INFLUENCE OF GENOTYPE, GROWTH STAGE AND TEMPERATURE VARIATIONS ON ANTI-NUTRITIVE PROPERTIES OF POTENTIAL FODDER SORGHUM IN KENYA

LILIAN ATIENO OUMA

A Thesis Submitted to the Graduate School in Partial Fulfilment of the Requirements for the Doctor of Philosophy Degree in Agronomy of Egerton University

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DECLARATION AND RECOMMENDATIONS

Declaration

This thesis is my original work and has not been presented for any award in this or any other institution of higher learning.

Signature: ______Lilian______ Date: 09/09/2024

Ouma Lilian Atieno
KD121/16513/18

Recommendations

This thesis has been submitted with our recommendation as university supervisors.

Signature: ________________________ Date: 09/09/2024

Prof. Erick K. Cheruiyot, PhD
Department of Crops, Horticulture and Soils
Egerton University

Signature: ________________________ Date: 09/09/2024

Prof. Joshua O. Ogendo, PhD
Department of Crops, Horticulture and Soils
Egerton University

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DEDICATION

I dedicate this work to my husband Narkiso Ochieng' for his endless love, patience, support and for always praying for me, and to my children Leynar Joy, Jael Mshindi and Joe Bezalel.

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ABSTRACT

Sorghum [*Sorghum bicolor* (L.) Moench] is a fodder crop that thrives well in tropical climates and can offer solution to the feed shortage in Kenya. The aim of this study was to evaluate the influence of genotype, stage and temperature variations on major anti-nutritive quality attributes of selected fodder sorghum genotypes. Three experiments were conducted in the field, greenhouse and growth chamber to obtain data for evaluating the effect of temperature on lignocellulose and Hydrocyanic acid potential (HCN-p) of selected fodder sorghum cultivars. In the field experiment, twenty sorghum cultivars obtained from ICRISAT, commercial varieties and farmers' collections were evaluated in a randomized comple block design in 3 locations with vatying temperatures {(Egerton-cool), and (Marigat-hot) respectively} in 2019 nd 2020. Sorghum plants were sampled at booting and dough stages of development to determine the lignocellulose content and hydrocyanic acid potential (HCN-p). The second experiment, which was conducted in and outside a greenhouse under natural light was laid out in a CRD with three replicates, was set up to determine the effect of varying temperature on the HCN-p of sorghum genotypes using five sorghum genotypes. The third experiment consisted of three potted sorghum genotypes: IS11442, IESV91105LT and E6518 grown in the greenhouse then later transferred to a growth chamber. The three genotypes were subjected to controlled temperature and relative humidity. The temperature settings were $35^{o}C/15^{o}C$, $25^{o}C/10^{o}C$ and $18^{o}C/8^{o}C$). Analysis of variance was done through generalized linear model in R statistical software version 4.3.0. Treatment means were separated through Tukey's honest significant difference (HSD) test at $P \leq 0.05$. There was a significant increase in HCN-p and lignin content from a cooler region (Egerton), to a warmer region (Rongai) through to a hotter region (Marigat). HCN-p in the test material ranged from 83 – 147 ppm. The lowest values of HCN-p were obtained from the brown mid-rib (*bmr*) genotypes; IS 23787, EST 36 and EST 37 whose content were 86.58, 86.24 and 84.43 ppm, respectively, in 2019 and 85.13, 85.73 and 83.79 ppm in 2020. Temperature had a significant effect on HCN-p of sorghum as shown by higher HCN-p in sorghum that was grown in the greenhouse (117.5 ppm) compared to that grown outside the greenhouse (101.5ppm) as well as sorghum held at high temperature ($35/15^{o}C$) in the growth chamber. Based on lignin content and HCN-p, EST 20, B 35, IS23787 and EST 37 sorghum lines are recommended for safe sorghum fodder provision when harvested at dough stage and grown in cool or warm environments.

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LIST OF ABREVIATIONS AND ACRONYMS

FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
HCN	Hydrocyanic acid
ICRISAT	International Crop Research Institute for Semi-Arid Tropics
RCBD	Randomized Complete Block Design
EST	Entry number
ANOVA	Analysis of variance
MoA	Ministry of Agricuture
GoK	Government of Kenya
KALRO	Kenya Agricultural and Livestock Research Institute
NCPB	National Cereals and Produce Board

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Sorghum (*Sorghum bicolor* (L.) Moench) is cultivated in most tropical countries including Kenya where temperatures are warm all year, averaging 25 to 28 degrees Celsius (77 to 82 degrees Fahrenheit). The crop is ranked fifth after the world major cereals: wheat, rice, maize and barley. According to Capps *et al*. (2023), sorghum production worldwide is approximately 44.91 million hectares which translates to 65.59 million metric tons of grain with average yield of 1.46 tons ha^{-1}. The US is the leading producer of sorghum in the world, while in sub-Saharan Africa; sorghum is majorly produced in the Sahel and East Africa regions. In 2020/2021, the United States was the largest producer of sorghum worldwide, producing about 11.4 million metric tons of sorghum while in Africa that year, Nigeria was leading in production with 5 million metric tons of sorghum (Shahbandeh, 2021). Sorghum is used both domestically as human food and feed and industrially as biofuel and for producing alcoholic beverages. In the recent past, sorghum has gained an increasing demand as a feed crop in the semi-arid tropics where livestock production is widely practiced. The high demand for sorghum as feed crop has been accentuated by the increasing water shortages as a result of global warming and hence pushing for a need for drought tolerant crop (Qadir, 2016).

In Kenya, sorghum is grown as a staple food crop for many low-income households who are resource-poor for home consumption in the often drought-prone marginal agricultural areas of Eastern, Nyanza and Coast Provinces (Muui *et al*., 2013). In recent years, production of sorghum has been spurred by emerging industrial demand, particularly from the brewing industry lead by the East African Breweries Limited (EABL). The huge market created by EABL provides income to farmers who grow and deliver sorghum grain for manufacture of low-cost beer (Chimoita, 2020). Sorghum has a rich genetic diversity and this has contributed to the availability of different genotypes for multiple products for both domestic and industrial uses. In view of the expanding dairy and livestock sector in Kenya, sorghum is one of those crops with the potential to offer solution to the feed challenges. Kenya's livestock sector especially the dairy sub-sector has relied on maize as a good source of feed. However, maize has production challenges among them being emerging pests, diseases andclimate change related limitations as well as human competition for food. Sorghum has a wide range of ecological adaptability and can grow anywhere from sea level to about 2,500 meters above sea level and with as little rainfall as 250 mm per year and on extreme temperatures of 10°C and 31°C (Kilambya & Witwer, 2019). Sorghum is tolerant to drought (Cothren *et al*., 2000), most of the diseases and is less prone to striga weed compared to maize and is one of the crops ideal for the climate smart agriculture (CSA). Most sorghum production in Kenya is concentrated in Eastern, Nyanza, Western and Rift Valley regions which account for about 43, 41, 9 and 7 percent of Kenya's total production (Kilambya & Witwer, 2019).

Sorghum remains the most suitable alternative to maize as a source of fodder for livestock. However, the use of sorghum as livestock feed is limited by relatively high crude fiber (2.1-2.3%), very low crude protein (8.9-15%) concentrations and by anti-feedant/anti-nutritive factors such as hydrocyanic acid and lignocellulose (Getachew *et al.,* 2016). The concentration of HCN and lignocellulose in sorghum fodder depend on variety, agronomic practices and environmental factors (Karthika & Kalpana, 2017). The presence of lignocellulose in fodder reduces feed digestibility in the rumen while HCN is lethal to animals when consumed in quantities above acceptable limits. Panasuik and Bills (2006) recommend 200 mg/kg on dry matter basis and 500 mg/kg on fresh weight basis as the permissible/safe threshold limit for HCN-p in sorghum fodder in ruminants. Studies have shown that production of HCN lowers the nutritive value of fodder due to its toxic effect on feeding livestock. Pandey *et al*. (2011) reported that there are many examples of death and serious illness from consumption of sorghum fodder in cattle, goats and other grazing animals.

Sorghum *bicolor* contains both cultivated and races of wild relatives, and has a substantial amount of genetic diversity for traits of agronomic importance. This wide genetic diversity provides an opportunity to select genotypes with desirable characteristics for fodder use (Tesfaye, 2014). Thus, the objective of this research was to evaluate potential fodder sorghum genotypes for lignocellulose content and hydrocyanic acid potential as affected by temperature in different locations and harvested at different stages of development.

1.2 Statement of the Problem

Good quality feed supply to the tremendously growing livestock sector in Kenya is a challenge. In the face of climate change, sorghum can be used as an alternative source of fodder to maize for both dairy and beef livestock in the arid and semi-arid zones where climatic and soil conditions do not favour maize production. However, the use of sorghum as fodder has been limited by high concentration of lignin and cellulose which is not easily digestible by livestock. Forage sorghums are also known to accumulate moderate to very high amounts of hydrocyanic acid which is lethal to livestock hence limiting its use as feed.

1.3 Objectives

1.3.1 General Objective

To contribute to food security and improved livelihood by selecting and identifying fodder sorghum genotypes with desirable attributes for increased livestock productivity.

1.3.2 Specific Objectives

This study endeavoured to determine the following:

i. Variation of lignocellulose and hydrocyanic acid content in selected fodder sorghum genotypes.
ii. Effect of environmental temperature on lignocellulose and hydrocyanic acid content in selected fodder sorghum genotypes.
iii. Effect of developmental stage of sorghum on lignocellulose and hydrocyanic acid content in selected fodder sorghum genotypes.

1.4 Hypotheses

i. There is no significant variation among selected fodder sorghum genotypes for lignocellulose and hydrocyanic acid content.
ii. Environmental temperature has no significant effect on lignocellulose and hydrocyanic acid content of selected fodder sorghum genotypes.
iii. Developmental stage has no significant effect on lignocellulose content and hydrocyanic acid content of selected fodder sorghum.

1.5 Justification

Sorghum is a versatile crop cultivated for its grain for food and for fodder. The importance for sorghum as fodder is significant in addressing global challenges of livestock feed quality and quantity for sustainable agriculture. In regions where other forage crops fail because of environmental constraints, sorghum serve as a crucial feed resource for livestock (Bashir & Rahman, 2021). This is due to sorghums ability to survive and thrive in arid and semi-arid regions of the world. During periods of drought, sorghum remains a reliable asset for enhancing feed security by increasing livestock productivity. Sorghum fodder is also rich in nutrients such as proteins, vitamins and minerals that are important in animal growth, health and development. Other than high nutrient content, digestibility when enhanced by addition of feed supplements, sorghum compares well with that of other forage crops hence sorghum is suitable for fodder use in places where such crops cannot grow or are costly to produce (Reddy & Reddy, 2018). In low input agricultural systems, sorghum is well-adapted as it requires fewer inputs in terms of water and fertilizers compared to other forage crops. Therefore, the cultivation of sorghum fodder supports sustainable agricultural practices due to reduced pressure on water resources and minimized environmental degradation/pollution. As a result, sorghum cultivation allows for sustainable land management and reduces the carbon footprint associated with livestock feed production (Van Sandt & Korth, 2007). Use of sorghum as fodder provides a cost-effective and reliable feed source, thereby lowering feed costs, and in-turn increase livestock productivity, and improve the economic stability of farming enterprises (Van Sandt & Korth, 2007). Additionally, the availability of dual-purpose sorghum varieties of sorghum (for both grain and fodder) boosts its economic value and supports varied income streams for farmers.

Fodder production in Kenya is still below the demand from the livestock sector due to the rapidly growing livestock numbers in the country. The livestock industry in Kenya has experienced tremendous growth over the past one decade with high numbers of livestock:15.8 million cattle, 26.7 million goats, 18.9 million sheep, 3.2 million camels, 44.6 million poultry, 1.9 million donkeys and 0.5 million pigs. Despite this large number of livestock in the country, the productivity of this industry has been fluctuating due to a number of constraints including seasonality in production, inadequate quality and quantity feed, limited use of manufactured animal feeds among others. Most of the fodder available in Kenya, both on-farm and on-sale, are from maize and low energy and little crude protein grasses like Napier and Rhodes grasses. Maize is the main crop used as fodder, particularly for making silage, despite its poor adoptability to various climatic conditions. The crop is mainly grown in the high potential agro-ecological zones in Kenya which cover about 20% of the total landmass. However, 70-80% of the total livestock is produced in the expansive arid and semi-arid zones where climatic and soil conditions do not favor maize production. Furthermore, maize production currently faces too major biotic stresses: the maize lethal necrotic disease (MLND) and the fall army worm, that limits dependence on the crop as the source of livestock feed.

Sorghum is among the suitable alternative to maize owing to its wide adaptability to the diverse agro-climatic zones of Kenya. Besides, it is resilient to low soil moisture and high temperatures which is characteristics of the ASALs. However, sorghum as livestock feed is limited by high concentration of lignocellulose which is not easily digestible by livestock. Grain and forage sorghum are also known to accumulate moderate to very high amounts of cyanogenic glucosides which is lethal to livestock. Screening for low lignocellulose and HCN-p in sorghum genotypes will provide a safe and well adapted alternative feed source to the growing livestock population. Since sorghum is also widely adapted to various climatic conditions in Kenya where livestock production is an economic activity, enhancing its use as fodder will increase its production and thereby increase household income. For a profitable livestock production, year-round supply fodder is a necessity.

1.6 Scope and limitation of the study

The current study focused on the effect of environmental temperature on HCN-p and lignocellulose content of potential sorghum genotypes. After ascertaining that increased growing temperature increases HCN-p and lignocellulose content in fodder sorghum, the mechanism involved or the metabolic chain of reaction for the observed responses were not investigated.

CHAPTER TWO
LITERATURE REVIEW

2.1 Agricultural sector in Kenya

Agriculture is the backbone of economic growth in Kenya and it contributes 33% of the GDP (Auma, 2023). Another 27% portion of the GDP is contributed by Agriculture through linkages with other sectors (Nyoro, 2019). The sector also provides livelihood and nutrition for a larger percentage of the Kenyan population of poor households as it employees 70% of the world populace (FAO, 2022). Other sectors which are driven by agriculture include manufacturing, inputs provision and markets, building/construction, transportation, tourism, education, and other social services (FAO, 2022). There are five main sub-sectors under agriculture in Kenya; crops, livestock, fisheries, aquaculture, and agroforestry. The crop sub-sector contributes approximately 6 million tons of food crops, and 4.2 million tons horticultural crops while the industrial crops give 500 tons per annum (Kangethe, 2004). Agriculture also plays a significant role in reducing poverty and hunger, as well as meeting the aspirations encapsulated in various Kenya Government policy documents such as "the big four agenda" (GoK, 2021). The major food crops produced in Kenya include maize, beans, potatoes, wheat, and sorghum among others.

2.1.1 Sorghum production globally and regional

Sorghum [*Sorghum bicolor* (L.) Moench] is useful as forage due to its adoptability to dry conditions and availability of high fodder yielding varieties as well as its adaptability to various agro– climatic conditions, of the world (Van Rooyen, 2001). The crop is ranked 5th in importance among cereal crops in both area of production and metric tons harvested (Bakari *et al.*, 2023). The area under sorghum production is approximately 42 M ha in the 99 producing countries. The place of origin for sorghum is believed to be in equatorial Africa while its first domestication was done in Ethiopia between 5000 and 7000 years ago, and from where it was distributed to other regions in the African continent along trade and shipping routes (Harlan, 1969). The US is the leading producer of sorghum in the world with 8.07 Metric tonnes, while in sub-Saharan Africa; it is majorly produced in the Sahel and East African regions (Table 2.1).

Table 2.1 Global sorghum production statistics in 2023/2023

Market	Global Production (%)	Total Production (2023/2024, Metric Tons)
United States	14%	8.07 Million
Nigeria	11%	6.4 Million
Brazil	8%	4.91 Million
India	8%	4.4 Million
Mexico	7%	4.28 Million
Ethiopia	7%	4.01 Million
Sudan	5%	3.06 Million
China	5%	3 Million
Argentina	4%	2.5 Million
Australia	4%	2.2 Million

FAO (2022)

2.1.2 Sorghum sub-sector in Kenya

Sorghum is a crucial crop in Kenya as it contributes to food security and due to its resilience to drought and its adoptability to various agroecological zones (AEZs). Kenya has a diversified landscape ranging from humid to arid conditions making some regions unsuitable for production of most crops (Mugo, 2020). Thus, sorghum is a great fodder crop that is suited in most of the AEZs. In kenya sorghum is produced on a 197,403-ha piece of land and as of 2023, the yield was 225,000 metric tonnes (Kiambi & Mugo, 2016). Compared to other African countries, sorghum production in Kenya is still low. Nigeria and Ethiopia are the leading producers in Africa with 6,725,000 metric tonnes and 4,450,000 metric tonnes respectively. In Kenya, sorghum is grown in the drier parts of Eastern, Nyanza, and Coast Provinces (Mwandu & Mwangi, 2013). Its production is predominantly done by smallholder resource limited farmers who mainly grow it for food (Kiambi & Mugo, 2016). In recent years, production of sorghum has been spurred by emerging industrial demand, particularly from the brewing industry lead by the East African Breweries Limited (EABL). The huge market created by EABL provides income to farmers who grow and deliver sorghum grain for the manufacture of low-cost beer. Th baking industry has also embraced sorghum grain for the manufacture of gluten free bread- an initiative by Egerton University researchers.

Sorghum has a rich genetic diversity and this has contributed to the availability of different genotypes for multiple products for both domestic and industrial uses.

2.2 Economic importance of sorghum

Sorghum plays a significant role in Kenyan economy especially in the rural sep up in generating income for small holder farmers and also providing food to the evergrowing rural population in Kenya (Mango *et al.,* 2019). Since sorghum is resilient to adverse weather conditions, therefore its production ensures a stable yield and cussions farmers against the everchanging climatic conditions (Ochieng *et al.*, 2012). In the livestock sector, sorghum can be a sustainable source of fodder for the ever-growing livestock sector in Kenya, and can be an alternative to maize which is not drought resistant and is susceptible to pests and diseases. Maize is also a staple food source in Kenya hence sorghum can supplement maize in providing fodder.

2.2.1 Sorghum market and value chain

The sorghum value chain in Kenya comprises of farmers, processors and marketers. Sorghum value chain is scanty compared to other cereal crops. Challenges in the subsector include; limited value addition, poor infrastructure and inadequate processing activities and limited use of sorghum as fodder (FAO, 2021). The sorghum value chain can be strengthened through market linkages and input subsidies to farmers as well as awareness creation on other uses of sorghum such as potential use as fodder (Nyangweso *et al.,* 2013).

2.3 Opportunities for development in the sorghum

Since sorghum is a versatile crop in the arid and semi-arid regions where crop production is a challenge, it offers several opportunities for development. Firstly, sorghum provides great opportunity for adoption of climate smart agricultural practices. As climate change has posed significant challenges in the agricultural sector in Kenya, sorghum ability to thrive in drought and harsh condition makes it give a great opportunity (Ochieng *et al.,* 2012). Practices such as improved water management and conservation, tillage and agroforestry can help mitigate climate risks while increasing sorghum yields (Thierfelder & Lammerding, 2014). Secondly, value addition and processing can enhance sorghum use in Kenya. Initiatives by EABL to use sorghum as raw material in the production of cheap liquor to low-income group of people has improved sorghum production by small scale farmers in parts of Eastern and Western Kenya (Mwangi & Kihoro, 2021). Partnerships formed between

EABL and small holder farmers involved providing them with technical support, high-quality seeds, and training to improve sorghum yields and quality (Mwangi & Kihoro, 2021). Furthermore, development and adoption of improved varieties of food, fodder and industrial use can significantly boost sorghum productivity. Opportunities for sorghum development through improved varieties exist in research and development, seed quality and accessibility and extension services. Breeding of improved sorghum varieties and distributing seeds to farmers can enhance sorghum production and use in Kenya (Nyamwamu *et al.,* 2021). The focus of research has majorly been the development of high yielding and drought tolerant sorghum varieties. Organizations that concentrate on sorghum research include KALRO, ICRISAT, PACA (partnership for aflatoxin control in Africa). Improved dual purpose sorghum that are suitable fodder use in Kenya include Ikinyaruka, E6518, KARI, mtama, Dura, Tigon (developed by KALRO) and E1219 (developed by Kenya Seed Company). Research on sorghum fodder quality focusing on digestibility and safety is still a gap to be explored.

2.4.1 Factors influencing sorghum production in Kenya

a) Sorghum adaptability to drought

The Kenyan economy depends on agriculture especially crop production in order to feed the ever-growing population, currently at 50 million people (KNBS, 2020). The proportion of Kenyan land that is high potential and suits most staple food crops is only 20% and receives above 900 mm of rainfall per annum (MoA, 2018). The high potential agroecological zones (AEZ I, II and III) have not only attracted a high human settlement but is also under the production of cash crops including tea and coffee. This has led to a reduction in the area under food crop production in the regions, hence there is a need for expansion of food crop production area in the ASALS (KALRO, 2020). Since sorghum is well adopted to diverse climatic conditions and can thrive in low rainfall environments, including the arid and semi-arid areas in Kenya and is therefore a suitable crop that can increase food production in Kenya (Fukai & Cooper, 1995; Kizito *et al.,* 2014). Sorghum has a deep root system that enables it to survive in various soil types since it can absorb water from deeper soil layers during drought. The deep root system also enables sorghum to improve soil health by reducing erosion and improving soil structure, providing ground cover and in soil stabilization. The genetic diversity of sorghum also allows for improvement through breeding and development of more drought resilient varieties (Wang *et al.,* 2018). Sorghum also has low input requirement compared to other crops in terms of fertilizer and pesticides use making it a cost-effective crop to smallholder farmers in Kenya who are resource constrained (Gougoulias, 2017). Due to the challenges posed by climate change, sorghum is one of the climate-smart crops that can help in maintaining sustainable agricultural practices (Trostle, 2010).

b) Sorghum as a food source

The main staple food in Kenya is Ugali (thick porridge) which mainly prepared using maize flour. Maize production in Kenya is done in an estimated at 2.2 m hectares with annual production of about 3.1-3.3 m tonnes of grain while the consumption is estimated at 4.3 m tonnes (KNBS, 2021). Maize is also the main ingredient in livestock feed manufacture and also a major fodder source to the livestock sector. Furthermore, maize production faces agronomic challenges besides being a food crop major ones being the maize lethal necrosis disease (MLND) and the fall armyworm. Controlling fall armyworm increases cost of maize production as it is widespread and farmers have to spray expensive pesticides (KNBS, 2021).

c) Sorghum rich nutrient profile

Sorghum nutrient profile ranks high as its grain contain protein, fibre, vitamins, and minerals. It can therefore provide a nutritious alternative to maize and improve food security and nutrition in Kenya (FAO, 2019). One hundred grams of uncooked sorghum contains 329% calories, 11 grams protein, 3 grams fat, 72 gras carbohydrates, 7 grams fibre as well as other micronutrients including vitamins, copper, iron and magnesium (Miller *et al.,* 2012). Sorghum can also be used in various forms, from food products to beverages, supporting diverse uses in Kenyan diets and economies. This versatility nature of sorghum enhances its value as a staple crop (Miller, 2012). The industries that can adopt sorghum as a value chain include the feed industry (grain for feed concentrates), brewing industry (grain for malting and brewing), confectionary industry (breakfast cereals (flakes, pops), bakeries (Bread, cakes, biscuits, cookies) and the agrochemical industry (Ethanol from sugar-rich stalks in sweet sorghum).

d) Sorghum as a feed source

Due to sorghum rich nutritional profile, it is increasingly recognized as a valuable feed for livestock in Kenya. Livestock production in Kenya concentrated in the ASALs where growing conditions do not favour production of other fodder crops such as maize. Sorghum being resilient to various growing conditions, its production is beneficial to the livestock sector and can increase fodder availability to the growing livestock sector. As a fodder crop, sorghum is a good source of protein, which is vital requirement for animal growth and production (Zhang *et al.,* 2015). Depending of variety, management practices and growing conditions, the protein content in sorghum ranges from 8% to 12% (Zhang *et al*., 2015). Fodder sorghum varieties also provide a significant amount of energy for the maintenance and growth of livestock. Sorghum energy content is comparable to other cereal grains like maize (Johnson *et al.,* 2017). In a study by Adeyemo *et al*. (2019), use of sorghum as a substitute for maize in poultry feed, provided similar nutritional benefits and supported growth and egg production. Widyaratne *et al.* (2016) also concluded that sorghum can be included in swine diets as a cost-effective energy source, with considerations for its protein and fiber content. In another study by Minson *et al.* (2018,) it was concluded that sorghum silage and grain can be used as feed for ruminants providing a balanced diet if supplemented. Sorghum also contains a moderate level of fiber which can surpass that of maize. The high sorghum fiber content supports digestive health in animals (Mertz *et al.*, 2016).

Sorghum has low mycotoxin contamination risk compared to other cereal crops hence the use of sorghum as fodder reduces health risk on livestock (Marin *et al.,* 2015; Mungasavalli *et al.,* 2018). The low mycotoxin risk is associated with the presence of physical and biochemical barriers on sorghum seed that can deter fungal growth as well as the presence of a tough outer hull and high tannin content in some varieties which help in hindering the growth and development of fungi and mycotoxin production (Kapachula *et al.,* 2021; Voss *et al*. (2019).

e) Government policies that support sorghum production

The government of Kenya has policies that can promote and enhance sorghum production. The national cereals board plays a central role in procurement, storage, and distribution of cereals hence policies that can stabilize sorghum prices can enhance sorghum production in Kenya. Policies that promote the establishment of strategic reserves which aim at protecting farmers against price manipulation will ensure sorghum market stability (MoA, 2017; MoA, 2018). Another government policy that aligns with sorghum production is the agricultural sector transformation and growth policy (ASTGP) which defines strategies for improving agricultural productivity and income. The policy also outlines the need for increasing the production of drought resistant crops including sorghum (GoK, 2018). Some of the strategies outlined in this policy also promote research, innovation, and extension services to boost sorghum cultivation (MoA, 2017; MoA, 2018).

Vision 2030 also emphasizes on agricultural development as a key component of economic growth and promotes the need for crop diversification value addition in agriculture through crop value chains (GoK, 2018). Vision 2030 includes agricultural development as a key component of economic growth. It emphasizes the diversification of crops and the promotion of value-added agriculture, including sorghum. The components of vision 2030 that emphasize on market access, infrastructure development and research initiatives can benefit sorghum farmers. The big four agenda by the Kenyan government focuses on enhancing food security through increased agricultural productivity (GoK, 2018). Among the food security goals, sorghum is included as a drought tolerant crop and there are initiatives aimed at improving research, irrigation, and extension services for sorghum farmers. Furthermore, the National Agricultural and Rural Inclusive Growth Project (NARIGP) provides support to smallholder farmers through market linkages, access to inputs, and capacity building (MoA, 2017; MoA, 2018). In this project, sorghum is one of the crops with projects aimed at improving production practices, access to quality seeds, and establishing value chains. Lastly, the Agricultural Finance Corporation (AFC) offers financial products custom-made for agricultural initiatives, including sorghum production. Through loans and credit facilities, the AFC helps farmers access capital for inputs, technology, and infrastructure development (Szebini *et al.*, 2021)

The sorghum research and development program initiated by the Kenyan government concentrates on advancing sorghum production through soil management, sustainable crop management practices and breeding. Partnership between this program and the international research organizations helps in accessing cutting-edge technologies and practices (GoK, 2015). National research organization like the Kenya Agricultural and Livestock Research Organization (KALRO) conduct research that targets at improving varieties for disease and pest resistance and sustainable agronomic practices. KALRO develops and disseminates improved sorghum varieties to farmers for improved productivity. This research in sorghum has promoted the developments of sorghum varieties for various environments and for different uses including for brewing, bakery, fodder and improved nutrition provided by fortified sorghum varieties (KALRO, 2020). The provision of extension services to farmers which involve educating farmers on the practices for sorghum production has also promoted sorghum production. The services include best pest and disease management practices, modern farming techniques as well as sustainable soil management techniques. Knowledge is also passed to farmers through farmers field schools where farmers receive hands-on training on sustainable agricultural practices and through participatory practical learning on demonstration plots.

The initiative by the government to invest on rural roads and storage facilities such as the National cereals and produce board (NCPB) has improved market access to farmers. The improvement on infrastructure led to reduction in post-harvest losses and a seamless movement of produce to markets (NCPB, 2023). Additionally, the initiatives by the government to create market linkages for sorghum through farmer cooperatives and market information systems helps farmers bargain for fair prices and access larger markets (GoK, 2011). Tax incentives that target the sorghum value chain to agribusinesses involved in sorghum production have also contributed to the growth of the sector. The provision of subsidies by the government on seeds and fertilizers, reduces the cost of production for sorghum farmers. The subsidies make farm inputs more affordable hence an increase in adoption of innovative technologies and increase in sorghum yield.

2.4 Sorghum crop morphology

Sorghum (*Sorghum bicolor* (L) Moench) is a cereal crop that belongs to *Poaceae* family, genus *sorghum* and order Poales (Kumar, 2016). The current classification of the *Sorghum bicolor* Sub spp. bicolor is based on morphological characteristics and contains all of the cultivated sorghum varieties (Kumar, 2016). Sorghum sub-species bicolor spp *bicolor* is further classified into five basic races: guinea (G), caudatum (C), kafir (K), durra (D) and Guinea (Kumar, 2016) (table 2.1).

Table 2.2. Cultivated sorghum race characteristics

Race	Distinct characteristics
Bicolor	Open inflorescences with pendulous branches Long, clasping glumes Elliptic grain
Kafir	Moderately compact, cylindrical inflorescences Elliptic spikelets Tightly clasping, long glumes
Caudatum	Compact to open inflorescences Grains with one side flat, opposite side curved Shorter glumes that expose grains
Durra	Compact inflorescences Flat, ovate shaped sessile spikelets Middle-creased lower glume Distinct texture on tip of lower glume
Guinea	Large, open inflorescences with pendulous branches Long, separated glumes that expose grains Obliquely twisted grains

Source: Doggett (1988).

2.4.1 Root characteristics

Sorghum has a fibrous root system that comprise of deep primary root and widely spread lateral roots. The extensive root system with long roots enables sorghum to thrive in various stress conditions such as water stress, drought and low soil nutrient (Kholova *et al.,* 2010). The extensive root system makes sorghum adaptable with high plasticity while responding to varying soil conditions (Vadez *et al.,* 2014). Sorghum root growth and distribution is largely affected by soil texture and soil moisture levels.

2.4.2 Stem Characteristics

Sorghum possesses cylindrical stem which ranges from 1.2-4 meters in height and are coved with nodes and internodes (Vadez *et al.,* 2014). There is variation in height and diameter of sorghum stem among different varieties and cultivars. The role of sorghum stem is to support the plant, store nutrients and water (Rooney *et al.,* 2005). In windy growing conditions, a strong stem helps in conferring lodging resistance which helps in maintaining yield stability (Thurber *et al.,* 2013). Breeding works have focused on sorghum stem modification in order to produce strong and thick stem stems and improve on lodging resistance in sorghum (Setimela *et al*., 2007). Research has also been done on sorghum stem sugar content particularly for forage and sweet sorghums in order to increase stem sugar content (Kawahigashi *et al.*, 2013). High biomass in forage sorghum is also affected by stem size and stalk characteristics (Mganga *et al.*, 2021)

2.4.3 Leaf Morphology

Sorghum has elongated liner leaves which posses a bulging midrib, with leaves ranging in length from 30-60 cm while the width depends on variety (Badu-Apraku *et al.,* 2008; Tafari & Kumar, 2016). Sorghum leaves are modified in order to optimize on photosynthesis and water use. The leaves are covered by numerous stomata and a layer of cuticle that regulates photosynthetic rate and water loss (Hassan & Gohar, 2003; Miller & Schertz, 1982). Waxy leaf cuticle and reduced stomatal density are crucial in conferring adaptation to drought and windy conditions and ensure a high water use efficiency (Jones *et al.,* 2002; Midega *et al.,* 2002). Sorghum leaves also contain anti-nutritive portions that are either harmful or reduces the quality of fodder (Lata & Redden, 2010; Rao & Reddy, 1985). The antinutrients include tannins, lignocellulose, phytates, oxalates, nitrites and cyanides among others.

2.4.4 Sorghum flower structure

Sorghum has small flowers that consist of lemma, palea and stamens with the pistil consisting of a single ovary, two styles and a stigma (Singh *et al.,* 2017; Tollenaere *et al*., 2010). The flowers are wind pollinated, a trait that enables the crop to achieve efficient pollen dispersal and maximum seed pollination. The inflorescent morphology and characteristics are key considerations while breeding for high seed yield and quality (Khalifa *et al*., 2014). Flowering in sorghum takes 45-75 days after planting depending on cultivar and the prevailing environmental conditions. The period taken by sorghum plants to achieve full bloom (flowering) affects time to maturity and grain and fodder yield (Gao *et al*., 2020). Breeding has focused on optimizing panicle structure in order to improve on seed yields and, quality and to synchronize harvest (Gao *et al*., 2020). Breeding programs can also be used to alter flowering time in different varieties to match different environmental conditions and achieve early maturity. Time taken by sorghum to attain 50% flowering affects grain and fodder for quality and yield.

2.5 Classification of sorghum

Sorghum bicolor (L) Moench has 52 species which are composed of 31 cultivated, 17 wild and 4 weed species (House, 1978; Venkatesh & Lee, 2012). The 52 species were combined into a single species by Harlan (1969) and Shewale (2008) and reported the absence of genetic barriers among sorghum races in *Sorghum bicolor* species (Harlan & de Wet, 1972). The species bicolor has further been divided into 5 races: bicolor (B), guinea (G), Caudatum (C), Kafir (K) and Durra (D) (Kumar, 2016). Based on sorghum use and morphological characteristics, sorghum can be classified as grain sorghum (used for human consumption), forage sorghum (stalk and leaves used as animal feed for grazing and silage) and sweet sorghum (the stalk has high sugar content used for producing syrup, molasses and biofuel) (Kanbar, 2019; Kumar & Johnson, 2009). Forage sorghum include sudan grass and sorghum-sudan grass hybrids that have been improve for good fodder quality and high fodder yield (Rooney & Mitchell, 2005). For fodder use, sweet, forage and grain sorghums are of importance and their utilization is highly dependent on genotype and phenotype variability (Kanbar, 2019). Sorghum can also be classified based on varieties and cultivars which have been bred over time for traits such as pest resistance and yield (Kanbar, 2019; Paterson *et al.*, 2009). In Kenya, dual purpose varieties of sorghum that are suitable for food and fodder are Ikinyaruka, E1291, BJ28 and BM30.

2.6 Sorghum developmental stages

The time taken by sorghum from planting to emergence is 5 to 10 days depending on the growing conditions such as soil temperature, seed vigor, depth of planting and moisture the depth of planting. After emergence, the plant progresses in a predictable manner in three distinct growth stages i.e GS I, GS II and GS III (Miller & Cormick, 2017). Each stage takes approximately 32 to 35 days a process that largely depends on the hybrid and environmental conditions (Gerik *et al.*, 2004). Sorghum developmental stages are further classified based on structures and changes that occur as the crop grows hence the 3 stages are further divided into nine stages starting with emergence denoted as stage 0 and ending with the physiological maturity stage which is denoted as stage 9 as shown in table 2.2 (Bean & Vanderlip, 2003). The stages are also widely grouped into two vegetative and grain filling stages with vegetative stage differentiated according to the number of leaves while the grain filling stage commence after booting stage (Norby, 2004). Table 2.3 shows all the nine stages together with their descriptive characteristics.

Growth stage I (GS I) is characterized by vegetative growth where the plant develops its vegetative structures, leaves and tillers, which inturn supports grain formation and growth (Beres & Frey, 2021). The duration of GS I largely depends on air temperature and is directly proportional to the number of leaves that form on the main stalk and longer time to maturity leading to a greater potential to produce forage and grain (Bett & Hines, 2015; Gerik *et al.*, 2004). Early-maturing hybrids produce 15 leaves per plant, medium maturing hybrids produce 17 leaves while late-maturing hybrids 19 leaves at GS I. GS II on the other hand is marked by the stage reproductive structures form on the pinnacles form and ends when the maximum number of seed per plant are set (Gerik *et al.*, 2004). This is the most critical period of sorghum crop growth and for maximizing grain and fodder production. The last stage of sorghum growth and development, GS III is grain filling which begins with flowering and ends when dry matter accumulation in the grain stops when a black layer forms near the point of the seed attachment in the floret (Gerik *et al.*, 2004).

Table 2.3: Sorghum developmental stages

Stage	**Name**	**Days from emergence**	**Description**
0	Emergence	_	The plant is visible when the first leaf, known as the coleoptile leaf, breaks through the soil surface.
1	Three-leaf	10-14	The collar of the third leaf is visible.
2	Five-leaf	20-25	The collar of the fifth fully expanded leaf is visible, and the growing point is at or just below the soil surface.
3	Growing point differentiation	30-40	The plant is entering a rapid period of growth. The growing point can be found just above the ground by splitting the stalk,
4	Flag leaf visible	45-50	The plant is entering a rapid period of growth. The growing point can be found just above the ground by splitting the stalk.
5	Boot	50-60	The boot stage is when the sorghum panicle, also known as the head, is in the flag leaf sheath and can be seen as a bulge or swelling. Leaf collars of all leaves are visible, and the panicle is pushed up through the flag leaf collar by the upper stalk, known as the peduncle.
6	Flowering	60-70	Flowering or blooming is the most critical stage in the life of the sorghum plant. A plant begins flowering from the top of the panicle and progresses downward. A field of sorghum is in the flowering stage when blooming has progressed halfway down the panicle in 50% of the plants.
7	Soft dough	70-85	Sorghum reaches the soft dough stage when the grain can be crushed between the thumb and index finger but no longer contains a milky liquid
8	Hard dough	80-95	The grain can no longer be crushed between the thumb and index finger. The seed coat is no longer green and has turned its final color, which can be

		white, tan, bronze or red.
9	Physiological maturity	Physiological maturity is recognized by a dark spot or black layer on the bottom of the kernel. Grain moisture content typically ranges between 25-35%

Source: Bean and Vanderlip (2003)

2.7 Sorghum uses

Sorghum uses include domestic (food, feed, fodder), bio-fuel and other environmental benefits. The plant has a wide set of varieties which enables the diverse uses as food in grain sorghums, forage sorghums, hay and fodder sorghums and broomcorn used in making brooms and brushes (Visarada & Aruna, 2019). Grain sorghum is an important source of nutraceuticals such as antioxidant, phenolics and cholesterol-lowering waxes, phenolic acids, tannins, anthocyanins are useful in the health (Aredt & Zannin, 2013; Ramatoulaye *et al*., 2016). Processing of sorghum into floor, malt and syrups informs its utilization in baking and demonstrates its usefulness as an alternative to wheat floor and as a source of gluten free foods. Some chronic diseases such as cancer, diabetes and cardiovascular complications can also be reduced by sorghum consumption. Bioethanol and other processing industries also depend on sorghum to produce products such as kafrin-prolamin proteins and the pericarp wax which is used in bioplastics films and, food coating (Ramatoulaye *et al*., 2016). Traditionally, sorghum stalks have also been used as building materials. Sorghum being resilient to harsh environmental conditions makes it an important crop for sustainable agricultures (Sullivan, 2002). The crop has a deep root system that helps in improving soil health by rejuvenating the soil and in reducing environmental impact of farming practices (Hede *et al.*, 2004).

2.6.1 Sorghum use as fodder

Forage sorghums are used in feeds as hay, silage or as green chops. This category of sorghum is classified into four types: the grain sorghum (*Sorghum bicolor* (L). Moench), sweet sorghum, sudan grass (*Sorghum vulgare* var. *sudanese*) and/or commercial fodder sorghum (Kumar, 2016). Grain sorghum can be used as fodder in form of green chops or as straw and stubble fed to livestock after harvesting of grains (Heuze *et al*., 2015). On the other hand, the sweet sorghum has sugar-rich stems which are usually used in small scale sugar making, ethanol manufacture and also grown for fodder especially in the USA. Grain and sweet sorghums are normally crossed to obtain fodder cultivars that are high in sugar and less likely to cause HCN poisoning.

