PHYSIOLOGICAL MECHANISM ASSOCIATED WITH REPRODUCTIVE-PHASE COLD TOLERANCE IN SORGHUM [*Sorghum bicolor* (L.) Moench] GENOTYPES

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented to this university or any other university for any degree or other award.

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Recommendation

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DEDICATION

This thesis is dedicated to Almighty God, to my husband Victor Ababu, son Ethan Amari and daughter Erin Amara.

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ABSTRACT

The low-temperature effect has limited the cultivation of sorghum with desirable traits in the highlands. This project was set up to evaluate and characterize sorghum genotypes for reproductive-stage cold tolerance, determine the lipid fractions associated with cold sensitive and cold tolerant sorghum genotypes, and determine and identify lipid fraction associated with cold tolerance in sorghum. Field experiments were conducted at Egerton University, Njoro (0° 22" S:35° 35″E), and Marigat (0°46' N:35°98'E) while growth chamber, fatty acid extraction and profiling was conducted at Egerton laboratories. Two hundred and fifty (250) genotypes were grown in three replications using Randomized Complete Block Design (RCBD) and evaluated for time taken to 50% heading, plant height, panicle length, panicle weight, panicle harvest index and grain weight. Lipid fraction were extracted using n-hexane a by Soxhlet extraction and methylated into individual Fatty Acid Methyl Esters (FAMES) using Boron trifluoride and separated using Gas Chromatography-Mass Spectrometry (GC-MS). Cluster analysis conducted for the two hundred and fifty genotypes placed sorghum genotypes into three clusters. Genotypes in cluster one remained vegetative, cluster two headed and had productive panicles while those in cluster three had sterile panicles at Egerton site. The genotypes took between 125-205 days to attain heading at Egerton while in Marigat the genotypes took between 49-80 days to heading. The number of tillers was increased for Egerton grown genotypes compared to Marigat. Plant height, panicle length, panicle weight and seed weight was reduced at Egerton in comparison to Marigat. Sorghum grown at Egerton had a mean post-harvest index (PHI) of 0.688 and with a range from 0.322-0.916 while the mean in Marigat was 0.750 with a range from 0 to 0.907. A total of fifteen fatty acids both saturated and unsaturated were identified in sorghum genotypes grown under field and growth chamber conditions. The saturated fatty acids included; palmitic acid, lauric acid, myristic acid as well as pentadecanoic acid. Unsaturated fatty acids included oleic, linoleic, linolenic, Cis vaccenic acid, methyl linolenate and arachidonic acid. The Genotypes grown at Marigat showed an increase in the number of saturated fatty acid while, the genotypes that were productive at Egerton had predominantly unsaturated fatty acids. Methyl linolenate was profiled in cold tolerant genotypes grown in Marigat and Egerton, an indication that this fatty acid can be used as a marker for cold and heat tolerance. Basing on characterization and fatty acid data, further research on GBK 000075 and BM 29 is recommended since they exhibit potential use as breeding materials for cold tolerance genotypes for the highland. Fatty acids if quantified can help understand the response mechanisms towards cold tolerance.

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LIST OF ABBREVIATIONS AND ACRONYMS/ SYMBOLS

ASL	Above sea level
AHE	Automated Hydrolysis and Extraction
ANOVA	Analysis of Variance
ASALS	Arid and semi-arid lands
CAN	Calcium Ammonium Nitrate
FAME	Fatty Acid Methy Esters
GCMS	Gas Chromatography Mass Spectrophotometer
HPLC	High Performance Liquid Chromatography
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IPCC	Intergovernmental Convection Climate Change
KPHC	Kenya Population and Housing Census
N	Nitrogen
NPK	Nitrogen, Phosphorus and Potassium
P_2O_5	Phosphorus pentoxide
PHI	Panicle Harvest Index
RCBD	Randomized Complete Block Design
ROS	Reactive Oxygen Species
SA	South Asia
SAF-LAB	Safe Foods Reference Laboratory
SSA	Sub-Saharan Africa

