vapor pressure deficit (VPD) test (Table 3.1) performed by the Centre d'Etude Régional pour l'Amélioration de l'Adaptation à la Sécheresse (CERAAS) in Senegal. Varieties were grouped into tolerant, medium, and sensitive to drought VPD-groups.

For each water regime (separated by 4 meters) there were four blocks separated by 2m, and the 20 genotypes randomized within each block. Each experimental plot was 4.5 x 4 meters, and plants spacing was 90 cm x 30 cm (5 rows for a total of 65 plants per plot). During sowing, 5 to 6 seeds were planted in each hole. At two weeks after emergence, hills were thinned to one plant. Nutrients were provided to plants by pre-plant addition of 150 kg ha⁻¹ of 15-15-15 fertilizer and two split applications of urea (50 kg urea ha⁻¹ three weeks after sowing, and 50 kg urea ha⁻¹ at heading).

In all analyses, genotypes were analyzed individually and then combined with those in the similar VPD group to produce a mean value for the group.

5.2 Seed nutritional quality analysis

Pearl millet spikes were harvested in 2 m² (9 spikes) in each experimental plot. After harvest, pearl millet spikes containing seed were dried to a constant moisture (13 %) at 60 °C using a stove, and then seed were separated from the spike. From each experimental plot grain samples were collected for physical and biochemical analysis. Weight of 100-grains was measured using a precision scale (Ohaus corporation, Switzerland). Pearl millet grain test weight was determined by fully filling a tube of known volume (7.1 cm³), then weighing.

Whole pearl millet grain was analyzed for mineral nutrients (Ca, Fe, Na, K, Mg, Mn), starch, protein, and soluble protein using near infrared spectroscopy (NIR) (FOSS XDS, Eden Prairie, MN). After NIR analysis, grain was ground to pass through a 30-mesh sieve.

5.3 Soluble sugars

Pearl millet flour was extracted and analyzed for glucose, fructose, sucrose, raffinose, stachyose, and maltose. A 0.1 g (accurate to 0.0001 g) flour sample was mixed with 1.0 ml of deionized water and then shaken for 45 minutes at room temperature. The sample tubes were centrifuged at 17,000 Xg for 15 minutes. A volume of 0.5 mL supernatant was then transferred into another centrifuge tube and 0.7 mL acetonitrile (58 % ACN) added. The mixture was left to sit for 1 hour at room temperature and centrifuged again at 17,000 Xg for 15 minutes. After centrifugation, a volume of 200 μ L of supernatant was mixed with 800 μ L of 65% ACN, and then the sample filtered with a 0.2 μ m syringe filter (dia. 13 mm nylon) into a high performance liquid chromatography (HPLC) sample vial. Five microliters of liquid sample was injected for chromatographic separation of sugars, which was performed using an Agilent 1260 HPLC, equipped with an evaporative light scattering detector (ELSD) (Agilent Technologies, Santa Clara, CA), and guard (4.6 \times 10 mm) and analytical (4.6 \times 250 mm, 5 μ m) columns (Supelco apHera NH₂ polymer). An isocratic elution with 65% acetonitrile at a flow rate of 1 mL per minute was used (Cicek et al., 2006).

Three calibration mix standards (100, 50, and 15 μ g ml⁻¹) were made and one standard check (50 μ g mL⁻¹). The formula used to calculate the concentrations in percent of free sugars in each sample was (Cicek et al., 2006):

$$\text{Sugar (\%)} = X \times D_{s1} / V_{f1} \times D_{s2} / V_{f2} \times V_{in} / W_s \times 10^{-4} \times (1 + H_2O)$$

Where X (μ g ml⁻¹) is the sugar concentration in the sample; D_{s1} first sample dilution volume (0.5 ml filtrate + 0.7 ml 58% ACN); V_f is the volume of filtrate (0.5ml) used to dilute the sample in 58% ACN, D_{s2} second sample dilution volume (0.2 ml filtrate + 0.8 ml 65% ACN); V_f is the volume of filtrate (0.2ml) used to dilute the sample in 65% ACN; V_{in} is the volume (ml) of water

added to powdered sample during the first step of the extraction; W_s is the weight (g) of the sample, and finally 10^{-4} is the conversion factor to percent (%); H_2O is the moisture content of samples in fraction after air-dried.

5.4 Amino acids

To extract amino acids, 0.1 g of pearl millet flour was placed into a 12 ml Pyrex glass vial tube, to which was added 4.985 mL of 6N hydrochloric acid and 15 μ l of 250 mM of L-norleucine as internal standard. The tube was flushed with N_2 gas for 10 seconds, and immediately closed with a PTFE-lined cap. The tubes were then incubated at 110-115°C in a bead-bath for 24 hr (González-Castro et al., 1997). After incubation (hydrolysis) and cooling, samples were filtered (Whatman No. 2) into 10 mL volumetric flask then rinsed with DI water three times and brought to volume with Millipore DI water. A volume of 50 μ L of the filtrate was mixed with 4950 μ L of Millipore DI water to decrease the acidity of the sample. The sample was filtered a second time with 1 mL of the filtrate with a PDVF 0.2 μ m filter, add 20 μ l of reagent 2 (Waters AccQ-Fluor Reagent (Fluorescent 6-Aminoquinoly-N-Hydroxysuccinimidyl Carbomate)) to the tube and immediately cap to vortex up down for 20 seconds to prevent from hydrolyzing the derivatizing reagent, and then pipette in a separate tube 10 μ L of the filtrate with 70 μ l of borate buffer. After mixing the solution using the vortex for 1 minute, tubes were centrifuged at 10,000 Xg for 1 minute. The tubes were incubated at room temperature for 1 minute, then at 55 °C for 10 minutes.

Amino acid (AAs) stock standard was prepared by mixing 400 μ L of a mixture of 1.25 mM of 20 amino acids (Aspartic acid, Serine, Glutamic acid, Glycine, Histidine, Arginine, Threonine, Alanine, Proline, Tyrosine, Valine, Methionine, Lysine, Leucine, Isoleucine, and Phenylalanine) with 4600 μ L 6N HCl. The standard was incubated in the same condition as samples. The stock

standard was then used to prepare four standard (std) solutions, std 1 (AAs standard 0.5 μM and 7.5 μM L-Norleucine), std 2 (AAs standard 2.5 μM and 7.5 μM L-Norleucine), std 3 (AAs standard 5 μM and 7.5 μM L-Norleucine), and std 4 (AAs standard 10 μM and 7.5 μM L-Norleucine) that were derivatized as samples. The HPLC used for analysis was the Agilent 1260 HPLC/FLD system (with analytical column: Waters XTerra MS C18 column (2.1mm \times 150mm, 3.5 μm particle size, Cat# 186000408, Waters Corporation, USA)). The HPLC conditions were:

- mobile phase A: 140mM sodium acetate, 17mM TEA and 0.1% (g/L, w/v) EDTA-2Na, pH 5.05
- mobile phase B: 60% ACN in water (v/v)
- flow rate: 0.35mL/min; column temperature: 50°C; injection volume: 5 μL
- FLD condition: $\lambda_{\text{ex}}=250\text{nm}$; $\lambda_{\text{em}}=395\text{nm}$

The concentration of the amino acid was calculated based on the peak area of the amino acid and its' standard curve.