Another category of forage sorghum comprises the commercial hybrid fodder sorghums such as Sudan grass (*Sorghum vulgare* var. *Sudanese)* and Columbus grass (*Sorghum* × *almu*). These sorghums retain the multi-cut qualities with relatively higher yield potential (Balole *et al.,* 2006). Furthermore, fodder sorghum includes the brown midrib (bmr*)* sorghums that are less lignified in the stems and leaves and therefore have a higher cell wall digestibility (Contreras-Govea *et al.,* 2010). However, fodder sorghums have not been widely adopted due to their high susceptibility to pests and diseases, less tolerance to harsh weather conditions and have less biomass yield (Ouda *et al.,* 2005).

2.8 Desirable characteristics of fodder sorghum varieties

2.8.1 Tillering ability

Tillering is an important growth attribute in sorghum which influences growth, development and crop yields (ICRISAT, 2012). Sorghum with high tillering ability has desirable attributes such as higher grain forage and DM yields and also rank high in digestibility (Mwangi & Kihoro, 2021). Improved sorghum varieties compared with local landraces have higher grain and fodder yields and this was related to increased number of tillers (Kim *et al*., 2010). Tillering in sorghum is mainly dictated by genetics but could also be as a result of carbon supply demand balance of the plant (Hae *et al.,* 2010).

2.8.2 Sorghum stay-green trait

Stay green is a phenomenon of delayed senescence where the plant remains green and the stems and leaves maintain freshness for a long period even after physiological maturity (Ejeta *et al.,* 2009; Gentinetta *et al*., 1986). This phenomenon enables the plant to stay green long after the dry spell sets in and thus extending green chop feeding and conservation of forage materials when they are still green and fresh. This trait is important while improving drought tolerance and yield stability in sorghum, for arid and semi-arid regions. Sorghum cultivars with stay green trait retain their green leaf color and continue photosynthesis for a longer period under stress, growth and productivity (Ejeta *et al.,* 2009).

The stay-green trait in sorghum is a complex, quantitative trait controlled by multiple genes located on various chromosomes (Ejeta *et al.,* 2009; Zhang *et al.,* 2005). Varieties with the stay green trait that are drought tolerant have been identified using molecular markers linked to quantitative trait loci through marker-assisted selection (Jordan *et al.,* 2011).

Physiological mechanisms that contribute to drought tolerance such as photosynthesis and chlorophyll retention are enhanced by stay green trait and these mechanisms helps sorghum maintain chlorophyll and photosynthetic activity under drought conditions, and in sustaining carbohydrate production (Borrell *et al.,* 2006). The research has been done through techniques for evaluating stay-green traits under field and controlled conditions and by using remote sensing and other technologies to assess. Stay-green varieties have shown improved yield and performance under drought conditions, making them valuable for cultivation in dry regions (Borrell *et al.,* 2014). Evaluating stay-green traits involves various techniques such as visual assessments and quantitative measurements such as chlorophyll meters are used to evaluate stay-green traits (Murray *et al.,* 2012) and remote Sensing techniques (UAVs and satellite imagery) which provide advanced methods for large-scale monitoring of stay-green characteristics (Schroeder *et al*., 2020). Stay green varieties also possess improved root growth and high-water regulation efficiency, which allows for better water and nutrient uptake (Ejeta *et al.,* 2009). Under terminal drought, stay green phenomenon associated with sorghum genotypes increased both grain and forage yield (Borrell *et al*., 2006; Kassahun *et al.,* 2010). Besides genetic factors, leaf senescence has also been associated with demand for nitrogen by plants during grain filling as the plant breaks down chlorophyll and N is re-mobilized from the leaves to the grain which acts as a new N sink (Kassahun *et al.,* 2010).

2.8.3 Sorghum plant stature

Plant height is also another factor which has been reported to have an effect on sorghum grains and fodder yields (Peng *et al.,* 1999). Dwarf varieties of cereals showed increased grain yields during the green revolution (Daoura *et al.,* 2014). According to Quinby (1974), plant height in sorghum is mainly controlled by genetic factors both for shortness (dwarf) and tallness where tallness is being partially dominant to shortness. Dwarf crop varieties of cereals grow up to a height of less than 1 meter as compared to their tall counterparts that can attain a height of up to 3 to 4 meters (Sabadin *et al*., 2012). Dwarf locus is generally associated with higher grain yield compared to tallness locus which has been associated with higher biomass yield (Peng *et al*., 1999). Short type sorghum matures earlier hence the plants optimize their use of resources in the environment earlier before stresses set in (Laurie, 1997).

2.8.4 Growth regeneration

Regeneration ability in sorghum which refers to the plant's capacity to recover and restore growth after experiencing stress or damage with enhancement of processes like leaf re-growth, root system recovery, and overall physiological and genetic responses to stress (Borrell *et al.,* 2014). The root system recovery helps in maintaining plant health and productivity under drought stress (Kumar *et al.,* 2015; Kuraparthy *et al.,* 2008). Leaf recovery in sorghum varieties is made possible by continuous photosynthetic processes even during drought (Borrell *et al.,* 2014). Management practices such as minimizing mechanical damage and optimizing irrigation in sorghum enhance the regenerative processes of sorghum (Lee *et al.,* 2018). Reducing stress through integrated disease and pest management strategies can also enhance regeneration in sorghum (Smith *et al.,* 2019).

Regrowth is a heritable character in sorghum where the basal buds of sorghum stool are stimulated to regrow after the grains have been harvested and the above ground culm removed (Wilson, 2011). The regrowth takes advantage of the next season rains to give a second crop. The advantages of this trait include no need for land preparation, no new seed is required, reduced problems related to crop establishment and quick maturity compared to direct sown crops (Wilson, 2011). Good regeneration ability from stubble of the plant crop is a desirable trait, as it reduces overall inputs in terms of seed for planting and labor for field preparation (Shihab & Jaddoa, 2011).

2.8.5 Brown mid-rib trait

The brown midrib (BMR) trait in sorghum is a significant genetic modification aimed at improving forage quality. The BMR trait affects the lignin content in the plant, resulting in enhanced digestibility and nutritional value for livestock. The trait refers to a genetic mutation in sorghum that affects the biosynthesis of lignin, a major component of plant cell walls that results in a reduction in lignin content, specifically in the midrib of the leaf, which appears as a brown coloration (Miller *et al.,* 1992). Lower lignin content enhances the digestibility of the forage by improving the breakdown of cell walls in the digestive tract of livestock which in turn enhances nutrient utilization and overall feed efficiency (Hatfield *et al.,* 1999). Sorghum varieties with the BMR trait can increase the efficiency of feed utilization, which is beneficial for animals of all types (Casler *et al.,* 2004). Genes that mutate to form the BMR trait involve encoding enzymes such as cinnamate-4-hydroxylase (C4H), cinnamate-CoA ligase (CCL), and others involved in the phenylpropanoid pathway (Miller *et* *al.,* 1992; Wu *et al.,* 2013). The inheritance of the BMR traithappens in a Mendelian fashion, and selection for this trait involves identifying homozygous plants with lower lignin content and improved forage quality (Rosenow *et al.,* 1996). While BMR sorghum typically has improved forage quality, the trait is associated compromise on fodder yield which also depends on environmental conditions and field management practices (Casler *et al.,* 2004).

Adoptability of sorghum fodder with BMR trait is largely influenced by the specific genetic background and agronomic practices (Rosenow *et al.,* 1996). Most breeding programs focus on incorporating the BMR trait into high-yielding and disease-resistant sorghum varieties in order to obtain well adopted and high yielding varieties (Wu *et al.,* 2013). Techniques such as marker-assisted selection and genetic engineering, are used to enhance the expression of the BMR trait and integrate it into desirable sorghum varieties (Miller *et al.,* 1992; Rao *et al.,* 2018). In order to maximize the benefits of BMR trait, proper management of harvest timing is essential to maximize to ensures that the forage maintains high digestibility and nutritional value (Rao *et al.,* 2018). Additional genes associated with BMR trait as well as regulatory elements have been identified through breeding mechanisms providing deeper insights into the molecular mechanisms and potential for further improvement (Rao *et al.,* 2018). Ongoing field trials and research are also evaluating the performance of BMR sorghum under various environmental conditions and management practices to optimize its use in different regions (Kumar *et al.,* 2010).

2.9 Chemical composition of sorghum fodder

The feed value of a fodder crop is measured in yield quantity and quality values such as palatability and nutritional value. The two main important components for quality in fodder sorghum are protein content and digestibility (Karthikeyan *et al.,* 2017). A suitable livestock feed should be high in dry matter digestibility and low in lignin content. These fodder quality attributes are, however, affected by genotype, stage of harvesting and the environmental conditions during the growth period of the fodder crop (Carmi *et al.,* 2005). BMR mutations disrupt the normal lignin biosynthetic pathway, leading to altered lignin composition with reduced levels of guaiacyl and syringyl lignins. This modification improves fiber digestibility and reduces the plant's overall lignin concentration (Hatfield *et al.,* 1999).

Previous research evaluating yield potential and nutritional quality of sorghum genotypes, reported that different sorghum genotypes recorded significantly different amounts of fibre and non-fibre contents. Chakravarthi *et al.* (2018) in a study on nutritive value and chemical composition of 50 fodder sorghum varieties reported a range of 12.42-

15.95% crude protein, 70.13-82.19% NDF, 47.87-78.86% ADF and 1.32-22.18% ADL, 13.85-45.57 cellulose and 0.34-28.38 hemicellulose. In a different study, Karthikeyan *et al.* (2017a) in a study to determine nutritive value of different sorghum cultivars also reported varying amounts of crude protein as follows; TKSV 1171 (12.78%), TKSV 1182 (12.43%), TKSV 1126 (11.94%), and TKFS 1161(11.73%).

2.10 Fibre content in sorghum fodder

Fibre is relevant in fodder sorghum as it determines the digestibility of the feed and its potential in supporting the livestock. The typical fibre content in the fodder sorghum varies from 20-30% of the total dry matter cotent of the feed (Harris *et al*., 2007). Crude fibre comprises of tissues such as cellulose, hemicellulose and lignin that are vital in the digestion of forage. Presence of high fibre levels in fodder lowers the feed value as fibres are least digestible as compared to other components of feed (Harris *et al*., 2007). Imbalanced fibre in feeds also affects satiety and rumen fill and results to feed intake reduction or rather under consumption. In the cases of fibre content, cellulose component often forms a major part of it while the lignin contributes to the fibre part that is largely indigestible (Rao *et al.*, 2018). As a result of this, the level of fibre in fodder sorghum varies with maturation, variety of the type, and growing condition the crop underwent. Total fibre content in fodder sorghum varieties may range from 30 to 50 percent of the total dry matter of the fodder (Harris *et al*., 2007).

Recent studies demonstrate that the fibre content in sorghum is generally high in mature plants probably due to high deposition of lignin and cellulose in mature plants (Reddy *et al.*, 2014). Cutting stage has also been found to influence the fibre content in fodder and so, cutting at an intermediate heading facilitates a right balance of both fibre values and nutrient contents thereby increasing the feed digestibility and efficacies (Blummel *et al.,* 2003). Fibre content also differ among sorghum varieties with more digestible fibre and low lignin being the main focus of most breeding program. Reducing the fibre content especially the lignin fraction of forage increases fodder digestibility and quality (Singh *et al*., 2017). Fiber content can be influenced by environmental factors which include temperature, rainfall and type of soil (Queiroz *et al.,* 2015). High levels of fibre have also been observed in plants grown in stress conditions for instance water stress (Paiva *et al.,* 2017). The increase in fibre content in fodder due to water stress has been associated with retarded growth and early maturity (Vadez *et al.*, 2014).

2.11 Energy content in sorghum Fodder

Energy value of fodder sorghum has been found to be an important component in selecting animal feed. Energy content in fodder is divided into digestible energy and metabolizable energy and both may differ with growth stage, processing technique and cultivar with crops at earlier stages of development exhibiting higher digestible energy (DE) content due to high moisture content and low fibre/ lignin content (McDonald *et al.,* 2002). On the other hand, overmature crops have higher fiber content and reduced digestibility, leading to a significant decrease in energy content (McDonald *et al.,* 2002). Digestible Energy is the percentage of the energy in the feed that is absorbed and used by the animal's digestive system after deducting the energy lost in feces and is calculated as; DE=Gross Energy−Energy in Feces (Mertens, 2005). Metabolizable energy on the other hand is the energy available to the animal after subtracting energy lost in urine, gases, and fermentation and what the animal uses for maintenance, growth, reproduction, and lactation (Mertens, 2005). Metabolizable energy is calculated as; ME=DE− {Energy in Urine+Energy in Gases}

Recent research demonstrated that digestible energy in fodder sorghum is affected by its fibre content i.e., neutral detergent fibre (NDF) and acid detergent fibre (ADF), as well as proteins content (Bean *et al.,* 2013). Digestible energy ranges between 6-9 megajoules per kilogram of the total dry matter while metabolizable energy (ME) ranges from 5-8 megajoules per kilogram of the total dry matter. Higher neutral detergent fibre (NDF) and acid detergent fibre (ADF) decreases energy content of feed (Rao *et al.,* 2018). This means that the feed gets less and less digestible as the level of fibre rises, and therefore the feed value also drops (Karthikeyan *et al.,* 2017 a). Operations such as grinding, chopping or ensiling may also have certain effects on the energy levels due to change in extent of digestibility of fibre (Carmi *et al.,* 2005). Selection for varieties with higher energy content and adding supplements to feed is necessary in order achieve high quality fodder for maximum productivity of animals (Carmi *et al.,* 2005).

2.12 Mineral content of fodder sorghum

The mineral content of fodder sorghum is an important factor to consider when choosing livestock feed, as minerals play vital roles in various physiological functions. The mineral content in a feed resource can vary based on soil fertility, cultivar, and environmental conditions (Osama *et al.,* 2019). Fodder sorghum has mnerals such as Calcium (Ca), Phosphorus (P), Potassium (K), Magnesium (Mg), Sulfur (S) and trace elements in varying quantities. Calcium forms 0.4 to 0.6% of the total dry matter and is helps in bone development, blood clotting and muscle function (Mourtzinis *et al.* (2018). In some sorghum cultivars, calcium level in fodder sorghum is relatively low compared to other feed sources, hence it is important to supplement calcium in order to achieve animal daily requirement in feed (Mourtzinis *et al.,* 2018). Phosphorous is also an important mineral in feed as it helps in bone formation, growth and energy metabolism. Its concentration in fodder sorghum generally ranges between 0.2 to 0.4% of the total dry matter (Osama *et al.,* 2019). Fodder sorghum has moderate to low phosphorus levels hence supplementation is required depending on the overall diet (Osama *et al.,* 2019). Potassium (K) on the other hand can range between 1-2% of the total dry matter and its roles in the animal body include maintaining fluid balance, nerve transmission and cellular function (Delin *et al.,* 2020). Sorghum generally has adequate potassium content, but it's important to balance it with other minerals to prevent imbalances. Magnesium (Mg) is also present in fodder sorghum at a range of 0.2-0.4% of the total dry matter and helps in muscle and nerve function, bone health and enzyme function (Munir *et al.,* 2021). Magnesium is present in moderate amounts in fodder sorghum as compared to other minerals. Sulfur (S). Sulphur is present in sorghum fodder at low concentration of 0.1-0.2% of the total dry matter in content. Sorghum fodder also contains trace elements such as Sodium (Na) ranging between 0.01-0.1% of the total dry matter and helps in nerve functioning and in maintaining fluid balance (Nemecek *et al.,* 2018). Since sodium concentration in sorghum fodder is low, a salt supplement to sorghum feed might be necessary for the livestock. Other trace elements such as Iron (Fe), copper (Cu), zinc (Zn) and Manganese (Mn) are also present in small amounts in fodder sorghum and ranges between (50-100 mg/kg 5-15 mg/kg, 20-50 mg/kg and 30-60 mg/kg of the total dry matter respectively) (Griffiths *et al.,* 2022; Tiwari *et al.,* 2021). The mineral content of fodder sorghum is influenced by the soil's mineral profile and the type of fertilizer applied as well as frequency of application. It is important to ensure that soils are properly fertilized in order to maintain adequate mineral levels in the plants.

2.13 Dry matter content

While evaluating fodder nutritional quality, dry matter content is key as it affectcts suitability of animal feed as well as storage. Dry matter is the portion that is left after all moisture has been dried up and it's the portion that helps in determining the exact concentration of of nutrients after removing water (Van Soest, 1994). A high dry matter content indicates high nutrient levelswhile low dry matter indicates low nutrient levels per unit basis and these measurements help during feed formulations (Miller, 2006). High dry matter is also required during forage storage in order to reduce spoilage and microbial degradation (Miller, 2006). Fodder dry matter content is affected by stage of maturity, variety and genetics, environmental conditions and management practices (Harris *et al.,* 2007; Haug & Ledesma, 2013; Jones *et al.,* 2001; Vadez *et al.,* 2014). In fodder sorghum, Dry matter ranges between 25%-50% depending on genetic and environmental factors. Generally, forage sorghum has high dry matter content compared to grain sorghum due to breeding programs that target high biomass in sorghum fodder (Reddy *et al.,* 2014). Time to maturity also affects dry matter content in fodder sorghum as early maturing varieties have been found to have low dry matter content compared to late maturing varieties (Reddy *et al.,* 2014). In order to achieve a higher DM in fodder sorghum, breeding programs have focused on improving varieties for high dry matter content combine with other traits (Meyers *et al.,* 2011). Another way to maximize dry matter content is proper timingfor harvest and proper manage (Rao *et al.,* 2018).

2.14 Crude protein content

Crude protein is the total protein content present in fodder, and it is calculated from the total Nitrogen present in feed including true protein and non-protein nitrogen sources (Sarfraz *et al.,* 2012). Crude protein should be in adequate quantity in every feed formulation in order to ensure maximum growth, high milk yield and good health of animals (Etuk *et al.,* 2012). It is also crucial to understand the crude protein content in feed formulation for different animal species, purpose and age (Etuk *et al.,* 2012). In fodder sorghum, crude protein ranges from 8% to 15% and is affected by environmental conditions, variety and growth stage (Utama *et al*., 2023).

Rainfall, soil fertility and temperature impact on crude protein content in fodder sorghum. When the soil moisture content is adequate, crude protein content is high while water stress conditions lead to a reduction in crude protein content as a result of reduced plant growth (Vadez *et al.,* 2014). It has also been reported that crude protein content decreases as plant matures as younger plants at vegetative stage usually have high crude protein content (Gul *et al.,* 2008). It is important to harvest at optimal maturity stage in order to balance crude protein content with other fodder nutritional aspects (Gul *et al.,* 2008). Varietal differences due to breeding and natural selection causes varying crude protein content among different sorghum varieties. Breeding targets the production of sorghum varieties with high crude protein content for improved fodder quality (Muraya *et al.,* 2014*;* Rao *et al.,* 2018).

Crude protein content in sorghum is also affected by crop management practices such as irrigation and fertilizer application. Application of enough nitrogen fertilizer as well as proper irrigation leads to high crude protein content in sorghum fodder while poorly mange crops have low crude protein content (Kaplan *et al.,* 2019). High crude protein content is a vital quality in fodder sorghum as it improves on fodder digestibility affecting both feed efficiency and livestock performance (Harris *et al.,* 2007).

2.15 Anti-nutritive qualities of fodder

Anti-nutritive factors are substances that when present in animal feeds, or water they either by themselves or through their metabolic products reduce the availability of one or more nutrients (Benjamin, 2006). These substances may be toxic to livestock. They include substances such as nitrates, oxalates, hydrocyanic acid and tannins (Patel *et al*., 2013). Previous research has shown that fodder crops such as Sudan grass, pearl millet and oats accumulate nitrates at toxic levels (Kumar, 2016; Singh *et al*., 2000). It has also been reported that tropical grasses such as buffel grass (*Cenchrus ciliaris* L.), pangola grass (*Digitaria eriantha*), giant setaria (*Setaria sphacelata* (Schum). and kikuyu grass (*Cenchrus clandestinu*) contain oxalates to levels that can cause calcium deficiency in animals (Patel *et al.,* 2013). Extended period of feeding livestock to feeds containing oxalates leads to mobilization of bone minerals which eventually leads to hypocalcemia (Rahman & Kawamura, 2011).

The levels of oxalates and nitrates poisoning in livestock depend on factors such as seasonal variation, type of forage, genotype, animal species, energy level of the forage and part of the plant fed to the animal. Sigh *et al.* (2000) reported that stems accumulate most nitrates, followed by leaves while seeds accumulate very little. Animals fed on poor diet also exhibit more susceptibility to nitrate poisoning (Benjamin, 2006).

Seasonal variation also affects the levels of oxalate concentration in feeds as observed in salt bush plant and in Strandline plant (*Triplex buchananii)* (Abu-Zanat *et al*., 2003; Rahman *et al.,* 2006). Oxalate level in feeds is also affected by stage of plant at harvest as observed in Napier grass which showed a decrease in oxalates as plants matured when the crop was regrown four times and also differed with increased harvest intervals in early summer (Rahman *et al*., 2006). Young shoots of bamboo exhibited three times more oxalate levels than older shoots in a report by Patel *et al*. (2013). On the contrary, Rahman *et al*. (2009) reported that leaves and stem tissues obtained in summer have equal amounts of oxalates on different parts of the plant. Sheep/goats prefer young leaves to older ones and are therefore more susceptible to oxalate and nitrate poisoning than large ruminants.

2.15.1 Tannins

Tannins are a type of polyphenolic compounds with significant impacts on animal nutrition, influencing the digestibility of proteins and other nutrients and found in many plant materials, including fodder sorghum (Makkar, 2003). According to Makkar (2003), tannins are majorly classified hydrolyzable tannins and condensed tannins. Hydrolyzable tannins are esters of gallic or ellagic acid with sugars, while condensed tannins are polymerized flavonoids. Tannins bind to proteins through the formation of tannin-protein complexes thereby reducing the digestibility f proteins and leading to lower nutrient absorption. Tannins also form insoluble complexes when they bind to essential minerals like iron, zinc, and calcium and as a result interfere with their absorption (Tiemann *et al.*, 2008). Due to the decreased digestibility of proteins and other nutrients in fodder with high tannin content, livestock feeding on such fodder exhibit slower growth rates (Falcao *et al.,* 2011). When Chronic exposure to high levels of tannins can lead to health issues such as liver damage and reduced reproductive performance in animals (Tiemann *et al.*, 2008). This binding is Despite their anti-nutritive effects, tannins also have antioxidant properties that can offer health benefits and improve overall animal well-being (Yang *et al.,* 2010). Fodder varieties with lower tanins have been developed through breeding and have demonstrated improved feed quality and animal performance (Tiemann *et al.*, 2008). Reduction of tannin content in cereal feeds can be done through various processing techniques, such as soaking, fermentation, or chemical treatment, in order to mitigate the negative effects on animals (Singh *et al.*, 2012). Additionally, the effects of tannins in animals can be counteracted by adding certain feed additives or supplements into feeds to improve on nutrient availability (Falcao *et al.*, 2011).

2.15.2 Saponins

Saponins are glycosides composed of a hydrophobic aglycone (sapogenin) and a hydrophilic sugar moiety and are classified into triterpenoid and steroidal saponins based on their aglycone structure (Francis *et al.,* 2002). Saponins lower the feed value by forming complexes with proteins and lipids, affecting their digestibility and reducing protein availability, which has negative impacts animal growth and feed efficiency (Makkar *et al.*, 2007). Saponins also form insoluble complexes with essential minerals such as calcium, phosphorus, and zinc hence reducing their absorption into the blood stream (Makkar *et al.*, 2007). High levels of saponins in sorghum fodder can also lead to decreased feed intake due to irritation of the gastrointestinal tract leading to slower growth rates in livestock. Additionally, chronic consumption of high-saponin fodder can cause gastrointestinal issues and affect overall health of livestock. In rare cases, saponins in feed may lead to reduced reproductive performance and altered immune responses.

At the cellular level, saponins disrupt cell membranes, leading to alterations in gut permeability and altered nutrient absorption impacting on digestion and health. However, some saponins possess antioxidant properties, which can provide health benefits by reducing oxidative stress and inflammation in animals (Francis *et al.,* 2002). Developing sorghum varieties with reduced saponin levels through breeding programs can help improve feed quality and animal performance (Liener *et al.,* 2001). In order to reduce saponins in feeds, soaking, fermentation, and heat treatment techniques can be applied to help alleviate their negative effects. Supplementation of feeds can also help counteract the effects of saponins and improve nutrient utilization (Kannan *et al.,* 2015). Studies that targeted selection of sorghum varieties with lower saponin content led to screening of varieties with improved animal performance and health (Liener *et al.*, 2001).

2.15.3 Oxalates

Oxalates are naturally occurring compounds that significantly impact the nutritional value of fodder by interfering with mineral absorption and affecting animal health (Banjeree *et al.*, 2011). Oxalates are classified into soluble and insoluble forms based on the extent of solubility with soluble oxalates being easily absorbed and directly influence mineral absorption, whereas insoluble oxalates bind with calcium, magnesium and zinc forming insoluble complexes that reduce calcium availability for absorption (Ali *et al.*, 2012). The binding process can lead to calcium deficiencies and reduced bone health in livestock which causes impaired growth rates and poor reproductive performance in livestock due to reduced mineral availability (Phelan, 1994).

The absorption of oxalates and their impact on mineral absorption is influenced by factors such as diet composition and the presence of other compounds that can affect oxalate absorption (Phelan,1994). Development of sorghum varieties with low oxalate content can lead to better livestock performance and reduced incidence of mineral deficiencies (Mc Allister *et al.*, 2013). Secondly, techniques such as soaking, fermentation, or heat treatment can reduce the oxalate content in fodder and remove its negative effects (Ali *et al.*, 2012). Including supplementary sources of calcium and other minerals in feed can also help offset the negative effects of oxalates in the diet and improve mineral availability (Prasad *et al.*, 2014). The absorption of oxalates and their impact on mineral absorption is influenced by factors such as diet composition and the presence of other compounds that can affect oxalate absorption.

2.15.4 Goitrogens

Goitrogens are substances that can interfere with the synthesis of thyroid hormones, potentially leading to thyroid gland enlargement or goiter and are categorized as thiocyanates, glucosinolates and goitrins (Laurberg *et al.*, 2002). These compounds are found in various plants and inhibits iodine uptake by the thyroid gland (which synthesizes thyroid hormones) in both humans and animals when consumed in significant quantities (Singh & Beigh, 2013). Commonly found in cruciferous vegetables such as cabbage, broccoli, and kale (Laurberg *et al.*, 2002). Goitrogens are divided into glucosinolates and Progoitrin. During digestion, glucosinolates are converted into isothiocyanates and other goitrogenic compounds which inhibit the enzyme iodothyronine deiodinase thus affecting thyroid hormone metabolism (Verkerk & Dekker, 2004). Progoitrin on the other hand is a specific type of glucosinolate that can be hydrolyzed to form goitrin that are found in certain seeds and roots. Goitrin interferes with thyroid hormone synthesis and also inhibit the incorporation of iodine into thyroid hormones thereby blocking iodine uptake by the thyroid gland (Peng *et al.*, 2008). In humans with insufficient iodine intake goitrogenic foods can contribute to the development of goiter and hypothyroidism, and in the long run affect overall thyroid function and metabolic health (Zimmermann, 2008). Selective breeding or genetic modification can help in developing crops with lower goitrogen content in order to improve the safety and nutritional value of plant-based feeds (Zimmermann, 2008).

2.15.5 Lectins

Lectins are a diverse group of carbohydrate-binding proteins with a wide range of biological activities that can impact human and animal by binding to carbohydrates (sugars) without altering them (Ebere *et al.*, 2016). When lectins bind to carbohydrates, they affect immune responses, cell-cell communications, and nutrient absorption (Ebere *et al.*, 2016). Lectins are classified based on their structural characteristics, plant and carbohydrate-binding behaviour. Common types include legume lectins (e.g., phytohemagglutinin), cereal lectins (e.g., wheat germ agglutinin), and plant agglutinins (Goldstein & Poretz, 1986). They bind to carbohydrates in the gut epithelium, potentially leading to gastrointestinal discomfort or altered nutrient uptake (Yau *et al*., 2016). Those lectins that modulate immune responses bind to glycoproteins on immune cells, interfering with immune function (Ebere *et al*., 2016). As a result, high levels of lectins in the diet cause gastrointestinal distress, low nutrient absorption, and immune system activation. Cooking and processing typically reduce lectin levels, mitigating these effects. Some lectins exhibit antitumor and antiviral activities, offering potential health benefits despite their toxicological risks (Yau *et al*., 2016). Lectins can also affect growth and feed efficiency in animals, often leading to reduced performance if not managed properly (Kluge *et al.,* 2014). Processes such as cooking, boiling, fermentation and roasting can significantly reduce lectin levels in foods, improving their safety, nutritional quality and digestibility (Liener, 1994). Genetic modification and selective breeding can produce crops with reduced lectin content, enhancing their safety and nutritional value (Nishida *et al.,* 2007). Evaluating the economic impact of reducing lectin content through various strategies helps inform agricultural practices and feed management (Roberts *et al*., 2016).

2.15.6 Cyanogenic glycosides in forage sorghum

Cyanogenic glycosides are naturally occurring compounds found in various plants that can release hydrogen cyanide (HCN) upon enzymatic hydrolysis. They are classified based on their aglycone moiety as dienyl glycosides (Linamarin and Lotaustralin), and aryl glycosides: (Amygdalin and Prunasin) (Moller, 2010). HCN inhibits cytochrome oxidase in the electron transport chain, leading to cellular hypoxia and potentially causing severe health issues such as respiratory failure (Makkar, 2003) Ingesting large quantities of cyanogenic glycosides or their hydrolysis products can cause acute poisoning, characterized by symptoms such as headache, nausea, and respiratory distress (Makkar, 2003). Cyanogenic glycosides in animal feed can cause cyanide poisoning, affecting livestock health and productivity. Symptoms include lethargy, convulsions, and sometimes death. Fermentation processes can also lower cyanogenic glycoside content, making foods safer for consumption (Makkar, 2003).

In sorghum, cyanogenic glucosides exist as dhurrin which occurs in different concentration depending on plant age, environmental factors and genotype. The concentration of dhurrin in sorghum seedlings increases after emergence of sorghum up to 30% of shoot dry mass (Morrison & Wray, 1991; Rosati *et al.,* 2019). It is widely established that high concentration of dhurrin is at early vegetative growth stages (Karthika & Kalpana, 2017; Vinutha *et al.,* 2017; Zagrobelny, 2008). Metabolism of dhurrin is attributed to the two cytochrome P_{450} enzymes (CYP79A1 and CYP71E1) due to the surge in the quantity of these enzymes in sorghum at seedling stage (Siegień & Bogatek, 2006).

Evidence of dhurrin in sorghum is widely reported (Busk & Møller, 2002; Jones *et al.*, 2000; Karthikeyan *et al.*, 2017; Karthika & Kalpana, 2017; Neilsen *et al.*, 2016). Its production has been traced and found in sorghum species which include Sudan grass (*Sorghum bicolor* cv. Sudanese), Johnson grass (*Sorghum halepense* (L.) Pers) and Columbus grass (*Sorghum x almum* Parodi). Research has demonstrated the existence of variation in dhurrin content among genotypes, their stage of crop development, and climatic conditions (Busk & Moller, 2002; Panasuik & Bill, 2006; Vetter, 2000).

Dhurrin is enclosed in the cell vacuole and it becomes toxic when it comes in contact with β-glucosidases and α-hydroxynitrilase enzymes (Bolarinwa, 2016). The two enzymes are located in the chloroplast and cytosol of mesophyll cells, respectively. During foraging on sorghums by animals, plant cell is disrupted leading to the coming in-contact of dhurrin and the enzymes. Dhurrin is hydrolyzed by β-glucosidases and α-hydroxynitrilase enzymes to release hydrogen cyanide (HCN) (Figure 1) (Bolarinwa, 2016). This reaction can also take place in the rumen after the masticated sorghum gain entry into the rumen. The generated HCN is absorbed into the rumen and is rapidly detoxified in the liver by the action of rhodanase enzyme which converts cyanide to thyocyanate (SCN). Rhodanase is a mitochondrial enzyme which converts cyanide to thiocyanate and also facilitates transfer of a sulfur atom to cyanide from a sulfane-sulfur donor (Figure 1). The cyanide ion then inhibits cytochrome oxidase thereby stopping Adenosine Triphosphate (ATP) formation leading to energy deprivation to the tissues and rapid death in animals (Robson, 2007).

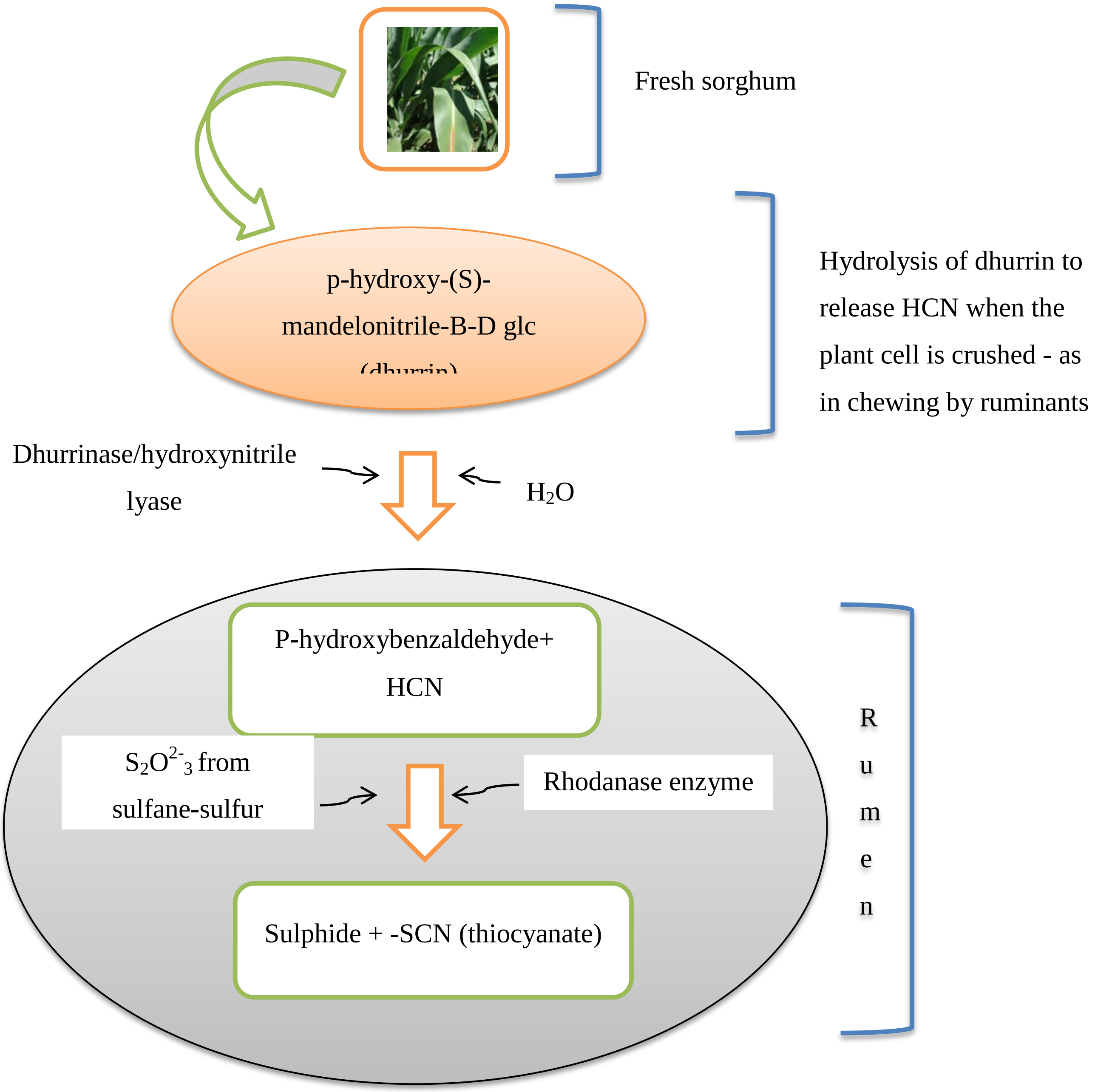

Figure 2.1: Hydrolysis of dhurrin to liberate HCN and dissociation of HCN in the rumen to release thiocyanate ion. Source: Busk and Moller (2002).

2.15.7 Dhurrin synthesis

The synthesis of dhurrin begins from amino acid tyrosine as the precursor and the process is catalyzed by cytochrome P_{450} enzymes: CYP79A1 and CYP71E1 (Blomsted *et al.*, 2012). The biosynthesis follows a general pathway which involves conversion of tyrosine to hydroxyphenyl acetaldoxime as shown in Fig. 2. (Gleadow *et al.*, 2021).

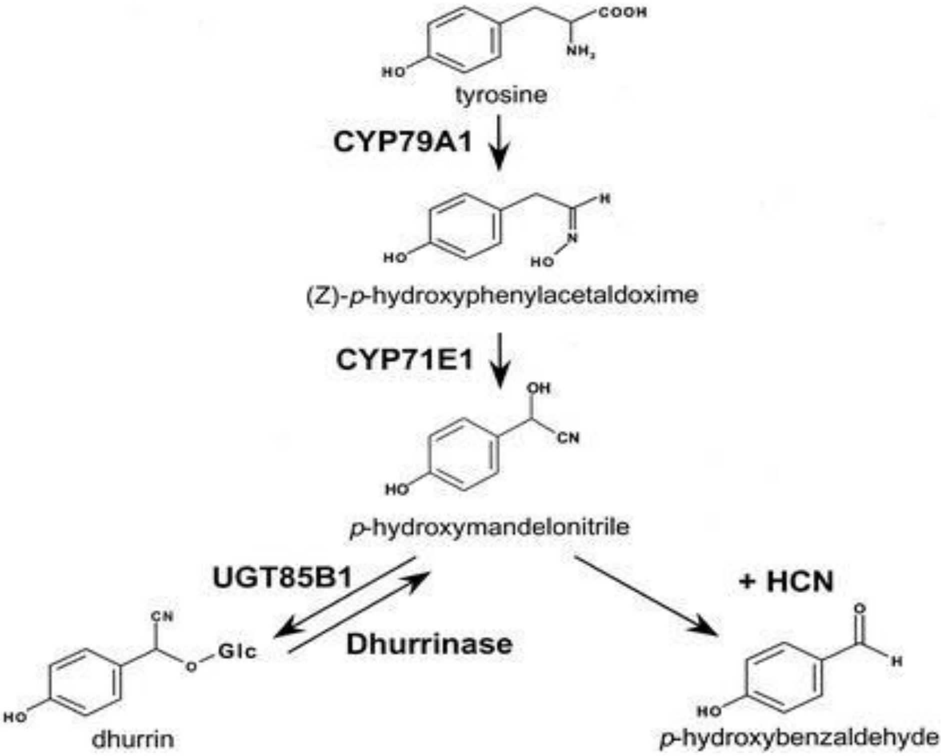

Figure 2.2: The biosynthetic pathway for the cyanogenic glycoside dhurrin in sorghum. Source: Busk and Moller (2002).

The second step involves subsequent conversion of (Z)-p-hydroxyphenylacetaldoxine to *p*-hydroxy-mandelonitrile by CYP71E1, an enzyme with low substrate specificity (Moller, 2002). In the last step of dhurrin synthesis p- hydroxy-mandelonitrile is converted to dhurrin by a soluble UDP-Glc p-hydroxi-mandelonitrile glycotransferase UGT85B1 (Clausen, 2015; Halkier & Gershenzon, 2006) (Figure 2). These reactions take place in the cytosolic surface of the endoplasmic reticulum, followed by transfer of dhurrin to the vacuole of the epidermal cells where it is stored (Clausen, 2015). In the vacuole the pH is low hence help in reducing self-toxicity of dhurrin (Clausen, 2015) Tissue disruption in sorghum facilitates contact of vacuolar dhurrin with glucosidase from the chloroplast of mesophyll cells and hydroxynitrile lyase from the cytosol of mesophyll cell where dhurrin is broken down to hydrogen cyanide and p- hydroxybenzaldehyde (Clausen, 2015).