CHAPTER ONE
INTRODUCTION

1.1 Background information

Sorghum is one of the staple crops for millions of people in Sub-Saharan Africa (SSA) and South Asia (SA), and is an important dryland crop for food, feed, and fuel (Motsi & Phiri, 2022). It is ranked 5th global major cultivated cereal for feed and food after maize, millet, wheat and rice in reference to production (Dahlberg, *et al.,* 2012; Hao *et al*., 2021; Ndlovu *et al*., 2021). In Kenya, sorghum ranks as the third most important cereal after maize and wheat, and it has been reported to perform well across diverse soil types (Kazungu *et al*., 2023). The crop is cultivated in a range of ecological zones including the moist mid-altitudes, semi-arid lowlands, cold semi-arid highlands, and the humid coastal regions. Among these, the semi-arid eastern zone accounts for the largest area under sorghum cultivation (Kazungu *et al*., 2023; Orr *et al*., 2022). Commonly grown varieties in the country include Gadama, Silla, Kari Mtama 1, Kari Mtama 2, IS76, E1291, E6518, BJ28, Ikinyaruka DP, Serena, and Seredo (Infonet, 2025). According to FAOSTAT (2024), the sorghum production area in Kenya covers approximately 207,739 hectares, with an average yield of 0.957 t/ha and an annual output of 198,921 tonnes.

While sorghum is primarily used as animal feed in the developed world, sorghum is mainly used as a staple cereal in the Sahelian region of Africa and South Asia. Moreover, the use of the crop as industrial raw material is on the rise with substantial amount of sorghum grain going to brewery and biofuel industries (Mundia *et al*., 2019). Due to its tolerance to drought and low-input conditions, it represents an essential staple crop and commodity especially in semi-arid regions of Africa, India, Australia, and Americas (Chakrabarty *et al*., 2021). However, as a tropical C4 plant, its sensitivity to temperatures below 15^{o}C is a substantial obstruction to successful implementation in both high-latitude temperate climates and tropical high-altitude areas (Singh, 1985). Though sorghum is noted for tolerance to hot and dry semi-arid environments, its gradual introduction into regions characterized by low temperatures has led to the evolution of cold tolerant lines (Burke *et al*., 2019; Burrow *et al*., 2011; Maulana & Tesso, 2013). Possible sources of cold tolerance have been linked to landraces that have evolved in the temperate regions of China and the highlands of Ethiopia, Rwanda and Uganda (Burow *et al*., 2011; Emendak *et al*., 2021).

Temperature of below 15^{o}C affects early seedling growth through reduction of seed germination, seedling emergence and vigour, thus limiting production (Parra-Londono *et al*., 2018). The reproductive stage of sorghum is considered the most cold-sensitive growth phase

as cold stress at this stage leads to delayed panicle initiation and flowering attributed by the insensitivity to florigen-related genes $SbCN_8$, $SbCN_{12}$ and SbEHD1 and possibly their up-stream regulators SbCo and SbEHD (Abdul-Awal *et al*., 2020). Low pollen germination is usually attributed to anther indehiscence caused by low temperatures (Rao *et al*., 2019). The inability of the pollen to form a pollen tube leads to pollen sterility and thus poor grain set (Rao *et al* 2019; Razzaq *et al.,* 2019).

At the cellular level, lower temperatures slow the velocity of enzymatic reactions as well as reduce the solubility of gases, increases the rigidity of membranes which negatively affect photosynthesis and respiration (Źróbek-Sokolnik, 2012). When photosystem II is downregulated, it promotes the accumulation of reactive oxygen species (ROS) that further damages the photosynthesis process (Yu *et al*., 2022; Zhuang *et al*., 2019). Reactive oxygen species leads to the destruction of plant biomolecules such as proteins, lipids and carbohydrates which leads to untimely cell death (Wang *et al*., 2013).

However, exposure of crops to chilling environments triggers a series of cold adaptation strategies to maintain the integrity of the cell (Atayee & Noori, 2020). Fatty acids are molecules that store energy (Ding, 2015) in the plants and are important for cellular biology and metabolism (Lee *et al*., 2003). Fatty acid helps the plants withstand biotic and abiotic stresses (Okazaki & Saito, 2014). The fluidity and stability of membranes are closely related to plant cold resistance, and is important for membrane function that depends on the interaction between lipid and protein compositions. Unsaturated fatty acids are generally considered to be beneficial for maintaining the stability of membranes (Bai *et al*., 2019; Upchurch, 2008). Unsaturated fatty acids contain one or more double bonds, which make it difficult for molecules to pack tightly, thus increasing the fluidity of the membranes. The increased fluidity of the membrane can maintain liquid form under low temperature stress, so the stability is increased and the cold resistance of the plant is enhanced. Linolenic acid (C18:3) and hexadecatrienoic acid (C16:3) have been found to maintain the fluidity and stability of the chloroplast membrane under cold stress (Routaboul *et al*., 2000). Therefore, increasing the contents of unsaturated fatty acids in membrane lipids can enhance cold resistance in low temperature environments (Wang *et al*., 2019). The unsaturation of membrane lipids is positively correlated with the activity of the enzyme fatty acid desaturase. When the activity of this enzyme increases, the membrane lipid unsaturation and fluidity increase, and the stability of the plant membrane system is improved at low temperatures (Falcone *et al*., 2004; Tian *et al*., 2022; Xiao *et al*., 2022; Zhang *et al*., 2023).