AAs (μmol) = Concentration of AA (μM) / 100000 * 5 / 0.01 * 10 / 0.05 / Weight of used Millet

AAs (mg/g) = Amino acids (μmol) * Molecular weight of the AA / 1000

5.5 Riboflavin

To determine pearl millet grain vitamin B2 (riboflavin) content, 0.1 g of sample was placed into a 1.25 mL of sulfuric acid (0.1 M H_2SO_4) solution and autoclaved for 30 minutes at 120 °C. Once cooled to room temperature, 200 μL of sodium acetate trihydrate (2.5 M NaOAc) was added to the tubes adjusting to pH 4.5. A volume of 100 μL of Takadiastase enzyme solution (2.5 mg ml⁻¹) was also added to the tubes. The sample mixtures were then incubated at 35 °C overnight. Next, samples were centrifuged at 12,000 g for 15 minutes. One hundred μL of supernatant was diluted into 10 mL of deionized water (100 times dilution), and the diluted samples were filtered through

0.2 μm pore-size PTFE filter into a HPLC vial (Sami et al., 2014). Samples were immediately refrigerated. Five μL of filtrate was injected into the Agilent 1290 UPLC system, coupled with an Agilent 6490 mass spectrometer (Agilent Technologies, Santa Clara, CA) with the sample introduction of electric spray ionization (ESI) (Byrd et al., 2012). The chromatography separation was achieved on an Agilent Zorbax SB C₁₈ column (2.1 \times 5 mm, 1.8 μm). A gradient elution with mobile phase A (0.1% formic acid in water) and B (methanol) was used: 0% B for 0 – 2 minute, 0 – 100% B within next 5 minutes, then 100% B for 2 minutes, and finally 100 – 0% B within 0.5 minute, followed by a 3-minute equilibration. The flow rate was 0.2 mL per minute. The analytes were detected by the following MRM (multiple reaction monitoring) method: transitions \rightarrow 172 (CE = 45) and 243 (CE = 21) m/z vitamin B₂; and positive ionization mode.

The quantification of riboflavin content was accomplished by comparison to vitamin B standards. Vitamin B₂ (C₁₇H₂ON₄O₆) was obtained from Sigma-Aldrich. Standard stock solutions for riboflavin (500 ppm), was prepared by weighting 50 mg of B₂ in a volumetric flask in 100 mL of deionized water. The standard stock solution was stored in plastic bottles and kept in the dark in a refrigerator (4 °C) to protect vitamins from light-induced oxidation. The final concentration of the five standards (std) made for B₂ were std 1 (B₂=5 ng/ml), std 2 (B₂=2.5 ng/ml), std 3 (B₂=0.5 ng/ml), std 4 (B₂= 0.25 ng/ml), std 5 (B₂=0.05 ng/ml). These working standard solutions were prepared fresh daily from stock solutions when needed. A sample blank was added to all calibration standards for matrix matching purpose. The formula used to calculate the vitamins content in samples was (Sami et al., 2014):

$$\text{Vitamin (mg/kg)} = C_s \times D_s / V_f \times V_{in} / W_s \times 10^{-3} \times (1 + H_2O)$$

Where, C_s is the vitamin concentration (ng ml⁻¹) in the sample, D_s sample dilution volume (10 ml water), V_f is the volume of filtrate (0.1ml) used to dilute the sample in water, V_{in} is the total

volume (ml) of added solution ($\text{H}_2\text{SO}_4 + \text{NaOAc} + \text{takadiastase}$) to extract vitamins from a sample, W_s is the weight (g) of sample, and finally 10^{-3} is the conversion factor to mg kg^{-1} , H_2O is the moisture content of samples in fraction. The moisture content was determined by oven dry as the ratio of $(W_2 - W_3) / (W_3 - W_1)$, where W_1 is the weight of the dry aluminum tray use to contain sample, W_2 is the weight of fresh sample and the tray, W_3 is the weight of sample and tray after oven dry.

5.6 Statistical analysis

Analysis of variance was performed using PROC GLM of SAS v 9.3 (SAS Institute, 2011) to evaluate the impact of millet drought tolerance grouping on grain composition. When significant differences were identified by ANOVA, mean separations using Tukeys test with a probability level of ($P < 0.05$) was used to compare differences between groups.

6. RESULTS

6.1 Pearl millet physical and mineral nutrient characteristics

Across all cultivars, regardless of VPD group, there was no effect of water regime on either kernel weight or test weight (Table 3.2). The weight of 100-grains and test weight for tolerant varieties measured 0.75 g and 769 kg m^{-3} , respectively, while those of sensitive varieties were 0.82 g and 773 kg m^{-3} , respectively (Table 3.3). Manga and Yadav (1995) reported a strong relationship between pearl millet seed size and drought tolerance, with larger seed selected from one cultivar having greater tolerance. They also report variation in kernel weight among cultivars. Because

genotype is the major determinant of kernel size and test weight, it is not surprising to find a greater effect of VPD-group compared to water regime in our studies.

Pearl millet grain nutrient content varied due to water regime, VPD-group or both for Ca, Fe, K, and Na (Table 3.4). There was a significant interaction of water regime and VPD-group for Ca, Fe, and Na (Table 3.4). Calcium content in pearl millet grains varied between VPD-groups only under water stressed ($P < 0.1$) conditions, and the sensitive group accumulated more Ca than the tolerant and medium VPD-group under stress condition (19.1, 18.2, and 18.3 mg g⁻¹, respectively) (Figure 3.1). Sodium concentration was highest for the drought tolerant group and lowest for drought-sensitive lines under water stress (Figure 3.2). Only the main effects of VPD-group and water regime were significant for K (Table 3.4). Averaged over VPD-groups, K concentration in pearl millet grain was lower in the water stressed environment compared to well-watered ($P < 0.05$) (Figure 3.3). Independently of water regime, differences in K content between VPD-groups was registered, with cultivars in the sensitive group accumulating significantly higher K concentration compared to tolerant lines (Figure 3.4). Grain iron content response exhibited an interaction of VPD-group and water regime (Table 3.4). The highest Fe content was in sensitive and tolerant groups under water stress compared to sensitive lines under well-watered conditions (Figure 3.5). Neither Mg nor Mn concentrations were affected by irrigation regimes nor did pearl millet VPD-group (Table 3.4).