2.15.8 Dhurrin as defense mechanism against phyto-pathogens and herbivores

The toxic HCN produced during hydrolysis of dhurrin act as phyto-anticipin in plants which is believed to provide immediate defense against pathogens or insects attack (Jones *et al.*, 2000). In a study by Moller (2010), induced cyanogenesis in barley leaf led to a significant reduction of powdery mildew colonization which suggests that hydrogen cyanide in sorghum could hinder fungal diseases in plants. In another study, Krothapali *et al.* (2013). observed that rapid cyanide release prevents insects from feeding on sorghum. In the same study, the fall armyworm (*Spodoptera frugiperda*) preferentially fed on sorghum plants that had low release of cyanide. Blomstedt *et al.* (2016) also reported that a cyanogenic sorghum mutant (dhurrin 2-1 mutant), which is deficient in dhurrin biosynthesis was preferred by insects (*S. frugiperda*) due to low HCN release (Kumar & Yadav, 2020; Spooner & Toxopeus, 1990). These evidences demonstrate that some cyanogenic plants do not release enough cyanide making them non-toxic to insect pests. Despite evidence supporting the deterrent effect of cyanogenic glycosides, a number of studies show instances of little or no effect on herbivores (Miller & Papadopulos, 2001). In *Turnera ulmifolia* (chanana shrub) high concentration of cyanogenic glycosides favored its use as an oviposition site by American butterfly (*Euptoieta hegesia)* (Ralph & Hatfield, 1991; Schappert & Shore, 1999). In another study by Sadasivam and Thayumanayan (2003) *Atherigona socata* and *Chilo partellus* preferably populated susceptible sorghum cultivars CSH-1, Swarna and IS 10795 showing dhurrin as an oviposition activator to insect pests. An inverse correlation was also reported between cyanogenic glucoside concentration and the amount of damage by herbivores in young eucalyptus seedlings (*Eucalyptus cladocalyx*) (Gleadow & Woodrow, 2000; Harborne & Williams, 2000; Seigler, 2003).

These contradicting findings point to some underlying role of cyanogenic glycoside dhurrin in sorghum other than defense against insect and pathogen attack, and that their synthesis could be triggered by some factor(s). It is not wholly convincing that sorghum produce the compound for defense against insect and pathogen attack. The herbivores or sucking insects still find the plants palatable. The herbivores only succumb to chemical effect after ingesting and any other animal or insect will still forage on the target, oblivious of the dangers. A defense chemical would be one that deters the pest probably through chemical emissions that can be perceived as unpleasant or harmful and can be avoided by the intruder.

2.15.9 Regulatory role of hydrogen cyanide in nitrate assimilation

The hydrogen cyanide produced when cyanogenic glycoside-dhurrin is hydrolyzed plays a key role in regulation of photosynthetic CO_2 fixation and nitrate reduction (Zagrobelny *et al*., 2004). This occurs under conditions of high intracellular $[O_2]/[CO_2]$ ratio when phosphoglycolate is generated in the reaction of Ribulose-1,5-bisphosphate carboxylase oxygenase during carbon fixation (Falkowski & Raven, 2007; Kleiner & Gout, 2006). The generated HCN can inactivate nitrite reductase when the rate of CO_2 fixation is low (Siegień & Bogatek, 2006). Cyanogenic glycosides also play a key role as a storage form of renewable N, C and glucose for seed germination and latex production in *Hevea brasiliensis* (Kongsawadworakul *et al.,* 2009). The findings agree with the results by Picmanova *et al.* (2015) which show retarded growth, slender stems, long internodes, small leaves and poor root development in cassava when cyanide content in plant was reduced to ≤25%. The growth of cassava was partly restored when N was added to the growth medium, signifying the influence of cyanide in internal N supply (Rao & Davis, 2001). Busk and Moller (2002) also demonstrated a link between dhurrin increase in sorghum seedlings with increase in nitrate supply. Their results showed that the amount of dhurrin in the shoot tip increased with an increase in application of nitrate showing that dhurrin serves as a nitrate sink (Kranner & Colville, 2007).

At seedling stage, dhurrin constitutes up to 30% of shoot tip dry mass but this amount reduces as the plant advances in age (Rosati *et al*., 2019; Smith & Lee, 2019). In an experiment with a cyanogenic sorghum line, Jorgensen *et al.* (2005) observed a significant reduction in plant metabolism when cyanogenic glycoside was removed at a certain crop growth stage, which confirms that dhurrin is a source of N for metabolism (Fink & Koch, 2011; Weber & Cutler, 2005). However, concluding that dhurrin in sorghum mainly functions as a metabolic compound would be biased owing to the fact that its concentration only surges at a specific crop developmental stage. A compound that acts as a source of stored N and C would be expected to be available in sufficient quantities at any developmental growth stages to serve as a buffer during adverse conditions when the plant cannot access sufficient N and C. There is also evidence that the production of dhurrin and other cyanogenic glycosides is triggered by external/environmental factors (Naikoo *et al.,* 2019; Sahebi *et al.,* 2017). This evidence could suggest that cyanogenic glucoside has a role in plant response to environmental stress.

2.15.10 Role of hydrogen cyanide in plant responses to stress

Physiological release of ethylene in plants is associated with biotic stress such as pathogenic attack and abiotic stress which include mechanical injury, flooding and/or drought (Naikoo *et al.*, 2019). Siegień and Bogatek (2006) argued that since hydrogen cyanide is a co-product of ethylene synthesis, it could be playing a significant role in responding to stress stimuli alongside ethylene. Enhanced synthesis of cyanogenic glycosides as observed in plants under stress conditions suggests a strategy by the plant to use it as source of N for metabolism when photosynthesis is low as occasioned by soil water deficit (Bjarnholt *et al.*, 2018). Furthermore, hydrogen cyanide possesses some properties that are akin to those of signaling molecules, which includes; quick and efficient production, induction of specific responses at low concentration and its ease to diffuse from cyanogenic plant to the immediate environment. These properties are akin to those exhibited by ethylene, methyl jasmonate and bacterial substances which are signaling molecules (Siegień & Bogatek, 2006). Similarly, Bjarnholt *et al.* (2018) reported that hydrogen cyanide plays a regulatory function in many physiological processes; seed germination, nitrate assimilation or in plant responses to some environmental stimuli.

Cyanide production in stressed plants is stimulated by 1-aminocyclopropane-1-carboxyllic acid synthase (ACS) activity. The stimulated ACS activity leads to elevated levels of endogeneous cyanide in plants which leads to a temporary increase in HCN concentration. The increase in cyanide levels only occur in a small region of plant tissue serving as a signaling cellular molecule in the plant and triggers a phenomenon that consequently leads to acquisition of stress tolerance (Siegień & Bogatek, 2006). Non- lethal concentration of cyanide has also been reported to induce resistance to diseases such as tobacco mosaic virus (Chivasa and Carr, 1998), turnip vein clearing virus (Wong *et al.*, 2002). Cyanogenic glucosides are also involved in modulating oxidative stress through scavanging of reactive oxygen species such as hydrogen peroxide in chlorotic leaves (Nahrstedt & Rockenbach, 1993). The role of cyanide as an enhancer of stress tolerance in mitigating oxidative stress by providing a ready source of N, through remobilization under stress has been reported (Rosati *et al.*, 2019).

The signaling role of cyanogenic glycosides in inducing resistance or tolerance to abiotic stress such as high temperature is not fully understood. The close relationship between drought frequency and elevated seasonal temperatures indicates that dhurrin production in plants could be triggered by increased temperature during growth period. While investigating the correlation between drought frequency and severity, Swain (2021) reported that the average return period and magnitude of deficiency of drought events have outstanding relationship with the range of temperature differences during the year in a specific location. There is need for further research to determine the possibility of temperature as a modulating factor in dhurrin production in sorghum.

2.15.11 The signaling molecule for dhurrin in sorghum

Dhurrin production in sorghum is regulated genetically, developmentally and environmentally (Rosati *et al.*, 2019). The enzymes that are involved in its synthesis (Figure 2) are coded for by 3 structural genes in a gene cluster containing sb MATE2 (Darbani *et al*., 2016). The biosynthetic gene cluster has gene encoding a MATE transporter (sobic 001G012500) and a glutathione S transferase (GST) which was named as sb GST1 (sobic 001 G012500) of the plant specific phi sub family (Darbani *et al.*, 2016). The genes that code for dhurrin are also reported to be involved in conjugating the reactive molecules including; tripeptide glutathione, xenophobic compounds and anthocyanins (Darbani *et al.,* 2016). Such reactive molecules protect plants against various biotic and abiotic stresses (Borges *et al.,* 2014) partially due to their powerful antioxidant properties. It can therefore be alluded that dhurrin, also function to protect plants against abiotic stress as its production is triggered by same factors that initiate the production of other plant antioxidants. However, more work is needed to confirm the involvement of dhurrin in protecting the plant against abiotic stress such as extreme temperatures during growth as such data is scanty in the available literature.

2.16 Lignocellulose component of sorghum

Plant cell walls represent a vast reservoir of reduced carbon in the form of biopolymers, mostly cellulose, hemicellulose and lignin which are bound together in a complex network. Cellulose is the most abundant biopolymer on earth and provides structural rigidity to plant cell walls (Fabio *et al.,* 2017). Hemicellulose is also a heterogeneous biopolymer that adds strength to cell walls by linking cellulose microfibrils. On the other hand, lignin is an amorphous high-molecular weight organic polymer with a three-dimensional network structure composed of carbon–oxygen and carbon–carbon bonds (Xu *et al.,* 2020). It intertwines cellulose and hemicellulose to givee good strength for the cell wall. The composition of the three biopolymers is affected greatly by plant genotype, plant age and weather conditions such as temperature (Ralph & Ralph, 2010). Nutritional value of fodder plays a critical role on its utilization and consumption by ruminants and depends upon the critical growth stages, cultivars and weather condition during fodder growth (Vogler *et al*., 2009). The quality of any forage crop is determined primarily by the degradability of the vegetative tissue which is affected by the property of its cell wall structure (Vogler *et al*., 2009). Lignocellulose refers to structural carbohydrates (polysaccharides) that form part of plant cell wall and are the ones responsible for cell wall strength, rigidity and shape (Vanholme *et al.,* 2010). The cell wall has several layers: middle lamella, the primary cell wall and three layers of secondary cell wall (Fromm, 2013). Polysaccharides are predominantly found on the primary cell wall together with lignin and hemicellulose (Fromm, 2013). Cellulose consists of a linear chain of glucose units with β-1, 4 linkages and is broken down by cellulase enzyme produced by microorganisms in the rumen. Hemicellulose on the other hand is a more complex polysaccharide with different types of sugars bound by α-1, 4 linkages and is closely associated with lignin. The digestibility of hemicellulose in the gastrointestinal system of ruminants is slowed down by the presence of lignin (Moore & Jung, 2001).

Lignin is a polymer that is usually deposited from monolignols derived from the phenyl-propanoid pathway in plants during the process of cell maturation (Moore & Jung, 2006). Lignin is an antinutritive fodder quality that act as a physical barrier to microbial enzymes hence has a direct and often important component on the digestible energy (DE) value of forage (Weng *et al*., 2010; Zhang & Wei, 2019). Lignin content in a forage plant depends on the plant maturity whereas as the plant matures, lignin content increases and the location of deposition changes (Moore & Jung, 2001). There are variations in lignin concentration and composition among diffent genotypes whih is caused by differences in genetic makeup of the plants (Waters *et al.,* 2013). Lignification in a forage crop also depends on the plant part used as fodder as the process tends to be most intense in structural tissues such as xylem and sclerenchyma. Plant organs such as stems are less digestible since they contain high concentrations of these tissues, than other plant parts such as seeds (Moreira *et al.,* 2013; Wu & Zhou, 2019).

Environmental and edaphic factors such as temperature, soil moisture, light, and soil fertility also affect the process of lignification and fodder in digestibility (Gatechew *et al*., 2018). The effect of environmental temperature on the accumulation of lignin cellulose and hemicellulose is not fully documented thus this study was aimed at evaluating the effect of growth temperature on lignocellulose content of twenty sorghum genotypes grown in different locations in Kenya. Sorghum being C4 plant accumulates lignocellulose more than other forage crops and this lowers its digestibility. The main parts used as fodder in sorghum are the leaves and the stems which form the main lignocellulose component of the plant**.** Since lignification has negative impacts on forage quality, this study therefore evaluated potential sorghum genotypes for lignin, cellulose and hemicellulose content and selects those with the lowest levels of the three cell wall components.

2.17 Lignin in forage sorghum

Recent studies have highlighted the presence of lignin in plants in within the *poaceae* family (angiosperm monocots). The presence of lignin in sorghum forage lowers its digestibility especially of the leaves compared to stem (Contreras-Govea *et al*., 2010). Acid detergent lignin (ADL) concentration in forage sorghum leaves increases with maturity (Cummins *et al.*, 1981). Previous studies have suggested that increasing fiber digestibility of leaves and stem of forage sorghum through plant breeding would make this crop more competitive in nutritive value to other forages like corn (Contreras-Govea *et al*., 2010). Since the 20th century, wild mutants with low lignin have been identified in barley, maize, sorghum, rice (Christensen & Rasmussen, 2019). According to Frei (2013), three major approaches have been applied in reducing lignin levels in crops: (i) use of naturally occurring or induced brown midrib mutations, (ii) mapping of quantitative trait loci (QTL) influencing the lignin content, which can be applied in marker assisted selection; and (iii) genetic modification of lignin biosynthetic genes to generate transgenic crops with altered lignin content or composition.

The extent of lignification tends to vary with variation in certain factors including plant age, genotype and abiotic and biotic stresses. According to Kramer *et al.* (2012), lignification has been reported to increase with increasing plant age especially in grasses. In addition, large genotypic differences in lignin content within the same species have been reported. For example, studies on genotypic variation in rice straw, the values reported on lignin range from 1 to almost 12 percent (Jahn *et al*., 2011). Moreover, abiotic and biotic stress factors tend to affect the lignification of crops. It is therefore important to evaluate different sorghum genotypes in order to understand the variation in lignin content as affected by the factors. The current study focuses on the effect of temperature, maturity stage and genotype on the lignin content of twenty sorghum genotypes comprising of sweet sorghum, *bmr* gene sorghum, commercial fodder sorghum and locally landraces.

2.18 Lignin biosynthesis

Lignin biosynthesis is an important process as it helps in plant growth, organ development, lodging resistance and also in response to a variety of biotic and abiotic stresses (Huang & Xu, 2021; Liu *et al.*, 2018). The structural unit of aromatic lignin includes three monolignols namely p-caumaryl, coniferyl and synapyl alcohols which are polymerized into p-hydroxiphenyl (H), guaicyl (G) and Syringyl (S) lignin (Harris & Trethewey, 2010; Wang *et al.*, 2020). The biosynthesis of lignin is via the phenylpropanoid pathway, a process where phenylalanine is deaminated to form cinnamic acid. Cinnamic acid is then hydroxilized, followed by *O*-Methilations and side chain modifications (Frie *et al*., 2013). The biosynthetic process requires a total of ten enzymes including; peroidases (pox), laccases (LAC) or other polyphenol oxidases that transfer electrons from monolignols to electron receptors. There are three main phases of lignin synthesis: (i) synthesis of monolignols in the symplastic shikimate and phenyl-propanoid pathway, (ii) export of monolignols to the apoplast, and (iii) activation of monolignols by enzyme-mediated formation of monolignol radicals in the apoplast and their polymerization to form complex lignin polymers (Frie *et al*., 2013). Oxidized monolignol radicals then couple onto each other to form three dimensionally cross-linked structures through polymerization (Chen & Dixon, 2007; Frei *et al.*, 2013). This constitutes the last step of lignin biosynthesis in plants. The enzymes that are involved in lignin synthesis interact with reactive oxygen species such as hydrogen peroxide or superoxide that accumulate excessively when plants undergo a period of abiotic and biotic stresses (Christensen & Rasmussen, 2019).

a) Plant growth and development

The role of lignin in plants is plant growth and development and regulation of seed propagation. Plant growth was shown to be inhibited and deformed when the biosynthesis of H units of lignin was interfered with (Bonawitz *et al.*, 2010). In some plants, deposition of lignin in seed coat can protect the seeds from external adverse factors before germination (Derikvand *et al.*, 2008; Liljegren *et al.*, 2000; Moura *et al.*, 2010). In a low-lignin *Arabidopsis thaliana* mutant, reduced seed germination rate, stunted growth and reduced number of seeds was reported in comparison to wild type Liu *et al*. (2018).

b) Role of lignin in plant lodging resistance

Lodging in crops negatively affects growth and yield if not prevented. Numerous studies have shown that lodging resistance in crops is related to plant height, biomass, stem diameter, composition and characteristics of stem cell walls (Islam *et al.*, 2007). Lignin accumulates in cell wall and in-turn significantly improves the mechanical strength of plant stalks and prevents lodging (Islam *et al.*, 2007; Matsumoto-Kitano & Nakamura, 2017). Previous work has highlighted the important roles that lignin content and activities of lignin biosynthesis enzymes (PAL, 4CL, CAD and POD) in lodging resistance in *Fagopyrum esculentum* Moench varieties (Hu *et al*., 2017; Li & Wang, 2020).

c) The link between lignin biosynthesis and plant stress adaptation

Plant cell wall is the first hurdle against external dangers and generally plants under biotic and abiotic stresses react by accumulating reactive oxygen species, coupled with an increase in lignin accumulation (Moura *et al.*, 2010). It is therefore obvious that lignin metabolism has a certain relevance with plant disease resistance, insect resistance and tolerance of drought, salt, heat, cold, heavy metals and other stresses (Moura *et al*., 2010). According to Frei (2013), interaction of lignin with abiotic stresses in crops can be categorized in two ways: (i) by abiotic stresses influencing lignin biosynthesis and therefore affecting the lignin content of crops, and (ii) lignification of crop tissues affect plant fitness and can confer tolerance to abiotic stresses. Regarding biotic stresses, lignin provides a physical barrier against initial ingress (Bonello *et al.*, 2003), and in most plant species lignin and lignin-like phenolic polymers are induced and rapidly deposited in cell walls in response to both biotic stresses, which may limit further growth and/or confine invading pathogens (Bonello *et al*., 2003; Menden *et al*., 2007; Wuyts *et al.*, 2006).

d) Roles of lignin biosynthesis in plant insect pests and diseases resistance

Lignin accumulation plays an important role in the process of plant resistance to insect pests (Frei, 2013; Jannoey *et al*., 2015; Santiago *et al.*, 2013). Abiotic or biotic stresses including pathogens have been shown to induce the expression of genes encoding monolignol biosynthetic enzymes in many plant species such as; thale cress (*Arabidopsis thaliana*), rapeseed (*Brassica napus* subsp. Napus) and rice (*Oryza sativa*) (Duan *et al.*, 2014; Olsen *et al.*, 2008; Zhao *et al*., 2009). In research by Duan *et al.* (2014), rice *PAL*, *C4H* and pathogenesis-related 9 (*PR9*) genes that associated with lignin biosynthesis were significantly up-regulated in brown plant hopper-infested insect-resistant rice varieties. These results suggest that these genes may synergistically participate in lignin biosynthesis which regulates the insect resistance of rice. Working with *Chrysanthemum* sp, Wang *et al.* (2020) found that an aphid penetration-induced transcription factor CmMYB19 and enhanced the expression of lignin biosynthesis genes and lignin accumulation, which limited the invasion of aphids and increased aphid tolerance. It can therefore be summarized that lignin can be used as a barrier directly or through the associated hormone signal pathway to increase insect resistance of plants.

e) Role of lignin in plant drought and salt stress tolerance

Drought and high salt are two of the abiotic stresses that are severely harmful to plants, and these stresses often cause irreversible damage (Duan *et al.*, 2014). The two phenomena (extreme drought and high salt stress) usually occur simultaneously and induce osmotic stress that causes plant cells to lose water and eventually die if the factors are not controlled. This significantly affects plant growth and development resulting in serious losses of crop yield (Agarwal *et al.,* 2012; Chaves *et al.*, 2009). Lignin alters the damage by reducing cell wall water penetration and transpiration which in-turn helps to maintain cell osmotic balance and protective membrane integrity (Abu-Zanat *et al.*, 2003). Enhancement of lignin biosynthesis under drought stress has been shown by many studies in *A. thaliana* (Zhang & Zhou, 2011), apple (Yang *et al.*, 2012) and tomato (Duan *et al*., 2014). In a study by Hu *et al.* (2009), the expression levels of lignin biosynthesis related genes (*CAD* and *COMT*) and lignin content in leaves of inbred maize lines under drought stress were significantly positively correlated with their drought tolerance. In another study by Fan *et al*. (2006), a significant up-regulation of *CCR* -a key gene in lignin monomer biosynthesis pathway, under the drought stress condition, was reported in the root elongation region of maize. Recent studies have also shown involvement of lignin deposition in plant salt tolerance. Shafi *et al*. (2015) found that tolerance of transgenic *Arabidopsis thaliana* was enhanced by overexpression of the *Potentilla atrosanguinea* superoxide dismutase (*SOD)* and *Rheum australe* ascorbate peroxidase (*APX*) gene by increasing lignification process. In another study by Park (2011), potatoes (*Ipomoea batatas) IbLEA14* genes also boosted transgenic callus salt and osmotic stress tolerance by enhancing lignin accumulation. Therefore, an impermeable lignin-rich barrier on the cell wall confers plants tolerance to the drought and high saline environment.

2.18.1 Hemicelluloses in sorghum forage

Hemicelluloses are components of plant cell wall that are extracted by dilute alkali then precipitated by ethanol (Ismail, 2017). Chemically, hemicelluloses are a class of heteropolymers consisting of side chains and are composed of pentose, mannose, galactose, glucose, and five-carbon sugars (Brunner, 2014). Structurally, hemicellulose is not related to cellulose because it has an amorphous structure with little strength while cellulose has a strong crystalline structure (Machmudah & Goto, 2017). However, some studies suggest that hemicellulose are intermediates in cellulose biosynthesis (Ismail, 2017). Each hemicellulose unit is conneted to another through covalent and H bonds to cellulose via H bonds and to lignin via cinnamate ester linkages (Ismail, 2017) It also has shorter side chains and an extensive cross-link to other cell wall constituents (Machmudah & Goto, 2017). Figure 3 below shows the structure of hemicellulose

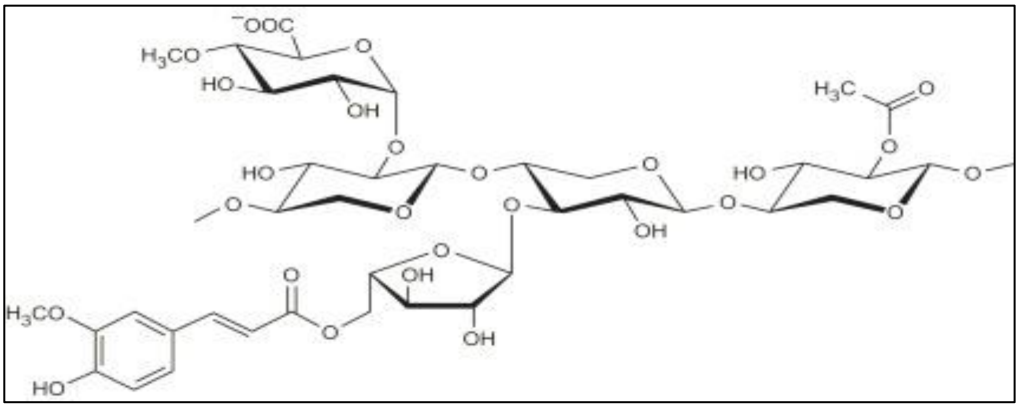

Figure 2.3: Hemicellulose structure

The main role of hemicellulose in plants is to provide rigidity and flexibility on the cell wall and to to offer water retention in the plant cell wall (Betts *et al.,* 1991). Hemicellulose is classified according to the chemical structure and three predominant types are found, distributed through various species (Betts *et al.,* 1991). They are classified as xylans, glucans and mannans with xylans being more predominant type in angiosperms (Betts *et al.,* 1991) whereas mannans are found widely in gymnosperms (Betts *et al.,* 1991).

2.19 Synthesis of hemicellulose

Biosynthesis of hemicelluloses occurs in the golgi apparatus and transmitted to the plasma membrane by vesicles (Scheller & Ulvskov, 2010). The process begins by the synthesis of NDP sugars including d-xylose, d-mannose and d-galactose in the hemicellulose backbone chain and l- arabinose, d-galactose, and 4-O-methyl-d-glucuronic acid in the side chains. The synthesis is by the action of golgi membrane localized glycosyl-transferases and are transported to the cell membrane by golgi derived vesicles. The final step is integration of hemicellulose in the cell wall (Liew *et al.,* 2019).

2.19.1 Role of hemicellulose in plants

The main role of hemicellulose in higher plants is to maintain cell wall organization, cell wall expansion, providing cell wall strength (Scheller & Ulvskov, 2010). Xyloglucan the main hemicellulose in cereals serves as a physical barrier to pathogens preventing them from invading, colonizing the plant, and protecting plants from aluminum toxicity. Xylem deficient mutants of plants exhibit collapsed xylem vessels and severely impaired growth, fertility and have decreased mechanical strength. This is an indication that hemicellulose is important in providing strength to the secondary cell wall (Kuki *et al*., 2020). McLaughlin (1980) also reported that in the cell walls of *Quercus alba* (white oak), xylem cell walls may function as food reserves. According to Scheller and Ulvskov (2010), hemicelluloses also contribute to the strength of the plant cell wall by forming network with cellulose fibres and also function in maintaining cell wall stability and rigidity.

Hemicelluloses are branched and intertwined to cellulose microfibrils thus they fill the spaces between the cellulose molecules and strengthen the cell wall. As the cell grows, hemicellulose helps in modulating cell wall extension as they loosen the cell wall thereby allowing for expansion (Cosgrove., 2005). As the cell expands during crop growth and development period, they adjust their size and shape in order to respond to the prevailing internal and external conditions. According to Scheller and Ulvskov (2010), hemicelluloses also help the plants coordinate communication between cells and between cells and the environment and also modulate cell wall characteristics.

Hemicelluloses also help plants to build defense mechanisms towards, pathogen attack and extreme environmental factors (Van Sandt *et al.,* 2007). In the event of pathogen attack, alterations in hemicellulose composition and structure can augment cell wall modification, hence making it more difficult for pathogens to enter the cell wall. This alteration helps in the plant's resistance to diseases. Hemicelluloses also function as biochemical signaling pathways that affect various physiological processes and influence plant responses to environmental stimuli and stress conditions (Hayashi, 1989).

2.20 Cellulose

Celluloses are deposited on the primary cell wall as polysaccharides during cell growth (Griffins & North, 2017). Structurally, cellulose is crystalline in nature and is cross-linked by hemicellulose (Zhu *et al*., 2019). Cellulose comprises of D-glucose residues linked via B B (1-4)-glycosidic bonds (Nishiyaa, 2009). Based on the nature of the crystalline structure, cellulose is grouped as cellulose Iα, 1β, II, III, and IV (Nishiyama, 2014) but only 1α and Iβ are synthesized naturally.

a) Cellulose biosynthesis

In plants, cellulose biosynthesis occurs in the plasma membrane where it is also organized into microfibrils which then undergo crystallization and into glucan chains (Polko & Keiber, 2019). UDP glucose reacts to form cellubiose residues that are added to the reactive ends of glucan chains. These chains are the ones that form globules which eventually form microfibrills (Betts *et al.,* 1991; Morreel & Ralph, 2014). The biosynthesis process is stimulated by a membrane bound enzyme glucosyl-transferase the same enzyme responsible for the synthesis of callose (Figure 2.2). Cellulose biosynthesis is influenced by environmental perturbations hence its content in plants is also dependent on environmental factors.

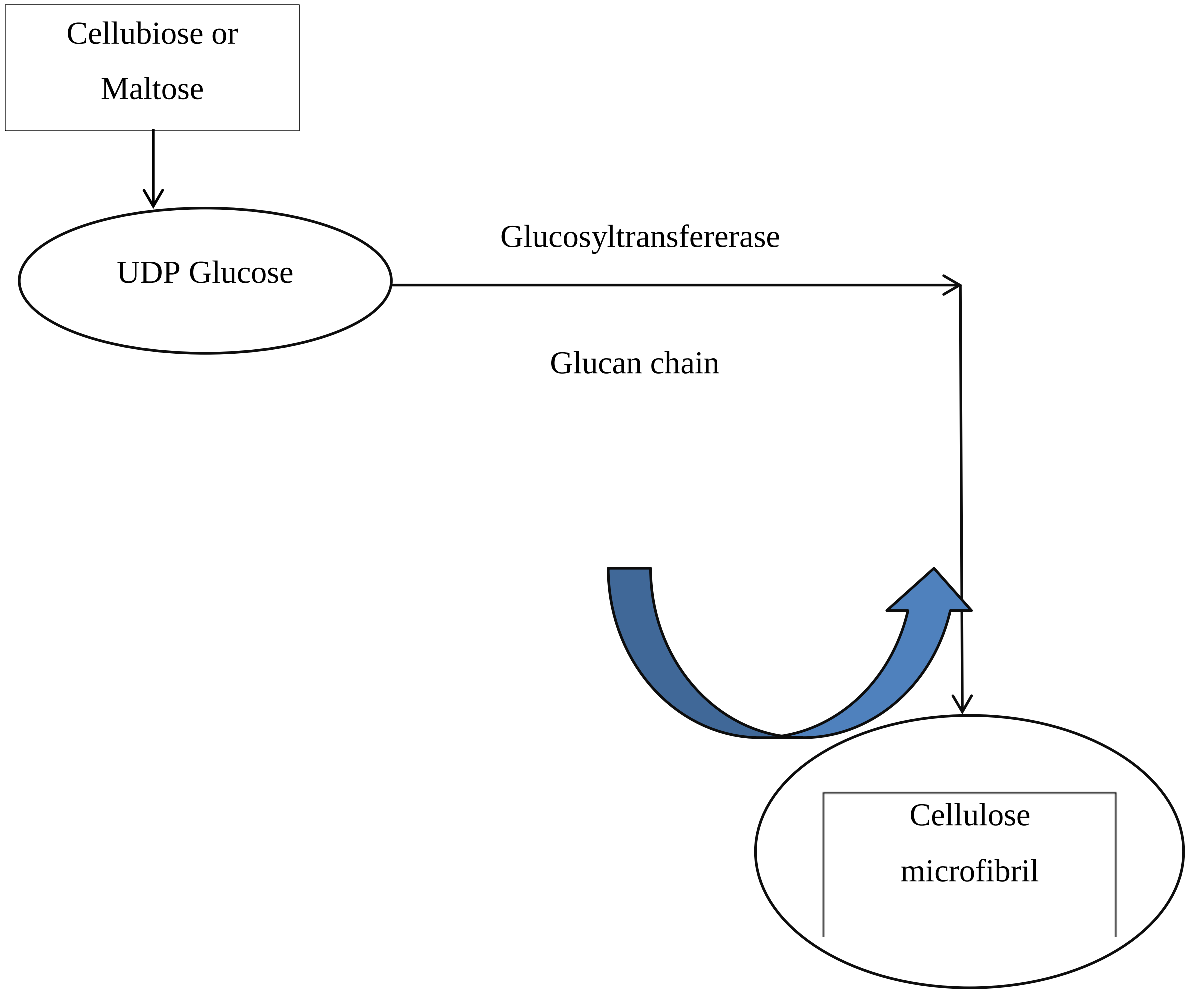

Figure 2.4: Cellulose biosynthesis

b) Role of cellulose in plants

Cellulose helps in cell expansion and growth which is accomplished through wall relaxations (Bewley *et al.,* 1994). The cell wall relaxations result in decreased water potential in the cell leading to water uptake and increased turgor. The cell wall expansion process is through the activities of expansin enzyme (Kang *et al.,* 2013).

2.21 Factors affecting fodder yield and quality of sorghum fodder

Fodder quality is a multifactorial attribute that is dependent on crop species, environmental conditions, plant maturity stage and plant part among others. Plant processes such as growth and development, photosynthesis and respiration respond to changes in environmental factors which in turn influence fodder quality. Environmental factors that affect fodder quality include temperature, precipitation, light and soil chemical properties (Bilalis *et al.,* 2017).

2.21.1 Effect of temperature on fodder quality

Temperature requirements for each crop are unique and therefore the temperature of a location dictates where a crop will grow within the agroecological zone (Ulukan, 2008). Temperature variation affects plant physiological processes such as photosynthesis, respiration and transpiration (Ahmad *et al.,* 2009). Warm temperatures hasten plant growth and development and reduce leaf/stem ratios and forage digestibility (Andueza *et al.,* 2010). Sugar accumulation in plants is also dependent on temperature as highly digestible sugars will accumulate at temperatures below the optimum for growth. High ambient temperatures bring about rapid rate of maturity of forages and a rise in cell wall content in the stems and leaves of both temperate and tropical grasses. Kumar and Tuti (2016) reported that alfalfa grown at 17°C took 52 days to reach early bloom but only 21 days at 32°C. High temperature also causes a decrease in fodder digestibility by increasing lignocellulose deposition in the cell wall.

2.21.2 Effect of soil chemical properties on fodder quality

Soil chemical properties include essential elements, Cation Exchange Capacity (CEC), base saturation, soil pH and liming, buffer pH and soil organic matter (Scheepers & du Toti, 2016). Availability of essential elements in the soil affects vegetative growth of fodder crops. Higher supply of available nitrogen to plants increases photosynthetic ability and vigorous growth of green parts of fodder plants (Colapietra & Alexander, 2005). Ball *et al*. (2001) found that 30 pounds per hectare of nitrogen added to a switch grass field raised forage crude protein from 5.3% to 6.4% and also increased forage intake by animals. Potassium is also vital to fodder growth and quality in enzyme activation, enhancing water absorption and use and in protein synthesis.

Soil pH affects soil nutrient availability and hence directly affect fodder quality and yield (Colapietra & Alexander, 2005). Harischandra *et al*. (2015) reported that the use of inorganic and organic fertilizers improved soil nutrient level and yield of fodder sorghum. Increase in potassium levels in calcareous clay loam soil increased protein content and crude fibre content of maize fodder (Ahmad *et al*., 2009). Sorghum is produced in different agro-ecological zones of Kenya with varying soil chemical properties thus it is important to evaluate various potential sorghum genotypes for nutritional quality and yield in different soils.

2.21.3 Effect of genotype on fodder quality

Plant physiological processes which are responsible for fodder quality aspects are genetically controlled hence there are variations in plant morphological and quality traits within genotypes of the same species. Interaction between genotype and environment may also be contributing to the variation in quality of different genotypes. For example, higher nutritive value in legumes as compared to C_4 grasses is due to higher crude protein content which leads to their higher digestibility (Wallau *et al*., 2022). Temperate or forage plants such as rye and ryegrass, are also higher in nutritive value than tropical or warm-season forage plants such as Bermuda grass and Bahia grass (Wallau *et al.*, 2022).

Similarly, there are so many variations in forage nutritive value among plants of the same genus and between genotypes of the same species (Wallau *et al.*, 2022). In an experiment to determine the effects of corn genotypes and plant densities on the forage and dry matter yield of corn**,** Yilmaz (2007) reported a significant forage yield effect by genotypes. Turgut *et al*. (2005) also reported on significant effects of maize hybrids on forage leaf ratio, stem ratio, ear ratio, green herbage yield and dry herbage yield significantly. Tokas *et al.* (2021) also found that different single cut sorghum genotypes showed variations in dry matter yield, crude protein yield and dry matter digestibility when subjected to different levels of phosphorous and nitrogen application. In order to achieve optimum fodder quality from sorghum, selection of genotypes with the best nutritive fodder quality is an option that can be explored by scientists.

2.21.4 Effect of stage of harvesting on sorghum fodder quality

Fodder yield and nutritional quality are both affected by stage of maturity (Amin, 2011). As fodder crops advance in maturity, quality of proteins, lipids, and soluble carbohydrates decreases while other indices such as DM content increases (Kim *et al*., 2001). Lignification of the cell wall also increases with fodder maturity (Kim *et al*., 2001) thus it is important to harvest fodder at a stage that gives more yield but also optimizes nutritional quality. According to Firdous and Gilani (2001) maize fodder should be harvested between eighth and ninth week after planting in order to target the optimum nutritive and digestible quality feed for livestock. Optimum sorghum fodder yield and quality was also achieved at flowering stage as observed by Firdous and Gilani (2001) while millet fodder harvested at pre-booting stage yielded more DM and nutritive value (Grewal *et al*., 2003) compared to other earlier stages.

In research to determine the effect of maturity on production efficiency, nutritive value and *in situ* nutrients digestibility of three cereal fodders, Khan *et al.* (2007) found that Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF), Acid Detergent Lignin (ADL), Water Soluble Carbohydrate (WSC) contents and Dry matter significantly increased with advancing age. These findings are in agreement with Atis *et al*. (2012) results which showed that sorghum fodder should be harvested at physiological maturity and dough stages in order to maximize on yield and nutritional quality. Most studies on sorghum fodder quality have concentrated on in-vitro and in-situ digestion aspects and how hydrocyanic acid affects livestock performance. However, there is still a gap of knowledge on how different site factors affect the concentration of prussic acid and its relationship with lignin content in forage. Thus, the proposed study aims at evaluating the influence of genotype, stage and temperature variations on major anti-nutritive quality attributes and biomass of selected fodder sorghum genotypes.

CHAPTER THREE

LIGNOCELLULOSIC COMPONENT OF POTENTIAL FODDER SORGHUM GENOTYPES AS INFLUENCED BY DEVELOPMENTAL STAGE AND GROWING TEMPERATURE

Abstract

Sorghum presents a good alternative to maize as green fodder for the livestock sector in Kenya. However, its use as livestock feed is limited by high concentration of lignocellulose that leads to low digestibility. This study was conducted to evaluate the influence of environment and stage of crop development on lignin, cellulose and hemicellulose content of selected fodder sorghum genotypes. The field experiment was arranged in RCBD replicated three times at Egerton University, Rongai and in Marigat in the years 2019 and 2020. Twenty sorghum genotypes obtained from ICRISAT, Kenya genebank and farmers' collections were evaluated and sampled at booting and dough stages of development. The samples were evaluated for lignin, cellulose and hemicellulose content using methods according to Vansoest (1991). Analysis of variance was done through generalized linear model using *lm* function of R version 4.3.0 (R) statistical software. Treatment means were separated through Tukey's honest significant difference (HSD) at $P \leq 0.05$. Cellulose, hemicellulose and lignin content varied among genotypes across the three environments. In 2019, sorghum lines IS11442, IS25547 and IS11612 recorded the highest cellulose (39-40%), hemicellulose (14-21.6%) and lignin (23-24%), respectively. Lines B35, EST 37 and EST 20 exhibited lower percentages 21%, 14.16% and 15% compared to 26.23%, 19.92% and 15.54% in the control line E6518. Cellulose, hemicellulose and lignin content significantly increased as crops matured. At booting stage, crops had lower values of cellulose, hemicellulose and lignin compared to dough stage. Location alone had significant ($p \leq 0.05$) effect on the three parameters with sorghum grown at Egerton University exhibiting the lowest lignocellulose content followed by Rongai and Marigat, respectively.