Previous research has delved more on cold tolerance in sorghum at the seeding stage (Chopra *et al*., 2017; Parra-Londono *et al*., 2018; Schaffasz *et al*., 2019), reproductive cold tolerance has not been extensively researched especially on the highlands. Krishnamurthy *et al*. (2014) evaluated the cold tolerance in Postrainy sorghum by looking at the panicle harvest index as a measure for cold tolerance. Amandin *et al*. (2020) evaluated the effect of low temperature on the flowering stage of sorghum without explaining the mechanisms surrounding the cold tolerance and cold sensitivity in sorghum. While attempts have been made to understand the cold tolerance at the phenotypic and genotypic level, little research has been made to understand the mechanism of cold tolerance at the field level. The role of fatty acid in cold tolerance in other crops has been extensively evaluated under controlled environment while for sorghum; the research has aimed at the juvenile development of the crop. The role of fatty acids in cold tolerance in the field for sorghum needs to be evaluated to develop markers for cold tolerance.

1.2 Statement of the problem

Sorghum is a warm climate crop as it is sensitive to temperatures below 15 ºC. Introduction of sorghum in the highlands is a challenge as some genotypes with desirable attributes for grain, fodder and brewing properties do not transition to grain filling due to low temperature. Research has shown that some of these sorghum materials do not produce panicles and those that do, will either have sterile panicles or shrivelled grain. Consequently, the expansion and promotion of sorghum to the arid and semi-arid lands, especially the dry highlands are limited. Plants respond to changes in temperature by employing different physiological mechanisms among them fatty acid molecules. Fatty acids are known to confer tolerance against cold in some plants; and it is believed that some of the fatty acid could contribute to low temperature tolerance in sorghum. Currently, a clear association between fatty acid fractions and cold tolerance in sorghum remains unestablished. Therefore, identifying the fatty acid molecules involved in cold acclimation is essential to guide crop improvement efforts aimed at enhancing sorghum's tolerance to low temperatures. If sorghum genotypes adapted to these conditions are developed, the crop could be introduced into an additional farm area. Given Kenya's current sorghum yield of about 0.96 t/ha (FAOSTAT, 2023), this expansion could translate into an additional production from current 198,921 tonnes per year.

1.3 Objectives

1.3.1 General objective

To enhance sorghum production in the high-altitude agro-ecologies of Kenya, by evaluating and promoting suitable sorghum varieties with improved adaptation to low-temperature conditions.

1.3.2 Specific objectives

i. To characterize sorghum genotypes for reproductive cold tolerance in the tropics.
ii. To determine the lipid fractions associated with cold sensitive and cold tolerance in sorghum genotypes.
iii. To determine and identify lipid fraction(s) responsive to temperature change in sorghum genotypes.

1.4 Hypotheses

i. Sorghum genotypes has no significant variation in response to temperature changes in their growth cycle at reproductive phase.
ii. Sorghum lipid fractions has no association with cold tolerance in sorghum genotypes.
iii. Lipid fraction(s) are not responsive to temperature changes in sorghum genotypes.

1.5 Justification

Sorghum is the fifth most important cereal crop globally after wheat, rice, maize, and barley, serving as a staple food for over 500 million people in semi-arid and arid regions. Its resilience to drought and ability to thrive in marginal environments makes it a strategic crop in addressing food security under climate change. Globally, sorghum is not only valued for food but also for fodder, brewing, and biofuel production, with growing industrial demand driven by its nutritional profile and versatility. At the regional level, particularly in Sub-Saharan Africa, sorghum plays a critical role in food and nutrition security, ranking as one of the top cereals consumed daily. It is widely used for food products, livestock feed, and traditional beverages, and is increasingly promoted in climate-smart agriculture programs due to its adaptability. Despite its importance, production is often constrained by environmental stresses, including low temperatures in highland areas, which limit expansion into potentially productive regions. In Kenya, sorghum production is largely market driven, and the contributory factors are desirable grain or stalk traits. The industries that are now emerging as main consumers of sorghum prefer specific sorghum varieties due to their inherent grain or stalk attributes. Most of the desirable sorghum varieties are currently grown in mid- and low-land regions of Kenya. However, these same varieties are not tolerant to low temperatures and are therefore not

adapted to the cold highlands, thus limiting the expansion of sorghum cultivation in response to specific markets. Some sorghum genotypes are known to tolerate low temperature. Identifying the tolerant genotypes and understanding the physiological mechanisms for tolerance could ease in identification of the responsible gene(s), which can be used to transfer the desirable trait from mid/low-land sorghums to the cold highland adapted genotypes.