6.2 Protein, soluble protein and starch

Neither water regime nor the interaction of water regime and VPD-group affected pearl millet grain protein, soluble protein, or starch (Table 3.2). These constituents were only affected by VPD-groups (i.e. tolerant, medium, and sensitive to drought genotypes). Thus, for the concentrations of soluble protein and starch the sensitive VPD-group contained a greater

concentration compared to tolerant and medium VPD-groups. Whereas, protein concentration in grain was highest in tolerant genotypes (Table 3.3). The trend in protein and starch content for the medium VPD-group tended to follow the tolerant VDP-group. Ash content in pearl millet grain was not affected by water regime, VPD-groups, and their interaction (data not shown).

6.3 Amino acids, sugars, and vitamins

Sixteen amino acids were analyzed in pearl millet grain. Water regime did not affect amino acid content of the different VPD-groups (Table 3.5). The analysis of variance showed that tolerant, medium, and sensitive VPD-groups were able to maintain constant amino acid content in grains under the water stressed environment as compared to well-watered condition (Table 3.5). The only differences detected with amino acids in grain were due to variations based on VPD-groups (i.e. difference in genetic potential) (Table 3.5). In fact, for individual amino acids such as arginine, proline and serine, sensitive varieties accumulated higher content (approximately 1.6 to 2 g/100g of protein) than tolerant and medium VPD-groups (Table 3.6). The tolerant VPD-group however, had higher concentrations of lysine and glutamic acid (respectively, 1 and 4 g/100g protein) than medium and sensitive VPD-groups, and more aspartic acid (2 g/100g protein greater) than the sensitive VPD-group (Table 3.6). For the other amino acids, tolerant and sensitive VPD-groups did not show differences in the accumulated concentration, but the medium VPD-group tended to have lower content for these amino acids when differences were detected.

There was no significant effect of water regime or an interaction of water regime with VDP group for pearl millet grain sugar or riboflavin content (Table 3.7). Nonetheless, VPD-groups showed differences in concentration of the various sugars (Table 3.7). Tolerant varieties contained less (approximately 1 mg g⁻¹ less) glucose, fructose, maltose, raffinose, and stachyose than the

sensitive group (Table 3.8) Sucrose content was similar across VPD-groups (Table 3.8). The medium VPD-group sugar content was generally similar to that of the sensitive group, but contained less fructose and raffinose (Table 3.8).

Riboflavin (vitamin B2) accumulation in pearl millet grains was not affected by either the water regime or the interaction of water regime and VDP-group. As observed with amino acids, differences in vitamin B2 content of grain was only affected by the VPD-group. The drought tolerant VPD-group accumulated more vitamin B2 (1 mg kg⁻¹ more than the two other groups) (Figure 3.6), while B2 levels in the medium and sensitive VPD-groups were similar (Figure 3.6).

7. DISCUSSION

Mechanisms underlying differences in grain protein and nutrient accumulation during grain filling of pearl millet grains under normal or drought conditions have received relatively little study. Most studies on drought adaptation in pearl millet have mainly centered their research in mechanisms around pearl millet adaptation to drought through measurements taken on roots, stems, and leaves (Kandpal and Rao, 1985; Mugo et al., 2000; Kholová, et al., 2010bc).

For cereal crops, the final step of development is grain filling, and the length and rate of the grain fill period determines final grain weight and thus yield (Bieler et al., 1993; Yang and Zhang, 2006). During grain maturation, grain functions as a sink, and the sink strength determines the capacity of grains to fill with assimilates. The strength of a sink is based on two components according to Ho (1988): (1) the size of the sink, characterized by physical constraints (conditions affecting cell division), and (2) sink activity as the physiological constraint (enzymes activity). Both influence sink assimilation, but this also has implications for good seed germination and

seedling stands (Rose et al., 2015). Under drought condition, decreased water availability generally results in decreased photosynthesis, which, during the grain-filling period favors the translocation of reserves from plant stems and leaves into the grains (Blum, 2005; Barnabas et al., 2008). Therefore, abiotic stresses such as drought stress during vegetative stages or during the final stage of plant maturation can induce changes in overall plant growth processes.

Drought sensitivity of the genotypes used in this study was determined by screening at the vegetative growth stage under vapor pressure deficit test (VPD test). Not much is known about the response of these lines to drought induced after flowering and its impact on grain composition. However, mechanisms underlying late season drought tolerance of plants was investigated for many field crops including pearl millet. The main mechanism of pearl millet dehydration control under water stress is through rapid senescence of leaves below the spikes of reproductive tillers and all leaves on tillers without spikes (Do et al., 1996). These authors reported that leaf area of pearl millet plants subjected to late season water deficit decreased by 50% five days after irrigation ceased. In our study, we observed rapid senescence of pearl millet leaves on plants deprived of water after anthesis, similar to what is frequently described in the literature. Some authors reported that leaf senescence is required prior to translocation of nutrients to the developing grains in monocarpic crops (Yang and Zhang, 2006; Guoth et al., 2009).

In a wheat experiment, Yang and Zhang (2006) reported that when subjected to a controlled drought during grain filling, plants exhibited faster and higher nutrient translocation from stem and sheath into grains due to a shortened leaf senescence period. A delay in senescence could then possibly result in poor grain fill (Yang and Zhang, 2006). These findings imply that water stress during grain filling reduces the duration of grain maturation due to rapid plant senescence, but can also increase the remobilization rate of assimilates.

The energy used for grain filling comes either from photosynthates directly transferred to the grain, or from the redistribution of reserve pools of assimilates stocked during pre- or post-anthesis (Schnyder, 1993). Yang et al. (1998) and Yang and Zhang (2006) found that in stressed wheat plants, 18 days after anthesis, 81.3% of $^{14}\text{CO}_2$ previously applied to plants was found in grains, and only 9.6% of $^{14}\text{CO}_2$ and 8% of sugars were found in the stem, whereas for well-watered plants, 41.3% of the applied $^{14}\text{CO}_2$ was found in grains and 40.5% of $^{14}\text{CO}_2$ and 29% of sugars remained in the stem. Grain maturation between the two environments was 10 days apart.

In our study, pearl millet plants were deprived of water after anthesis; which obviously induced soil drying. We could conclude from the above statements that this would have caused the observed rapid senescence of plants coupled with a rapid remobilization of assimilates into the grains of stressed plants. In our study, water regime generally did not influence grain nutrient concentration. Tolerant, medium and sensitive VPD-groups maintained relatively similar nutrient concentrations in grains across both water environments. Saini and Westgate (1999) stated that even during severe drought, grains maintain sufficient water potential to allow metabolic processes in the kernel to be completed, even if metabolism stops in vegetative parts. Protein, soluble protein, starch, soluble sugars and free amino acids were similarly not affected by water stress treatment. This could reveal stability in the mechanism underlying nutrient translocation and metabolism in pearl millet for the previously listed nutrients. In fact, pearl millet has also been reported to possess ability to recover from assimilate deprivation caused by water stress during grain maturation through the mobilization of stored carbohydrate and soluble sugars (Fussel et al., 1991; Yadav et al., 2012). A study with rice also did not reveal water stress effect on grain amino acid content (Nam et al., 2014). This is an important finding, revealing the stable character of protein and amino acid levels in pearl millet under both well-watered and water stress conditions.