3.1 Introduction

Lignocellulose refers to structural carbohydrates (polysaccharides) that form part of plant cell wall and are responsible for cell wall strength, rigidity and shape. The cell wall has several layers which are visible under the microscope. The layers include the middle lamella, the primary cell wall and three layers of secondary cell wall (Kirk, 1983). The primary cell wall is composed almost exclusively of polysaccharides (complex carbohydrates) including neutral detergent fibres which are primarily lignin, cellulose and hemicellulose. Cellulose consists of a linear chain of glucose (sugar) units with β-1, 4 linkages which are broken by cellulase enzyme produced by microorganisms in the rumen (Palackal *et al.*, 2007). Hemicellulose on the other hand is more complex polysaccharide with different types of sugars bound by α-1, 4 linkages and is closely associated with lignin (Scheller & Ulvskov, 2010). The digestibility of hemicellulose in the gastrointestinal system of ruminants is slowed down by the presence of lignin (Moore &Jung, 2001).

Plant cell wall is mainly composed of lignin- a polymer deposited from monolignols derived from the phenylpropanoid pathway in vascular plants as part of the process of cell maturation (Moore & Jung, 2001). Lignin is an anti-quality component in forages due to its hindrance to the digestion of cell-wall polysaccharides and therefore has negative impact on the nutritional availability of plant fiber (Pond *et al.,* 2005). Lignin acts as a physical barrier to microbial enzymes hence has a direct and often important impact on the digestible energy (DE) value of forage (Moore & Jung, 2001). Lignin content in a forage plant depends on the plant maturity whereas as the plant matures, lignin content increases and the location of deposition changes (Moore & Jung, 2001). Since lignification is under genetic control, there are considerable differences in lignin concentration and composition among species and even genotypes within species (Moore & Jung, 2001). Lignification in a forage crop also depends on the plant part used as fodder as the process tends to be most intense in structural tissues such as xylem and sclerenchyma. Plant organs such as stems are less digestible since they contain high concentrations of these tissues, than other plant parts such as seeds (Boerjan *et al.,* 2003). Lignification process responds to environmental factors that affect the extent to which fodder is digestible. Factors like temperature, soil moisture, light, and soil fertility can have either direct or indirect effects on lignification (Moore *et al.,* 2020). The effect of environmental temperature on the accumulation of lignin, cellulose and hemicellulose is not fully documented thus this study was aimed at evaluating the effect of growing temperature on lignocellulose content of twenty sorghum genotypes grown in three locations in Kenya.

In view of the expanding dairy and livestock sector in Kenya, sorghum is one of those crops with the potential to offer solution to the feed challenges. Kenya's livestock sector especially the dairy sub-sector has relied on maize as a good source of feed despite the challenges facing maize production, which includes emerging pests, diseases, climate change related limitations and human competition for food. Sorghum is a suitable alternative to maize as fodder for the livestock sector in Kenya. However, its use as livestock feed is limited by the high concentration of lignocellulose (Getachew *et al.,* 2016). Sorghum being a C_4 plant accumulates lignocellulose more than other forage crops and this lowers its digestibility. The main parts used as fodder in sorghum are the leaves and the stems which form the main lignocellulose component of the plant**.** This study was conducted to test the hypothesis that environmental temperature and crop developmental stages have no significant effect on lignocellulose fodder sorghum genotypes.

3.2 Material and Methods

3.2.1 Study Site Description

These experiments were conducted between March and September, 2019 and repeated between May and October in 2020. In 2019 the experiments were conducted in two sites (Egerton University and Marigat) in Nakuru and Baringo Counties with varying soil chemical properties and temperature conditions. In the year 2020 the studies were conducted in three regions; Egerton University, Rongai and Marigat. Egerton University (0º 22' S; 35º 55' E) is at an elevation of 2286 m above sea level and is located in agro-ecological zone (AEZ) III classified as an upper highland (UH) zone. The area has a bimodal pattern of rainfall with both long and short rains. The long rains occur around March and August while the short rains occur between October and December. The mean annual temperature is 14.9 ^{o}C while average minimum and maximum daily temperatures are 7.9 ^{o}C and 21.9^{o}C, respectively. The total annual rainfall ranges between 1000-1200 mm per annum. The soils are deep sandy loam classified as *Mollic andosols* with sandy loam texture and good water holding capacity with pH range of 5.5 to 6.5 and high organic matter content (Jaetzold *et al*., 2007).

On the other hand, Rongai (0^{o}23'N; 35^{O} 51'E) is at an altitude of 1890 m above sea level is located in AEZ IV in lower highland (LH4) in Nakuru county. The annual temperature ranges between 12.6-18.8^{o}C while the total annual rainfall is 900 mm. The soils are *Vitric andosols* with a silt-loam texture with pH range of 5.5-5.8 and moderate organic matter content and moderate water holding capacity. The third site was in Marigat (0°46'

N;35°98’E) at an altitude of 1066 m above sea level is categorized as an ASAL in AEZ V (Lower midland 5) with low and variable rainfall. The mean annual temperature is 24.5 °C while minimum and maximum daily temperatures are 16.8 °C and 32.3 °C. The total annual rainfall ranges between 590-652 mm. The soils in the area are Cambisols which are mainly moderately to poorly drained, very deep, strongly calcareous, saline and sodic and the texture is fine sandy loam to clay. The soil pH varies between acidic to slightly neutral with moderate organic matter content (Jaetzold *et al.*, 2007).

3.2.2 Experimental procedure (layout, design and crop management)

The fields in all the three sites were prepared to a suitable tilth for sorghum seed prior to the onset of long rains. Twenty sorghum genotypes including two brown-midrib gene varieties (bmr37 and bmr36), 1 stay green variety, 1 Genebank variety (GBK013132), 10 potential fodder genotypes from ICRISAT (IS11612, IS11613, IS11721, IS11838, IS11442, IS2331, IS25557, IS9201, IS25563 and IS25547), 5 farmers’ collections and crosses (nguugu, EST 20, S79, EST 49 and IESV91105LT) and 1 commercial fodder variety from KALRO (E6518- control) were evaluated. The genotypes and their respective characteristics are outlined in Table 3.1. The treatments were arranged in a RCBD and replicated three times per site. Each experimental unit measured 4 m x 5 m which accommodated five rows of sorghum plants. Each experimental block had twenty experimental units which corresponded to the number of treatments.

Sorghum seeds were sown at the rate of 10 kg ha^{-1} and at a spacing of 60 cm by drill at a depth of 2 cm and later thinned at 20 cm height to spacing of 60 cm x 15 cm giving a plant population of 111,111 per hactare. Phosphorous fertilizer was applied during sowing at the rate of 60 kg of P_2O_5 ha^{-1}. Top dressing was done by applying nitrogen at the rate of 60 kg N ha^{-1} when the crops were 4 weeks old. Weed control was manually done by in-plant tillage cultivation and Thunder 145 O-TEQ (Imidacloprid 100 g/l + Beta-cyfluthrin (45 g/l, Triachem, city/state/country) was applied to control aphids and fall armyworm when it was necessary at the rate of 0.2 L/Ha. Bird scaring was done through engagement of bird scarers. Standard laboratory procedures were followed in analyzing chemical properties of the soil. Sampled soils were air dried then sieved with 2 mm mesh and analyzed for pH (Soil: H_20), total N (Kjedahl method), total carbon (Walkley & Black, 1934), CEC (Chapman, 1965), and available P according to Okalebo *et al.* (1986). Exchangeable K was extracted with 1.0 M-ammonium acetate at pH 7 and measured by atomic adsorption spectrophotometer (©Analytik jena).

In 2020, another experiment was set up using 10 sorghum cultivars (marked with a superscript “a” in Table 1 selected during 2019 season based on initial HCN-p (low, intermediate, or high HCN-p). From this experiment, growth parameters (days to 50% heading and plant height at dough stage) and air temperature measurements were taken. Plant height was measured from the crown level to the leaf collar of the flag leaf using a graduated pole. Days to 50% heading were counted from the date of emergence to the date when 50% of the plants in each experimental unit had emerged panicles. Daily maximum and minimum temperatures were measured using a digital maximum and minimum thermometer, and the data were later converted to average daily temperature.

Table 3.1: Selected sorghum genotypes grown in Egerton, Rongai and Marigat

Sorghum genotype	Source	Attributes
IS11612	ICRISAT	High biomass
IS11442	ICRISAT	High bomass
IS25547	ICRISAT	Tall with thick stems
IS25557	ICRISAT	High tillering ability
IS11721	ICRISAT	High biomass
IS11838	ICRISAT	High regenerative ability, High biomass
IS2331	ICRISAT	High regenerative ability, High biomass
IS25563	ICRISAT	High regerative ability
IS9201	ICRISAT	High biomass
EST 36	ICRISAT/INDIA	*Bmr trait
EST 37	ICRISAT/INDIA	Bmr trait
IS23787	ICRISAT/INDIA	Bmr trait
EST 20	Cross at Egerton	High stem sugar content
E6518	Kenya Seed	Commercial sorghum variety
IESV9110	ICRISAT	High regenerative ability, High tillering ability
GBK01313	Genebank	High biomass
Nguugu	Rwanda	Landrace
S79	ICRISAT	High tillering ability
B35	ICRISAT	Stay green trait
EST 49	Cross at Egerton	High biomass

*Bmr – brown midrib

3.2.3 Plant tissue sampling for laboratory analysis

Harvesting of plants for analysis was done at two developmental growth stages of sorghum as described below;

i. Booting stage: when the leaf sheath of flag was swollen, indicating an emerging panicle.
ii. Dough stage: when sorghum grain had a dough-like consistency, ideal stage for sorghum fodder harvesting as is associated with optimum dry matter and nutritive value (Lyons *et al*., 2019).

Harvesting of samples at each of the maturity stages above was done in the three middle rows of each experimental unit. From each of the three rows, four plants with the same height were harvested by cutting at the base, shredded using a fodder chopper and placed in a paper bag. The samples were labeled in the field and taken to the laboratory for drying and subsequent analysis.

Harvesting of plants for analysis was done at two developmental growth stages of sorghum as described below;

i. Booting stage: when the leaf sheath of flag was swollen, indicating an emerging panicle.
ii. Dough stage: when sorghum grain had a dough-like consistency, ideal stage for sorghum fodder harvesting as is associated with optimum dry matter and nutritive value (Lyons *et al*., 2019).

Harvesting of samples at each of the maturity stages above was done in the three middle rows of each experimental unit. From each of the three rows, four plants with the same height were harvested by cutting at the base, shredded using a fodder chopper and placed in a paper bag. The samples were labeled in the field and taken to the laboratory for drying and subsequent analysis.

3.2.4 Determination of lignin, cellulose, and hemicellulose

Lignin, cellulose, and hemicellulose contents of biomass samples were analyzed using acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL) methods (Vansoest., 1991). During NDF analysis, approximately 1.0 g of dried biomass sample was transferred into a crucible and 100 ml of neutral detergent solution (30 g sodium lauryl sulphate, 18.61 g EDTA disodium salt, 6.81 g sodium borate decahydrate, 4.56 g disodium hydrogen phosphate and 10 ml 2-ethoxy ethanol dissolved in 1L distilled water) at room temperature with 0.5 g of sodium sulfite and drops of n-octanol. The mixture was washed with boiling water then rinsed twice with acetone followed by drying for 8 hours at 105 °C and refluxed for 60 minutes after which it was filtered and washed three times with boiling water then rinsed twice with acetone. Calculations; neutral detergent fiber:

$$\text{NDF}\,(\%) = \frac{(\text{weight of crucible} + \text{weight of residue}) - (\text{weight of crucible})}{\text{weight of sample}} \text{x100}$$

Neutral detergent solution:

$$\text{NDS}\,(\%) = 100 - \text{NDF}\,(\%)$$

The ADF analysis was similar to that of NDF but using a different detergent solution, which is acid detergent solution (20 g cetyl trimethyl ammonium bromide) which was then dissolved in 700 ml distilled water. 27.56 ml of 96.7% Sulphuric acid was then added to the solution and then topped up to 1 L with distilled water. The ADL analysis started from covering the residues from ADF analysis with 72% H_2SO_4 (at 15 °C) solution and stirring three times at an hourly interval. The mixture was then filtered, washed with hot water and then dried at 105 °C for 3 hrs and then cooled down to room temperature. The residue was heated in furnace set at a temperature of 500 °C for 2 hrs. The crucibles were then transferred into a 100 °C oven for an hour before cooling in a desiccator to room temperature and then weighed. The percentage of weight loss was calculated from the initial and final weight difference.

The percentages of cellulose, hemicellulose and lignin were calculated as follows;

$\text{Cellulose}\ (\%) = \text{ADF} - \text{ADL}$

$\text{Hemicellulose}\ (\%) = \text{NDF} - \text{ADF}$

$\text{Lignin}\ (\%) = \text{ADL}$

3.3 Statistical Model and data analysis

Data was subjected to analysis of variance through generalized linear model using *lm* function of R statistical software. Effects were considered significant in all statistical calculations if the *P*-values were ≤ 0.05. Using *HSD.test* function of agricolae package in R, means were separated based on Tukeys HSD test. The following statistical model was fitted during the analysis of experimental results.

$Y_{ijklm}= \mu+ B_i +G_j +BG_{ij} +L_k + BE_{ik}+ S_l + GL_{jk} + eSG_{jl} + SL_{lk}+GLS_{jkl} +\varepsilon_{ijklm}$

where; μ= Overall mean; Bi=effect due to the ith blocking; G_j=effect due to the j^{th} genotype; BG_{ij} = effect due to interaction between ith block and jth genotype; L_k= effect due to the k^{th} location; BL_{ik} = effect due to interaction between ith block and kth location; S= effect due to the l^{th} stage of harvesting; GL_{jk}= effect due to the interaction between j^{th} genotype and kth location; $SG_{jl=}$ effect due to the interaction between j^{th} harvesting stage and l^{th} genotype.

SL_{kl}= effect due to the interaction between k^{th} location and l^{th} harvesting stage

SGL_{jkl}= interaction effect between j^{th} harvesting stage, l^{th} sorghum genotype and k^{th} location;

ε_{ijkl}= random error term normally distributed with mean zero.

3.4 Results

3.4.1 Average air temperature during crop growth period

The average air temperatures during the growing season ranged as follows; 24.21-27.29 ^{O}C, 21.75-22.92 ^{O}C and 16.52-19.53 ^{O}C for Marigat, Rongai and Egerton, respectively. Data on temperature is presented on Table 3.1

Table 3.2: Average air temperature in Egerton, Rongai and Marigat during crop growing period in 2020

Location	Average air temperature (^{O}C) (2020 cropping season)
Egerton	16.52-19.53
Rongai	21.75-22.92
Marigat	24.21-27.29

3.4.2 Influence of genotype and location on growth parameters and lignocellulose content in selected fodder sorghum genotypes

a) Influence of genotype and location on days to 50% heading

Number of days taken by sorghum plants to attain 50% heading were significantly influenced by interactive effect of location and genotype (Table 3.2). Plants grown in Marigat took shorter time to attain 50% heading followed by those grown in Rongai and Egerton, respectively. Sorghum variety E6518 took the longest time (121 days) to attain 50% heading across the three locations while B35 took the shortest time (85 days) across the three locations.

Table 3.3: Day 50% heading of selected sorghum genotypes grown in Egerton, Rongai and Marigat in 2020

Genotype	Location		
	Egerton	Rongai	Marigat
B35	111.33g	80.00h	64.00h
E6518	143.67c	120.00c	99.67ab
EST 37	133.00d	101.33e	101.67a
EST 20	149.00b	119.33c	95.67cd
IS25557	124.67f	87.67g	75.55g
IS11721	149.0b	126.33b	97.33bc
IS11838	133.33d	100.33e	80.33f
IS2331	154.0a	134.00a	98.33a
IS25547	129.67e	114.33d	93.33d
IS9201	131.00de	93.67f	84.00e
CV (%)	0.46	1.85	1.09
HSD test	2.28	1.91	3.63

Values in a column, followed by different superscript letters are significantly different at p≤ 0.05. CV =coefficient of variation

b) Influence of genotype and location on plant height

Plant height was significantly (P≤0.05) affected by location and sorghum genotype in the year 2020 (Table 3.3). The tallest plants were registered in Marigat followed by Rongai and Egerton respectively. Genotypes E6518, IS1125547, IS1125557, 1S11721 and IS2331 had tallest (above 190 cm) plants while genotype B35 had the shortest (below 100 cm) plants across the three locations.

Table 3.4: Height of selected sorghum cultivars grown in Egerton, Rongai and Marigat at dough stage (cm) in 2020

Genotype	Plant height (cm)		
	Location		
	Egerton	Rongai	Marigat
B35	88.93^{h}	91.96^{j}	92.80^{h}
E6518	205.24^{b}	226.0^{a}	258.6^{a}
EST37	111.44^{g}	119.49^{i}	114.19^{g}
EST 20	155.40^{f}	168.47^{h}	173.80^{f}
IS25557	192.67^{d}	223.22^{b}	242.90^{b}
IS11721	192.9^{d}	213.3^{d}	219.3^{c}
IS11838	198.10^{c}	217.83^{c}	219.79^{c}
IS2331	196.9^{c}	196.83^{f}	208.84^{d}
IS25547	218.43^{a}	205.7^{e}	216.63^{c}
IS9201	183.47^{e}	191.53^{g}	186.57^{e}
CV (%)	0.46	1.85	1.09
HSD test	1.39	1.91	3.63

Values in a column, followed by different superscript letters are significantly different at $p \leq 0.05$. CV (%) =coefficient of variation

Table 3.5: Correlation Coefficient of lignin with days to 50% heading and plant height in 2020

	Lignin	Days to 50% heading	Plant height
Lignin	-	-	-
Days to 50% heading	0.692	-	
Plant height	0.749	0.869	-

**Correlation coefficient*

Results of lignin nd growth paramenters indicated that with 1 cm increase in palnt height, there is a 1% increase in lignin content in sorghum. Taller plants also had higher lignin content hence the positive correlation between plant height and lignin. Also, the longer the plant took in the field the higher the lignin content thus there was a positive correlation of lignin with days to 50% heading. Correlation coefficient in Table 3.5 indicate that 74.9% and 69.5% of variations in sorghum lignin content is due to plant height and days to 50% flowering respectively.

c) **Cellulose content**

Results showed that genotype, growth stage, location, year and their interactions had a significant (P≤.001) effect on cellulose content except for growth stage by year interaction (Table 3.6). Results from the study clearly indicate variation on cellulose content among sorghum lines and across growing environments (Table 3.7). The cellulose content among the genotypes ranged from 21.03% to 40.83%. The outstanding genotypes within the cellulose content range in 2019 were lines IS11442 (39 – 40 %) and B35 (21 – 23%) (Table 3.7). The trend was more or less similar in 2020 with IS11442, giving the highest cellulose content and

B35 recording low cellulose content (Table 3.7). These results are in agreement with previous researches in sorghum as shown by Miron *et al.* (2007), Carmi *et al.* (2005), Firdous and Gilani (2001) and Khan *et al.* (2007) in sorghum and millet who all reported variation in cellulose content among genotypes.

Table 3.6: Anova table output showing the mean sums of square values for the effect of genotype, location, stage, year and their interactions on cellulose (percent)

**Significant at P≤.001, NS not significant

Source	DF	Cellulose (percent)
Genotype	19	767.57**
Location	2	1276.53**
Stage	1	2178.17**
Year	2	1212.41**
Genotype*Location	1	95.61**
Genotype*Stage	38	32.6**
Genotype*Year	19	67.72**
Stage*Year	19	NS
Location*Stage	1	54.76**
Location*Year	2	677.39**
Genotype	19	767.57**

Table 3.7: Mean Cellulose content (%) of selected sorghum cultivars grown in Egerton, Rongai and Marigat I 2019 and 2020

Sorghum Genotype	2019	2020
IS11442	40.83 ^{a}	39.50 ^{bc}
IS25547	40.11 ^{ab}	40.74 ^{a}
IS11612	39.20 ^{b}	39.70 ^{b}
IS11721	36.81 ^{c}	36.76 ^{e}
EST 49	32.48 ^{d}	38.65 ^{cd}
IS11838	32.29 ^{d}	38.42 ^{d}
IS2331	31.51 ^{d}	34.16 ^{f}
IES9110	31.24 ^{d}	33.31 ^{f}
NGUUGU	31.06 ^{d}	30.87 ^{h}
IS25557	28.01 ^{e}	30.92 ^{h}
GBK01313	27.80 ^{ef}	32.15 ^{g}
EST 36	27.26 ^{ef}	31.71 ^{gh}
IS25563	27.14 ^{ef}	31.75 ^{gh}
IS9201	26.89 ^{ef}	31.65 ^{gh}
E6518	26.23 ^{f}	21.70 ^{l}
IS23787	24.44 ^{g}	27.04 ^{i}
EST 20	23.86 ^{g}	25.04 ^{k}
EST 37	23.76 ^{g}	26.18 ^{ij}
B35	21.03 ^{h}	25.77 ^{jk}
CV (%)	5.97	12.11
HSD test	1.80	4.15

Values in a column, followed by different superscript letters are significantly different at p≤0.05. CV (%) =coefficient of variation

Stage of development had a highly significant (p≤0.01) effect on cellulose levels of the sorghum genotypes with the general trend showing increased cellulose content as the crop matured. In most genotypes, sorghum lines harvested at booting stage exhibited lower levels of cellulose compared to those harvested at dough stage across the study environments during the two years (Figures 3.1 and 3.2). These results conform to previous work using different sorghum cultivars and different growing conditions as shown by Firdous and Gilani (2001) who observed a rapid rise in cellulose content up to flowering stage and a slight increase at milk and dough stages. However, Carmi *et al*. (2005) and Miron *et al.* (2006) who worked on sorghum and Filya (2004) who worked on maize reported contrary results, that cellulose content decreased with advancement in plant age.

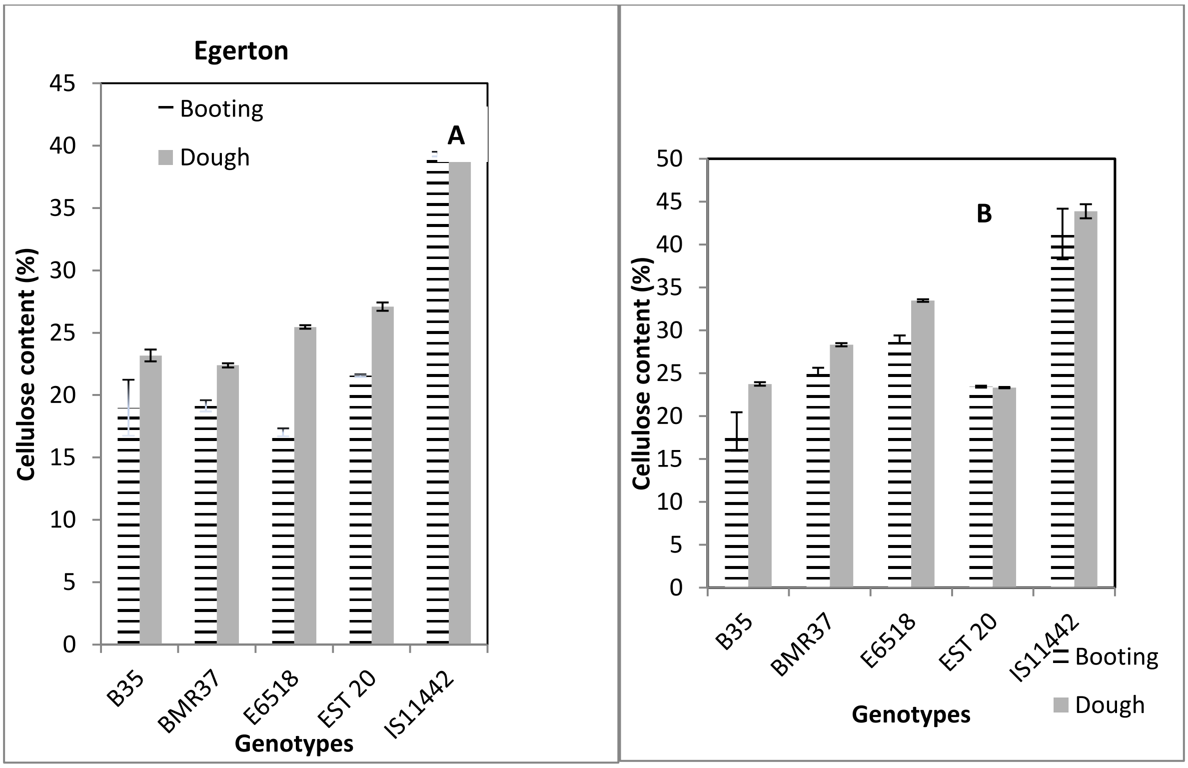

Figure 3.1: Cellulose content (%) of sorghum cultivars grown at (A) Egerton and (B) Marigat in the year 2019 at booting and dough stages

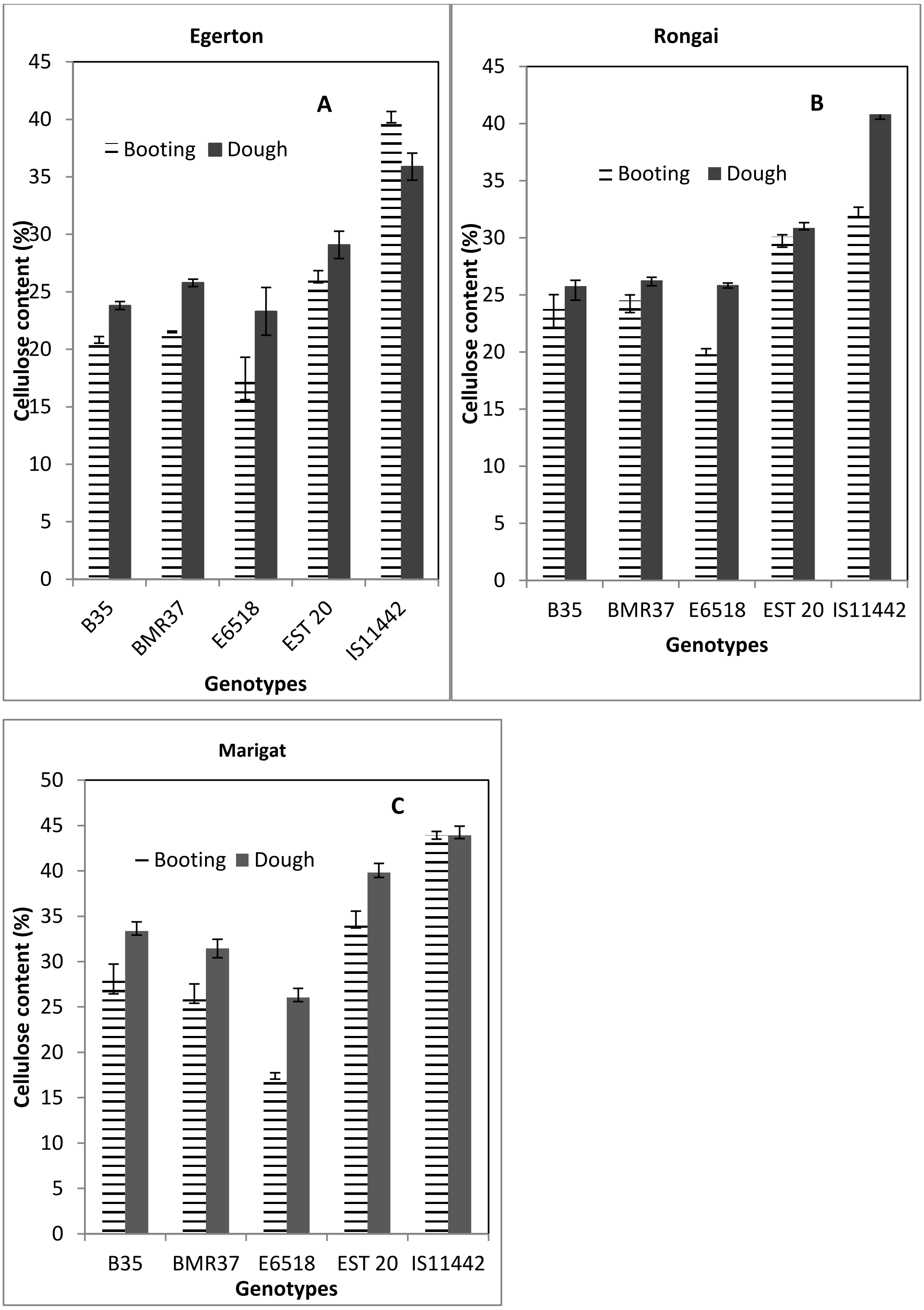

Figure 3.2: Cellulose content (%) of sorghum cultivars grown at (A) Egerton, (B) Rongai and (C) Marigat in the year 2020 at booting and dough stage

Results presented in Tables 3.8 to 3.11 and Figures 3.1 and 3.2 showed that sorghum genotypes grown in Marigat had the highest cellulose content followed by Rongai and

Egerton, respectively in both years. In the year 2019 season, the cellulose content ranged from 17.02 (E6518) to 39.34 (IS11442) at Egerton and from 18.74% (B35) to 43.14% (EST 49) at Marigat at booting stage (Table 3.8). The trend was more or less similar in the 2020 season, when the cellulose levels ranged from 20.03% (E6518) to 46.26%(IS11721) and from 26.06% (E6518) to 44.58% (EST 49) at booting and dough stages, respectively in 2020 (Table 3.8 and 3.9). Sorghum grown at Egerton had the lowest levels of cellulose which ranged from 17.40% (E6518) to 37.43% (IS25547) and 23.36% (E6518) to 42.01% (IS25547) at booting and dough stages, respectively in 2020 (Table 3.10 and 3.11). These results are in agreement with those of Okwaro *et al.* (1978) which aimed at determining the effect of genotype and location on the nutritive value of nine sorghum varieties grown under Kenyan conditions and reported a significant effect of location on cellulose content of sorghum. Interaction between location and genotype had significant effect on the cellulose content of sorghum genotypes. Similar results were also reported by Schetter *et al*. (2021) in a study of genotype-environment-management interactions on biomass yield and feedstock composition of photoperiod-sensitive energy sorghum. Schetter *et al*. (2021) reported that genotype-environment interaction significantly influenced cellulose content in sorghum.

Table 3.8: Cellulose content (%) of selected sorghum cultivars grown in Egerton and Marigat at booting stage in 2019

Sorghum genotypes	Egerton	Marigat
B35	18.99^{f}	18.74^{g}
EST 36	23.83^{d}	24.74^{ef}
EST 37	19.15^{f}	25.18^{ef}
E6518	17.02^{g}	28.95^{cde}
EST 49	18.31^{g}	43.14^{a}
GBK11313	24.55^{d}	25.12^{ef}
IESV9110	20.19^{e}	36.17^{f}
IS11442	39.34^{a}	41.22^{c}
IS11612	37.11^{b}	37.65^{e}
IS11721	30.99^{c}	38.47^{d}
IS11838	18.12^{g}	42.96^{a}
IS2331	21.70^{d}	32.78^{bc}
IS23787	20.50^{ef}	25.87^{ef}
IS25547	37.68^{b}	38.68^{a}
IS25557	26.60^{c}	27.44^{def}
IS25563	24.30^{d}	24.30^{ef}
IS9201	24.27^{d}	24.90^{ef}
NGUUNGU	21.13^{e}	32.06^{bcd}
EST 20	21.57^{e}	23.42^{f}
S79	24.15^{c}	24.74^{ef}
CV (%)	4.23	10.51
HSD test	1.92	4.84

Values of cellulose in a column, followed by different superscript letters are significantly different at $P \leq 0.05$. CV (%) =coefficient of variation

Table 3.9: Cellulose content (%) of selected sorghum cultivars grown in Egerton and Marigat at dough stage in 2019

Sorghum genotype	Egerton	Marigat
B35	23.17^{i}	23.74^{i}
EST 36	29.96^{ef}	30.52^{ef}
EST 37	22.39i	28.32^{i}
E6518	25.47^{h}	33.47^{g}
EST 49	32.97^{d}	35.51^{c}±
GBK11313	30.55^{e}	30.99^{ef}
IESV9110	26.85^{g}	41.76 ^{c}
IS11442	38.87^{d}	43.87^{a}
IS11612	40.35^{a}	41.67^{c}
IS11721	25.33^{h}	41.70^{c}
IS11838	35.33^{c}	32.78^{f}
IS2331	32.19^{d}	39.37^{d}
IS23787	22.64^{i}	28.76^{i}
IS25547	41.38^{a}	42.70^{b}
IS25557	27.41^{g}	30.58^{fg}
IS25563	29.74^{ef}	30.21^{fg}
IS9201	27.91^{g}	30.49^{fg}
NGUUNGU	29.77^{ef}	41.29^{c}
EST 20	23.33^{i}	27.10^{g}
S79	29.11^{f}	29.67^{gh}
CV (%)	2.34	1.81
HSD test	1.18	1.02

Values of cellulose in a column, followed by different superscript letters are significantly different at $P \leq 0.05$. CV (%) =coefficient of variation

Table 3.102: Cellulose content (%) of selected sorghum cultivars grown in Egerton, Rongai and Marigat at booting stage in 2020

Sorghum genotypes	Egerton	Rongai	Marigat
B35	20.82^{f}	24.77^{fg}	28.07^{g}
EST 36	25.56^{e}	30.30^{de}	34.95^{ef}
EST 37	21.51^{f}	24.52^{fgh}	26.47^{g}
E6518	17.46^{g}	17.46^{j}	20.03^{i}
EST 49	22.18^{f}	37.99^{hij}	$40.79\ ^{b}$
GBK11313	26.19^{e}	30.33^{de}	35.54^{efd}
IESV9110	21.13^{f}	33.06^{bc}	$39.72\ ^{bcd}$
IS11442	32.25^{d}	40.19^{a}	43.93^{a}
IS11612	36.39^{b}	39.06^{a}	$38.50b^{d}$
IS11721	33.19^{c}	34.41^{b}	46.26^{a}
IS11838	22.06^{f}	40.63^{a}	37.46^{cde}
IS2331	29.67^{d}	30.36^{de}	33.41^{f}
IS23787	22.45^{f}	26.60^{f}	27.00^{g}
IS25547	37.43^{b}	39.33^{a}	$40.09\ ^{bc}$
IS25557	25.87^{e}	29.57^{e}	34.38^{f}
IS25563	26.53^{e}	$30.64d^{e}$	34.63^{ef}
IS9201	26.31^{e}	30.01^{de}	34.85^{ef}
NGUUNGU	22.51^{f}	25.29^{f}	33.38^{f}
EST 20	21.76^{f}	22.63^{h}	23.17^{h}
S79	26.75^{e}	30.7^{cde}	34.01^{f}
CV (%)	7.21	5.05	8.67
HSD test	2.08	2.42	3.02

Values of cellulose in a column, followed by different superscript letters are significantly different at $P \leq 0.05$. CV (%) =coefficient of variation

Table 3.11: Cellulose content (%) of selected sorghum cultivars grown in Egerton, Rongai and Marigat at dough stage in 2020

Sorghum cultivars	Egerton	Rongai	Marigat
B35	23.80^{i}	23.77^{gh}	33.38^{i}
EST 36	28.98^{d}	30.27^{d}	40.22^{de}
EST 37	25.78^{h}	26.82^{f}	31.96^{j}
E6518	23.36^{i}	25.87^{fg}	26.06^{k}
EST 49	41.94^{a}	44.37^{a}	44.58^{a}
GBK11313	29.52^{fg}	30.62^{cd}	40.73^{de}
IESV9110	29.77^{fg}	32.65^{c}	43.52^{ab}
IS11442	35.89^{d}	40.82^{a}	43.93^{ab}
IS11612	35.14^{d}	40.97^{a}	42.04^{c}
IS11721	30.96^{fg}	35.14^{b}	40.56^{de}
IS11838	41.76^{a}	44.17^{a}	44.40^{a}
IS2331	31.40^{e}	36.32^{b}	36.33^{e}
IS23787	26.34^{h}	27.41^{ef}	32.40^{ij}
IS25547	42.01^{a}	42.08^{a}	42.26^{bc}
IS25557	28.76^{g}	29.80^{d}	37.18^{g}
IS25563	29.23^{fg}	29.64^{de}	39.82^{ef}
IS9201	29.08^{g}	30.18^{d}	39.47^{ef}
NGUUNGU	29.89^{fg}	30.77^{cd}	43.39^{ab}
EST 20	29.08^{g}	29.70^{d}	32.09^{ij}
S79	29.08^{g}	29.08^{d}	39.31^{f}
CV (%)	9.7	3.93	2.06
HSD test	1.49	2.41	1.32

Values of cellulose in a column, followed by different superscript letters are significantly different at $P \leq 0.05$. CV (%) =coefficient of variation.

Results showed that cellulose content sorghum sampled at dough stage was greater than those sampled at booting stage as shown by higher median values (Figures 3.3 A, B and C). Tukey analysis revealed that there was a significant difference between sorghum sampled at booting stage and dough stage as shown by the different letters above the whiskers. Overall, sorghum planted in Marigat had the highest mean cellulose, followed by Rongai while sorghum grown in Egerton had the lowest cellulose content as indicated by higher median values in Figures 3.3 A, B and C. Mean separation showed a significant difference among sorghum sampled in Egerton, Rongai and Marigat.

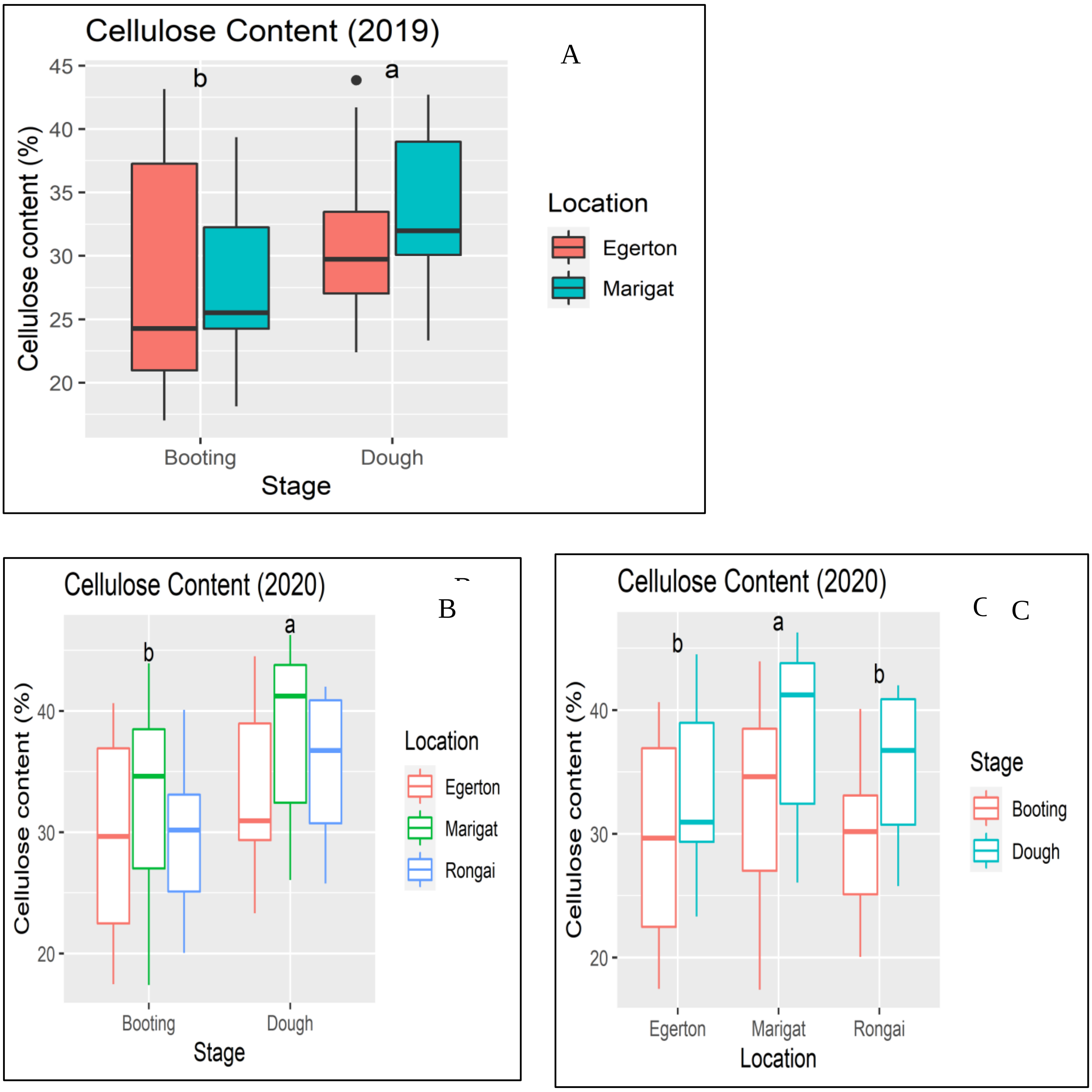

Figure 3.3 A, B and C: Box plot of cellulose (%) content of sorghum cultivars grown at Egerton, Rongai and Marigat in the year 2019 and 2020 respectively. The letters in the figure show significant differences at α = 0.05. Each whisker contains 25

d) **Hemicellulose content**

Genotype, location, crop developmental stage, year and their interactions had a highly significant (P≤0.001) effect on hemicellulose content except for the interaction between stage and year (Table 3.12). The results from the study clearly indicated variation on hemicellulose content among sorghum lines and across growing environments. The hemicellulose content among the genotypes ranged from 14.16% to 21.67% (Table 3.13). The outstanding cultivars within the hemicellulose content range included IS11442, IS25547, and IS11612 which gave hemicellulose content above 20% and B35, EST 37 and EST 20 with hemicellulose below 13.5% in 2019 (Table 3.13). The result trend was recorded in 2020 with cultivars IS11442, IS25547 and IS11612, EST 49 and IS11838 giving the highest hemicellulose content and E6518, B35, EST 20 and EST 37 recording the lowest hemicellulose content (Table 3.13). Similar results were reported by Diallo *et al*. (2019), Miron *et al*. (2007), Carmi *et al.* (2005), Firdous and Gilani (2001) and Khan *et al.* (2007) in sorghum who all confirmed that hemicellulose content differs among sorghum genotypes.