Selecting and availing cold-tolerant sorghum genotypes with similar attributes to lowland sorghum genotypes will expand the benefits of sorghum to the highland regions. The accrued benefit will be increased productivity, enhanced livelihoods for farmers, and promotion of agro-based industries with specific market demand.

CHAPTER TWO
LITERATURE REVIEW

2.1 Origin and cultivation of sorghum

According to archaeological evidence, *Sorghum bicolor* originated from its wild progenitor *Sorghum bicolor* (L.) Moench subsp. verticilliflorum (Steud.) which is commonly distributed in Africa (De Wet & Harlan, 1971; De Wet, 1978; Doggett, 1988), the evidence supports domestication in eastern Sudan around 3000 BC (Fuller & Stevens, 2018). The next hypothesis is that the origin of sorghum could be in eastern Sahara, around 9700- 6200 BC (Ehret, 2014), and the final hypothesis relies on the evidence of the race durra in India back in 4000 BC. From its first ancestor in Africa, domesticated sorghum was distributed across the globe by various means most commonly along trade routes. From East Africa, cultivated sorghum was moved across eastern and southern Africa as a result of human migration (Mann *et al*., 1983). It was then introduced to India via the Middle East trade routes (Mann *et al*., 1983). Doggett (1988) reported overland routes from East Africa and Somalia via Aden.

The earliest Sorghum species found in India was *S. bicolor* and evidence for domestication and cultivation dating back to c. 2000–1700 BC was found in the Indus Valley (Fuller & Stevens, 2018; Venkateswaran *et al*., 2019). Since then, sorghum has played a key role in agriculture in India (Kleih *et al*., 2008) and India is now considered to be its secondary centre of diversity (Appa-Rao *et al*., 1996). Sorghum was introduced to China from India, again via sea and overland trade routes. From China, sorghum was brought to the USA by the slave traders in the 19th century. According to Martin (1970), the first sorghum to be introduced to the United States of America (USA) was the Chinese Amber in 1853. Sorghum was introduced to Queensland, Australia, in the 1900s by Americans (Venkateswaran e*t al*., 2019). Since then, sorghum has become a major summer crop in Australia, accounting for 5% of the global export of sorghum globally (Venkateswaran *et al*., 2019).

2.2 Sorghum biology and anatomy

The genus Sorghum was first classified as Holcus by Linnaeus in 1753 and constituted three species; Holcus sorghum, Holcus saccaratus and Holcus tricolor. Sorghum was separated out from the genus Holcus by Moench in 1794 (Venkateswaran *et al*., 2019). Following these classifications, domesticated sorghum was formally recognized as *Sorghum bicolor* (L.) Moench (Hariprasanna & Patil, 2015; Venkateswaran *et al*., 2014). According to the current classification, sorghum belongs to the kingdom Plantae, division Magnoliophyta, class

Liliopsida, order Cyperales, family Poaceae, tribe Andropogoneae, subtribe Sorghinae, and genus Sorghum (Hariprasanna & Patil, 2015).

There exist 25 species of sorghum globally separated into five taxonomic units. The unit *Eusorghum* comprises of the cultivated species and their predecessors: S.× *almum*, S. ×*arundinaceum*, S. × *bicolor*, S. ×*drummondii*, S. ×*propinquum* and S. ×*halepense* are (apart from S. ×*halepense* and S. ×*almum*, which are considered the most advanced species in evolution. The units *Chaetosorghum* and *Heterosorghum* contains S. *macrospermum* and S. *laxiflorum*. Both of these species are and are natives of Australia with S. *laxiflorum* spread across Papua New Guinea and the Philippines. The unit *Parasorghum* have got seven species of Sorghum distributed across Africa, Australia, India and China.

There are several types of sorghum, including grain sorghums, forage sorghums (for pasture and hay), sweet sorghums (for syrups) and broomcorn for making brooms and whiskbrooms (Dahlberg *et al.,* 2012). The crop is suited to hot and dry regions where other food grains do not grow well. The roots are fibrous and adventitious; stomata occur on both leaf surfaces while vascular bundles are scattered around the stem. The panicle may be loose, compact or open with seeds containing tannins.