Neither kernel weight nor test weight of pearl millet was affected by water regime in our study. Wright et al. (1983) and Fletcher's (2003) findings related to kernel weight and yield of sorghum and birdseed millet, respectively, corroborate our observations. In these studies, drought did not affect these parameters and no genetic difference was reported. Fletcher (2003) also measured lower yield for birdseed millet varieties characterized by low osmoregulation, and according to these authors, this yield reduction for sensitive varieties under water stress was due to a reduction in the number of grains spike⁻¹. Santamaria et al. (1990) also reported similar causes of yield differences among drought tolerant and sensitive wheat genotypes. These findings on yield determinants and grain characteristics under stress and between tolerant and sensitive varieties support our observations of similar pearl millet grain test weight and kernel weight regardless of water stress. This likely indicates that all genotypes used in this experiment were able to efficiently translocate nutrient reserves to the developing grains even under stress.

Several studies indicated that drought stress increased amino acid and sugar content in plant leaves, stems, and grains in both tolerant and sensitive genotypes as a response to water stress (Kusaka et al., 2005; Saeedipour and Moradi, 2011, 2012; Sarafraz-Ardakani, 2014). Most attribute this increase to the need for plant osmotic adjustment in response to water deficit. Particularly, in Kukasa et al. (2005) pearl millet genotypes tolerant to drought accumulated more amino acids in leaves and stems than sensitive genotypes under stress. The opposite was true for sugars with greater accumulation in sensitive genotypes. Thus, one would suspect that during grain filling, tolerant and sensitive genotypes would accumulate respectively, more amino acid and sugars in grains when under water stress. Organic osmolytes such as sugars (mainly sucrose, hexose, glucose, but also raffinose and stachyose) (Gupta and Kaur, 2005; Taji et al., 2002), and amino acids (such as proline) (Kusaka et al., 2005; Saeedipour and Moradi, 2011) have been

characterized as having a protective effect in plants and also play a critical role as regulators of source and sink relationships and minimize desiccation of grains during development under stress. Nevertheless, Bieler et al. (1993) reported that grain growth parameters for pearl millet were not dependent on tolerance or susceptibility to drought. According to Ho (1988), the intrinsic ability of a sink to attract and unload assimilate is gene dependent, and nutrient unloading is maximal when there is abundant assimilate and that conditions for metabolic reactions in the sink are optimal. Therefore, determining the capability of a sink organ based on its absolute growth rate and/or net accumulation rate of dry matter would be a false assessment of the real strength of a sink organ to accumulate nutrients (Ho, 1988). Bieler et al. (1993) stated, that the decline in grain mass under stress was only a consequence of an early cessation of assimilate transfer to grains rather than a decline in grain storage capacity. Also, the findings of Kusaka et al. (2005) add possible proof that pearl millet has the ability to recover from assimilate deprivation during grain fill (Fussel et al., 1991; Yadav et al., 2012). These authors reported that the total amino acids accumulated by pearl millet drought tolerant and drought sensitive plants (leaves) became equal 9 days after drought was induced. These observations could partially explain the lack of significant differences in nutrient concentration between VPD-groups under stress conditions for most of the tested grain nutrients. Contrary to our findings, Saeedipour and Moradi (2011) reported a decrease in sucrose, glucose and fructose in wheat grain over time and this effect was greater for the sensitive cultivar under stress. Ahmed et al. (2013) reported an increase in amino acid content in a drought tolerant barley variety under water stress. Nutrient variations in this study were consistently observed among VPD-groups, and thus involve genetic variation. Overall, only mineral levels varied due to water regime.

The putative drought tolerant, medium and drought sensitive VPD-groups typically differed significantly for most of the determined nutrients. In Kusaka et al. (2005) sugar accumulation in pearl millet vegetative parts was higher in drought-sensitive lines under water stress. A similar result was reported for wheat (Saeedipour and Moradi, 2011). Our study also found higher sugar content for genotypes in the sensitive VPD-group compared to the tolerant or medium VPD-groups used in our study. Jones et al. (1980) stated that increased sugar content in plants could result from increased starch hydrolysis and synthesis through various pathways or a reduction transformation of sugars into other downstream products. In fact, cultivars in the sensitive VPD-group accumulated more starch in grains under both well-watered and water stressed condition. Amino acids accumulation in grain differed between VPD-groups. Regardless of irrigation regime, cultivars in the tolerant VPD-group accumulated more lysine, glutamic acid and aspartic acid whereas those in the sensitive VPD-group accumulated higher proline, serine and arginine. The former amino acids have also been found in high proportions in sorghum and sunflower leaves mainly under water stress (Jones et al., 1980). Most of these amino acids are constituents of dehydrin protein involved in inducing plant protective reactions against damage caused by dehydration (Allagulova et al., 2003; Rorat, 2006).

Starch is a complex carbohydrate made from tightly bound sugars. Relative starch accumulation in wheat grains was related to the rate of synthesis, through the activity of starch-synthase enzymes, and the duration of the accumulation process (Bhullar and Jenner, 1983; Ho, 1988). In wheat, the activity of starch-synthase enzymes as well as sugars in a sink is documented to be more related to genotype, nevertheless these components can be osmotically regulated (Ho, 1988). This reinforces the likelihood that our sugars and starch accumulation differed among the three VPD-groups due to genetics since they were unaffected by water stress. Fábíán et al. (2011)

found water stress resulted in decreased starch content in a drought sensitive wheat line, but no differences between tolerant and sensitive cultivars were observed under optimum moisture. Ahmed et al. (2013) found no effect of water stress on starch content of a drought-tolerant barley genotype.