Table 3.12: Anova table output showing mean square values of the effect of cultivar, location, stage, year and their interactions on hemicellulose

Source	DF	ms
Genotype	19	216.23**
Location	2	359.64**
Stage	1	613.66**
Year	2	341.57**
Genotype*Location	1	26.94**
Genotype*Stage	38	9.19**
Genotype*Year	19	19.08**
Stage*Year	19	NS
Location*Stage	1	15.43**
Location*Year	2	190.84**
Genotype*Location*Stage*Year	1	9.89**

*****Significant at P≤0.001, NS not significant, DF degrees of freedom***

Table 3.13: Hemicellulose content (%) of selected sorghum cultivars grown in Egerton, Rongai and Marigat

Sorghum cultivars	2019 cropping season	2020 cropping season
IS11442	21.67^{a}	20.97^{bc}
IS25547	21.29^{ab}	21.62^{a}
IS11612	20.80^{b}	21.07^{b}
IS11721	19.54^{c}	19.51^{e}
EST 49	17.24^{d}	20.51^{cd}
IS11838	17.14^{d}	20.39^{d}
IS2331	16.73^{d}	18.13^{f}
IES9110	16.58^{d}	17.68^{f}
NGUUGU	16.49^{d}	16.39^{h}
IS25557	15.89^{e}	16.42^{h}
GBK01313	14.76^{ef}	17.07^{g}
EST 36	14.47^{ef}	16.93^{h}
IS25563	14.40^{ef}	16.85^{gh}
IS9201	14.28^{ef}	16.80^{gh}
E6518	$19.92\ ^{l}$	11.52^{l}
IS23787	12.98^{g}	14.35^{i}
EST 20	12.66^{g}	13.29^{k}
EST 37	12.61^{g}	13.89^{ij}
B35	11.16^{h}	13.68^{jk}
CV (%)	5.42	4.24
HSD test	0.42	0.87

Values in a column, followed by different superscript letters are significantly different at $p\leq 0.05$. CV (%) =coefficient of variation.

A highly significant ($P\leq 0.001$) effect of crop growth stage on hemicellulose was also observed in sorghum cultivars from the three locations in both 2019 and 2020 with an increasing trend as the crops advanced in age. Similar to cellulose and in most cultvars at the three locations, sorghum harvested at booting stage had lower hemicellulose content than those harvested at dough stage in the two study years (Tables 3.14 to 3.17. These findings align with those of Zhao *et al*. (2009) indicating that hemicellulose yields changed between 1.6 and 6.6 t ha^{-1} from anthesis to 40 days after anthesis in sweet sorghum cultivars. However, Atis *et al*. (2012) reported contrary results that hemicellulose content seemed to reduce with advancement in age in some forage sorghum cultivars. Location also affected the levels of hemicellulose in sorghum genotypes at both booting and dough stages in the two study years. As shown in Tables 3.14 to 3.17, sorghum lines grown in Marigat had relatively higher hemicellulose content followed by Rongai and Egerton, respectively in both 2019 and 2020. In 2020 the levels in Marigat ranged from 9.23% (E6518) to 34.01 % (S79) while at Egerton the range was from 9.27% (E6518) to 21.57% (IS11838) and from 12.40 % (E6518) to 21.57% (IS11838) at booting and dough stages, respectively (Tables 3.16 and 3.17).

Table 3.14: Hemicellulose content (%) of selected sorghum genotypes grown in Egerton and Marigat at booting stage in 2029

Sorghum cultivar	Egerton	Marigat
B35	10.08^{f}	9.67^{g}
EST 36	12.65^{d}	13.13^{ef}
EST 37	10.17^{f}	13.37^{ef}
E6518	9.03^{f}	15.37^{cde}
EST 49	9.72^{f}	22.90^{a}
GBK11313	13.03^{d}	13.33^{ef}
IESV9110	10.72^{ef}	19.20^{ab}
IS11442	20.37^{a}	20.88^{a}
IS11612	19.70^{b}	19.98^{a}
IS11721	20.42^{b}	16.45^{cd}
IS11838	9.62	22.80^{a}
IS2331	11.52^{e}	17.40^{bc}
IS23787	10.88	13.73^{ef}
IS25547	20.00^{b}	20.53^{a}
IS25557	14.12^{c}	14.57^{def}
IS25563	12.90^{d}	12.90^{ef}
IS9201	12.88^{d}	13.22^{ef}
NGUUNGU	11.22^{e}	17.02^{bcd}
EST 20	11.45^{e}	12.43^{f}
S79	13.13^{cd}	12.82^{ef}
CV (%)	4.23	10.52
HSD test	1.02	2.57

Values in a column, followed by different superscript letters are significantly different at $P \leq 0.05$. CV (%) =coefficient of variation

Table 3.14: Hemicellulose content (%) of selected sorghum genotypes grown in Egerton and Marigat at dough stage in year 2019

Sorghum cultivar	Egerton	Marigat
B35	12.30^{i}	12.60^{j}
EST 36	15.90^{ef}	16.20^{fg}
EST 37	11.88^{i}	15.03^{i}
E6518	13.52^{h}	17.77^{e}
EST 49	18.85^{c}	17.50^{e}
GBK11313	16.22^{e}	16.45^{f}
IESV9110	14.25^{g}	22.17^{bc}
IS11442	19.20^{a}	20.63^{b}
IS11612	21.15^{a}	22.12^{c}
IS11721	19.15^{c}	22.13^{bc}
IS11838	18.75^{c}	17.40^{e} ±
IS2331	17.08^{d}	20.90^{d}
IS23787	12.02^{i}	15.27^{ih}
IS25547	21.97^{a}	22.67^{b}
IS25557	14.55^{g}	16.23^{fg}
IS25563	15.78^{ef}	16.03^{fg}
IS9201	14.82^{g}	16.18^{fg}
NGUUNGU	15.80^{ef}	21.92^{c}
EST 20	14.38^{g}	12.38^{j}
S79	15.45^{f}	15.75^{gh}
CV (%)	2.34	1.82
HSD test	0.63	0.54

Values in a column, followed by different superscript letters are significantly different at P≤ 0.05. CV (%) *=coefficient of variation*

Table 3.15: Hemicellulose content (%) of selected sorghum genotypes grown in Egerton, Rongai and Marigat at booting stage in year 2020

Sorghum cultivar	Egerton	Rongai	Marigat
B35	11.05^{f}	13.15 ^{fg}	14.90 ^{g}
EST 36	13.57^{e}	16.08 ^{ed}	18.55 ^{ef}
EST 37	11.42^{f}	13.02 ^{fgh}	14.05 ^{g}
E6518	9.27 ^{g}	10.63 ^{j}	9.23 ^{i}
EST 49	20.17^{b}	11.77 ^{hij}	21.65 ^{b}
GBK101313	13.90^{e}	16.10 ^{de}	18.87 ^{def}
IESV9110	11.22 ^{f}	17.55 ^{bc}	21.08 ^{bc}
IS11442	19.05 ^{b}	17.12 ^{bcd}	23.32 ^{a}
IS11612	19.31^{b}	20.73 ^{a}	20.43 ^{bcd}
IS11721	17.62 ^{c}	18.29 ^{b}	18.65 ^{a}
IS11838	21.57 ^{a}	11.71 ^{ij}	19.88 ^{cde}
IS2331	15.75^{d}	16.12 ^{de}	17.73 ^{f}
IS23787	11.92^{f}	14.12 ^{f}	14.33 g
IS25547	19.87 ^{b}	21.28 ^{a}	20.98 ^{bc}
IS25557	13.73 ^{e}	15.70 ^{e}	18.25 ^{f}
IS25563	14.08 ^{e}	16.27 ^{de}	18.38 ^{ef}
IS9201	13.97 ^{e}	15.93 ^{de}	18.50 ^{ef}
NGUUNGU	11.95 ^{f}	13.43 ^{f}	17.72 ^{f}
EST 20	11.55 ^{f}	12.02 ^{ghi}	23.17 ^{h}
S79	14.20 ^{e}	16.30 ^{cde}	34.01 ^{f}
CV (%)	4.49	5.05	5.34
HSD test	1.10	1.28	1.60

Values in a column, followed by different superscript letters are significantly different at $P \leq 0.05$. CV (%) *=coefficient of variation*

Table 3.16: Hemicellulose content (%) of selected sorghum genotypes grown in Egerton, Rongai and Marigat at dough stage in 2019

Sorghum cultivar	Egerton	Rongai	Marigat
B35	12.63 ^{i}	12.62 ^{hg}	17.72 ^{i}
EST 36	15.38 ^{g}	16.07 ^{d}	21.35 ^{def}
EST 37	13.68 ^{h}	14.23 ^{f}	16.97 ^{j}
E6518	12.40 ^{i}	13.73 ^{fg}	13.83 ^{k}
EST 49	23.67 ^{a}	22.67 ^{a}	23.55 ^{a}
GBK11313	15.67 ^{fg}	16.25 ^{cd}	21.62 ^{de}
IESV9110	15.80 ^{fg}	17.33 ^{c}	23.10 ^{ab}
IS11442	21.33 ^{d}	21.67 ^{a}	23.32 ^{a}
IS11612	22.32 ^{c}	21.67 ^{a}	21.88 ^{cd}
IS11721	17.62 ^{ef}	21.53 ^{a}	22.56 ^{h}
IS11838	23.57 ^{ab}	22.17 ^{a}	23.45 ^{a}
IS2331	16.67 ^{e}	19.28 ^{b}	23.35 ^{a}
IS23787	13.98 ^{h}	14.55 ^{ef}	17.20 ^{ij}
IS25547	22.87 ^{bc}	22.30 ^{a}	22.43 ^{bc}
IS25557	15.27 ^{g}	15.82 ^{d}	19.73 ^{g}
IS25563	15.73 ^{fg}	15.52 ^{de}	31.13 ^{ef}
IS9201	15.43 ^{g}	16.02 ^{d}	20.95 ^{ef}
NGUUNGU	15.87 ^{fg}	16.33 ^{cd}	23.03 ^{ab}
EST 20	14.37 ^{h}	12.48 ^{h}	32.09 ^{ij}
S79	15.43 ^{g}	15.77 ^{d}	39.31 ^{f}
CV (%)	4.49	5.05	5.34
HSD test	0.76	2.13	0.70

Values in a column, followed by different superscript letters are significantly different at p≤ 0.05. CV (%) *=coefficient of variation*

The same trend in the effect of location on hemicellulose was observed in 2019 with most sorghum lines grown in Marigat exhibiting the highest hemicellulose content at both booting and dough stages of development. The values ranged between 9.03% (E6518) and 20.37% (IS11442) and from 11.88 % (EST 37) to 21.97 (IS11838) at booting and dough stages, respectively in Egerton. In the same year, higher values of hemicellulose were recorded in Marigat during the same season with Egerton. The recorded values were 15.37% (E6518) and 19.20% (IS11442) at booting stage and 15.03% (EST 37) and 20.88% (IS11442) at dough stage (Tables 3.14 and 3.15). Singh *et al*. (2012), reported similar results that hemicellulose content varied significantly with locations.

Results hemicelluose content (percent) of sorghum sampled at dough stage was greater than those sampled at booting stage as shown by higher median values (Figures 3.4 A, B and C). Overall, sorghum planted in Marigat had the highest mean hemicellulose, followed by Rongai while sorghum grown in Egerton had the lowest hemicellulose content as indicated by higher median values except at booting stage in Figures 3.3 A, B and C. Mean separation revealed a significant difference between hemicellulose content of sorghum grown at Egerton, Rongai and Marigat and that sampled at booting and dough stage (indicated by the different letters above whiskers).

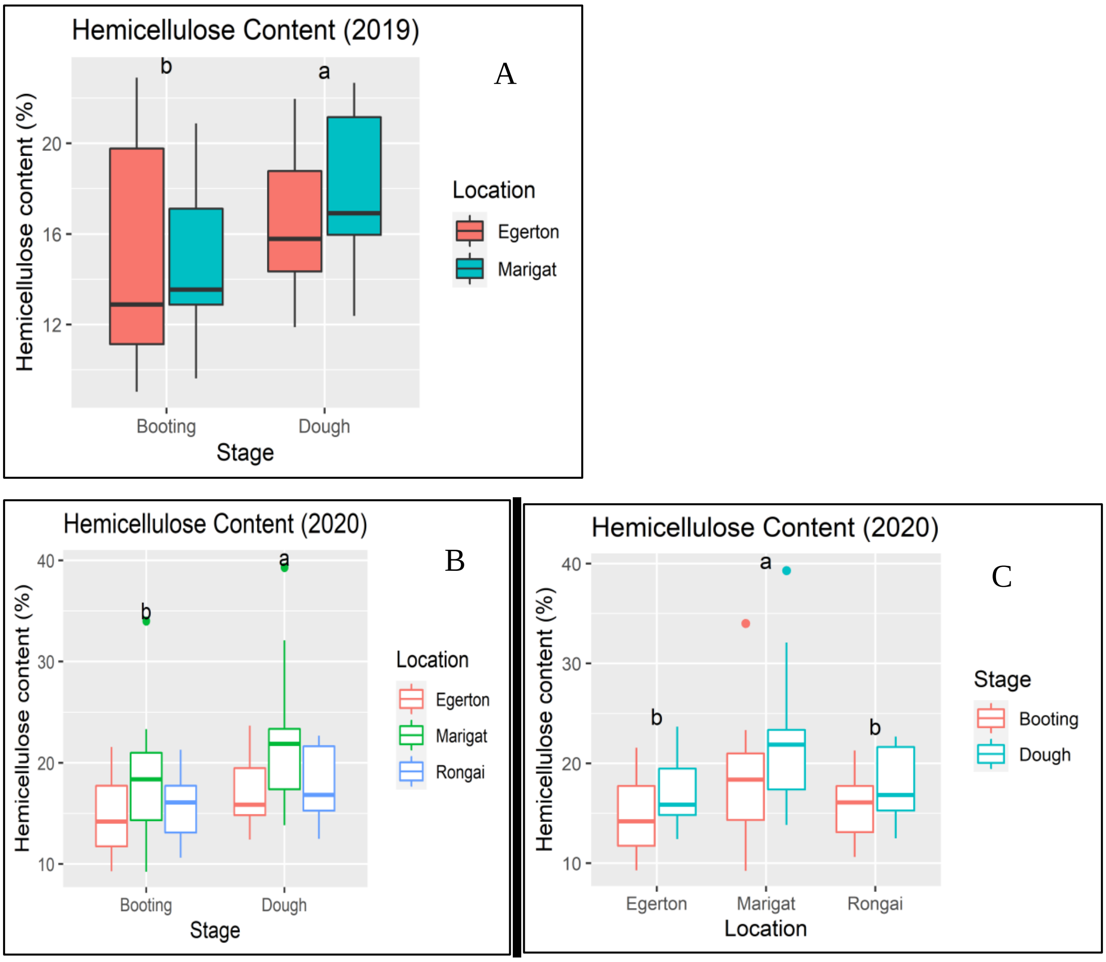

Figure 3. 3: A, B and C: Box plot of hemicellulose (percent) content of sorghum genotypes grown at Egerton, Rongai and Marigat in the year 2019 and 2020 respectively.

The letters in the figure show significant differences at α = 0.05. Each whisker contains 25% of lignin at each temperature level. The outer vertical bars represented the maximum and minimum values of the data. The different letters whiskers showed that HCN was significantly different at the three temperature levels.

e) **Lignin content**

Genotype, location, stage, year and their interactions except for stage by year had significant (P≤0.001) effects on lignin content in sorghum (Tale 3.18). The outstanding cultivars with high lignin content included lines IS11442, IS25547, and IS11612 which gave a range of 23-24% (Table 3.19). Cultivars with lower range included B35, EST 20, EST37 (bmr trait cultivar), E6518 and IS23787 with the lignin content below 15% (Table 3.19). Similar results were reported by Wahyuni *et al*. (2019) who did a comparative study on lignocellulose contents and composition of 30 Indonesian sorghum accessions and reported a significant variation among sorghum genotypes for lignin. De Almeida *et al*. (2019) also reported results consistent with the current research findings and confirmed that three brown midrib (bmr) sorghum mutant hybrids had lower lignin content compared to conventional hybrids.

Table 3.17: ANOVA output showing mean square values of the effect of genotype, location, crop developmental stage, year and their interactions on lignin (percent)

Source	DF	ms
Genotype	19	269.31**
Location	2	447.92**
Stage	1	764.29**
Year	2	425.42**
Genotype*Location	1	33.55**
Genotype*Stage	38	11.44**
Genotype*Year	19	23.76**
Stage*Year	19	NS
Location*Stage	1	19.22**
Location*Year	2	237.69**
Genotype*Location*Stage*Year	1	12.32**

*******Significant at p≤.001, NS not significant*

Table 3.18: Lignin content (%) of selected sorghum genotypes grown in Egerton, Rongai and Marigat

Sorghum cultivar	(2019) cropping season)	(2020) cropping season)
IS11442	24.18^{a}	23.40^{b}
IS25547	23.76^{ab}	24.13^{a}
IS11612	23.22^{b}	23.52^{b}
IS11721	21.80^{c}	21.77^{e}
EST 49	19.24^{d}	22.89^{cd}
IS11838	19.13^{d}	22.76^{d}
IS2331	18.67^{d}	20.24^{f}
IES9110	18.51^{d}	19.73^{f}
NGUUGU	18.40^{d}	18.29^{hg}
IS25557	16.59^{e}	18.32^{h}
GBK01313	16.47^{ef}	19.05^{g}
EST 36	16.14^{ef}	18.78^{hg}
IS25563	16.07^{ef}	18.81^{gh}
IS9201	15.93^{ef}	18.75^{gh}
E6518	15.54^{f}	12.85^{l}
IS23787	14.48^{g}	14.35^{i}
EST 20	12.66^{g}	14.83^{k}
EST 37	14.08^{g}	15.51^{ij}
B35	12.46^{h}	15.26^{jk}
CV (%)	3.38	3.73
HSD tet	0.42	0.52

Values in a column, followed by different superscript letters are significantly different at p≤ 0.05. CV (%) *=coefficient of variation*

A significant (p≤0.05) effect of stage on lignin was also observed in sorghum genotypes from the three locations with an increasing trend as the crops advanced in age (Figure 3.5 A, B and C). Similar to cellulose, sorghum cultivars harvested at booting stage had lower lignin content than those harvested at dough stage in most genotypes in all the three locations Figures 3.5 A, B and C. The increasing lignin concentrations with increasing forage maturation were expected, as this has been well-documented in alfafa (Lamb *et al.*, 2012; Marković *et al.*, 2012). When alfalfa was harvested across increasing forage maturities, Marković *et al.* (2012) reported increasing stem acid detergent lignin (ADL) with concentrations ranging from 84 to 201 g kg^{-1}.

Location also affected the levels of lignin in sorghum cultivars at both booting and dough stages in the two study years. As shown in Tables 3.20-3.22, sorghum cultivars grown in Marigat had higher lignin content followed by Rongai and Egerton, respectively in both 2019 and 2020. In 2020, the levels in Marigat ranged from 10.30% (E6518) to 27.41% (IS11721) and 15.44% (E6518) to 26.28% (EST 20) in 2020 at booting and dough stages, respectively. Sorghum grown in Egerton had lignin content ranging from 10.34% (E6518) to 24.07% (IS11838) and from 13.84% (E6518) to 26.41% (EST 20) at booting and dough stages, respectively (Tables 3.20 and 3.22).

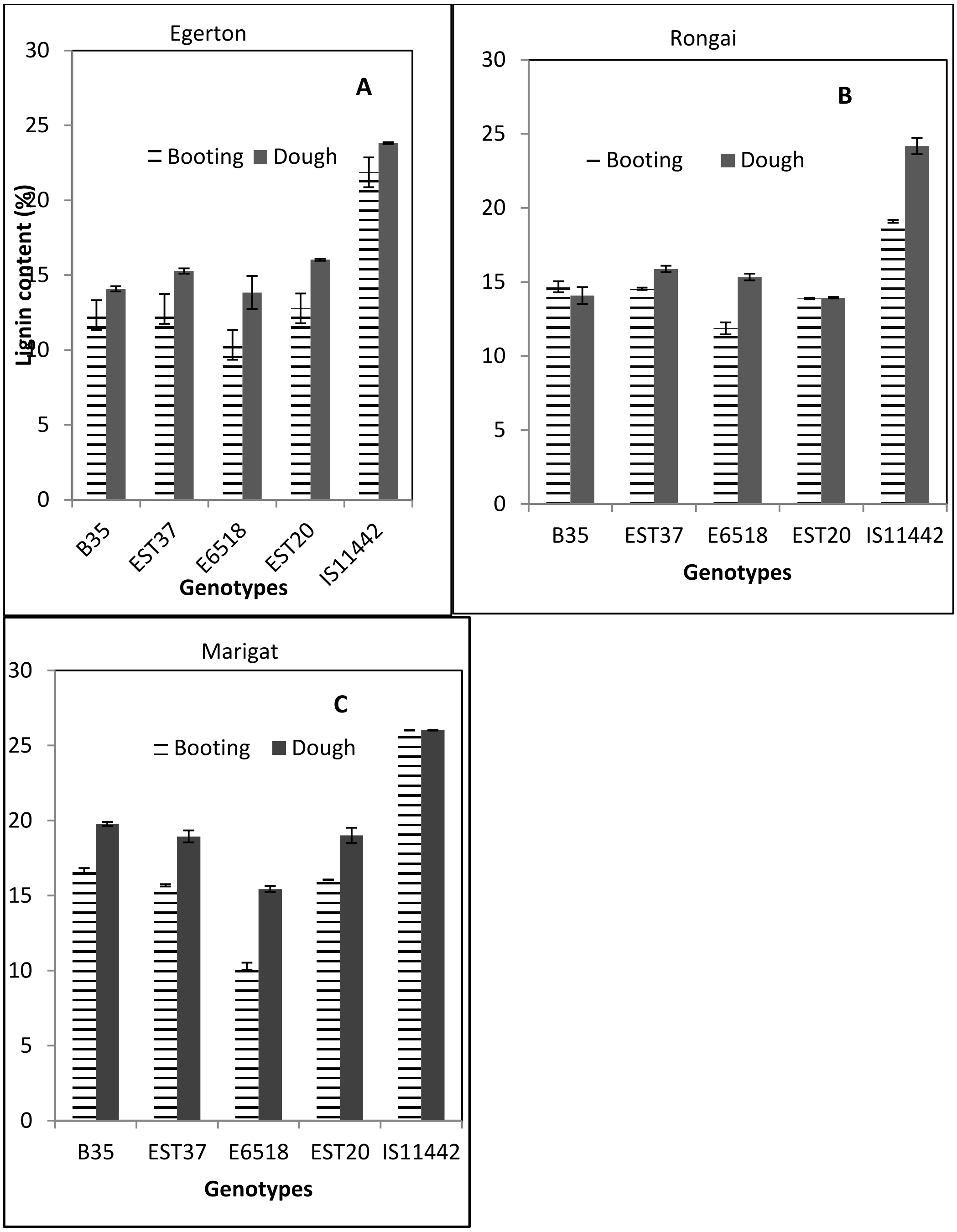

Figure 3.4: Lignin content (%) of selected sorghum genotypes grown at (A) Egerton, (B) Rongai and (C) Marigat in the year 2020 at booting and dough stages

Table 3.19: Lignin content (%) of sorghum genotypes grown in Egerton, Rongai and Marigat at booting stage in 2020

Sorghum cultivar	Egerton	Rongai	Marigat
B35	12.33 ^{f}	14.68 ^{fg}	16.63 ^{g}
EST 36	15.14 ^{e}	17.95 ^{ed}	20.70 ^{ef}
EST 37	12.74 ^{f}	14.53 ^{fgh}	15.68 ^{g}
E6518	10.34 ^{g}	11.87 ^{j}	10.30 ^{i}
EST 49	22.51 ^{b}	13.14 ^{hi}	24.16 ^{b}
GBK11313	15.51 ^{e}	17.97 ^{de}	21.06 ^{def}
IESV9110	12.52 ^{f}	19.59 ^{bc}	23.53 ^{bc}
IS11442	21.26 ^{a}	19.10 ^{bcd}	26.02 ^{a}
IS11612	21.56 ^{b}	23.14 ^{a}	22.80 ^{bcd}
IS11721	18.34 ^{c}	20.39 ^{b}	27.41 ^{a}
IS11838	24.07 ^{a}	13.07 ^{ij}	22.19 ^{cde}
IS2331	17.58 ^{d}	17.99 ^{de}	19.79 ^{f}
IS23787	13.29 ^{f}	15.76 ^{f}	15.99 ^{g}
IS25547	22.18 ^{b}	23.75 ^{a}	23.42 ^{bc}
IS25557	15.24 ^{e}	17.52 ^{e}	20.37 ^{f}
IS25563	15.72 ^{e}	17.32 ^{de}	20.52 ^{ef}
IS9201	15.59 ^{e}	17.18 ^{de}	20.65 ^{ef}
NGUUNGU	13.34 ^{f}	14.98 ^{f}	19.77 ^{f}
EST 20	12.89 ^{f}	13.41 ^{gih}	13.73 ^{h}
S79	15.85 ^{e}	17.59 ^{cde}	20.14 ^{f}
CV (%)	4.49	5.34	5.05
HSD test	1.23	1.43	1.78

Values in a column, followed by different superscript letters are significantly different at $P \leq 0.05$. CV (%) *=coefficient of variation*

Table 3.20: Lignin content (%) of sorghum genotypes grown in Egerton, Rongai and Marigat at dough stage in 2020

Sorghum cutivar	Egerton	Rongai	Marigat
B35	14.09^{i}	14.08^{hg}	19.77^{i}
EST 36	17.17^{g}	17.93^{d}	23.83^{def}
EST 37	15.27^{h}	15.88^{f}	18.94^{j}
E6518	13.84^{i}	15.33^{fg}	15.44^{k}
EST 49	26.41^{a}	24.85^{a}	26.28^{a}
GBK11313	17.48^{fg}	18.14^{a}	24.12^{de}
IESV9110	17.63^{fg}	19.34^{c}	25.78^{ab}
IS11442	23.81^{d}	24.18^{a}	26.02^{a}
IS11612	24.91^{c}	24.27^{a}	24.42^{cd}
IS11721	19.60^{ef}	24.03^{a}	20.81^{h}
IS11838	26.30^{ab}	24.74^{a}	26.17^{a}
IS2331	18.60^{e}	21.52^{b}	25.95^{a}
IS23787	15.61^{h}	16.24^{ef}	19.19^{ij}
IS25547	25.52^{bc}	24.89^{a}	25.03^{bc}
IS25557	17.04^{g}	17.65^{d}	22.02^{g}
IS25563	17.56^{fg}	18.15^{de}	23.59^{ef}
IS9201	17.22^{g}	17.88^{d}	23.38^{ef}
NGUUNGU	17.71^{fg}	18.23^{cd}	25.71^{ab}
EST 20	16.03^{h}	13.93^{h}	19.01^{ij}
S79	17.22^{g}	18.19^{d}	23.87^{f}
CV (%)	4.49	5.05	5.34
HSD test	0.85	1.26	0.78

Values in a column, followed by different superscript letters are significantly different at $P \leq 0.05$. CV (%) *=coefficient of variation*

The same trend in the effect of location on lignin was observed in 2019 with most sorghum lines grown in Marigat exhibiting the highest lignin content at both booting and dough stages of development. The values ranged between 10.79% (B35) and 23.3% (IS11442) and from 13.82% (EST 20) to 25.98% (IS11442) at booting and dough stages, respectively. In the same year, the highest values of lignin at Egerton were recorded in EST 49 (25.56%) and IS25547 (24.52%) at booting stage and dough stages, respectively. These results are shown in Tables 3.18 and 3.19 below. Lignin concentrations variations in different locations observed in the present study were comparable to previous work. Crasta *et al.* (1997) observed differences in maize forage quality aspects including lignin associated with soil water differences in different regions in northeastern USA.

Table 3.31: Lignin content (%) of selected sorghum genotypes grown in Egerton and Marigat at booting stage in 2019

Sorghum cultuvar	Egerton	Marigat
B35	11.25 f	10.79 ^{g}
EST 36	14.12 ^{d}	14.66 ^{ef}
EST 37	11.35 ^{f}	14.92 ^{ef}
E6518	10.08 ^{g}	17.15 ^{cde}
EST 49	25.56 ^{a}	10.84 ^{g}
GBK11313	14.55 ^{d}	14.88 $_{ef}$
IESV9110	11.96 ^{ef}	21.43 ^{ab}
IS11442	24.42 ^{a}	23.31 a
IS11612	21.99 ^{b}	22.30 ^{a}
IS11721	22.79 ^{b}	18.36 ^{cd}
IS11838	25.45 ^{a}	10.73 ^{g}
IS2331	12.85 ^{e}	19.42 ^{bc}
IS23787	12.15^{ef}	15.33 ^{ef}
IS25547	22.32 ^{b}	22.92 ^{a}
IS25557	15.75^{c}	16.26 ^{def}
IS25563	14.40 ^{d}	14.40 ^{ef}
IS9201	14.38^{d}	14.75 ^{ef}
NGUUNGU	12.52^{e}	18.99 ^{bcd}
EST 20	12.78^{e}	13.88 ^{f}
S79	15.85 ^{cd}	14.30 ^{ef}
CV (%)	4.23	10.52
HSD test	1.14	2.86

Values in a column, followed by different superscript letters are significantly different at P≤ 0.05. CV (%) =coefficient of variation

Table 3.42: Lignin content (%) of selected sorghum genotypes grown in Egerton, Rongai and Marigat at dough stage in 2019

Sorghum cultivar	Egerton	Marigat
B35	13.73^{i}	14.06^{j}
EST 36	17.74^{ef}	18.08^{fg}
EST 37	13.26 ^{i}	16.78 ^{i}

E6518	15.09^{h}	19.83^{e}
EST 49	21.04^{c}	19.53^{i}
GBK11313	18.10^{e}	18.36^{f}
IESV9110	15.90^{g}	24.74^{bc}
IS11442	23.03^{b}	25.98^{a}
IS11612	23.90^{a}	24.68^{c}
IS11721	21.37^{c}	24.70^{bc}
IS11838	20.93^{c}	19.42^{e}
IS2331	19.07^{d}	23.32^{d}
IS23787	13.41^{i}	17.04^{hi}
IS25547	24.52^{a}	25.30^{b}
IS25557	16.24^{g}	18.12^{fg}
IS25563	17.61^{ef}	17.89^{fg}
IS9201	16.53^{g}	18.06^{f}
NGUUNGU	17.63^{ef}	24.46
EST 20	16.05^{g}	13.82
S79	17.57^{f}	17.58^{h}
CV (%)	2.34	1.82
HSD test	0.70	0.60

Values in a column, followed by different superscript letters are significantly different at $p \leq 0.05$. CV (%) =coefficient of variation

Lignin content (percent) of sorghum sampled at dough stage was greater than those sampled at booting stage as shown by higher median values (Figures 3.6 A, B and C). Overall, sorghum planted in Marigat had the highest mean lignin, followed by Rongai while sorghum grown in Egerton had the lowest lignin content as indicated by higher median values in Figures 3.6 A, B and C. Mean separation showed significant difference among sorghum grown at Egerton, Rongai and Marigat and between sorghum sampled at booting and dough stages as indicated by different letters above whiskers.

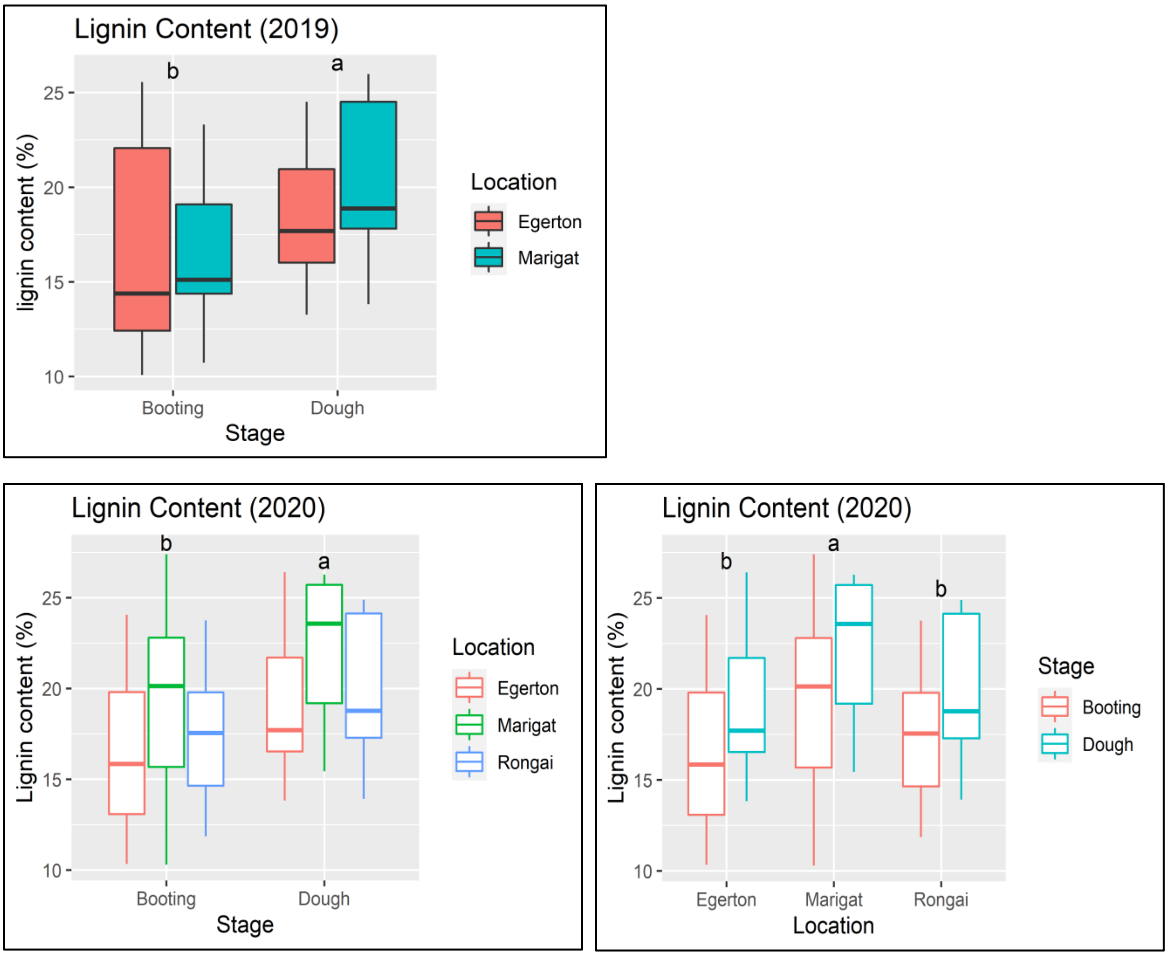

Figure 3.5: A, B and C: Box plot of lignin content of sorghum genotypes grown at Egerton, Rongai and Marigat in the year 2019 and 2020, respectively.

The letters in the figure show significant differences at α = 0.05. Each whisker contains 25% of lignin (%) of each stage of development. The central box contains half of the values and is divided by the median lignin at each temperature level. The outer vertical bars represented the maximum and minimum values of the data. The different letters whiskers showed that HCN was significantly different at the three temperature levels.

CHAPTER FOUR

INFLUENCE OF ENVIRONMENT AND STAGE OF DEVELOPMENT ON HYDROCYANIC POTENTIAL IN SORGHUM CULTIVARS

Abstract

Sorghum (*Sorghum bicolor* (L.) Monech) is an important fodder as well as food crop. However, a limiting factor with the forage sorghum is the presence of hydrocyanic acid which is toxic to livestock. The current study aimed at determining the effect of environment, genotypes and stage of development on hydrocyanic acid in potential fodder sorghum. Twenty sorghum lines were grown at Marigat, Rongai and Egerton University with different agroecological zones [AEZ]: [AEZ V-Lower midland, AEZ IV-Lower highland and AEZ III-Upper highland, respectively]. Field experiments were set up at two locations in 2019 and three locations in 2020 in a RCBD with three replications per site. Sorghum samples were taken at two developmental stages; booting stage and dough stage and analyzed for hydrocyanic acid potenitial (HCN-p) following the argentometric method with ISO 2164-1975 standards**.** Plant height, number of days to 50% heading and daily average temperature were also taken during crop growth period. Data were subjected to ANOVA using the GLM procedure of SAS package version 9.1. the treatment means were separated using Tukeys HSD test. Results revealed a significant ($P \leq 0.05$) effect of both two way and higher order interactions of cultivar (G), location (L) and stage (S) by Year (Y) i.e., G×L×S, G×L×Y, G×Y, G×S, and L×S, L×Y. Main factors (cultivar, location and stage) also had a significant effect on HCN-p of sorghum. Sorghum *cv* IESV9110 had the highest HCN-p (146.09 mg/L) while EST 37 had the lowest HCN-p (83.97 mg/L) across all locations despite the stage of development. There was a progressive increase in HCN-p from a cooler region, to a warmer and a hotter region, respectively. HCN-p on average was highest at Marigat followed by Rongai then lowest at Egerton. Sorghum sampled at booting stage had averagely higher HCN-p (133.94 mg/L) compared to dough stage (91.31 mg/L). Growing temperature needs consideration when recommending fodder sorghum with low HCN-p.

4.1 Introduction

Sorghum *(Sorghum bicolor* (L.) Moech) is a drought resilient crop that can be grown in tropical areas with varied soil types (Ottman & Olsen, 2009; Prakash *et al*., 2010). It is Africa's second most important cereal, being the primary food source for 300 million in sub-

Saharan African. In Kenya, sorghum is among the few crops that grow well under local conditions due to its drought and heat-tolerance. The crop is grown for food and fodder, mostly in the traditional, smallholder farming sector. However, the use of sorghum as fodder is limited by the presence of dhurrin which is accumulated in its shoot, particularly leaves during crop growth stages. When the plant tissue is damaged as it occurs through freezing, cutting or chewing, beta-glycosidase enzymes inherent in the plant cells come in contact with dhurrin and reacts to produce hydrocyanic acid (Alison & Baker, 2002). Hydrocyanic acid is poisonous when ingested by ruminants because the cyanide-releasing enzymes in the plant are more readily activated in the rumen (Al-beiruty *et al.,* 2020). Once absorbed in the rumen, HCN prevents production of energy for the cells (adenosine triphosphate or ATP) through inactivation of cytochrome oxidase enzyme and can lead to death of the animal.