2.3 Sorghum races

According to Hariprasanna and Patil (2015) sorghum has five races basing on the spikelet which are; bicolor, Guinea, Caudatum, Kafir and Durra. The race bicolor is considered a primitive race whose panicles are open; it has thick glums and elongated grains. This plant produces many tillers and produce low grain yield. Guinea race has long, glabrous and loose panicles which split open and exposes the grain when mature. The plants are light in colour and produce very low yields. Caudatum race is important as it produces high grains and yield of agronomic significance (Mann *et al*., 1983). Kafir plants have cylindrical erect panicles which are semi compact, medium in stature and fairly yielding while Durra the panicles are stiff, compact and dense with pigmented glumes; medium stature plants.

2.4 Sorghum production

Sorghum (*Sorghum bicolor* [L.] Moench) is the 5th major cereal after maize, millet, wheat and rice in reference to production globally (Dahlberg, *et al.,* 2012). The United States is the largest producer of sorghum, producing up to 11.5 million metric tonnes, in the areas of Texas, Oklahoma, South Dakota, Colorado among other regions. India is the second leader producing up to 7.5 million metric tonnes in the areas of Karnataka, and Maharashtra. In Africa, Nigeria

is the leading producer coming third globally with the production of up to 7.4 million metric tonnes (FAOSTAT, 2019). Sorghum production is mainly in Central America, Asia, and Africa, where in the latter two continents, it is used to promote food and nutrition security (Hassan *et al*., 2021). Africa produces about 20% of the world's total production (Dube *et al.,* 2020) which is approximately 20 million metric tonnes. Ethiopia, Tanzania Rwanda, and Uganda produce about 7, 42 and 4 percent, respectively while Kenya produces about 0.8 % of the Africa's total (Mitaru *et al*., 2012).

Global area under sorghum has been increasing while a decline is noticeable in Africa despite the research and emphases to increase the area under food production to feed the increasing population (Grovermann *et al.,* 2018; Orr *et al*., 2016). In Kenya, sorghum production is mainly in the coastal, Nyanza and Eastern provinces, at elevations of 184, 1190 and 1380 meters above sea level respectively (Muui *et al*., 2020). Sorghum production in Kenya is predominantly small scale and it has increased steadily from 195, 507 ha in 2015 to 229, 883 ha in 2018 with a production of 189,000 tonnes in 2015 to 206,234 tonnes in 2018 (FAOSTAT, 2020). The growing altitude ranges from 184 meters to 2500 metres above sea level with temperature of up to 37^{o}C (Vinutha *et al*., 2017).

2.5 Uses of sorghum

United States of American being the greatest producer of sorghum is also the greatest consumer majorly as feed, fodder and silage for livestock production (Hariprasana & Patil, 2015). Hence, sorghum is most preferred due to high biomass accumulation and high caloric value (Almodares *et al*., 2015). Dry matter content, yield, Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) of sorghum is higher in comparison to maize, crude protein was found not to be significantly different (p<0.05) to that of maize. This makes sorghum suitable for ensiling (Qu *et al*., 2021). Therefore, forage sorghum is a major contributor in the development of livestock industry in USA (Kaewpila *et al*., 2021). In developing countries such as Kenya, sorghum is used as a source of feed mostly in traditional, smallholder farming sector.

Sweet sorghum is an excellent feedstock for renewable energy used in production of biofuel, sugar-to-ethanol, starch to-ethanol and lignocellulosic or cellulosic-to-biogas production (Mathur *et al*., 2017; Ravichandra *et al*., 2022). Bagasse which is obtained after extracting the juice is used for the manufacture of paper and pulp (Whitfield *et al*., 2016). It is considered an ideal biofuel crop for the first- and second-generation bioethanol production, particularly having the advantages of exploitation of marginal land and avoiding competition for land for food crops. The conversion of sugar to ethanol requires less energy as compared to starch in

which more energy is used for depolymerization (Khawanja *et al*., 2021). In the USA, sweet sorghum has demonstrated potential to produce up to 6000L ha-1 of ethanol (Timucin, & Yucel, 2020).

Sorghum acts as a dietary staple for millions of people living in about 30 countries in the subtropical and semi-arid regions of Africa and Asia. It is a source of food and fodder, mostly in the traditional, smallholder farming sector in Kenya, sorghum grain is milled into flour and it used to prepare porridge commonly referred to *Uji*. In other countries like Sudan and India, they use sorghum as a whole grain similar like rice (Awika, 2017). Sorghum composite flour has also been used for production of therapeutic foods (RUTF) such as plumpy nuts (Qaku *et al*., 2020) and to increase energy, protein and minerals in 8 biscuits (Adebowale *et al*., 2020; Mridula *et al*., 2015). When 8% of sorghum flour is mixed together with wheat flour, the composite bread has better nutritional and sensory attributes (Mariera, 2018).