Protein accumulation in plant leaves, stems and grains is often affected by drought stress, but we found no effect of water regime on pearl millet grain protein. Guoth et al. (2009) reported similar results for wheat grown in pots. However, Ahmad et al. (2013) reported increased protein content of a drought tolerant barley variety under stress. Abscisic acid (ABA) is a regulatory hormone produced by plants that induces stomatal closure and gene expression (Lindberg et al., 2012). Under drying soil condition, increased levels of ABA accumulate not only in vegetative plant parts, but also in grains (Guoth et al., 2009). The maximum accumulation of protein and starch in each grain depends strongly on the number of endosperm cells, determined early in grain filling (Egli, 1998; Barnab et al., 2008). In grains, persistent increase in ABA (from maternal origin) was reported to impact grain development via a reduction of cell division in grain endosperm (Myers et al., 1990). This occurs due to the interruption of ^{14}C -sucrose transport (Borkovec and Prochazka, 1992), conversion of sucrose to starch (Ahmadi and Baker, 1999), and starch synthesis capacity (Ober et al., 1991). Yang et al. (2001) reported a positive effect of ABA accumulation on rice grain assimilate accumulation and grain growth rate. Wang et al. (2015) stated that whether ABA has a positive or negative effect on grain filling is dose dependent. A high concentration injected in rice grain during grain filling under well-watered condition decreased sink activity (sucrose synthase, starch synthase) similarly to the sink activity under severe water stress. In fact, Ahmadi and Baker (1999) and Xie et al. (2003) reported that high ABA levels depressed wheat grain starch, while it increased grain protein. Physiological screening for

sensitivity to drought for pearl millet genotypes have shown that tolerant varieties expressed more ABA content in well-watered and water stressed conditions than sensitive varieties. Therefore, we suspect that this higher level of ABA in tolerant varieties reduces sink activity of grains, resulting in less accumulation of starch, possibly sugars, and higher protein than sensitive varieties as observed in this study. Also, studies have reported that yield increase was usually accompanied by lower total protein percentage in grain (Busch et al., 1969; Al-Tahir, 2014), and sensitive VPD-group performed better for yield in well-watered condition in our study.

In our study, grain mineral concentration was the only constituent consistently affected by water regime, specifically Fe, K, Ca, and Na. Pearl millet drought tolerance has been linked to stomatal closure (Winkel et al., 2001), and potassium plays an important role in plant osmotic changes (Nieves-Cordones, 2012; Kant et al., 2002). Drought induces decreased K production from chloroplasts. The accumulation of ABA is also partly responsible for the decrease in K under stress by activating K extrusion, and inhibiting K intrusion channels (Pospíšilová, 2003). Hu and Schmidhalter (2005) reported decreased K availability to plants under reduced soil moisture due to lowered K mobility. The decrease of K in vegetative plant parts under drought could be responsible for the concurrently lower K accumulation in pearl millet grains, as found in this study. Radhouane (2013) reported a decrease in K in pearl millet leaves under stress. However, Kusaka et al. (2005) reported the opposite trend in K in pearl millet leaves and stems under stress. In their work, tolerant varieties had higher K in both well-watered and stressed conditions. In this study, the sensitive VPD-group accumulated higher K content in grain regardless of water regime. Potassium has numerous functions in the growing plant. It functions in protein synthesis, enzyme activation (e.g. for starch synthesis), as well as in sugars and transport of some amino acids (glutamine) needed for growth and storage (Wakeel et al., 2011). From the above information, we

hypothesize that greater K accumulation in the sensitive VPD-group occurred via the accumulation of starches and sugars which were found in higher concentration in the sensitive VPD-group. Potassium accumulation in pearl millet grains decreased under water stress in all VPD-groups, however sensitive the VPD-group stored more K in grains.

Increased Ca concentration in plant cytosols under stress is also caused by ABA, since ABA activates entry channels of Ca and the availability of intracellular stored Ca (Pospíšilová, 2003). Accumulation of Ca (considered as a second messenger) in cytosols stimulates other plant defense mechanisms (gene expression) to adapt to the stress condition (Sadiqov et al., 2002; Krugman et al., 2010). Nayyar (2003) stated that Ca affects plant osmoregulation by inducing proline and Gibberellin accumulation, and this effect was more pronounced in drought-sensitive wheat seedlings. Rose et al. (2015) reported an increase in Ca content in wheat grain under water stress. No change in pearl millet leaf or stem Ca concentration between drought tolerant and drought sensitive cultivars under water stress has been reported (Kusaka et al., 2005). Calcium was highest in grain of the sensitive VPD-group under water stress in our study. Since Ca also functions in osmoregulation, it is likely that plants with greater ability to mobilize Ca would also be those with better tolerance to drought.

Sodium content in grain decreased under drought stress for the sensitive VPD-group compared to the other VPD-groups. Sodium was reported to be a substitute of K in some metabolic processes not specific to K, and therefore may reduce plant K requirement (Wakeel et al., 2011). Accumulation of Ca can also cause Na displacement from plant cells (Chen et al., 2007; Adams and Chin, 2014). This could explain why Na levels were lower under stress for sensitive varieties. Magnesium (Mg) and manganese (Mn) content in our millet varieties were not affected by either water regime or VPD-groups and their interaction, nonetheless, Kusaka et al. (2005) found no

changes in Fe, Mn, and Mg concentration in leaves and stems of two pearl millet varieties with contrasting sensitivity to drought. Bashir et al. (2014) reported differences in pearl millet varieties' grain Fe content due to genotype effect and the interaction of genotype \times environment; which corroborate with our findings.

Riboflavin (vitamin B2) is an important constituent in energy, fats, carbohydrates and proteins metabolism (Deng et al., 2013, 2014). A number of vitamins produced by plants have amino acids as precursors, which is true for riboflavin (Miret and Bosch, 2014). According to Miret and Bosch (2014) biotic and abiotic stresses increase vitamin concentration in plants, and more importantly in genotypes that are tolerant to these stresses. Riboflavin content was higher in the tolerant VPD-group in our study. Riboflavin influences plant growth, development and defense responses (Li et al., 2012). Tobacco plant tolerance to drought was increased via addition of riboflavin (Deng et al., 2014); however, it was clarified that high riboflavin levels could impair drought tolerance (Deng et al., 2013). Drought did not induce changes in vitamin B2 in this study. More experimentation is needed to elucidate the role of B2 in pearl millet drought tolerance.

8. CONCLUSION

Water stress after flowering affected only Ca, Na, Fe and K. Other minerals were not affected by either drought or VPD-group. For Ca and Na, the sensitive VPD-group was most affected by soil moisture deficit with Ca most affected and Na least for the sensitive VPD-group. Calcium and Na levels were similar among VPD-groups under optimum moisture conditions. Fe accumulation in sensitive VPD-group increased under water stress. Grain K was less in all VPD-groups under water stress. These results reveal that tolerant, medium and sensitive VPD-groups are not different in mineral content in well-watered conditions and maintain their concentration across the two environments except for K. Under stress, the sensitive VPD-group will tend to

accumulate more Ca and less Na than other VPD-groups. Selection for drought tolerance likely will affect mineral content in grains. Selection could affect grain content of sugars, amino acids, starch, and soluble protein. In this study, the sensitive VPD-group accumulated more sugars, starch, and soluble protein. For different amino acids concentrations were higher in the sensitive VPD-group for some and higher in tolerant VPD-group for others. This finding merits further research into the mechanisms for these differences. Vitamin B2 (riboflavin) was also different among VPD-groups with the highest concentration found in the drought tolerant lines. This study also reinforced the finding that pearl millet has the ability to recover from assimilate deprivation under water stress during grain filling, since drought stress did not affect accumulation of most of the measured constituents. Further studies integrating additional comparators for pearl millet genotypes with varying sensitivity to drought are needed to provide further insight into grain constituent components under normal and drought conditions.