The production and accumulation of dhurrin in sorghum is dependent on plant part, crop growth stage, genotype, management and prevailing weather conditions. Understanding the role of environment, crop growth stage and genotype in the hydrocyanic acid content in fodder sorghum could assist in reducing the negative impact of HCN in livestock. Considering that sorghum is a genetically diverse crop and that hydrocyanic acid is a heritable trait (Kaur *et al*., 2020; Singh *et al.*, 2008), it is important to evaluate and select sorghum genotypes with safe levels of HCN for fodder use. Sorghum growth stage is also a factor that influences both yield and nutritional quality of fodder (Kim *et al*., 2001). In regard to hydrocyanic acid, there is a need to identify the developmental stage when fodder sorghum is safe for livestock consumption and when biomass is at maximum. Any factor that reduces the size and vigor of plants increases the production of secondary metabolites such as dhurrin (Vicari *et al*., 1993). Since high temperature is detrimental to plant growth and vigor, increased production of HCN in crops growing in such environment could point to some plant response mechanism to temperature stress.

Relationship between hydrocyanic acid and genotype

The concentration of dhurrin in sorghum differs among genotypes. The genetic makeup of the sorghum genotypes and differences in leaf characteristics might be responsible for the differences in HCN (Kaur *et al.,* 2020; Singh *et al.,* 2008). The HCN contents in sorghum range from as low as ≤16 ppm to as high as >347 ppm depending on growth conditions and crop growth stage (Moaveni, 2010; Sarfraz *et al.,* 2012). Mohanraj *et al.* (2006) studied the effect of six genotypes of sorghum and the extent of the resulting hybrid inheritance (24 hybrids) from genotypes in inheriting the hydrocyanic acid content after 32 days of planting, they found a significant difference between genotypes and their hybrids for plant content of HCN. In another study, Iyanar (2001) indicated an expression of the characteristic of plant content of HCN as referring to the act of an additional gene. Mohanraj *et al.* (2006) and El-Obeid *et al.* (2006) found a significant contrast between sorghum genotypes in their HCN content. When studying 25 genotypes of sorghum, Chaturvedi (1994), indicated that 20 of them contained high HCN concentrations in the flowering stage and reached 606 $mg^{-1}kg^{-1}$ and are unsafe for animal feeding, while five genotypes were reported to be suitable for feeding animals where HCN levels reached 230 mg^{-1} kg^{-1}. Other studies reported that the genetic factor has a significant impact on the determination of the HCN concentration in the plant include Abusuwar and El zilal (2010), Khan *et al*. (2013), Pandey *et al*. (2011) and Sarfraz *et al.* (2012)

Decrease in hydrocyanic acid as crops advance in age is due to changes in plant architecture as plants grow. As plants grow taller, the canopy grows denser and thicker leading to a reduction in temperature and increase in humidity beneath the foliage and ultimately slowing down the dhurrin accumulation in the leaves. Pistoia *et al*. (2003) reported that the HCN proportion decreases as the height of the plant increases. Sher *et al.* (2016) also reported that high nitrogen application resulted in good vegetative growth and dense canopy of plants thereby, affecting forage sorghum quality. In another study by Kaur *et al.* (2020) the HCN content in sorghum genotypes were higher at early stages of maturity, however, with the advancement in the maturity the HCN content was decreased. Similarly, Muthuswamy *et al.* (1976) also estimated HCN content of five hybrids of sorghum at different growth stages and observed that the HCN content in the vegetative part was very high (650 mg kg-1 fresh weight) after 18 days of planting and gradually decreased to its lowest concentration (7 mg kg^{-1} fresh weight) after 49 days of planting. In a study conducted by Al-Beiruty *et al.* (2020) a significant effect of the cutting stages in the concentration of hydrocyanic acid was reported. He reported that sorghum harvested at vegetative growth stage had the highest HCN concentration of 336.9 ppm, while the stage 100% flowering gave the lowest concentration of 201.7 ppm.

Role of hydrogen cyanide in plant responses to stress

Large quantities of dhurrin may be produced quickly when the sorghum is under the influence of environmental stresses (drought and frost) and when leaf tissues are disrupted (Mohanraj *et al.*, 2006) and increased soil acidity (Oten, 2017). According to Pushpa *et al.* (2019) the amount of the dhurrin content responsible for the production of HCN in sorghum leaves not only varies depending on the genotype, but it is also influenced by the environmental conditions. Khatri *et al. (*1997) reported that different genotypes of sorghum showed a change in their HCN content when growing in different environments, and they attributed the change to climate factors, plant growth stage, soil type, quantity and quality of added fertilizers. In the contrary, Wheeler *et al.* (1984) found that the HCN, of sorghum stem was reduced when plants were grown at a higher temperature. In white clover (Trifolium repens), high light intensity, higher temperature and phosphorus fertilization significantly reduced the HCN potential. In the same study by Vickery *et al*. (1987) the authors reported that any factor that reduced the size and vigour of white clover plants increased the HCN. Since extremely high temperature is detrimental to plant growth, increased production of HCN in crops growing in such environment can be a mechanism by the crops to respond against temperature stress. Due to climate change and increases in environmental temperatures globally, production and the use of sorghum as a fodder crop is limited due increased production of hydrocyanic acid. Current researches have mostly dwelt on variation of HCN as triggered by biotic factors and abiotic factors such as moisture stress and frost but ignored the role of high environmental temperature on HCN content in sorghum leaves. The study aimed at determining the effect of growing temperature, genotypes and stage of development on hydrocyanic acid in potential fodder sorghum.

4.2 Materials and methods

4.2.1 Site description

The experiments were conducted during long rains seasons at Egerton University (0º 22' S; 35º 55’ E) which is at an altitude of 2286 m above sea level and Rongai (0^{o}23’N; 35^{O} 51’E) at an altitude of 1890 m above sea level. The third site was under irrigation at Kenya Agricultural and Livestock Research Organization (KALRO) in Marigat (0°46’ N; 35^{o}98’E) which is at an altitude of 1066 m above sea level. The experients were conduted in two seasons during the months of March and September in 2019 and repeated between May and

October in 2020. Soils at the three sites are classified as *Mollic andosols, Vitric andosols,* and Cambisols in agroecological zones (AEZ) III upper highland (UH), AEZ IV lower highland (LH4), and AEZ V lower midland 5 (LM5) for Egerton, Rongai and Marigat, respectively.

4.2.2 Soil sampling and analysis

Soil chemical properties of experimental sites at Egerton, Rongai and Marigat

Soil analysis results (Table 4.1) revealed that the soils were acidic with low available P and nitrogen contents. The total exchangeable bases and CEC were rated as medium.

Table 4.1: Chemical properties of the soil at Egerton, Rongai and Marigat sites

Soil properties	Egerton	Rongai	Marigat
N (%)	0.21	0.24	0.4
P (mg kg-$^{1)}$	3.2	2.9	4.2
K (cmol$_c$kg^{-1})	0.9	0.7	0.7
CEC (cmol$_c$kg^{-1})	21.5	9.6	6.2
pH	5.8	5.7	4.8
Organic carbon	1.9	1.6	0.3

4.2.3 Experimental design and treatment

The treatments were arranged in a RCBD with three replications. Treatments of twenty sorghum genotypes listed in Table 3.1 in chapter 3 were evaluated. Each experimental unit measured 4 m x 5 m which accommodated five rows of sorghum plants.

4.2.4 Planting and crop management

Sorghum seeds were sown at the rate of 10 kg ha^{-1}, at a spacing of 60 cm by drill and at a depth of 2 cm. Thinning was later done when the seedlings were at 20 cm height to spacing of 60 cm x 15 cm. Diammonium phosphate (DAP) fertilizer was applied during sowing at the rate of 60 kg P_2O_5 ha^{-1}. Top dressing was done once by applying Nitrogen at the rate of 60 kg N /ha when the crops were at vegetative growth stage. Weed control during growth period was manually done while thunder OD 45 (Imidocloprid 100g/l +Beta cyfluthrin 45g/l) was applied to control aphids and fall army worm when it was necessary at the rate of 0.2 L/ha. Birds scaring was be done through engagement of personnel.

4.2.5 Data collection

a) Growth parameters and temperature measurements

Ten sorghum lines were selected during the year 2019 trial based on HCN and evaluated in a separate experiment in the year 2020. Data on days to 50% heading and plant height at dough stage were taken during the crop growth period. Plant height was measured using a graduated pole from crown level to the leaf collar of the flag leaf. Days to 50% heading were counted from the date of emergence to the date when 50% of the plants in each experimental unit had emerged panicles. Average daily temperature was measured using a digital maximum and minimum thermometer.

b) Plant tissue sampling for laboratory analysis

Plants in the field experiment were harvested at booting: a stage when the sheath of flag leaf is swollen-enclosing an emerging panicle, and dough stage, when sorghum grain had a dough-like consistency. Simple random sampling was done in the three middle rows of each experimental unit and from each of the three rows. Flag leaf from four plants with the same height were clipped from the leaf sheath and in an air tight container and placed in a cool box immediately. The samples were then labeled in the field and taken to the laboratory for analysis of hydrocyanic acid.

c) Determination of hydrocyanic acid potential

Hydrocyanic acid was determined using the argentometric method according to ISO 2164-1975 standard**.** The leaves were cut into pieces approximately one-half inch in length with a pair of grass shears. Fifty grams of stripped leaves were used for a bulk sample. The finely cut leaves were thoroughly mixed by hand before sampling for distillation. Twenty grams of the finely cut sorghum leaves were macerated in an electric grinder with a small quantity of pure silica sand moistened with a few cubic centimeters of distilled water. After maceration the samples were transferred to an 800 ml Kjeldahl flask with approximately 300 ml of distilled water to wash out the mortar. Ten milliliters of sodium acetate was added into the flask. After transferring the macerated sorghum to the Kjeldahl flask, the flask was corked tightly immediately, using a large rubber stopper. The macerated sorghum in the tightly stoppered Kjeldahl flask was allowed to digest over night at room temperature. The HCN was distilled on the ordinary Kjeldahl distilling apparatus into a receiving flask containing 10 ml of 0.05 normal silver nitrate (AgNOa), 1 ml of nitric acid (HNO_3) (to keep the solution acidic). Fifty millilitres of distilled water was then added in to the receiving flask. The receiving flask was wrapped with alluminium foil in order to avoid reduction of silver nitrate solution by light. The silver nitrate remaining in solution was determined by titration with a 0.5 M standard potassium ·thiocyanate solution, using ferric ammonium sulphate as an indicator. The percent HCN was calculated from the titration on a fresh weight basis and converted to parts per million (ppm.) using the formula;

$$\text{HCN-p}\,\frac{(mg)}{(kg)}=0.54(\text{V2}-\text{V1}) \times \frac{500}{250} \times \frac{1000}{M} \qquad \text{Equation 4.1}$$

Where:

V1 is the volume of potassium thiocyanate required to neutralize the excess of silver nitrate in the sample test.

V2 is the volume of potassium thiocyanate required to neutralize the excess of silver nitrate in the blank test.

M is the weight (gram) of the test sample.

4.3 Statistical Model and Data analysis

Data on HCN-p was subjected to ANOVA using the GLM procedure of Statistical Analysis System (SAS) package (Statistical Software Version 9.1). Effects were considered significant in all statistical calculations if the *P*-values were ≤ 0.05. Treatment means were separated through Tukey's honest significant difference (HSD) at P≤0.05 using *HSD.test* function in *agricolae* package of R. The following statistical Model was fitted during the analysis of data;

$$Y_{ijklm}= \mu+ B_i +G_j +BG_{ij} + L_k + BL_{ik} +S_l +GL_{jk} +GS_{jl} + LS_{kl} + Y_m + GY_{jm}+ LY_{km} + SY_{lm} + GLSY_{jklm} +\varepsilon_{ijklm}$$

where; μ= Overall mean; B_i=effect due to the i^{th} blocking; G_j=effect due to the j^{th} sorghum genotype; BG_{ij}=effect due to the interaction between i^{th} block and j^{th} genotype; l_k= effect due to the k^{th} location; BL_{ik}= effect due to the interaction between i^{th} block and k^{th} location
S_l = effect due to the l^{th} stage of devlopment; Gl_{jk}= effect due to interaction between j^{th} sorghum genotype and k^{th} location; GS_{jl} =interaction between j^{th} genotype and l^{th} stage of development; LS_{kl} =interaction between k^{th} location and l^{th} stage of development; Y_m= effect

due to mth year, GY_{jm}= effect due to the interaction between j^{th} sorghum genotype and m^{th} Year , LY_{km} = effect due to the interaction between k^{th} location and m^{th} year, SY_{lm} = effect due to the interaction between l^{th} stage of development and m^{th} year. GLS_{jkl} = interaction between j^{th} genotype, k^{th} location and l^{th} stage

$\varepsilon ijkl$= random error term

4.4 Results

a) HCN-p in sorghum genotypes

Analysis of variance revealed a significant ($p \leq .001$) effect of genotype × location ×stage × year for HCN-p (Table 3) as well as significant effect of two-way interactions (G×L, G×S, G×Y, L×S and L×Y) except for S×Y. Higher order interactions of G×L×S and G×L×Y were also significant for HCN-p. The high significance observed due to G×L×S×Y could be attributed to high significance observed in all the two-way interactions effects and of main factors which had a high significant ($P \leq 0.001$) for cultivar and location and at $P \leq 0.01$ for stage (Table 4.1). The 4-way interaction contributed 18% of the contribution by fixed factors while GLS contributed 18% of the contribution by fixed factors. This is an indication that temperature significantly affected HCN-p of sorghum cultivars.

Table 4.2: Mean squares for HCN-p for 20 sorghum cultivars evaluated in the Rift valley region of Kenya in the years 2019 and 2020

Source of variation	**Df**	**Ms**
Genotype (G)	19	7675***
Location (L)	2	76199.5***
Stage (S)	1	182679 ***
Year (Y)	1	Ns
GL	38	2043**
GS	19	1068***
GY	19	885***
LS	2	23424.49***
LY	2	2299.12***
SY	1	Ns
GLS	39	1879.18***
GLY	18	5067.5***
LSY	2	Ns
G×L×S×Y	38	182679***
Error	388	226
Total	599	782476.79

*** Significance at P≤0.001, ** significance at P≤0.01, ns not significant, Df- degree of freedom, G-cultivar, L-location, S-growth stage, Y-year.

The hydrocyanic acid potential (HCN-p) on fresh weight of selected fodder sorghum genotypes indicated significant (P≤0.05) differences among the genotypes and across environments. The HCN-p in the test material ranged from 83 – 147 ppm (Table 4.3). The lowest values of HCN content were obtained from the brown mid-rib genotypes; IS 23787, EST 36 and EST 37 whose content were 86.58, 86.24 and 84.43 ppm, respectively, in 2019 and 85.13, 85.73 and 83.79 ppm in 2020. This observation corroborates work by Khan *et al.* (2013) who showed a *bmr* variety Sorghum 2011 with lower HCN-p compared to JS-2002 and JS-2002. HCN-p below 100 ppm on –fresh weight basis is considered safe for animals and should not cause toxicity (Patel *et al.,* 2013). However, levels ranging between 100-200 ppm are viewed as dangerous and toxic to livestock (Patel *et al.,* 2013). The genotypes with the highest HCN-p when the data was pooled across the growing environments were IESV9110, IS2331, IS25547, IS1142, IS 25557, IS 111838 and IS 11612 in 2019 with 147.6, 128.5, 128.5, and 127.8, 126.18, 125.30 and 124.77 ppm, respectively (Table 4.4). The same trend was observed in 2020 where IESV9110, IS2331, IS 11612, IS11838 and IS 25557 recorded 145.08, 140.02, 129.82, 127.70 and 125.97 ppm, respectively (Table 4.4).

The observed average HCN-p for the different sorghum lines/varieties is slightly below what has been reported elsewhere. Moaveni (2010) reported HCN-p values ranging between 216 and 243 ppm while Sarfraz *et al.* (2012) recorded HCN-p between 255 and 347 ppm in different sorghum varieties. However, at booting stage, some genotypes grown in Marigat had HCN-p above 200ppm that related closely to the values previously reported by Sarfraz *et al.* (2012) and Moaveni (2010). Results from the current study is also in agreement with previous research that hydrocyanic acid content of sorghum is a heritable trait and that it varies with crop genotype (Emendack *et al.,* 2018; Sun *et al.,* 2018; Zagrobelny *et al*., 2008;).

Table 4.3: HCN-p of sorghum genotypes grown in Egerton, Rongai and Marigat

	HCN-p (ppm) in year 2019	HCN-p (ppm) in year 2020
Sorghum cultivars		
IESV9110	147.60^{a}	145.08^{a}
IS2331	128.50^{b}	140.026^{b}
IS11612	124.77^{bc}	129.820^{b}
IS11838	125.30^{b}	127.701^{bc}
IS25557	126.18^{b}	125.973^{bc}
IS9201	116.27^{bcd}	122.849^{cd}
IS25547	128.50^{bcd}	117.445^{ed}
S79	119.17^{bcd}	116.79^{de}
GBK01313	124.29^{cb}	116.29^{e}
EST 49	119.90^{bcd}	115.67^{e}
E6518	108.24^{efd}	114.11^{ef}
NGUUGU	110.783^{efd}	109.06^{fg}
IS11721	112.05^{cde}	106.60^{gh}
IS25563	119.54^{bcd}	101.98^{hi}
EST 20	99.45^{f}	98.30^{ij}
IS11442	127.8^{b}	95.54^{jk}
B35	101.93^{ef}	91.58^{kl}
EST36	86.24^{g}	85.78^{lm}
IS23787	85.58^{g}	85.125^{m}
EST 37	84.23^{g}	83.79^{m}
CV (%)	5.97	8.46
HSD test	11.16	6.20

Values in a column, followed by different superscript letters are significantly different at P≤ 0.05. Values are means ±standard errors, CV (%) = coefficient of variation

b) Influence of sorghum developmental stage on HCN-p

A significant (P≤0.05) effect of crop developmental stage on HCN-p was observed in sorghum cultivars in both 2019 and 2020 with a decreasing trend as the crops advanced in age. In all the cultivars tested, sorghum harvested at dough stage had lower HCN-p than those harvested at booting stage in the two study years (Figures 4.1 A and B; 4.2 A, B and C). Haque and Bradbury (2002), reported a progressive decline in HCN-p in sorghum leaves (*S. vulgare* Pers.) sampled at the end of the 2nd, 3rd and 4th growth weeks as 280, 40 and 60 ppm, respectively. Abusuwar and El-zilal (2010) also reported a decrease in HCN-p with maturity in two cultivars of sorghum. Progressive decrease in HCN-p from booting to flowering and post anthesis in sorghum have been reported by Emendack *et al.* (2018). Similar results to the findings in the current work wer reported in several studies (Busk & Moller, 2002; Karthika & Kalpana, 2017; Nicholson, 2012; Sun *et al.*, 2018; Vinutha *et al.*, 2017; Zagrobelny, 2008).

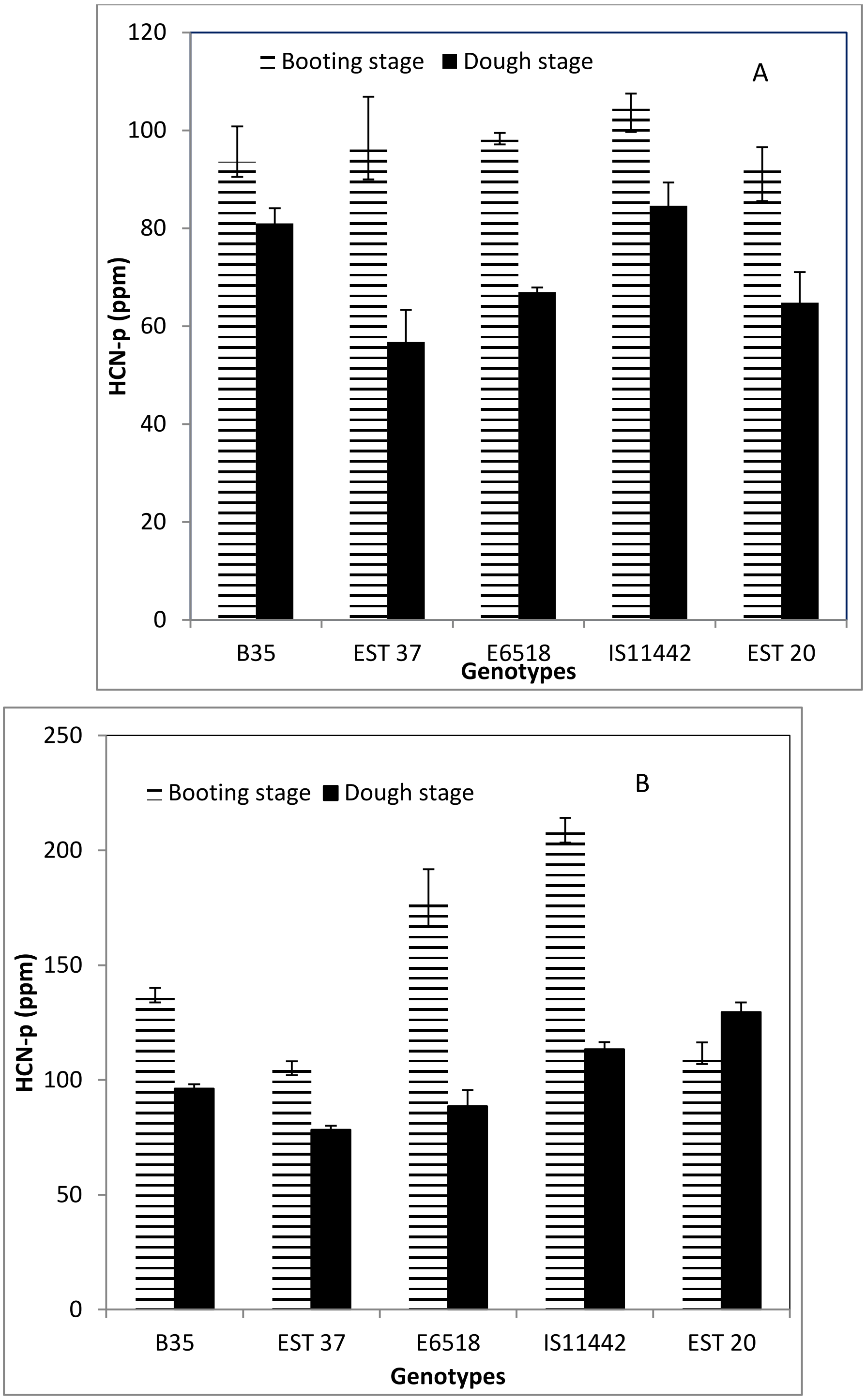

Figure 4.1: HCN-p (ppm) of sorghum genotypes grown at (A) Egerton and (B) Marigat respectively in the year 2019 at booting and dough stages

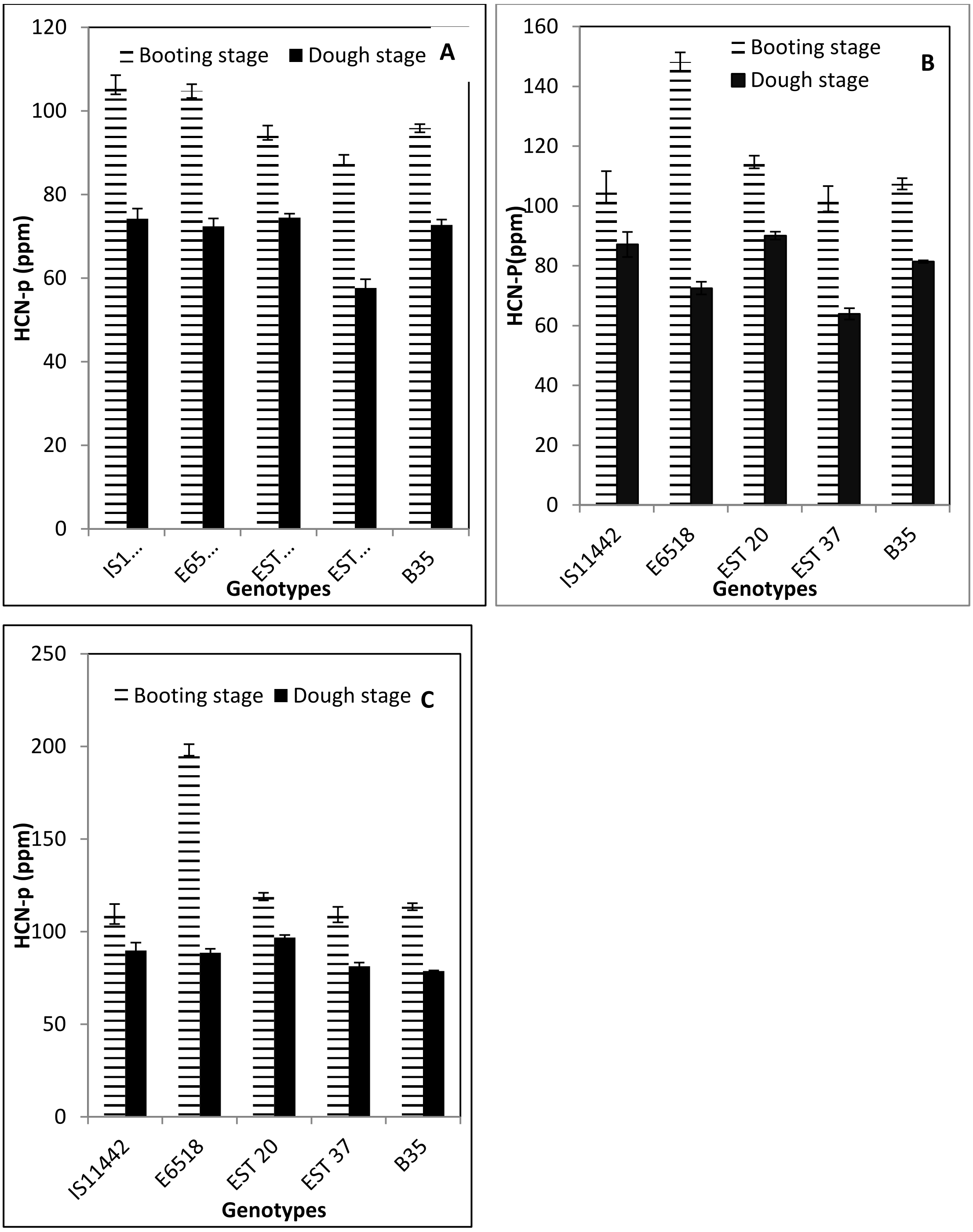

Figure 4.2: HCN-p (ppm) of sorghum genotypes grown at (A) Egerton, (B) Rongai and (C) Marigat respectively in the year 2020 at booting and dough stages

c) Influence of temperature on HCN-p in sorghum genotypes

The pooled data across the three environments showed a significant increase in HCN-p from a cooler region-Egerton to warmer region-Marigat. In both 2019 and 2020 sorghum harvested in Marigat had higher HCN-p. The HCN-p increased from a cool environment at Egerton, to a hotter environment at Marigat while the concentration was intermediate in Rongai. In this experiment, the effect of location was influenced by temperature (Tables 4.5-4.8). The results in the current study are in agreement with those by Bahrani and Deghani (2004) who reported that HCN levels were also significantly lower in second cut (during low temperature season) than in first cut fodder and higher during high temperature seasons. In another study that aimed at investigating relationship of drought frequency and severity with a range of annual temperature variation, Amrit *et al.* (2018) reported that drought events had notable relationship with temperature variation during the year at a given place. The results are also in agreement with observations by Dahler *et al.* (1995), Pushpa *et al.* (2019) and Ravindran (1993) who reported that nutritional factors, especially N supply, and sudden changes in temperature have pronounced effects on the actual level of cyanogenic glycosides.

Table 4.4: HCN-p of sorghum genotypes at booting stage as observed in Egerton, Rongai and Marigat in 2020

Sorghum cultivar	Egerton	Rongai	Marigat
B35	95.90^{ghi}	107.41^{de}	113.4^{hg}
EST 36	90.35^{hi}	104.89^{de}	111.69^{h}
EST 37	88.24^{i}	102.46^{de}	109.10^{h}
E6518	104.80^{efg}	148.22^{a}	198.00^{dc}
EST 49	89.91^{hi}	99.70^{e}	223.58^{a}
GBK11313	110.93^{cdef}	114.66^{bcde}	189.43^{bcd}
IESV9110	125.67^{ab}	145.59^{a}	220.68^{abc}
IS11442	106.29^{defg}	106.29^{de}	109.48^{h}
IS11612	100.67^{ghif}	112.91^{bcde}	222.83^{ab}
IS11721	97.26^{ghi}	108.88^{cde}	172.37^{e}
IS11838	121.83^{abc}	123.67^{bc}	198.00^{cd}
IS2331	117.55^{bcd}	161.09^{a}	216.42^{abc}
IS23787	89.65^{i}	104.09^{de}	110.85^{h}
IS25547	106.81^{defg}	109.05^{cde}	209.32^{abc}
IS25557	102.14^{fgh}	127.68^{b}	215.86^{abc}
IS25563	114.68^{bcde}	114.68^{bcde}	137.33^{fg}
IS9201	130.76^{a}	153.50^{a}	140.63^{f}
NGUUNGU	95.44^{ghi}	104.99^{de}	207.90^{abc}
EST 20	94.81^{ghi}	114.72^{bcde}	118.85^{fgh}
S79	109.93^{cdef}	116.51^{bcd}	175.32^{de}
CV (%)	7.21	5.34	8.67
HSD test	12.47	7.87	24.43

Values in a column, followed by different superscript letters are significantly different at $P \leq 0.05$. CV (%) =coefficient of variation

Table 4.5: HCN-p of sorghum cultivars at dough stage in samples obtained from Egerton, Rongai and Marigat in 2020

Sorghum cultivar	Egerton	Rongai	Marigat
B35	72.71 ^{fg}	81.43 ^{efgh}	78.63 ^{k}
EST 36	59.03 ^{h}	65.45 ^{ij}	83.26 ^{k}
EST 37	57.66 ^{h}	63.93 ^{j}	81.32 ^{k}
E6518	72.54 ^{fg}	72.54 ^{hij}	88.57 ^{jk}
EST 49	82.81 ^{cdef}	85.16 ^{defg}	112.84 ^{efg}
GBK11313	72.08 ^{fg}	82.27 ^{efgh}	119.35 ^{ef}
IESV9110	95.66 ^{ab}	110.83 ^{a}	172.06 ^{a}
IS11442	74.24 ^{efg}	87.16 ^{cdef}	89.78 ^{ijk}
IS11612	84.09 ^{bcdef}	97.97 ^{bc}	160.49 ^{ab}
IS11721	75.86 ^{defg}	83.08 ^{defgh}	102.13 ^{ghi}
IS11838	89.15 ^{abc}	95.23 ^{cd}	138.32 ^{cd}
IS2331	86.47 ^{abcde}	108.47 ^{ab}	150.15 ^{ab}
IS23787	58.58 ^{h}	64.95 ^{j}	82.62 ^{k}
IS25547	86.89 ^{abcd}	88.71 ^{cdef}	103.88 ^{gh}
IS25557	93.22 ^{abc}	91.02 ^{cde}	125.92 ^{de}
IS25563	74.05 ^{efg}	74.05 ^{higj}	97.08 ^{hij}
IS9201	93.81 ^{abc}	110.13 ^{ab}	108.27 ^{fgh}
NGUUNGU	69.63 ^{hg}	76.59 ^{hifg}	99.82 ^{ijhg}
EST 20	74.48 ^{defg}	90.13 ^{dce}	96.81 ^{ihj}
S79	96.69 ^{a}	81.72 ^{hfge}	120.53 ^{ef}
CV (%)	9.71	8.70	7.19
HSD test	12.59	12.30	13.14

Values in a column, followed by different superscript letters are significantly different at P≤ 0.05. CV (%) =coefficient of variation

Table 4.6: HCN-p of sorghum cultivars at booting stage in Egerton and Marigat in 2019

Sorghum cultivar	Egerton	Marigat
B35	93.60^{bcd}	136.87^{e}
EST 36	99. 05^{bcd}	107.61^{e}
EST 37	96.57^{bcd}	105.11^{e}
E6518	98.07^{bcd}	179.47^{bcd}
EST 49	81.00^{d}	212.40^{ab}
GBK11313	93.60^{bcd}	226.80^{a}
IESV9110	120.60^{a}	212.40^{a}
IS11442	104.40^{abc}	208.80^{abc}
IS11612	99.03^{bcd}	226.80^{a}
IS11721	79.20^{d}	194.40^{abcd}
IS11838	108.00^{ab}	201.60^{abcd}
IS2331	99.00^{bcd}	172.80^{d}
IS23787	98.23^{bcd}	106.79^{e}
IS25547	104.10^{abc}	212.40^{ab}
IS25557	107.94^{ab}	194.40^{abcd}
IS25563	94.79^{bcd}	198.00^{abcd}
IS9201	92.47^{bcd}	176.40^{cd}
NGUUNGU	86.77^{cd}	198.00^{abcd}
EST 20	91.80^{cd}	111.60^{e}
S79	105.87^{abc}	172.80^{d}
CV (%)	14.49	14.84
HSD test	20.17	34.79

Values in a column, followed by different superscript letters are significantly different at $P \leq 0.05$. CV (%) =coefficient of variation

Table 4.7: HCN-p of sorghum cultivars at dough stage in Egerton and Marigat in 2019

Sorghum cultiavr	Egerton	Marigat
B35	81.00^{abcde}	96.26^{fg}
EST 36	58.08^{i}	80.21^{h}
EST 37	56.73	78.34^{h}
E6518	66.93^{edihgf}	88.50^{hg}
EST 49	74.60^{cghdbe}	111.60^{de}
GBK11313	65.17^{fghie}	111.60^{cde}
IESV9110	91.80^{a}	165.60^{a}
IS11442	84.60^{abc}	113.40^{cde}
IS11612	$71.53^{fcghide}$	101.72^{ef}
IS11721	64.80^{efghi}	109.80^{ed}
IS11838	$72.80^{bcdefghi}$	118.80^{bcd}
IS2331	86.40^{abc}	118.80^{bcd}
IS23787	57.64^{ih}	79.60^{h}
IS25547	82.29^{abcd}	115.20^{cd}
IS25557	88.97^{ab}	113.40^{cde}
IS25563	61.20^{ihg}	124.20^{bc}
IS9201	$75.60^{abcdefg}$	120.60^{bcd}
NGUUNGU	63.30^{fghi}	95.07^{fg}
EST 20	64.80^{efghi}	129.60^{b}
S79	79.20^{abcdef}	118.80^{bcd}
CV (%)	14.49	14.84
HSD test	20.17	34.79

Values in a column, followed by different superscript letters are significantly different at $p\leq 0.05$. CV (%) =coefficient of variation

HCN-p (ppm) of sorghum sampled at booting stage was greater than those sampled at dough stage as shown by higher median values (Figures 4.3 A, B, C and D). Overall, sorghum planted in Marigat had the highest HCN-p (ppm), followed by Rongai while sorghum grown in Egerton had the lowest HCN-p as indicated by higher median values in Figures 4.3 A, B, C and D. There was a significant difference in HCN-p (ppm) of sorghum grown in Egerton, Rongai and Marigat and between those harvested at booting and dough stage.

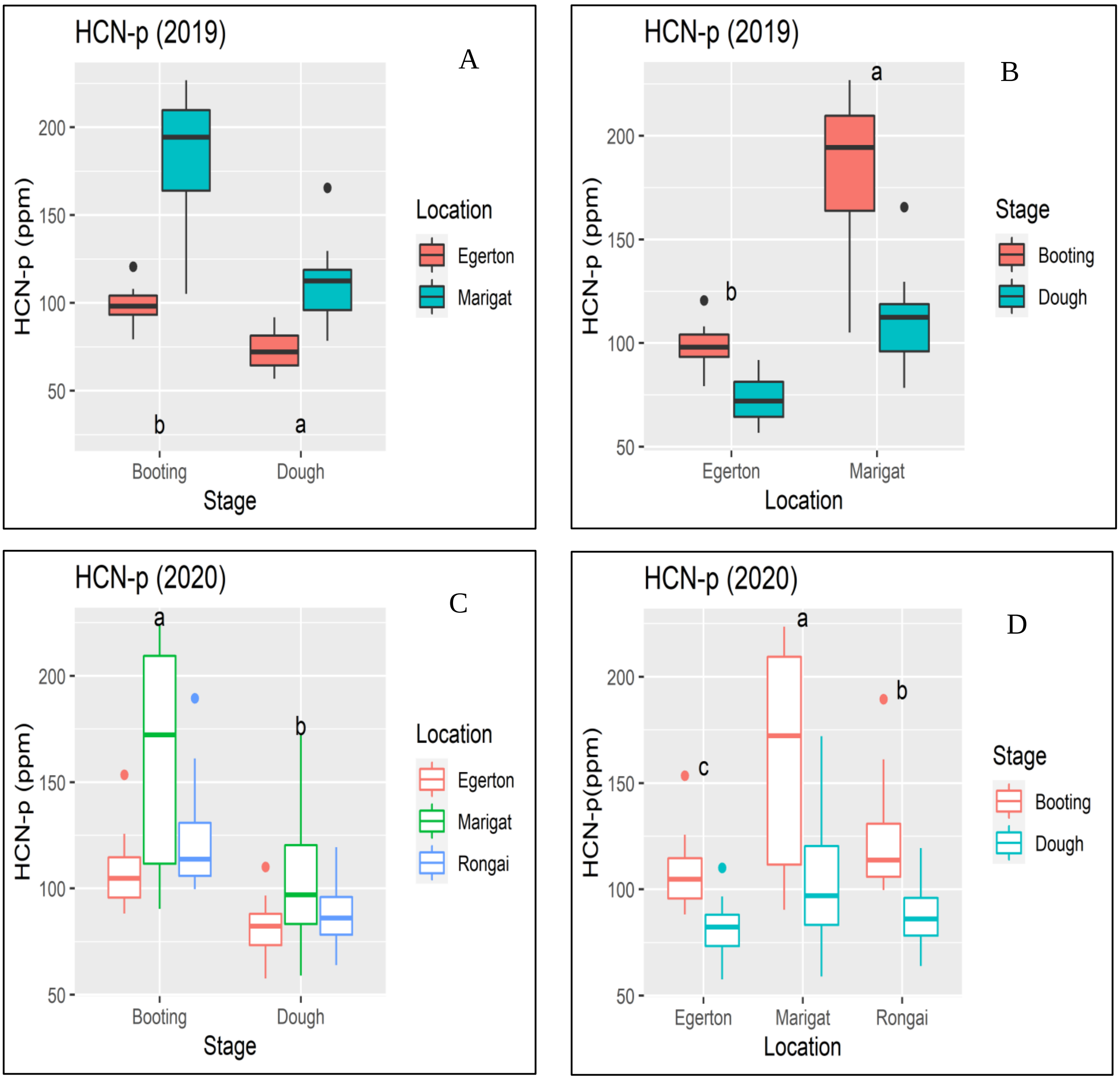

Figure 4.3: Box plot of HCN-p t of sorghum genotypes grown in the growth in Egerton, Rongai and Marigat in the year (A) 2019 and (B) 2020 respectively.

The letters in the figure show significant differences at α = 0.05. Each whisker contains 25% of prussic acid (ppm) of each stage of developoment. The central box contains half of the values and is divided by the median HCN-p at each temperature level. The outer vertical bars represented the maximum and minimum values of the data. The different letters whiskers showed that HCN-p was significantly different at the three temperature levels.