Sorghum grain is endowed with saturated and unsaturated fatty acids such as palmitic acid and linoleic acid which are essential in nourishing the body (Oso & Ashafa, 2021). It is rich in minerals and vitamins such as the potassium which amount up to (900-6957.67mg/kg) while Phosphorus is about 1498-3787 mg/Kg. These minerals are essential in maintaining a healthy nervous system and normal functioning of bones and teeth (Nnamchi, *et al*., 2022). Sorghum grain is gluten free and has high nutritional value due to the presence of several antioxidants and micronutrients. This crop can play a key role in food and nutritional security and its grain has potential to be used and promoted as nutritional and health food (Xiong *et al*., 2019).

Sorghum grain is a source of raw material in commercial food industry in making products such as potable alcohol, beer, starch, gruels, malt, adhesive core binders for ore refining, metal casting and packaging materials such as grits (Gopalakrishnan *et al*., 2020; Ochieng' *et al*., 2018; Ogeto *et al*., 2020; Saïed *et al*., 2023). Nutritional content of sorghum including total starch, amylopectin, proteins, tannins, germination energy and capacity are desirable attributed for malting and brewing (Kiprotich *et al*., 2015). In the African traditional set up, sorghum is used for malting for both alcoholic and non-alcoholic beverages. Sorghum was found to have the highest amylase enzyme activity after 96hours fermentation and the alcoholic beverage was found to have no significant differences with that produced from barley (Ratnavathi *et al*., 2016). In Kenya, Gadam and Serena have been utilized for malting by the East African Breweries Limited (EABL) company for brewing beer because of its good malting quality (Shinda, 2021).

On the other hand, sorghum contains antinutritional compounds, such as kafirins, phytates, and tannins, which can influence its processing and utilization (Zhang *et al*., 2025). For example, in the United States, around 25% of sorghum production is directed toward animal feed, typically characterized by low tannin levels. In contrast, Chinese sorghum, which generally has higher tannin concentrations, is mainly used in brewing applications (Zhang *et al*., 2025). Feed-grade sorghum usually contains approximately 5% tannins. Therefore, when high-tannin sorghum is included in swine diets, additional protein or amino acid supplementation may be required to meet the animals' nutritional needs (Pan *et al*., 2022). However, adverse effects on the standardized ileal digestibility (SID) of amino acids are generally observed only when tannin levels exceed 1% (Mariscal-Landín *et al*., 2004).

2.6 Effect of low temperature on growth and development of crops

The degree of crop damage caused by cold stress varies with plant species, stages of growth and development, conditions of irradiance as well as the mineral nutrition during the chilling stress (Kumar *et al.*, 2018). Sharma *et al.* (2012) reported that photo-oxidative injury is determined by formation of reactive active oxygen species which is greater under illuminated conditions. Reduced emergence, poor germination and reduced growth rate and poor seedling establishment are symptoms of lower temperatures during planting (Pill, 2020; Singh, 1985). Chloroplast function is impaired by lower temperatures hence a reduction in photosynthetic activity as observed in maize (Rymen *et al.,* 2007). Lower temperatures limits the growth and distribution of crops such as Sorghum where the temperature can fall below the optimum growth range of 18-28°C (Ercoli *et al.*, 2004). The temperature stress adversely affects many plant physiological processes including seed germination, seedling establishment, photosynthesis, flowering and the grain yields (Prasad *et al.*, 2008). Cold stress reduces the function of chloroplast thus severely affecting photosynthetic efficiency and respiration (Chongping *et al.,* 2022). Crops exposed to low temperatures leads to reduction in growth and development (Cakmak, 2005), which can lead to irreversible death of plant cells if the condition is not adjusted (Wilson & Roberts, 2012).

Lower soil temperatures slow down germination hence poor seedling emergence and seedling vigour which results to yield losses (Chiluwal 2018; Kapanigowda *et al*., 2013). Increase in spikelet infertility and delays in phenological development results to decreased crop yields (Gunawardena *et al.,* 2003; Shi *et al*., 2022). Low temperatures reduce growth and development of sorghum which can be attributed to the impairment of chlorophyll function and low photosynthetic activity hence reduction in yields (Rymen *et al*., 2007). Maulana and Tesso

(2013) reported that chlorophyll content was significantly affected by low temperature by up to 21% reduction in plants exposed to cold stress. Temperature of below 15°C was recorded to reduce photosynthetic efficiency in maize where sensitive genotypes showed greater reductions to photosynthetic efficiency (Wu *et al*., 2022).