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Table 3.1. List of pearl millet varieties used in the study and their vapor pressure deficit (VPD) group classification.

Variety	VPD-group
SL222	Tolerant
SL173	Tolerant
SL206	Tolerant
SL186	Tolerant
SL90	Tolerant
SL40	Tolerant
SL301	Sensitive
SOSAT C88	Sensitive
Souna 2	Medium
Gawane	Medium
Thialack 2	Medium
IBMV8402	Medium
ICMVIS89305	Medium
ICMVIS99222	Medium
ICMVIS99001	Medium
ISMI9507	Medium
ISMI9301	Medium
PEO2830	Medium
PEO3089	Medium
PEO8030	Medium

Adapted from CERAAS-Senegal, 2014

Table 3.2. Analysis of variance for the effect of water regime and VPD-group on pearl millet grain protein, soluble protein, starch, 100 grain weight, and test weight.

Source	Protein	Soluble Protein	Starch	Grain weight	Test Weight
	-----Pr>F-----				
Water Regime	ns	ns	ns	ns	ns
VPD-Group	***	***	***	*	**
WR x VPD	ns	ns	ns	ns	ns

* Significant at the 0.10 probability level.

** Significant at the 0.05 probability level.

*** Significant at the 0.01 probability level.

ns Not significant at the 0.10 probability level.

Table 3.3. Protein, soluble protein, starch, 100 grain weight, and test weight of pearl millet varieties as affected by VPD-group.

VPD-group	Protein		Soluble Protein		Starch		Grain weight		Test Weight	
	-----mg g ⁻¹ -----		-----		-----		g 100 grain ⁻¹		kg m ⁻³	
Tolerant	139.5	a	105.6	b	459.2	b	0.75	a	769.4	a
Medium	139.4	a	105.4	b	461.4	b	0.81	a	761.7	a
Sensitive	135.7	b	155.1	a	472.8	a	0.82	a	772.8	a

Within a column for each location, means followed by the same letters are not significantly different (P<0.05).

Table 3.4. Analysis of variance for effect of water regime and VPD-group on pearl millet grain Ca, K, Fe, Mg, Mn, and Na.

Source	Ca	Fe	K	Mg	Mn	Na
	-----Pr>F-----					
Water Regime	ns	**	**	ns	ns	***
VPD-Group	**	ns	**	ns	ns	***
WR x VPD	*	**	ns	ns	ns	*

* Significant at the 0.1 probability level.

** Significant at the 0.05 probability level.

*** Significant at the 0.01 probability level.

ns Nonsignificant at the 0.10 probability level.

Table 3.5. Analysis of variance for effect of water regime and VPD-group on pearl millet grain free amino acids.

Sources	Aspartic acid	Serine	Glutamic acid	Glycine	Histidine	Arginine	Threonine	Alanine
	-----Pr>F-----							
Water regime	ns	ns	ns	ns	ns	ns	ns	ns
VPD-group	***	**	***	ns	ns	***	***	*
Water *VPD	ns	ns	ns	ns	ns	ns	ns	ns
	Proline	Tyrosine	Valine	Methionine	Lysine	Isoleucine	Leucine	Phenylalanine
	-----Pr>F-----							
Water regime	*	ns	ns	ns	ns	ns	ns	ns
VPD-group	***	***	***	**	***	**	*	**
Water *VPD	ns	ns	ns	ns	ns	ns	ns	ns

* Significant at the 0.1 probability level.

** Significant at the 0.05 probability level.

*** Significant at the 0.01 probability level.

ns Nonsignificant at the 0.10 probability level.

Table 3.6. Amino acid content of pearl millet by VPD-group.

Amino Acid	VPD-group					
	Tolerant		Medium		Sensitive	
	-----g/100 g protein-----					
Essential						
Arginine	3.0	b	2.6	b	4.6	a
Histidine	1.4	a	1.3	a	1.6	a
Isoleucine	2.6	a	2.2	b	2.7	a
Leucine	6.5	a	5.8	a	6.3	a
Lysine	4.3	a	3.3	b	3.0	b
Methionine	1.5	ab	1.6	a	1.4	b
Phenylalanine	3.0	a	2.7	a	3.1	a
Threonine	2.7	a	1.6	b	2.8	a
Valine	3.4	a	2.2	b	3.9	a
Non-essential						
Alanine	6.3	a	6.1	a	5.5	a
Aspartic acid	7.9	a	7.3	a	5.8	b
Glutamic acid	14.0	a	11.9	b	9.9	b
Glycine	2.3	a	2.2	a	2.5	a
Proline	3.2	b	2.6	c	4.8	a
Serine	5.2	b	5.6	b	7.2	a
Tyrosine	1.8	a	1.5	b	2.1	a

Within a row, means followed by the same letters are not significantly different ($P < 0.05$).

Table 3.7. Analysis of variance for effect of water regime and VPD-group on pearl millet sugars and riboflavin.

	Fructose	Glucose	Sucrose	Maltose	Raffinose	Stachyose	Riboflavin
Sources	-----Pr>F-----						
Water regime	ns	ns	ns	ns	ns	ns	ns
VPD-group	***	**	ns	***	***	**	***
WR *VPD	ns	ns	ns	ns	ns	ns	ns

* Significant at the 0.1 probability level.

** Significant at the 0.05 probability level.

*** Significant at the 0.01 probability level.

ns Nonsignificant at the 0.10 probability level.

Table 3.8. Soluble sugar content of pearl millet grain by VPD-group.

	Glucose		Fructose		Sucrose		Maltose		Raffinose		Stachyose	
VPD-group	----- mg g ⁻¹ -----											
Tolerant	4.9	b	4.9	b	4.0	a	1.6	b	4.6	b	0.59	b
Medium	5.4	a	5.3	b	3.8	a	2.3	a	4.2	c	0.67	ab
Sensitive	5.8	a	6.5	a	4.2	a	2.3	a	5.2	a	0.75	a

Within a column for each location, means followed by the same letters are not significantly different (P<0.05).