4.5 Discussion

Effect of genotype on hydrocyanic acid content

The results of the current study showed a significant influence of sorghum genotype on hydrocyanic acid content. Genetic differences in sorghum on hydrocyanic acid content has previously been reported (El Obeid *et al.*, 2006; Emendack *et al.*, 2018; Jorgensen *et al.*, 2005; Kaur *et al.*, 2020; Mohanraj *et al.*, 2006; Singh *et al.*, 2008; Sun *et al.*, 2018; Zagrobelny *et al.*, 2008). The enzymes that are involved in dhurrin synthesis pathway are CYP79, CYP71E-P450 monooxygenases and UDP-Glc-glucosyl transferase enzymes they are influenced by 3 structural genes namely *CYP79A1, CYP71E1, UGT85B1and POR*. Since cyanogenesis is a genetically controlled process, natural selection and selective breeding for adaptation could indirectly cause variation in dhurrin content among sorghum cultivars. The 20 genotypes also had obvious phenotypic variation as observed in architecture, height and days to maturity. Stay-green is the maintenance of greenness of leaf and delayed senescence during growth, and has been associated with post-flowering drought tolerance in sorghum (Thomas and Howarth, 2000). In the current study, stay-green line B

35 had the lowest HCN content compared to other lines including the commercial fodder variety E6518. The findings can be attributed to the fact that line B35 matured early and probably did not have sufficient time to accumulate hydrocyanic acid. In another study, Muller *et al.* (2020) observed low HCN content in line B35 when 25 sorghum lines were evaluated for hydrocyanic acid potential. Though these two separate studies seem to suggest an association of stay green with low HCN, Burke *et al*. (2013) reported high dhurrin content in a stay green sorghum genotype. The authors observed a significantly higher dhurrin content of stay green line (BTx642) than in a senescent line (Tx7000) in both fully irrigated and water-stressed conditions.

The *bmr* lines EST 37 and EST 36 also had low HCN-p compared to other cultivars which had comparatively higher lignin content. The *bmr* trait in sorghum is associated with low lignin. The observed low HCN among the *bmr* could suggest a strong correlation between lignin content and hydrocyanic acid content in sorghum. Further research could be necessary to confirm this finding. Taller lines EST 20 and E6518 had lower HCN content which can be associated with a dense canopy that could have led to lowering of temperature at the overall plant canopy. The low temperature in this study has been shown to favor low dhurrin accumulation. Taller sorghum varieties having low HCN have been reported (Pistoia *et al.*, 2003; Sher *et al.*, 2016). The low HCN content in variety E6518 could be due to the fact that HCN content in sorghum is a heritable character and subject to modification by selection and breeding as well as by climate (Abusuwar & El Zilal, 2010). Variety E6518 is a commercial sorghum fodder and is expected to have been released due to its good quality characteristics, among which is the lower HCN content.

Effect of developmental stage on hydrocyanic acid

Sorghum developmental stages have shown a significant influence on HCN concentration. Sorghum fodder harvested at booting stage had higher HCN content compared to those harvested at dough stage. Similar observation indicating reduced HCN with advanced developmental stage in sorghum is available (Carlson & Anderson, 2013; Srinivasa *et al*., 2006). The results of this study showed that sorghum leaves harvested at booting stage had higher HCN content compared to those at dough stage due to recycling of dhurrin in a glutathione transferase-catalysed process. This is a process that takes place without simultaneous release of HCN (Bjarnholt *et al*., 2018) suggesting that other than being a defense mechanism, dhurrin could be having other roles in the plant growth and development. The high level of HCN at booting stage, which marks the end of vegetative phase and the beginning of reproductive phase has been linked to a possible role of dhurrin in reproductive fitness (drought tolerance) in preparation of plants against possible herbivore attack during reproductive phase (Busk & Moller, 2002). Dhurrin also serves as a source of reduced nitrogen during grain filling (Gleadow *et al.,* 2021) hence could be the reason for reduction of HCN content at sorghum dough stage in the current study.

Effect of environment on hydrocyanic acid

The results of this study have shown that environment had a significant effect on the concentration of HCN in sorghum genotypes as harvested at two different developmental stages. Clearly, there was a significant increase in HCN as we moved from a cool region (Egerton) to a relatively warm (Rongai) and hotter region (Marigat). There was a significant variation on temperature in the three sites. The average mean temperature during the growing season at Egerton (0º 22' S; 35º 55’ E), Rongai (0^{o}23’N; 35^{O} 51’E) and Marigat (0°46’ N;35^{o} 98’E) was 16.52-19.53 ^{o}C, 21.75-22.92 ^{o}C and 24.24-27.29 ^{o}C, respectively. In the current study, the high concentration of HCN in sorghum grown in Marigat is linked to high mean growing temperature. Egerton site had the lowest mean growing temperature hence the lowest concentration of HCN. Since extremely high temperature is detrimental to plant growth, increased production of HCN in crops growing in such environment could be a response mechanism by the crop against temperature stress. High temperatures increase the transpiration of plants, translocation of contaminants which may result in higher accumulation of HCN as a toxicant, which eventually play a phytotoxic role in the plant (Yu *et al.*, 2007).

Differences in soil chemical properties in the three locations could have also contributed to differences in HCN-p in the sorghum genotypes. According to soil analysis, the soil at Marigat site had pH varying between 5.8 to 6.0 with low-moderate organic matter content (appendix 3.3), whereas soil at Egerton site had a pH range of 5.5 to 6.5 and high organic matter content (appendix 3.1). Rongai site on the other hand, had soil with a pH range of 5.5-5.8 and moderate organic matter content (appendix 3.2). High organic matter content in the soil at Egerton led to high crop vigor with little environmental stress to crops hence little plant response and ultimately less dhurrin accumulation as compare to Marigat and Rongai whose soils had moderate organic matter content. There were no distinct differences in soils across the sites as soil ammendments were done by application of fertilizers at rates that were informed by soil analysis results. The observed variation in HCN-p could probably be due to temterature variation in the three sites.

CHAPTER FIVE

THE INFLUENCE OF TEMPERATURE ON HYDROCYANIC ACID POTENTIAL IN SORGHUM

Abstract

Sorghum as fodder is limited by high hydrocyanic acid (HCN) content which has been shown to vary with environmental growing condition and the crop's developmental growth stages. Two experiments were set up in and outside greenhouse and another in a growth chamber with temperature control to determine the effect of growing temperature on hydrocyanic acid potential (HCN-p) of sorghum cultivars. Five sorghum cultivars which were selected based on HCN-p content and were grown in and outside greenhouse and their HCN-p determined at different growth stages. The selected cultivars were IESV9110LT, IS11442, E6518 (Commercial fodder sorghum), EST 37 (*bmr* line) and Nguugu. Each of the genotypes was planted directly in the soil in the greenhouse in a single row measuring 2 m in a CRD and replicated three times both inside and outside the greenhouse. In the growth chamber experiment, three of the five sorghum cultivars selected basedon HCN-p content were used: E6518, IESV91105LT and IS11442. They were grown in pots in greenhouse for 1 month then transferred to be exposed to varied temperatures in the growth chamber. The temperature in the growth chamber was set at three levels; 35/15^{o} C, 25/12^{o} C and 18/10^{o} C (to represent high, intermediate and low temperature environments), relative humidity of 90%, 85% and 80% for the respective temperature levels. The crops were maintained in the growth chamber under the set temperature for 72 hours followed by leaf sampling for HCN-p analysis. The leaf samples were analyzed for HCN-p following the argentometric method according to ISO 2164-1975 standard**.** Results showed that plants grown in the greenhouse exhibited higher HCN-p as compared to the plants grown outside the greenhouse. Cultivars Nguugu and IS11442 had the highest HCN-p ranging from 180-97.20 ppm and 208.8-94.55 ppm respectively while E6518 had the lowest HCN-p in all the development stages ranging between 129.02-57.34 ppm. As expected, plants that were exposed to 35/15^{o}C in the growth chamber had the highest HCN-p in all the three genotypes while those held at 18/10 ^{o}C had the lowest HCN-p. The findings presented here support the hypothesis that increase in temperature leads to an increase in HCN-p in sorghum.

5.1 Introduction

Sorghum is an important crop with potential for forage production due to its tolerance to salinity, drought and extreme temperatures which are prevalent in the tropics (Ottman & Olsen, 2009; Prakash *et al.*, 2010). Sorghum has desirable characteristics that have led to the expansion of its production globally. Its high protein and carbohydrate content as well as tillering and ratooning ability makes it suitable for use as hay or silage. However, the use of sorghum as fodder is limited by the presence of cyanogenic glycoside (which produces hydrocyanic acid HCN) which reduces its nutritional value due to its toxic effects on livestock. All sorghum genotypes contain cyanogenic glucoside, dhurrin, together with glucosidase enzymes which break down the compound into hydrocyanic acid (HCN) (Oten, 2017). Sorghum plants contain dhurrin in high quantities that is toxic to ruminants except in mature seeds. The amount of dhurrin in sorghum is at its peak at the seedling stage and reduces gradually as the crop matures and reaches non-toxic levels after dough stage (Al-Beiruty *et al.*, 2020).

The accumulation of dhurrin in sorghum is affected by several factors which include environmental conditions, genotype, plant part and stage of development. Highest dhurrin levels are reached before the boot stage and as plants mature, the stalks make up a greater proportion of the plant, causing dhurrin content in the total forage to decrease (Srinivasa *et al.*, 2006). Severe drought and freezing have also been reported to favor high production of dhurrin in sorghum. Other factors that lead to high accumulation of dhurrin in plants include use of herbicides such as 2,4-D, and application of high rates of nitrogen fertilizer. Most studies that evaluate the effect of environmental factors on dhurrin content in sorghum have mostly focused on drought, freezing, light and soil factors (Pandey *et al.*, 2011). Due to global warming, environmental temperatures have increased to extreme levels beyond crops threshold requirements. Extremely high temperatures affect crop physiological processes negatively forcing crops to develop ways of mitigating the stress or reducing its effects. One of the ways plants employ during high temperature stress is by production of antioxidants, secondary metabolites, hormones, and osmoprotectants (Nahar *et al.*, 2015). Numerous studies have proved that osmoregulators, anti-oxidants, signaling molecules, polyamines, and trace elements confer high temperature stress tolerance in the organisms. The compounds that protect plants during high temperature stress include; low molecular mass aliphatic amines and organic polycations such as proline, polyamines, signaling molecules such as nitrogen oxide, glutathione, gibberellins, auxins and abscisic acid among others (Nahar *et al.,* 2015).

Dhurrin possesses some properties that are akin to the aforementioned signaling molecules, which include; quick and efficient production, induction of specific responses at low concentration and its ease to diffuse from cyanogenic plant to the immediate environment. In stressed plants, cyanide production is stimulated by 1-aminocyclopropane-1-carboxyllic acid synthase (ACS) activity (Rosati *et al.,* 2019). The stimulated ACS activity leads to elevated levels of endogeneous cyanide in plants which leads to a temporary increase in dhurrin concentration. The increase in cyanide levels only occur in a small region of plant tissue serving as a signaling cellular molecule in the plant and triggers phenomenon that consequently led to acquisition of stress resistance (Siegień & Bogatek, 2006).

When dhurrin increases to toxic levels in sorghum, the forage becomes poisonous to livestock when ingested. Hydrogen cyanide is synthesized from dhurrin from the action of two biosynthetic enzymes; CYP 79A1 and CYP 71E1. The levels of dhurrin inside the plant cells is regulated by the levels of the two enzymes coupled with their protein and mRNA (Busk & Moller, 2002). Dhurrin is a secondary metabolite that is non-toxic to the plant but only becomes toxic after hydrolysis process upon tissue disruption through the action of dhurrinase and β-glucosidase enzyme. This hydrolysis process releases HCN which provides a chemical defense system against herbivory and pathogens attack. Other than this proven role of cyanogenic glycosides in plant chemical defense, the compound could be possessing other physiological functions which are closely linked to phenotypic plasticity during environmental stress (Picmanova *et al.,* 2015; Sohail *et al.,* 2022). Amides that are formed during dhurrin turnover pathway in sorghum could be evidence that dhurrin is involved in reactive oxygen species reduction in stressed plants. Amides form part of the non-enzymatic antioxidant system in plant ROS scavenging system in plants.

Increased environmental temperature during crop growth period affects plant growth and development (Hatfield & Prueger, 2015). Due to climate change, warmer temperatures are expected which will impact on crop productivity. Nonetheless, the role of cyanide as enhancer of stress tolerance in plants has not received much attention in HCN studies. In order to assess the link between HCN and increase in temperature during sorghum growth, HCN potential (HCN-p) of five sorghum cultivars grown in and outside the greenhouse were determined and, in another experiment, three of the sorghum cultivars were subjected to three different temperature conditions in a growth chamber. The hypothesis of the current study was that plants grown in the greenhouse and those exposed to high temperature would have higher HCN-p than those grown outside the greenhouse and those exposed to low temperature.

5.2 Materials and Methods

5.2.1 Site description and experimental procedures

a) Greenhouse experiment

The experiment was conducted in and outside the greenhouse at Egerton university, Njoro campus. Egerton University (0º 22' S; 35º 55’ E) which is at an altitude of 2286 m above sea level with average temperature in the greenhouse ranging 25.95-38.44^{O}C while outside was 11.10 -18.50 ^{o}C. Soil in the greenhouse was deep sandy loam and the plants were grown directly on the ground. Natural lighting was used for plant growth and daily minimum and maximum temperature were taken using a digital minimum and maximum thermometer.

Experimental design and treatments

In a CRD experiment with three replications, sorghum seeds which were obtained from a field experiment conducted in 2020 to determine the effect of environment on HCN-p of sorghum were evaluated. The sorghum cultivars were IESV9110LT, IS11442, E6518 (Commercial fodder sorghum), EST 37 (*bmr* line) and Nguugu a locally grown line. The genotypes were selected based on HCN-p with high, moderate and low HCN-p. A similar experiment was set up in the field outside the greenhouse to act as a control.

Planting and crop management

Sorghum seeds were sown at the rate of 10 kg ha^{-1}, at a spacing of 60 cm by drill and at a depth of 2 cm. Thinning was later done when the seedlings were at 20 cm height to spacing of 60 cm x 15 cm. Phosphorous fertilizer was applied during sowing at the rate of 60 kg P_2O_5 kg ha^{-1}. Top dressing was done by applying Nitrogen at the rate of 60 kg N /ha. Hand weeding was done control the weeds. Irrigation was done daily by drip irrigation.

Sampling of plants

Plants in and outside the greenhouse were sampled at - growth differentiation point, flag leaf, booting, half bloom and at soft dough stages of development. Simple random sampling was done on the flag leaf from four plants with the same height by clipping from the leaf sheath. The samples were placed in an air-tight container, labelled and placed in a cool box immediately. The samples were then taken to the laboratory for analysis of HCN-p.

b) Growth chamber experiment

Planting and crop management

Three sorghum cultivars (*Sorghum bicolor* (L) Moench); E6518, IESV91105LT and IS11442 were grown in the greenhouse in pots and transferred to the growth chamber at vegetative stage of development (one month after planting). The plants were grown under greenhouse conditions in pots under natural light conditions at Egerton University during March and April 2022 period. The growth media consisted of pure sand and loam soil (3:1). Each cultivar was replicated 3 times in an CRD experiment. The plants were watered every two days to saturation while in the greenhouse and in the growth chamber.

Growth chamber temperature exposure

The plants in the pots were transferred to a growth chamber with three temperature levels; 35/15° C, 25/12° C and 18/10° C. The relative humidity was maintained at 90%, 85% and 80%. During the day, all the fluorescent tubes and bulbs were switched on to mimic day period while at night the bulbs and fluorescent tubes switched off automatically from 1800 hrs to mimic natural dark period. At each temperature level, three pots representing one replication from every genotype were placed in the chamber and left to stay for 72 hours before sampling leaves and placing in a cool box containing ice packs. The samples were transferred to the laboratory for analysis of HCN-p.

Sampling of plants

All the leaves from the plants were clipped using a pair of scissors and placed in an airtight container then put inside a coolbox filled with ice packs before transferring to the laboratory for analysis of HCN-p.

5.2.2 Determination of hydrocyanic acid potential

Hydrocyanic acid potential was determined using the argentometric method according to ISO 2164-1975 standard**.** The leaves were cut into pieces approximately one-half inch in length with a pair of grass shears. Fifty grams of stripped leaves were used for a bulk sample. The finely cut leaves were thoroughly mixed by hand before sampling for distillation. Twenty grams of the finely cut sorghum leaves were macerated in an electric grinder with a small quantity of pure silica sand moistened with a few cubic centimeters of distilled water. After maceration the samples were transferred to an 800 ml Kjeldahl flask with approximately 300 ml of distilled water to wash out the mortar. Ten millilitres of sodium acetate was added into the flask. After transferring the macerated sorghum to the Kjeldahl flask, the flask was corked tightly immediately, using a large rubber stopper. The macerated sorghum in the tightly stoppered Kjeldahl flask was allowed to digest over night at room temperature. The HCN was distilled on the ordinary Kjeldahl distilling apparatus into a receiving flask containing 10 ml of 0.05 normal silver nitrate (AgNOa), 1 ml of nitric acid (HNO_3) (to keep the solution acidic). Fifty millilitres of distilled water was then added in to the receiving flask. The receiving flask was wrapped with alluminium foil in order to avoid reduction of silver nitrate solution by light. The silver nitrate remaining in solution was determined by titration with a 0.5 M standard potassium ·thiocyanate solution, using ferric ammonium sulphate as an indicator. The percent HCN was calculated from the titration on a fresh weight basis and converted to parts per million (ppm.) using the formula;

HCN-p $\frac{(mg)}{(kg)}$=0.54(V2-V1) × $\frac{500}{250}$ ×$\frac{1000}{M}$ ……………………………………………. Equation 1

Where:

V1 is the volume of potassium thiocyanate required to neutralize the excess of silver nitrate in the sample test.

V2 is the volume of potassium thiocyanate required to neutralize the excess of silver nitrate in the blank test.

M is the weight (gram) of the test sample.

5.3 Statistical model and data analysis

Analysis of variance was done through generalized linear model using *lm* function of R version 4.3.0 (R) statistical software. Treatment means were separated through Tukey's honest significant difference (HSD) at $P \leq 0.05$. The following statistical model was fitted during data analysis;

Greenhouse experiment model

$Y_{ijk} = \mu + \beta_i + E_j + G_k + EG_{jk} + S_l + ES_{jl} + GS_{kl} + EGS_{jkl} + \varepsilon_{ijkl}$……………………………… Equation 2

μ= Overall mean; Bi=effect due to the i^{th} blocking; G_j=effect due to the j^{th} sorghum genotype; EG_{jk}=effect due to the interaction between j^{th} environment and k^{th} genotype; E_j= effect due to the j^{th} environment; ES_{jl}= effect due to the interaction between j^{th} environment and l^{th} stage
S_l = effect due to the l^{th} stage; GE_{jk}= effect due to interaction between j^{th} sorghum genotype and k^{th} location; GS_{jl} =interaction between j^{th} genotype and l^{th} stage; ES_{kl} =interaction between k^{th} environment and l^{th} stage; EGS_{jkl} = interaction effect between j^{th} eironment, k^{th} genotype and l^{th} stage
$\varepsilon ijklm$= random error term

Growth chamber experiment model

$Y_{ijkl} = \mu + G_i + + S_j + T_k + GSK_{ijk} + + \varepsilon_{ijkl}$………………………………………………………………… Equation 3

μ= Overall mean; G_i=effect due to the i^{th} sorghum genotype; S_j = effect due to the j^{th} stage; T_k = effect due to the k^{th} tempeterature GST_{ijk}=interaction between i^{th} genotype and j^{th} stage and k^{th} temperture; εijk= random error term

5.4 Results

5.4.1 Greenhouse experiment

Cultivar, stage of crop development, temperature and their interactions had a significant effect on HCN-p (Table 5.1).

Table 5.1: Anova table output showing effect of temperature and genotype and their interactions on HCN-p (ppm)

Source	Df	ms
Cultivar (C)	4	399.9***
Stage (S)	4	13854.9***
Environment (E)	1	8863.8***
G*S	16	712.8***
G*E	4	1134.55***
S*E	4	98.93***
G*S*E	16	490.01***

*, **, *** significance at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively, Df- degree of freedom

Hydrocyanic acid potential in sorghum cultivars

When the data on HCN-p was pooled, the five sorghum cultivars differed significantly in HCN-p ranging from 81.02-116.28 ppm (Table 5.2). Cultivars Nguugu and IS11442 had the highest HCN-p (116.28 ppm and 113.29 ppm, respectively) while E6518 had the lowest HCN-p 81.02 ppm (Table 5.2). These findings are in agreement with previous studies by Gupta *et al.* (2002) who reported varietal differences in HCN content in sorghum genotypes grown under similar conditions.

Table 5.2: Hydrocyanic acid potential (HCN-p) (ppm) of sorghum cultivars in the greenhouse experiment at Egerton Universty

Genotype	HCN-p (ppm)
Nguugu	116.28^{a}
IS11442	113.29^{b}
IESV9110LT	99.14^{c}
EST 37	83.56^{d}
E6518	81.02^{d}
HSD test	2.73
CV (%)	3.77

Values in a column, followed by different superscript letters are significantly different at $p \leq 0.05$. CV **(%)** =coefficient of variation

Effect of growth stage on hydrocyanic acid content of sorghum cultivars

Stage of development also has a significant effect on the HCN-p of sorghum. Plants sampled at the growth differentiation stage (GD) had the highest HCN-p and the levels reduced as crops advanced in maturity with the crops at the soft dough (SD) stage having the lowest levels of HCN-p in all the genotypes. The HCN-p values ranged from 137.15 ppm (at growth differentiation stage), to 64.12 ppm at soft dough stage (Table 5.3). In the control experiment, the values ranged between 215.13-108.33 ppm and 78.32-38.85 ppm at GD and SD stages respectively (Figure 5.2). Decrease in HCN-p as crops mature has been previously reported (Carlson & Anderson, 2013; Jadav *et al.,* 2019; Oten, 2017; Simili *et al.,* 2013).

Table 5.3: Hydrocynic acid potential (ppm) of sorghum cultivars sampled at different stages of development at Egerton University.

Stage of development	HCN-p (ppm)
Growth differentiation stage	137.15 ^{a}
Flag leaf stage	118.89 ^{b}
Booting Stage	99.17 ^{c}
Half bloom stage	73.96 ^{d}
Soft dough stage	64.12 ^{e}
HSD test	2.73
CV (%)	3.77

Values in a column, followed by different superscript letters are significantly different at $p \leq 0.05$. Values are means. CV (%) =coefficient of variation

Effect of temperature on hydrocyanic acid content of sorghum genotypes

As expected, temperature in the greenhouse was higher than that outside throughout the experimental period. Greenhouse temperature ranged between 25.95-38.44^{o}C while temperature in the field near greenhouse ranged between 11.10-19.50^{o}C (Figure 5.3). Pooled data showed a significant difference for HCN-p in sorghum grown outside and inside the greenhouse; 117.5 ppm and 101.5 ppm for greenhouse and outside, respectively (Table 5.4). Sorghum grown in the greenhouse had higher HCN-p at all growth stages compared to those in the control experiment except for Nguugu which had higher HCN content in the control experiment at growth differentiation stage (Figures 5.1 and 5.2). Different cultivars responded differently to the high temperature in the greenhouse for example at boot stage, the deviations in HCN-p in the greenhouse compared to outside were 34.65%, 26.53%, 7.4%, 12.05% and 8.4 % for Nguugu, IS11442, IESV9110, EST 37 and E6518 respectively (Figures 5.1 and 5.2). The direct effect of high temperature during crop growth period on HCN in sorghum has not been widely reported except in a few previous studies where sudden increase in temperature has been reported to increases levels of cyanogenic glycosides (Dahler *et al.,* 1995; Pushpa *et al.,* 2019; Ravindran, 1993).

Table 5.4: HCN-p (ppm) of sorghum cultivars grown inside and outside the greenhouse at Egerton University

Environment	HCN-p (ppm)
Inside greenhouse	117.5 ^{a}
Outside greenhouse	101.5 ^{b}
HSD test	1.17
CV (%)	3.37

Values in a column, followed by different superscript letters are significantly different at $p \leq 0.05$. CV (%) =coefficient of variation

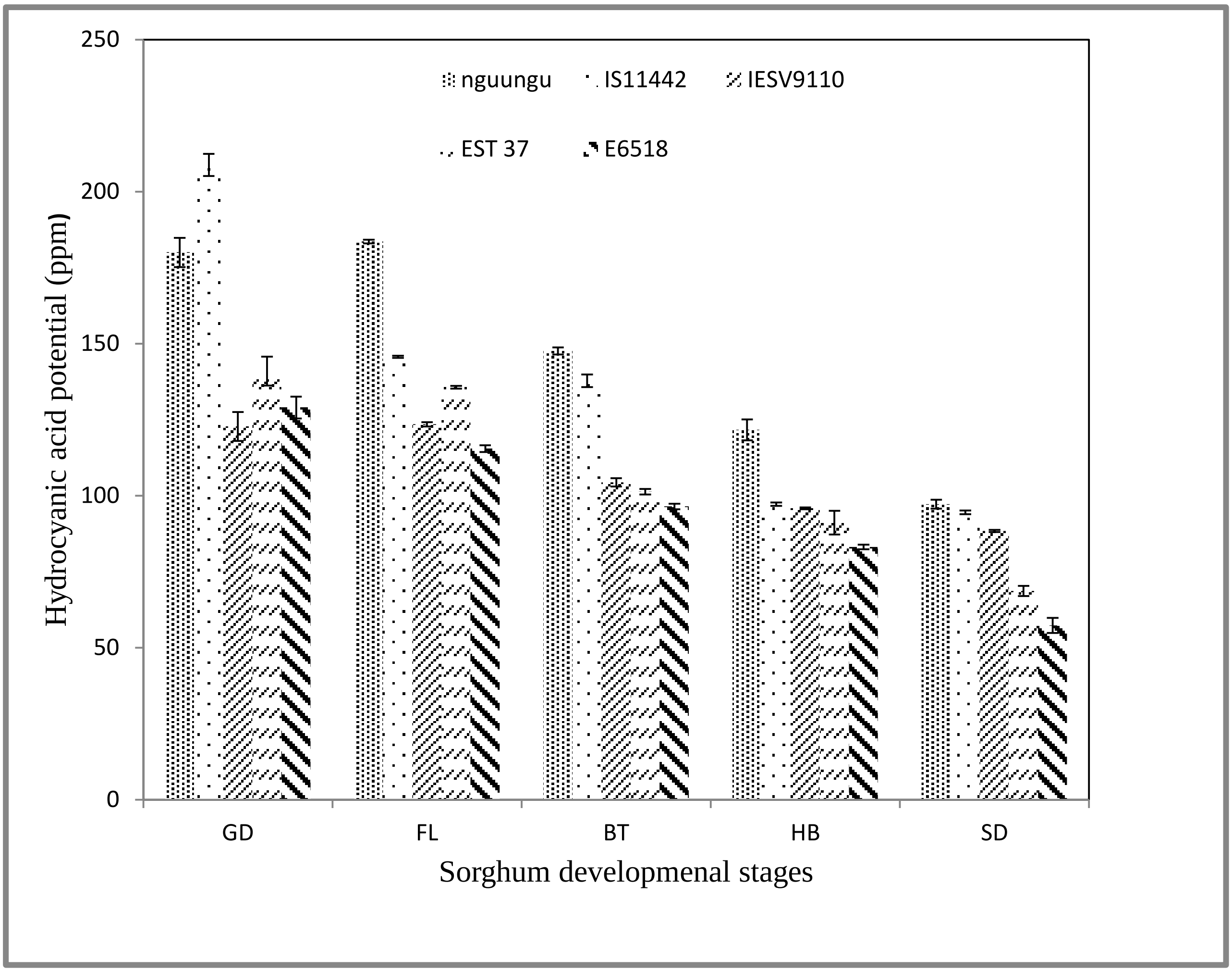

Figure 5.1: Hydrocyanic acid content (ppm) of sorghum genotypes grown in the greenhouse

*GD- growth differentiation point; FL- Flag leaf stage; BT- Boot stage; HB- Half bloom
SD- Soft dough stage

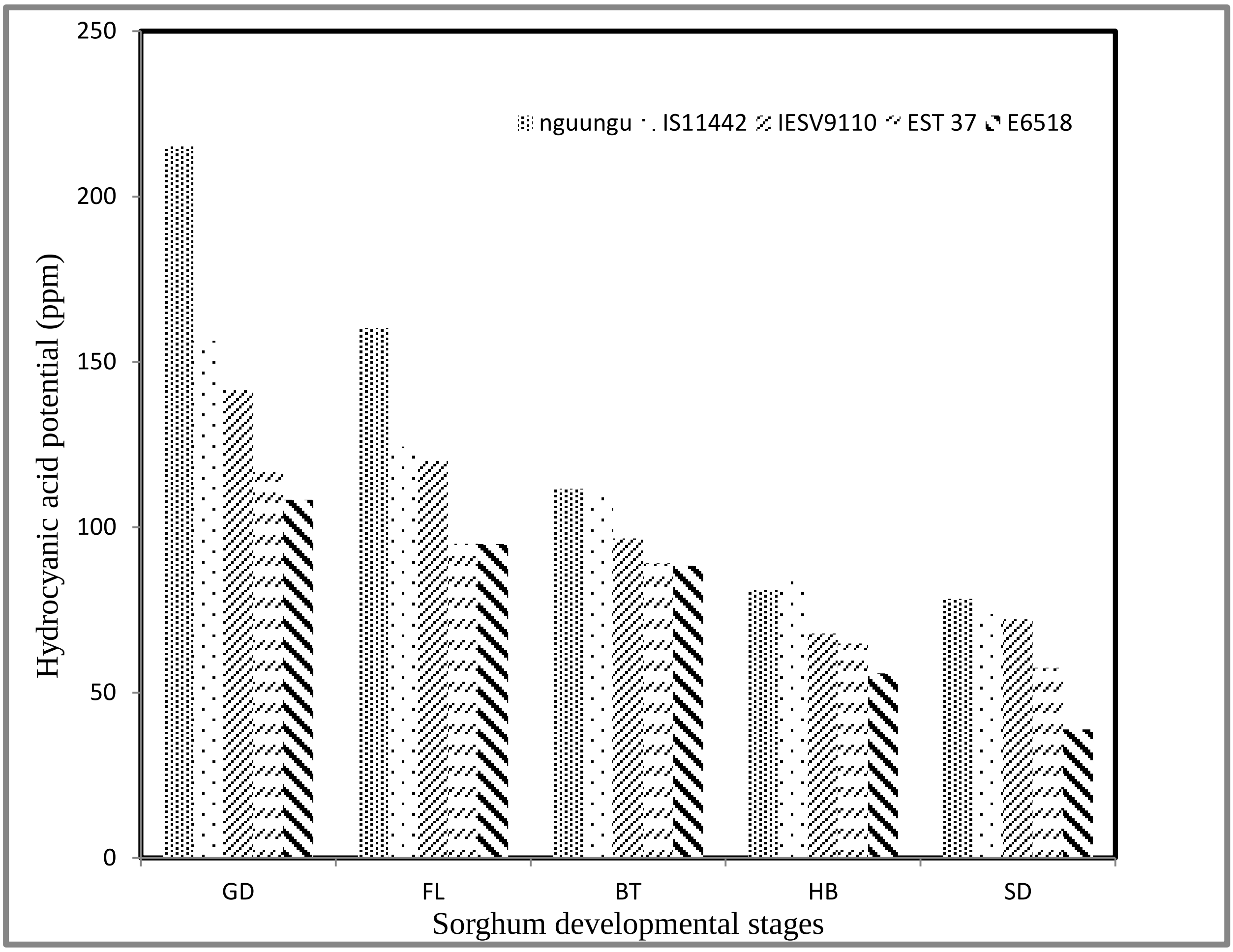

Figure 5.2: Hydrocyanic acid content (ppm) of sorghum genotypes grown in field outside greenhouse

*GD- growth differentiation point; FL- Flag leaf stage; BT- Boot stage; HB- Half bloom
SD- Soft dough stage

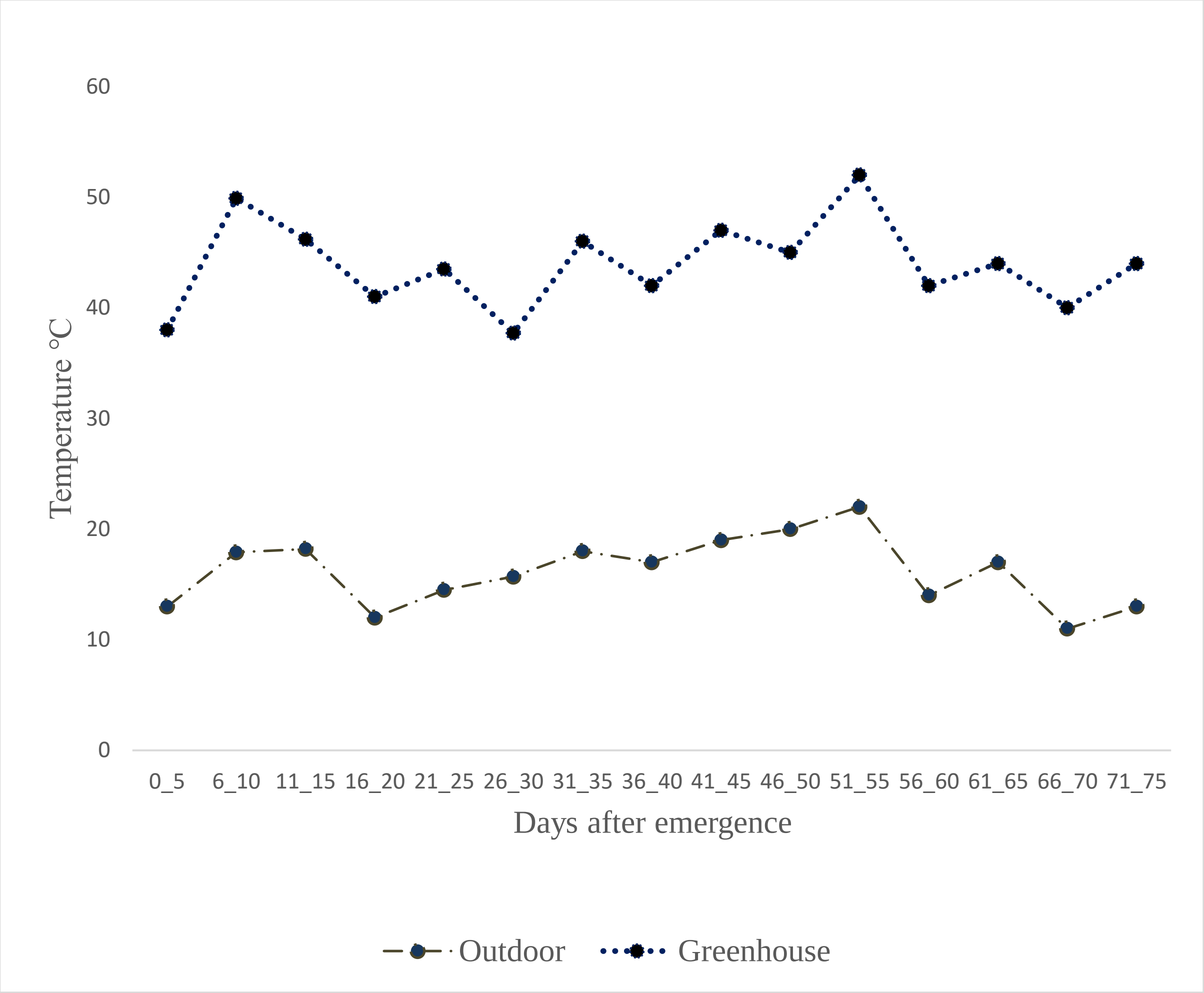

Figure 5.3: Average daily temperature (^{o}C) inside and outside greenhouse

HCN-p (ppm) of sorghum in the greenhouse was greater than those grown in the field outside the greenhouse indicated by higher median values (Figures 5.4 A and B). The greatest HCN-p was recorded at growth differentiation stage as indicated by higher median values in Figures 5.4 A and B. HCN-p of sorghum cultivars grown in the greenhouse had a significantly higher ($P \leq 0.05$) HCN-p compared to sorghum cultivars grown outside the greenhouse. (Indicated by different letters above the whiskers).

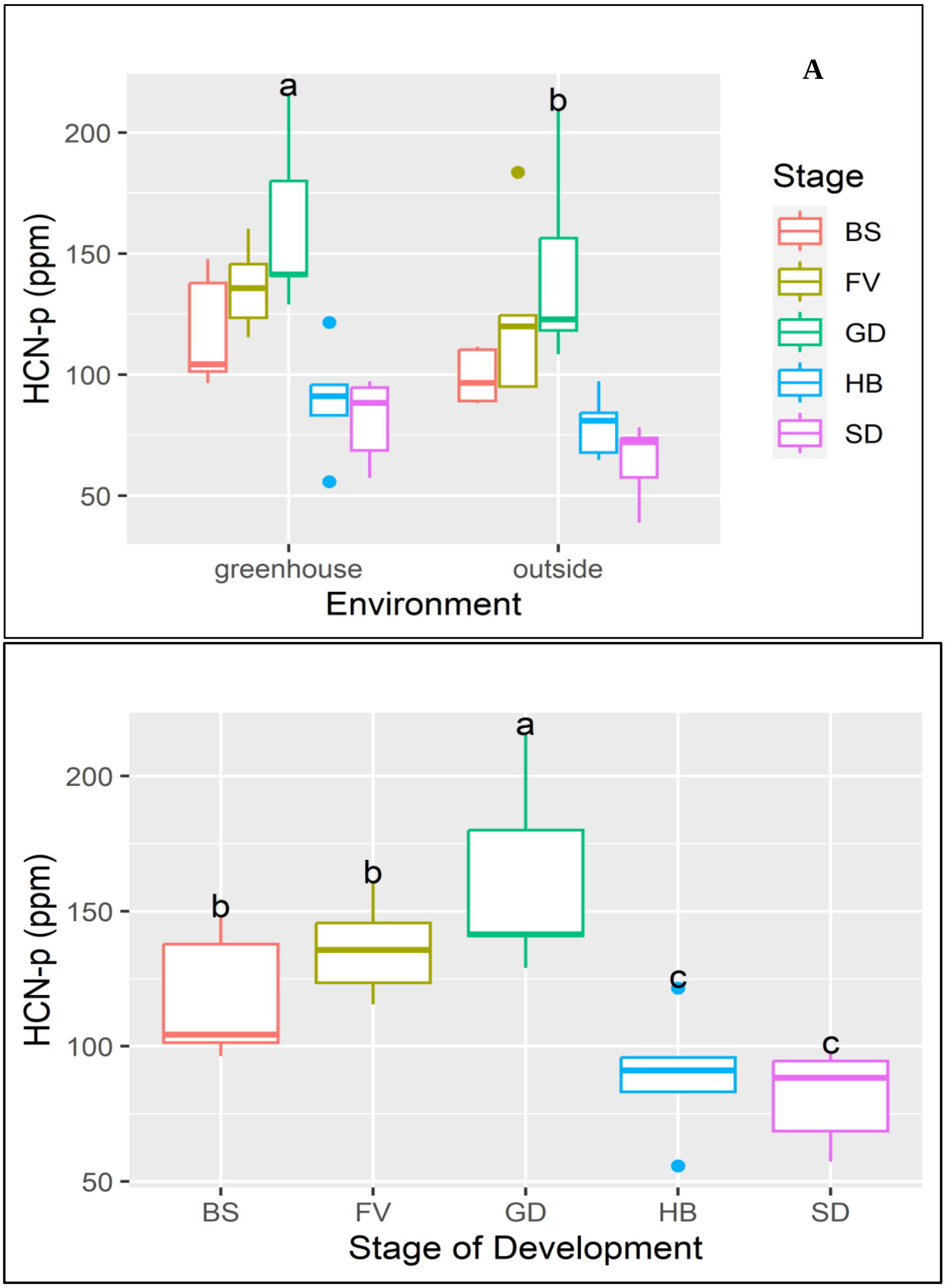

Figure 5.3: Box plot of HCN-p of sorghum genotypes planted inside and outside greenhouse.