2.6.1 Effect of low temperature during reproductive development of sorghum

The juvenile growth and pre-flowering reproductive stages are the most critical growth stages in sorghum (Sanchez *et al*., 2002). Sorghum crop is grown in diverse agro-ecologies and it is better adapted to regions of high temperature and requires a minimum temperature of 15°C. However, recent years has seen sorghum introduced into regions of high elevations with lower temperatures. Various researches have led to the development of some genotypes adapted to cold (Maulana & Tesso, 2013). The few adaptable genotypes developed for the highlands are mainly targeting fodder and forage or dual purpose (Ouma & Akuja, 2013). Lowland varieties of Eastern and Coastal areas are not suitable for growth in the highlands of Kenyan Rift valley as the lower night temperatures and occasional frosts limit seed set (Emendack *et al*., 2021).

There is no escape strategy at the moment for cold spells during periods of reproductive stages which induces male sterility leading to low or no seed and grain yield (Dolferus, 2014). Cold temperatures at flowering stage significantly reduce the mean panicle weight, number of seeds per panicle and seed weight (Maulana, 2011). Lower temperatures affect panicle initiation resulting in male sterility hence degrades proteins in anther cells (Nahar, 2011). These effects are primarily due to the impact of stress at flowering, pollination and fertilization. Mukri *et al*. (2016) reported that when temperatures dropped below 10 °C, panicle initiation was delayed by up to 78.48 days, which subsequently reduced seed set by 57.79%. Temperatures below 13°C expose the crop to male sterility as a result of pollen sterility leading to poor grain set (Hu *et al*., 2021). The meiosis mother spore cells were described as a possible reason for male sterility (Zanders & Unckless, 2019). In addition, low temperatures in the highland exposes the crops to a systemic disease named ergot (*Claviceps africana*) which attacks the ovaries of sorghum leading to poor quality seed and seed set (Dahlberg *et al*., 2001). Most of the sorghum genotypes that show tolerance to cold at seedling stage do not show tolerance at the reproductive stage (Singh, 1985).

2.7 Cold acclimation mechanisms in plants

After exposure to chilling stress, plants develop a series of strategies to deal with the cold, in a process called acclimation. Cold acclimation phase involves a series of intense changes in gene expression leading to alterations in the transcriptome, proteome and metabolome levels

(Chinnusamy *et al*., 2007; Fürtauer *et al*., 2019). The process is an energy demanding process which is provided by upregulation of carbohydrate catabolic process and downregulation of anabolic reactions (Kosova *et al*., 2012). Through unknown mechanisms, proline has been shown to enhance stress tolerance by improving membrane stability, ROS scavenging, and energy and nitrogen storage (Ashraf & Foolad, 2007; Szabado & Savoure, 2010). Some plants are reported to increase their soluble sugars content to scavenge against the ROS mechanisms (Ruelland & Zachowski, 2010). In other plants like Arapdopsis employ antishock proteins to help in cold acclimation. Lipids contribute greatly to the stability of cell membranes and proper functioning of the cells.

2.8. Fatty acid in plants

Based on the chain length of fatty acids, they are classified as: short-chain (aliphatic tails of up to 5 or even 7 carbons), medium-chain (aliphatic tails of 6–8 up to 12–14 carbons), long-chain (aliphatic tails of 13–18 up to 22 carbons), or very long-chain fatty acids (aliphatic tails longer than 22 carbons (Guo *et al*., 2019; Iskandarov *et al.,* 2020; Kamp & Hamilton, 2006; Reynolds *et al*., 2017; Wernig *et al*., 2020). Lipids of plant cell membranes are characterized by a high content of polyunsaturated fatty acids (Wang *et al*., 2006) that consists of carboxylic acids with hydro carbonated chain of between 4-36 carbons. In some, the hydro-carbon chain is totally saturated and non-ramificated; while in others the chain contains one or more double bonds. If a fatty acid has one or more double bond it is classified as unsaturated and if no double bond exists, then it is a saturated fatty acid. In addition, UFA can be divided into Monounsaturated (monoenoic) Fatty Acids (MUFA) and polyunsaturated fatty acids (PUFA) with exactly one or at least two double bonds, respectively (Castro *et al*., 2016; Wang, *et al*., 2013). Palmitic acid (C_{16}) is a case of saturated fatty acid having no bond at all while linolenic and linoleic are among the unsaturated fatty acids (Cruz *et al*., 2010).