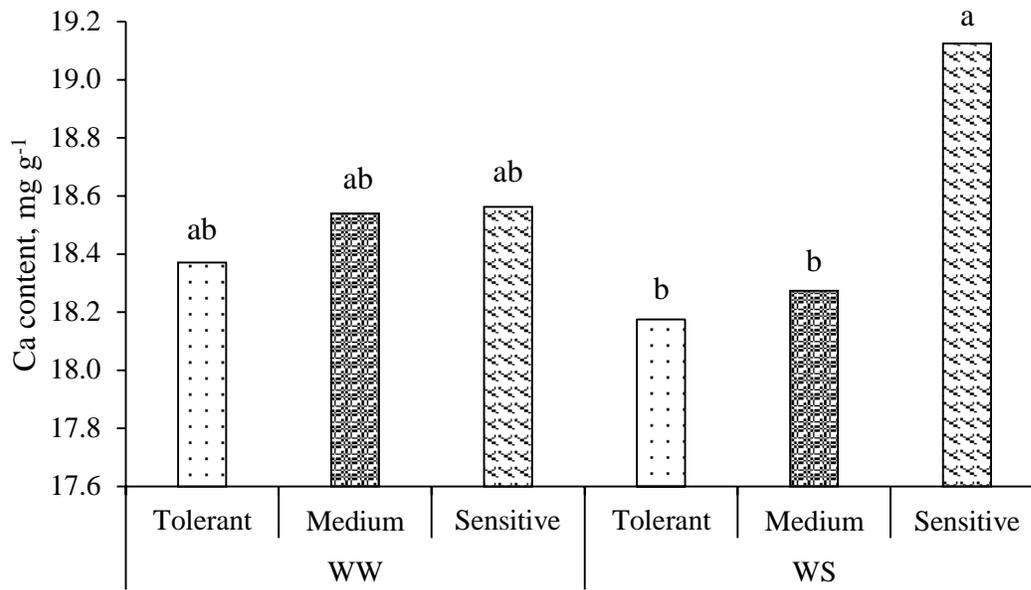


Figure 3.1. Calcium content of pearl millet grain by VPD-group and water regime.

WW = well-watered; WS = water stressed

Treatment means labeled with the same letters are not significantly different ($P < 0.05$).

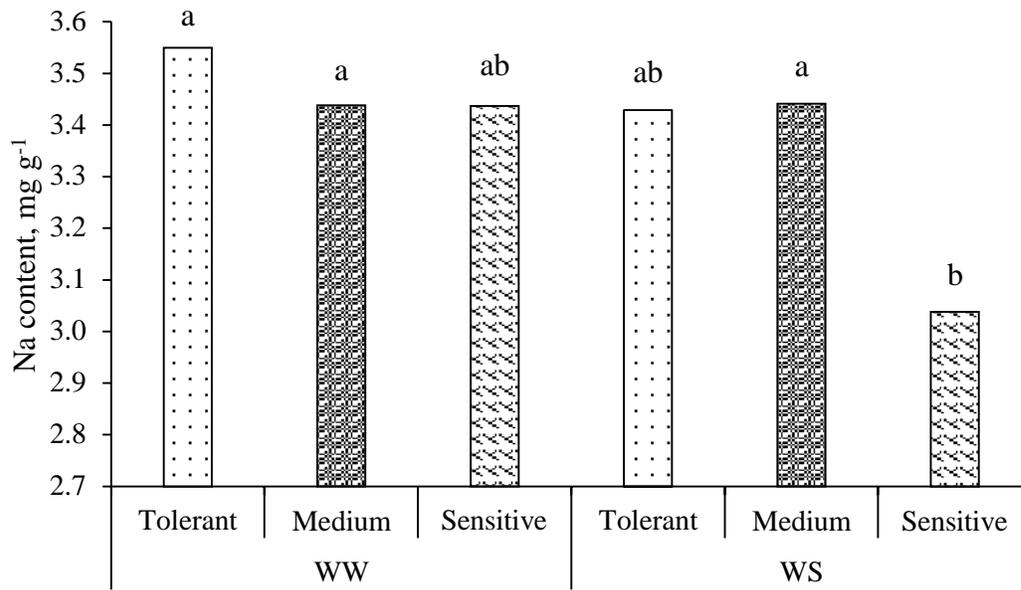


Figure 3.2. Sodium content of pearl millet grain by VPD-group and water regime.

WW = well-watered; WS = water stressed

Treatment means labeled with the same letters are not significantly different ($P < 0.05$).

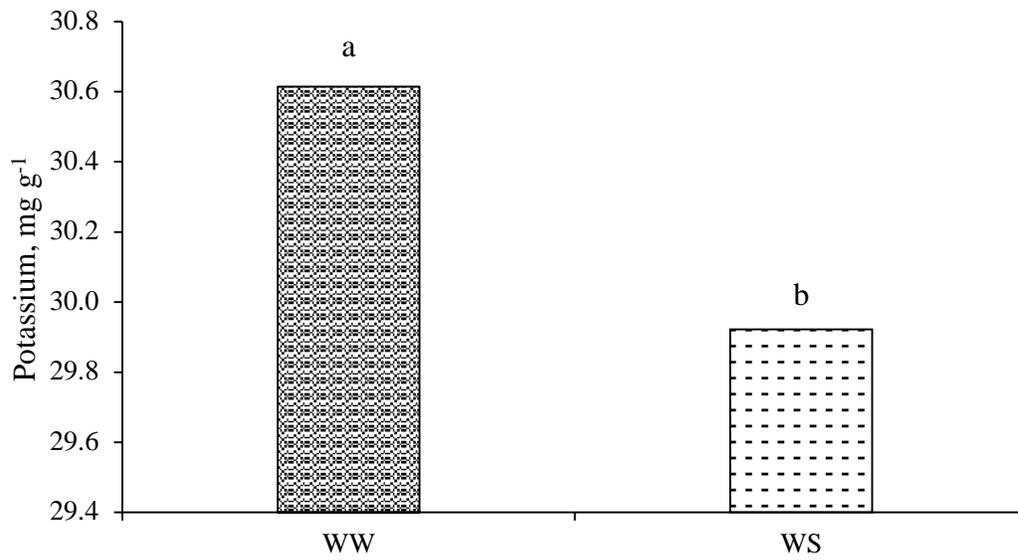


Figure 3.3. Potassium content of pearl millet grain as affected by water availability.

WW = well-watered; WS = water stressed

Treatment means labeled with the same letters are not significantly different ($P < 0.05$).

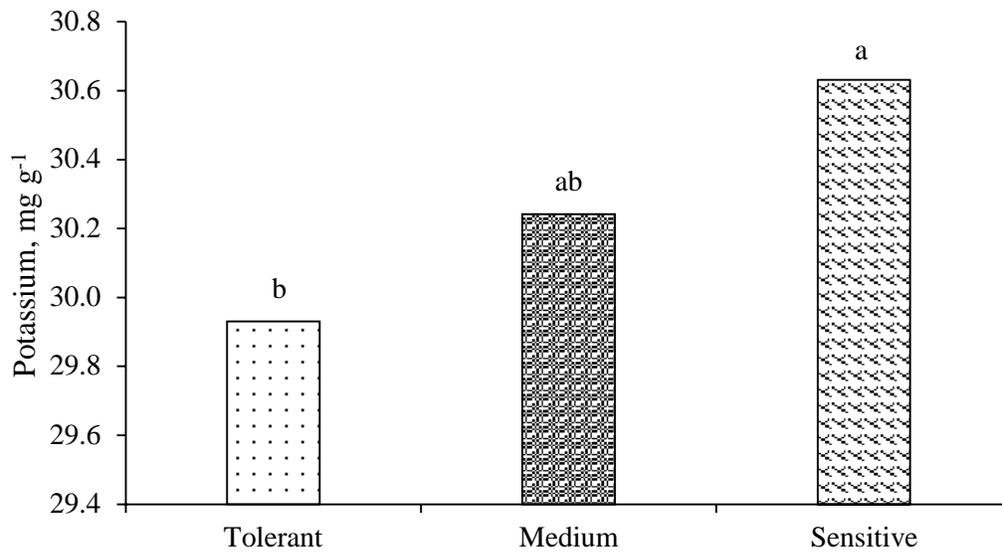


Figure 3.4. Potassium content of pearl millet varieties as affected by VPD-group.