The letters in the figure show significant differences at α $p \leqslant 0.05$, $p \leqslant 0.01$ and $p \leqslant 0.001$. Each whisker contains 25% of HCN-p (ppm) of each temperature level. The central box contains half of the values and is divided by the median prussic acid at each temperature level. The outer vertical bars represented the maximum and minimum values of the data. The different letters whiskers showed that HCN-p was significantly different inside and outside greenhouse.

5.4.2 Growth chamber experiment

From the results, differences in HCN-p due to temperature, genotype and interactions were highly significant (Table 5.5). As expected, plants that were exposed to 35/18^{o}C had the highest HCN-p in all the three genotypes while those exposed to 18/10 ^{o}C had the lowest HCN-p (Figures 5.6 and 5.7). Among the genotypes, IS11442 had the highest HCN-p followed by IESV91105LT and IS11442, respectively (Figure 5.5). Different letters on top of the bars showed statistical differences in HCN-p in three temperature conditions. The results also showed that the three genotypes were differently affected by increase in temperature displaying 63.16%, 44.44% and 49.41% change in HCN-p from control to the highest temperature level 35/18^{o}C (Figure 5.6). Results showed that HCN-p (ppm) of sorghum exposed at 35/15°C was greater followed by 25/12 °C and lowest at 18/10 °C as indicated by higher median values (Figures 5.8).

Table 5.5 Anova Table output showing the effect of temperature and genotype and their interactions on HCN-p (ppm)

Source	Df	HCN-p (ppm)
Genotype	2	1484.59**
Temperature	3	35101.87***
Temperature*Genotype	6	3572.5***

*, **, *** significance at P≤ 0.05, P≤0.01 and P≤0.001, respectively, Df- degree of freedom

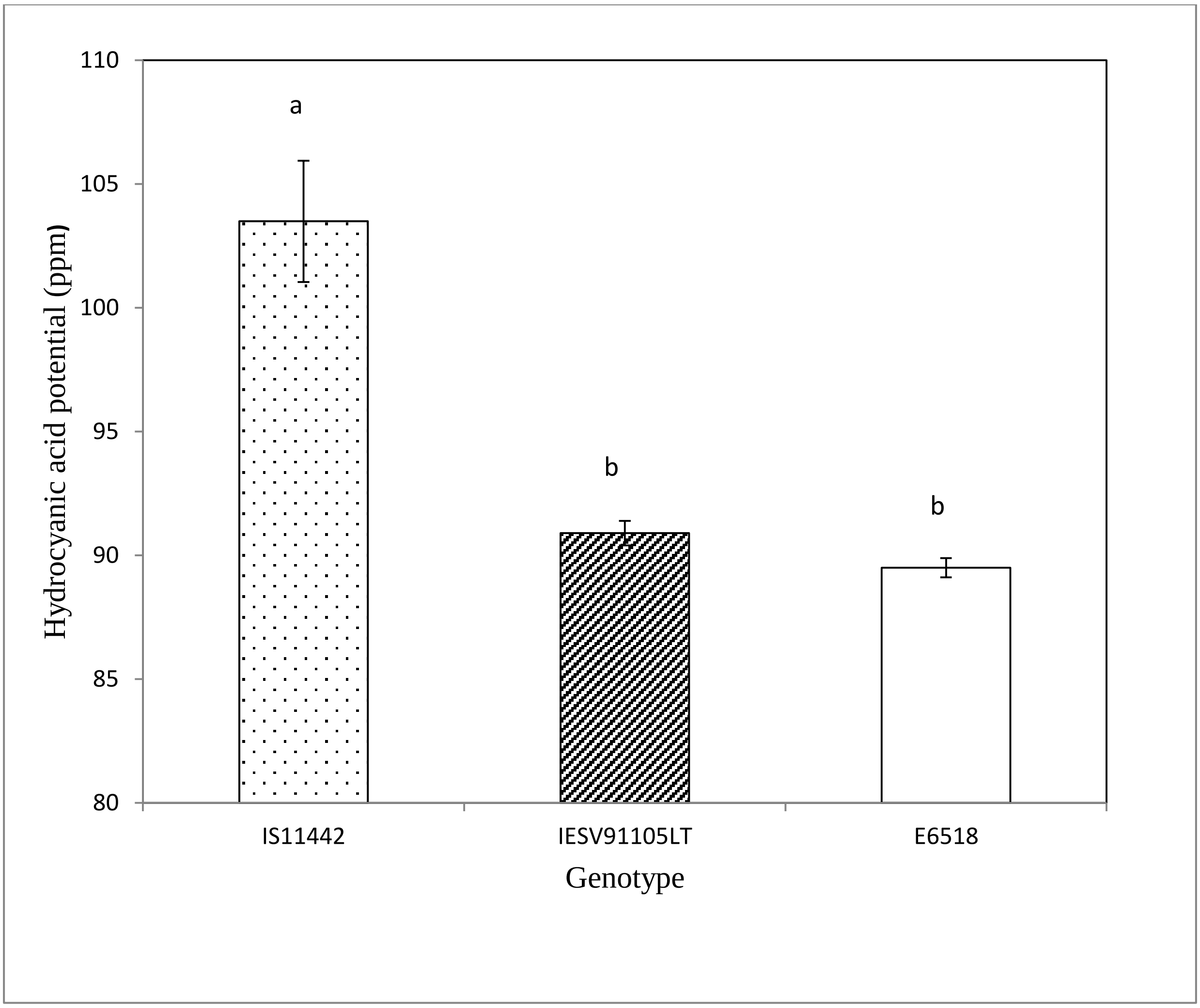

Figure 5.4: Hydrocyanic acid potential (ppm) of sorghum cultivars grown under different temperature levels in the growth chamber

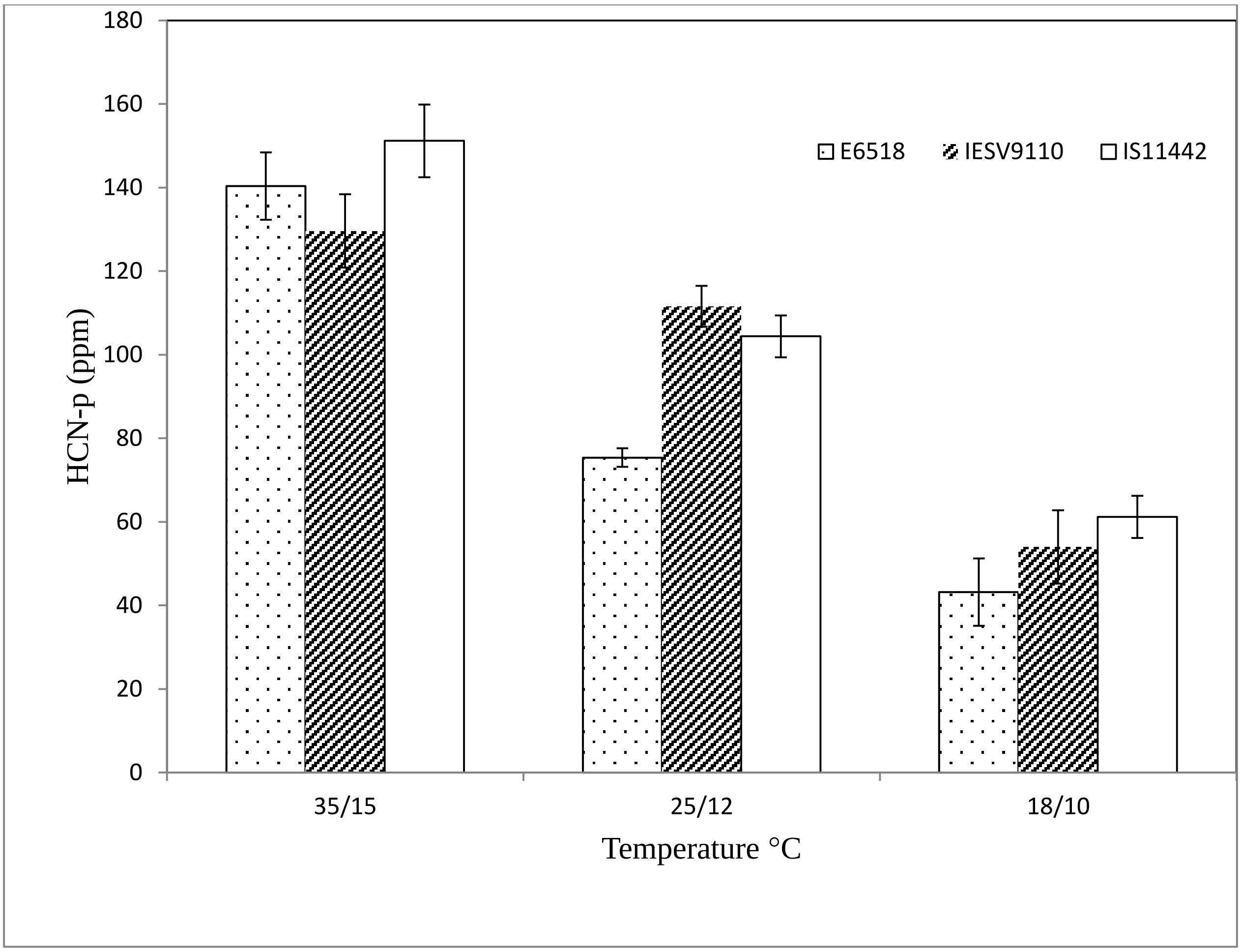

Figure 5.5: HCN-p (ppm) of three sorghum cultivars placed in growth chamber at different temperature levels

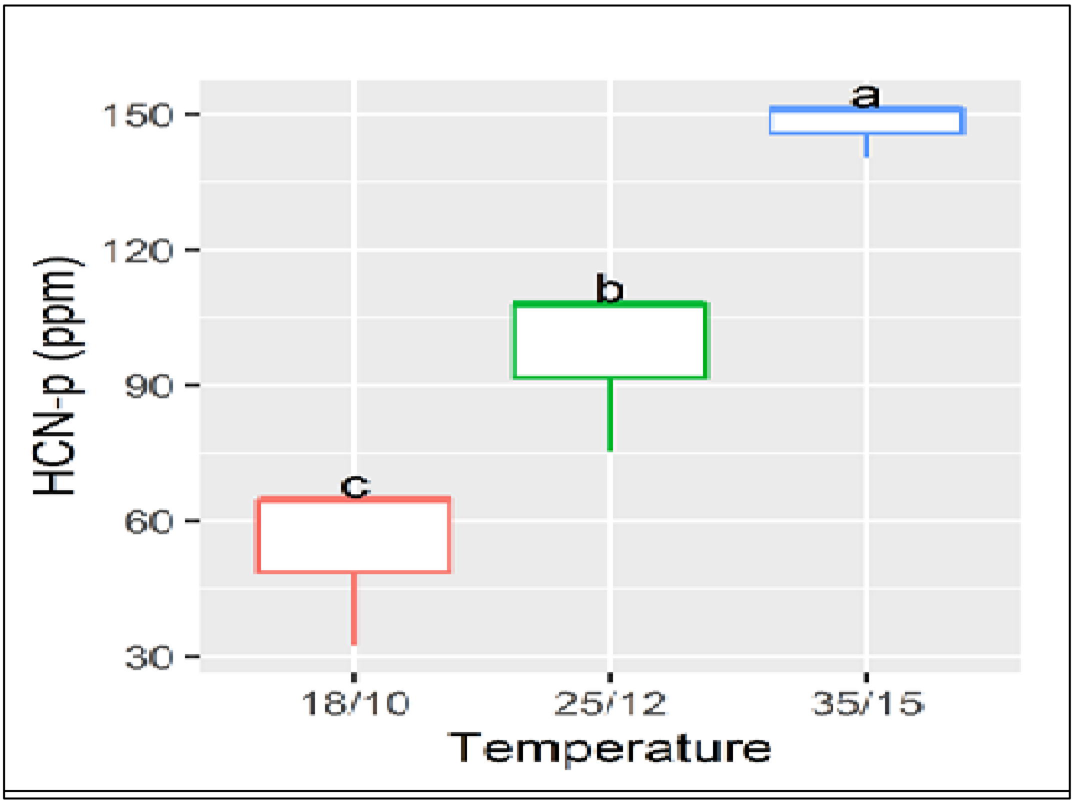

Figure 5.6: Box plot of HCN-p of sorghum genotypes exposed to various temperature conditions growth chamber.

The letters in the figure show significant differences at α p≤ 0.05, p≤0.01 and p≤0.001. Each whisker contains 25% of HCN-p (ppm) of each temperature level. The central box contains half of the values and is divided by the median prussic acid at each temperature level. The outer vertical bars represented the maximum and minimum values of the data. The different letters whiskers showed that HCN-p was significantly different at the three temperature levels.

5.5 Discussion

Effect of genotype on hydrocyanic acid content of sorghum

Dhurrin biosynthesis in sorghum is a genetically regulated process hence the different genotypes recorded different levels of HCN-p even under similar conditions (Gupta *et al.*, 2002). The biosynthesis of dhurrin is regulated by glucosidase enzymes which are also controlled by genetics (Darbani *et al.*, 2016). Differences in HCN-p in sorghum genotypes has been reported elsewhere (Iynar, 2001; Khan *et al.*, 2013; Oten, 2017; Sarfraz *et al.*, 2012). Sorghum variety E6518 is a commercial fodder in Kenya which has been bred and selected for feed. Since HCN content in sorghum is a heritable character, breeding and selection could have led to reduced HCN level in the fodder variety (Abusuwar & El-Zilal, 2010). EST 37 is a sorghum line with *bmr* trait which is associated with low lignin: the low HCN level in the current study could be suggesting a positive correlation between lignin and HCN. In data that is shown here, IS11442 and IESV110 are late maturing sorghum lines and propably the longer period of exposed to the environmental hazards like temperature could have triggered high accumulation of dhurrin in the leaves.

Effect of growth stage on hydrocyanic acid content of sorghum genotypes

In the current study, HCN-p in plants increased gradually with advancement in plant age from growth differentiation stage to soft dough stage, a trend that has been reported in previous studies (Pandey *et al.,* 2011; Srivansa *et al.,* 2006). In most studies, sorghum at pasta (soft dough) stage have the lowest HCN concentration (Calson & Anderson, 2013; Oten, 2017; Simili *et al.*, 2013). Simili *et al.* (2013) reported that HCN content in sorghum decreased with age of plants from 205-230.3mg/100g dry weight in the 2nd week of sowing to 5.9-69mg /100g dry weight in the fifth week from sowing. According to Jadav *et al*. (2019), 25% flowering stage is safe and appropriate stage that animals can be fed on sorghum fodder without any toxic effect. Cyanogenic glycosides play a key role as a storage form of renewable N, C and glucose for seed germination and explains why HCN is higher post germination to growth differentiation stage and reduces in later stages (Kongsawadworakul *et al.*, 2009).

Effect of temperature on hydrocyanic acid content of sorghum genotypes

Climate change and global warming have led to various environmental changes including increased temperatures. Global temperature has increased by 0.3-0.6 °C since the late 19th century and by 0.2-0.3°C over the last 40 years. An increase in environmental temperature leads to an earlier occurrence of crop phonological events including flowering and consequently shortening the crop growth phases (Moriondo *et al.,* 2010). Enzymatic mediated physiological processes like cyanogenesis are elevated with temperature increase. Moreover, when temperatures are high, evapotranspiration rate increases. This could lead to soil moisture deficit within the crop rhizosphere hence triggering processes leading to an increase in dhurrin production in plants (Mohanraj *et al.,* 2006). This may explain why in the current study, sorghum that was grown in the greenhouse had higher HCN-p compared to control. The accumulation of HCN in the greenhouse sorghum could be a strategy by plants to mitigate high temperature stress. The direct relationship between HCN production in plants and high environmental temperature has received less attention creating a need to illuminate triggers and processes responsible for dhurrin accumulation in sorghum with rise in temperature.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General Discussion

Sorghum is an important cereal crop grown for both grain and fodder production. It has high nutritive value which compares to that of maize, making it suitable for use even in feeding lactating dairy cows. Other than crude proteins, sorghum also contains non-dietary fibre fraction which entails; neutral detergent fibre, acid detergent fibre and acid detergent lignin. The fibrous fraction in sorghum fodder affects its forage quality as these fibres lowers forage digestibility. The current study aimed at determining the effect of genotype, stage of development and location variations on anti-nutritive properties of potential fodder sorghum as well as effect of temperature variations on HCN-p of selected fodder sorghum genotypes.

The results revealed that genotype, location, crop developmental stage and their interactions had a significant effect on lignin, cellulose, hemicellulose and HCN-p of sorghum. Cultivar EST 37 which contain a *bmr* gene had low lignin, cellulose and hemicellulose Previous research indicates both *bmr*-6 and *bmr*-18 forage sorghum have decreased acid detergent lignin (ADL) when compared to wild-type forage sorghum. Cultivar B35 is a stay-green dwarf sorghum which took a shorter period to achieve 50% heading (≤90 days). The cultivar had low lignocellulose content which may have been contributed by the shorter maturity period which led to reduced lignin and cellulose build up. Late maturing genotypes such as IS11442, IS25547 and IS11612 took more days to achieve 50% heading which translated to a longer period for lignocellulose material build up on the cell wall therefore they exhibited higher levels of lignin, cellulose and hemicellulose across the growing environments. Overall, lignin, cellulose and hemicellulose concentration increased with advancement in plant maturity such that sorghum harvested at dough stage had higher lignin, cellulose and hemicellulose content than those harvested at booting stage. There was also variation in lignin due to location with sorghum grown at Marigat exhibiting higher lignin, cellulose and hemicellulose content compared to Rongai and Egerton. The observed differences in lignin due to location have been attributed to environmental temperature during the crop growth period.

HCN-p was significantly affected by genotype, stage and temperature in the field, greenhouse and in the growth chamber environments. The *bmr* lines EST 37 and EST 36 and taller cultivars EST 20 and E6518 had lower HCN-p. The low lignin in the *bmr* lines could be associatedwith low HCN-p. In the taller cultivars, a dense canopy could have led to lowering of temperature at the overall plant canopy and low HCN-p in the flag leaves. Line E6518 had a low HCN-p content due to modification by selection and breeding because it is a commercial fodder variety in Kenya. Location affected HCN-p with an increase from cool region (Egerton) to a relatively warm (Rongai) and hotter region (Marigat) due to variation in the average mean temperature during the growing season. The temperature range was 16.52-19.53 ^{o}C, 21.75-22.92 ^{o}C and 24.24-27.29 ^{o}C, for Egerton, Rongai and Marigat, respectively.

The contributory effect of temperature on HCN-p was confirmed in both the greenhouse and growth chamber experiments. Sorghum grown in the green house and those grown at a higher temperature in the growth chamber exhibited the highest HCN-p compared to those grown outside the greenhouse and those grown at lower temperatures in the growth chamber. Increased production of HCN-p in crops under high temperature conditions could be a response mechanism by the crop against temperature stress. High temperatures increase the transpiration of plants, translocation of contaminants which may result in higher accumulation of HCN as a toxicant, which eventually play a phytotoxic role in the plant.

6.2 Conclusions

In summary, the results of the current study revealed that lignin, cellulose, hemicellulose content and HNC-p are a function of environment, stage of development and genotype. This was demonstrated by the significant variations in the anti-nutritive quality attributes of the tested 20 sorghum genotypes sampled at booting and dough stages of development despite the growing environment. The results are therefore useful for breeders and biotechnologists to easily screen and select for sorghum lines with acceptable levels of cellulose, lignin, hemicellulose contents and HCN-p. From the results of this study, it was therefore concluded that;

i. There is a variation among sorghum genotypes based on lignin, cellulose, hemicellulose content and hydrocyanic potential. Sorghum cultivars EST 20, B35, IS23787 and EST 37 have low hydrocyanic acid potential and low lignin content.

ii. High temperature leads to increase in lignin, cellulose, hemicellulose and HCN-p in sorghum.

iii. Sorghum harvested for fodder at booting stage has lower lignin, cellulose and hemicellulose content than those harvested at dough stage. Sorghum harvested for fodder at dough stage has lower HCN-p below the recommended threshold

6.3 Recommendations

Based on the results of the current study, following are recommendations;

i. Sorghum cultivars that have low lignin content and low hydrocyanic acid potential when grown in a cool to wrm environment are EST 20, B35, IS23787 and B37. However, when growing these sorghum cultivars for fodder, in high temperature environmments, the fodder should be left overnight after cutiing before feeding to livestock to avoid prussic acid poisoning.

ii. Cool and warm, rather than hot environment produce good quality and safe fodder sorghum. Caution has to be taken when growing sorghum for fodder in relatively high temperatures because of the likelihood of feeding livestock with elevated levels of HCN in feed. It is advisable that the fodder should be left overnight after cutiing before feeding to livestock to avoid prussic acid poisoning.

iii. The appropriate stage to harvest fodder sorghum that will guarantee maximum fodder digestibility is at booting stage owing to reduced lignin content.

iv. Further research is needed to determine the process that is activated by temperature which triggers increase in dhurrin in production as temperature rises

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APPENDICES

Appendix 1: Research permit

REPUBLIC OF KENYA

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Appendix 2: Analysis of variance (ANOVA) sample outputs

Appendix 2.1 Hydrocyanic acid (ppm) ANOVA output

Source of variation	D.f	S.S	M.S	V.r	Fpr
Genotype	20	141599.4895	7079.9745	38.84	≤.0001
Stage	1	227114.7982	227114.7982	1246.05	≤.0001
Rep	2	1710.0869	855.0435	4.69	
Location	1	348731.7866	348731.7866	1913.29	≤.0001
Season	1	1137.1623	1137.1623	6.24	0.0130
Genotype*Stage	20	23684.0784	1184.2039	6.50	≤.0001
Genotype*Location	19	58072.7093	3056.4584	16.77	≤.0001
Genotype*Year	20	9163.0448	458.1522	2.51	0.0004
Stage*Location	1	42783.8403	42783.8403	234.73	≤.0001
Stage*Year	1	232.9531	232.9531	1.28	0.2590
Location*Year	1	1191.2522	1191.2522	6.54	0.0110
Genotype*Stage*Location	19	33724.3937	1774.9681	9.74	≤.0001
Genotype*Stage*Year	19	3295.3086	173.4373	0.95	0.0418
Stage*Location*Year	1	559.6788	559.6788	3.07	0.0806
Residual	252	64158.438	182.268		
Total		1012982.219			

Appendix 2.2 Cellulose (percent) ANOVA output

Source of variation	D.f	S.S	M.S	V.r	Fpr
Genotype	20	13301.25	665.06	98.50	≤.0001
Stage	1	2071.63	2071.63	1246.05	≤.0001
Rep	2	9.66	4.83	0.72	
Location	1	1995.82	1995.82	295.59	≤.0001
Year	1	1031.57	1031.57	152.78	0.0130
Genotype*Stage	20	624.87	31.24	4.63	≤.0001
Genotype*Location	19	3001.67	3056.4584	16.77	≤.0001
Genotype*Year	20	1319.71	458.1522	2.51	0.0004
Stage*Location	1	138.96	138.96	20.58	≤.0001
Stage*Year	1	1.28	1.28	0.19	
Location*Year	1	654.26	654.26	96.54	0.0110
Genotype*Stage*Location	19	369.12	19.43	2.88	≤.0001
Genotype*Stage*Year	19	309.95	16.31	2.42	0.0009
Stage*Location*Year	1	50.34	50.34	7.46	0.007
Residual					
Total					

Appendix 2.3 Hemicellulose (percent) ANOVA output

Source of variation	D.f	S.S	M.S	V.r	Fpr
Genotype	20	3747.41	187.37	98.50	≤.0001
Stage	1	583.65	583.65	306.82	≤.0001
Rep	2	2.72	1.36	0.72	
Location	1	562.29	1995.82	295.59	≤.0001
Year	1	290.63	290.63	152.78	0.0130
Genotype*Stage	20	176.05	8.80	4.63	≤.0001
Genotype*Location	19	845.67	44.51	23.40	≤.0001
Genotype*Year	20	371.81	18.59	9.77	0.0004
Stage*Location	1	39.15	39.15	20.58	≤.0001
Stage*Year	1	0.36	0.36	0.19	
Location*Year	1	184.33	184.33	96.90	0.0110
Genotype*Stage*Location	19	184.33	184.33	96.90	≤.0001
Genotype*Stage*Year	19	309.95	4.60	2.42	0.0009
Stage*Location*Year	1	14.18	14.18	7.46	0.007
Residual					
Total					

Appendix 2.3 Lignin (percent) ANOVA output

Source of variation	D.f	S.S	M.S	V.r	Fpr
Genotype	20	4667.23	233.36	98.50	≤.0001
Stage	1	726.91	726.91	306.82	≤.0001
Rep	2	3.39	1.69	0.72	
Location	1	700.31	700.31	295.59	≤.0001
Year	1	361.96	361.96	152.78	0.0130
Genotype*Stage	20	219.26	10.96	4.63	≤.0001
Genotype*Location	19	1053.24	55.43	23.40	≤.0001
Genotype*Year	20	463.07	23.15	9.77	0.0004
Stage*Location	1	48.76	48.76	20.58	≤.0001
Stage*Year	1	0.45	0.45	0.19	
Location*Year	1	229.57	229.57	96.90	0.0110
Genotype*Stage*Location	19	129.52	6.82	2.88	≤.0001
Genotype*Stage*Year	19	108.76	5.72	2.42	0.0009
Stage*Location*Year	1	17.66	17.66	7.46	0.007
Residual					
Total					

Appendix 3: Soil data

Appendix 3.1: Chemical properties of the soil at Egerton site

Soil property	**Soil Depth**		
	(0-15)	**(15-30)**	**(15-30)**
% N	0.25	0.24	0.25
P (mg kg-$^{1)}$	2.8	2.9	3.0
K (cmol$_c$kg^{-1})	0.9	0.7	0.8
CEC (cmol$_c$kg^{-1})	9.5	9.6	9.6
PH	5.6	5.7	5.6
Organic carbon	1.7	1.6	1.6

Appendix 3.2: Chemical properties of the soil at Rongai site

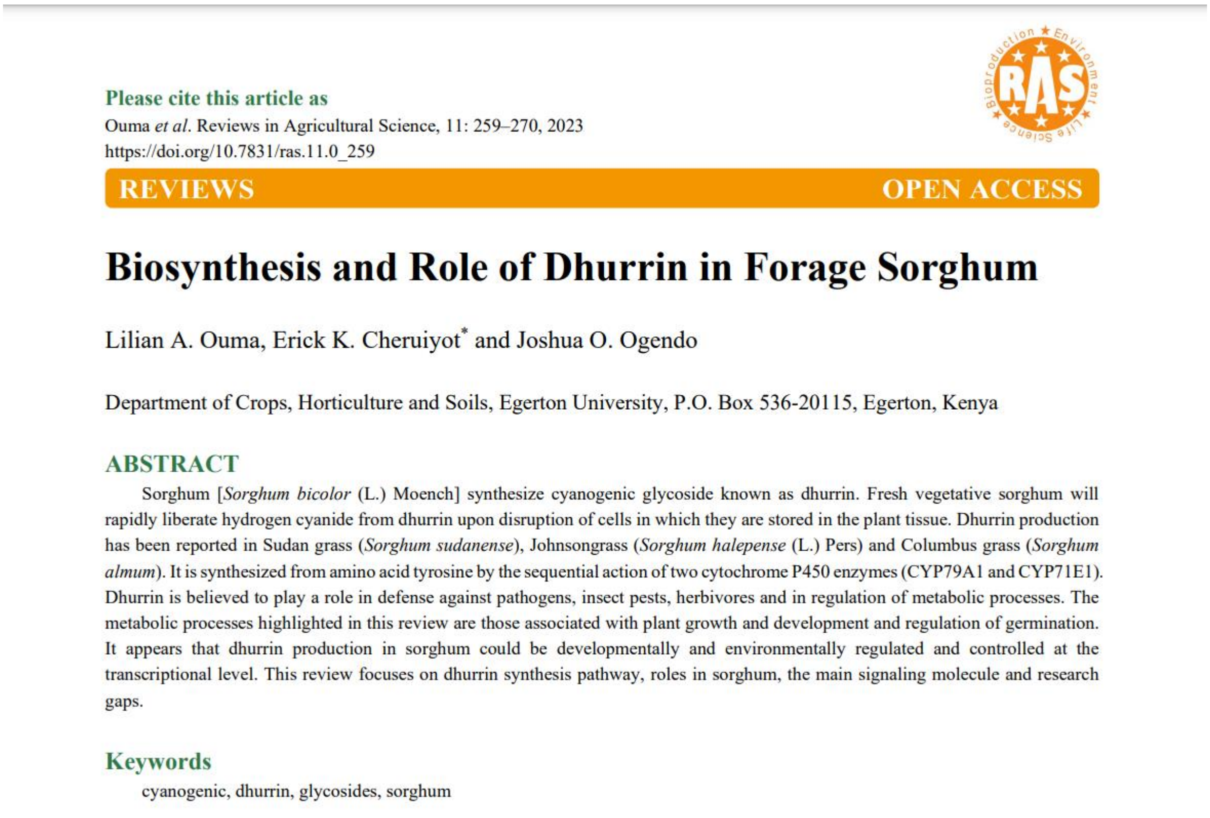
Soil property	**Soil depth**		
	(0-15)	**(15-30)**	**(15-30)**
%N	0.31	0.30	0.4
P (mg kg-$^{1)}$	4.2	4.1	4.2
K (cmol$_c$kg^{-1})	0.6	0.5	0.7
CEC (cmol$_c$kg^{-1})	6.5	7.8	6.2
PH	4.5	4.9	4.8
Organic carbon	0.3	0.4	0.3

Appendix 3.3: Chemical properties of the soil at Marigat site

Soil property	**Soil Depth**		
	(0-15)	**(15-30)**	**(15-30)**
%N	0.21	0.20	0.22
P (mg kg-$^{1)}$	3.2	3.1	3.2
K (cmol$_c$kg^{-1})	0.9	0.9	0.8
CEC (cmol$_c$kg^{-1})	21.5	20.8	21.2
PH	5.8	6.0	5.8
Organic carbon	1.7	1.6	1.6

Appendix 4: Author's Own Publications

a) Publications in Refereed Scientific Journals



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REVIEWS **OPEN ACCESS**

Biosynthesis and Role of Dhurrin in Forage Sorghum

Lilian A. Ouma, Erick K. Cheruiyot* and Joshua O. Ogendo

Department of Crops, Horticulture and Soils, Egerton University, P.O. Box 536-20115, Egerton, Kenya

ABSTRACT

Sorghum [*Sorghum bicolor* (L.) Moench] synthesize cyanogenic glycoside known as dhurrin. Fresh vegetative sorghum will rapidly liberate hydrogen cyanide from dhurrin upon disruption of cells in which they are stored in the plant tissue. Dhurrin production has been reported in Sudan grass (*Sorghum sudanense*), Johnsongrass (*Sorghum halepense* (L.) Pers) and Columbus grass (*Sorghum almum*). It is synthesized from amino acid tyrosine by the sequential action of two cytochrome P450 enzymes (CYP79A1 and CYP71E1). Dhurrin is believed to play a role in defense against pathogens, insect pests, herbivores and in regulation of metabolic processes. The metabolic processes highlighted in this review are those associated with plant growth and development and regulation of germination. It appears that dhurrin production in sorghum could be developmentally and environmentally regulated and controlled at the transcriptional level. This review focuses on dhurrin synthesis pathway, roles in sorghum, the main signaling molecule and research gaps.

Keywords

cyanogenic, dhurrin, glycosides, sorghum

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Growing Temperature Influence Lignocellulose in Sorghum and Could Lead to a Significant Variation in Feed Value of Fodder Sorghum Genotypes

Lilian Ouma[1], Erick Cheruiyot[1] & Joshua Ogendo[1]

[1] Department of Crops, Horticulture and Soils, Egerton University, Njoro, Kenya

Correspondence: Lilian Ouma, Department of Crops, Horticulture and Soils, Egerton University, P.O. Box 536-20115, Njoro, Kenya. E-mail: lilydoty2@gmail.com

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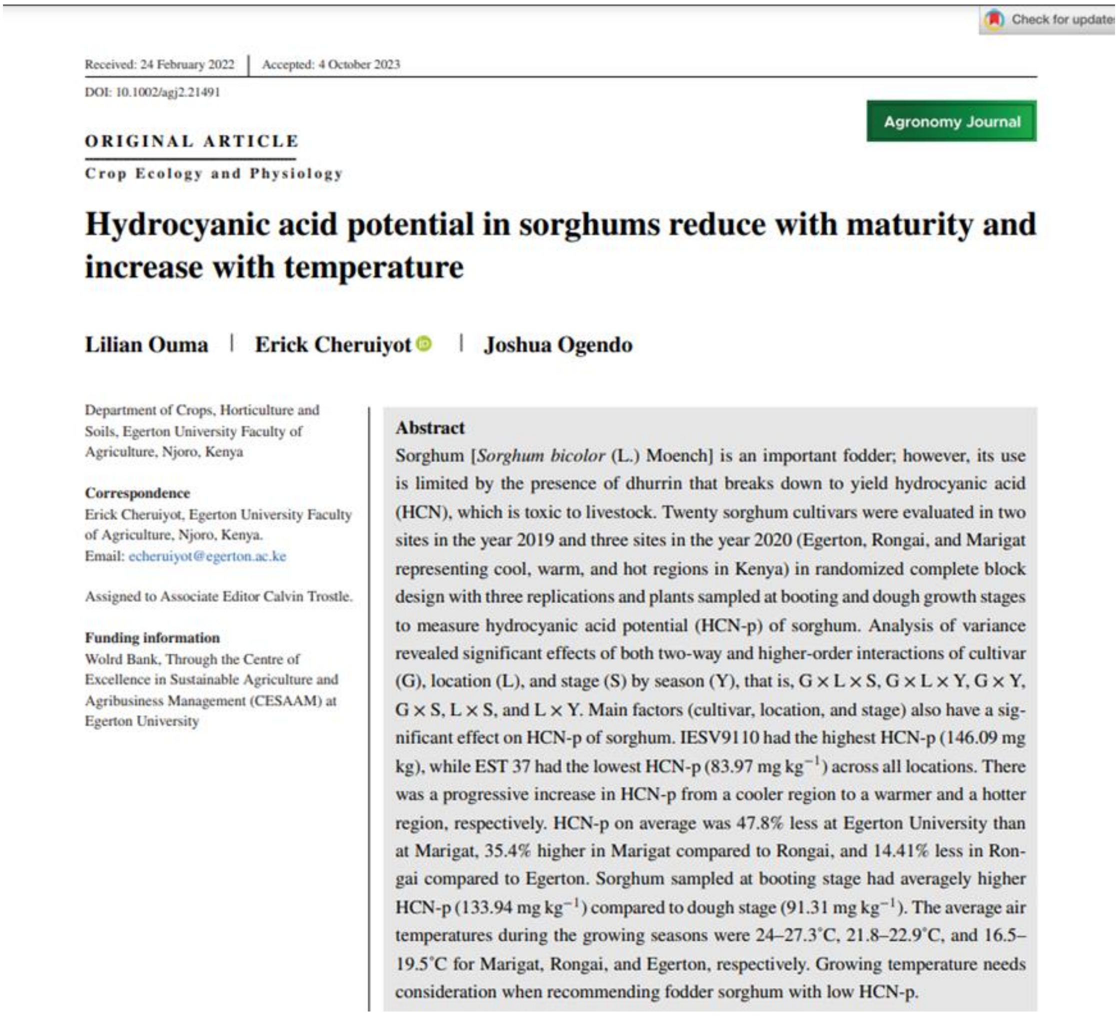
Abstract

Sorghum (*Sorghum bicolor* (L.) Moench) presents a suitable source of green fodder in the livestock sector. However, its use as livestock feed is limited by a high concentration of lignocellulose. A study was conducted to evaluate the influence of environment and developmental stage of harvesting on lignin, cellulose, and hemicellulose concentration of selected fodder sorghum cultivars. A field experiment was laid in a randomized complete block design and replicated three times at Egerton University (0°22′S; 35°55′E in Nakuru county), Rongai (0°23′N; 35°51′E in Nakuru county) and Marigat (0°46′N; 35°98′E in Baringo county) in Kenya in the years 2019 and 2020. Twenty different genotypes of sorghum were grown in a randomized complete block design and sampled at the booting and dough stages of development. The samples were analyzed for cellulose, lignin, and hemicellulose content. Plant growth, number of days to 50% heading, and daily average temperatures were recorded. Cellulose, hemicellulose, and lignin content varied among genotypes and across the three environments. The lowest cellulose content was recorded in line E6518 when sampled at the booting stage at Egerton (17.02%) while the highest concentration was recorded in IS11442 (43.87%) from Marigat at the dough stage. Lignin was highest in sorghum grown at Marigat than at Egerton and Rongai while sorghum harvested at dough stage had higher cellulose, hemicellulose, and lignin concentration than at booting stage. Location which distinctively varied on average daily temperature had a significant ($p > 0.05$) effect on the three parameters with sorghum grown at Egerton showing the lowest lignocellulose content followed by Rongai and Marigat, respectively. Lignin was positively correlated with plant height and days to 50% heading. However, regression analysis showed a negative relationship between days to 50% heading and the total sum of temperature. Crop developmental stage, genotype, and environment determine the lignin, cellulose, and hemicellulose concentration in fodder sorghum. The recommendation of suitable sorghum fodder for a region should consider local growing temperature and the developmental stage of harvesting.

Keywords: sorghum, lignin, cellulose, temperature effects



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Agronomy Journal

ORIGINAL ARTICLE

Crop Ecology and Physiology

Hydrocyanic acid potential in sorghums reduce with maturity and increase with temperature

Lilian Ouma | **Erick Cheruiyot** | **Joshua Ogendo**

Department of Crops, Horticulture and Soils, Egerton University Faculty of Agriculture, Njoro, Kenya

Correspondence
Erick Cheruiyot, Egerton University Faculty of Agriculture, Njoro, Kenya.
Email: echeruiyot@egerton.ac.ke

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Abstract

Sorghum [*Sorghum bicolor* (L.) Moench] is an important fodder; however, its use is limited by the presence of dhurrin that breaks down to yield hydrocyanic acid (HCN), which is toxic to livestock. Twenty sorghum cultivars were evaluated in two sites in the year 2019 and three sites in the year 2020 (Egerton, Rongai, and Marigat representing cool, warm, and hot regions in Kenya) in randomized complete block design with three replications and plants sampled at booting and dough growth stages to measure hydrocyanic acid potential (HCN-p) of sorghum. Analysis of variance revealed significant effects of both two-way and higher-order interactions of cultivar (G), location (L), and stage (S) by season (Y), that is, G × L × S, G × L × Y, G × Y, G × S, L × S, and L × Y. Main factors (cultivar, location, and stage) also have a significant effect on HCN-p of sorghum. IESV9110 had the highest HCN-p (146.09 mg kg), while EST 37 had the lowest HCN-p (83.97 mg kg^{-1}) across all locations. There was a progressive increase in HCN-p from a cooler region to a warmer and a hotter region, respectively. HCN-p on average was 47.8% less at Egerton University than at Marigat, 35.4% higher in Marigat compared to Rongai, and 14.41% less in Rongai compared to Egerton. Sorghum sampled at booting stage had averagely higher HCN-p (133.94 mg kg^{-1}) compared to dough stage (91.31 mg kg^{-1}). The average air temperatures during the growing seasons were 24–27.3˚C, 21.8–22.9˚C, and 16.5–19.5˚C for Marigat, Rongai, and Egerton, respectively. Growing temperature needs consideration when recommending fodder sorghum with low HCN-p.

b) Publications in Refereed Conference Proceedings

Ouma, Lilian (2022) "The Lignocellulosic Component of Potential Fodder Sorghum Genotypes as Influenced by Genotype Stage and Ambient Temperature: Lignin, cellulose and hemicellulose content in selected fodder sorghum genotypes", *Egerton University International Conference*. Available at: https://conferences.egerton.ac.ke/index.php/euc/article/view/68 (Accessed: 5 June 2023).