Treatment means labeled with the same letters are not significantly different ($P < 0.05$).

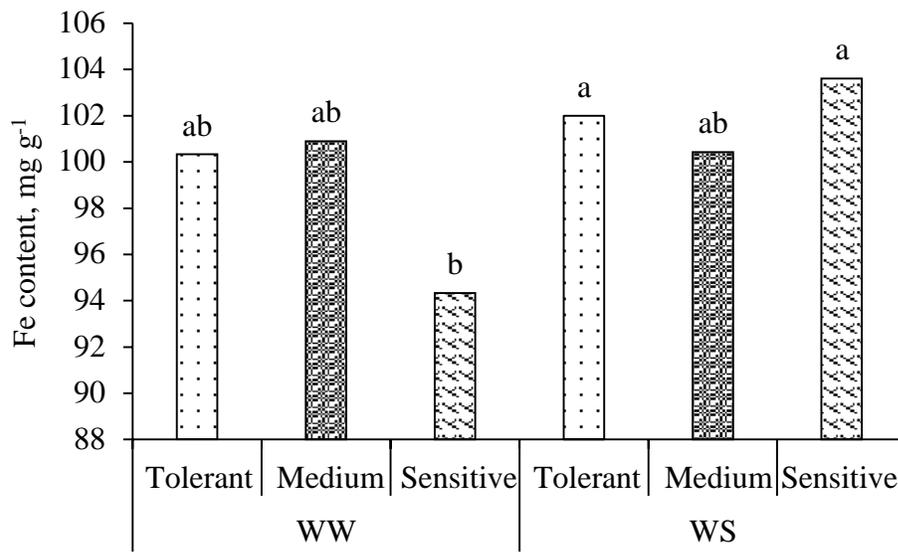


Figure 3.5. Iron content of pearl millet varieties by VPD-group and water regime.

WW = well-watered; WS = water stressed

Treatment means labeled with the same letters are not significantly different ($P < 0.05$).

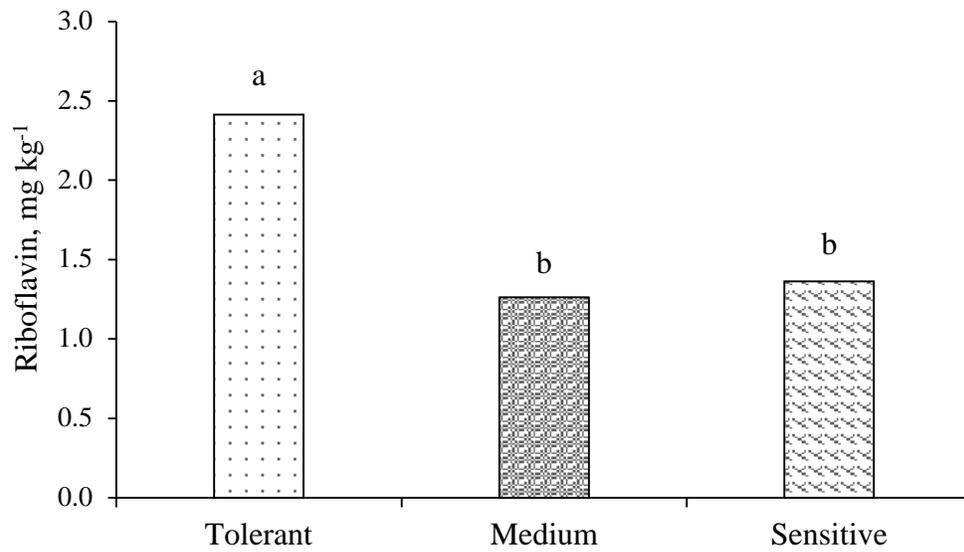


Figure 3.6. Vitamin B2 (riboflavin) content of pearl millet varieties by VPD-group.

Treatment means labeled with the same letters are not significantly different ($P < 0.05$).

CONCLUSION

Expanding and improving the efficiency of sweet corn production in Senegal could be beneficial to multiple parties. Currently, sweet corn production is centered in the Niayes area due to the abundant water resources there. Developing tools to optimize fertilizer use in sweet corn will not only serve to increase grower profits but also to minimize potential environmental concerns linked to over application of nutrients. Our study indicated that the GreenSeeker active crop canopy sensors estimate yield potential at the V9 growth stage, but the performance was less satisfactory with the chlorophyll meter and IEMs. In terms of the dose of applied fertilizer, neither the GreenSeeker® nor the SPAD meter detected differences in plant mass or nutrient uptake at V5 and V9 growth stage. This result does not incriminate the performance of the sensors since there was no fertilizer treatment effect on leaf mass N, N uptake, and some yield components. Ear yield was affected by N treatment at Sangalkam where the SPAD meter was used. IEMs did show differences in soil available N at V9 for NH_4^+ and at V5 for NO_3^- different removal at Ndiol and Sangalkam, respectively, but these results were not enough to affirm the utility of IEMs to measure available soil N. Further research is needed to evaluate the use of GreenSeeker, SPAD meter and IEMs for predicting in-season N needs, and the optimum rate of fertilizer.

Millet improvement for adaptation to drought condition is needed to maintain productivity; however, the nutritional quality of these improved genotypes is similarly important and may be affected by selection for drought tolerance. Comparison of nutrient concentration in grains of pearl millet genotypes contrasting in sensitivity to drought (VPD-group) and grown under water stressed and non-stressed condition confirmed our hypothesis that selection for drought tolerance in millet cultivars may indirectly influence grain nutritional composition. In fact, the nutritional composition of grains (soluble sugars, amino acids, protein, soluble protein, starch, riboflavin)

were found to be affected by genotype differences. Sensitive varieties had greater sugars, soluble protein, starch, and tolerant genotype had greater protein and riboflavin. Concentrations of individual amino acids varied among tolerant and sensitive genotypes. For mineral nutrients, Ca, Na, Fe, and K were also affected by genotype and responded to water stress. The 100-grain weight and test weight did not differ between drought sensitive and tolerant VPD-groups.