Plants fatty acids respond to different environmental stimulus differently. Under high temperature plants tend to accumulate more of saturated fatty acids such as palmitic acid (Cruz *et al*., 2010). Unsaturated fatty acids are accumulated under low temperatures (Tian *et al*., 2022; Wang *et al*., 2021). The modification of membrane fluidity is mediated by changes in unsaturated fatty acid levels, a function provided in part by the regulated activity of fatty acid desaturases which modulates the function of oleic acid and linolenic acid in response to low temperatures (Upchurch, 2008). In Arabidopsis, the accumulation of Linoleic and linolenic acid is triggered by LuFAD2A and LuFAD3A genes (Wang *et al*., 2021). The increased levels

of linolenic acid raise the content of Jasmonic acid, a phytohormone involves in modulating against abiotic stresses in plants such as cold (Wang *et al*., 2021).

Crops which are suggested to be cold tolerant have the levels of linoleic and linolenic acid increased as there is an increase in unsaturation by the fatty acid desaturase enzyme, while in cold sensitive the content of unsaturated fatty acid is reduced (Cruz *et al*., 2010; Guo *et al*., 2016; Tian *et al*., 2022). In rice cold tolerance was due to up-regulation of oleic acid molecule through chlorophyll remodelling (Ayelén *et al*., 2018). An increase in polyunsaturated lipids was observed under cold acclimation in *Arabidopsis* (Gao *et al*., 2015; Welti *et al*., 2002). Other lipid changes in *Arabidopsis* cold acclimation include remodelling of phospholipids in the extra-plastidic membranes and galactolipids [monogalactosyldiacylglycerol (MGDG)] in the plastid membranes (Moellering *et al*., 2010; Welti *et al.*, 2002). Under high temperatures, the level of saturated fatty acid is upregulated to reduce fatty acid peroxidation and an increase in the number of long chain fatty acids ($C_{22:1}$) to form cuticular wax to protect against heat shock (Prasertthai *et al*., 2022).

2.8.1 Role of fatty acids in cold tolerance in plants

The physical properties of fatty acids and of the substances that contain them are mainly determined by the length and degree of unsaturation of the hydro carbonated chain (Maghrebi *et al*., 2021). The non-polar chain is responsible for the low solubility of fatty acids in water. Under temperature of 25 degrees, C_{12}-C_{14} fatty acids have a cerous consistency while unsaturated fatty acids of the same carbon length are liquid oily (Tsiantas *et al*., 2022). Under chilling stress, lipids in cell membranes change from a lipid-crystalline condition to a solid state at a critical temperature that is determined by the ratio of saturated to unsaturated fatty acids (Quinn, 1988). This phase transition causes alteration of cell metabolism and leads to cell death and damage of sensitive plants (Ye *et al*., 2021).

Unsaturation of fatty acids maintains membrane integrity under chilling by lowering the melting temperature and increasing membrane fluidity. An increase in polyunsaturated lipids was observed under cold acclimation in *Arabidopsis* (Gao *et al*., 2015). Zhou *et al*. (1982) reports indicated that chilling stress induce changes in fatty acid composition, mainly in the content of linolenic acid (18:3), the increased production of 18:3 was found to accompany cold acclimation in plants, and a positive relationship was found between a higher degree of fatty acid desaturation and cold tolerance (Tian *et al*., 2022). Previous studies have attributed cold tolerance in plants to changes in lipid membrane composition which maintain membrane fluidity during cold stress (Liu *et al*., 2022). Increasing unsaturated fatty acids in membrane

lipids can alleviate photoinhibition of PSII in plants (Sui, 2015; Sui & Han, 2014). This suggests that increasing unsaturated fatty acids in membrane lipids could protect the photosynthetic apparatus, and stabilizes the photosynthetic processes.

2.9 Methods of fatty acid extraction and analysis

There exist many methods of plant lipids extractions but the choice of method is based on the characteristics of the sample being tested. Plant lipids are mostly extracted based on the type of lipids being analysed (neutral or polar solvents for extraction) which leads to extractions of other components such as carbohydrates and proteins. Most of these extraction protocols are adaptations of protocols developed for the extraction of lipids from animal tissues such as (Bligh & Dyer 1959) method. However, these may not be as effective in extracting plant lipids since the lipid composition of plant tissues is unique and different from that of animal. Other methods involve the Soxhlet n-hexane extraction by (AOAC, 2012), Microwave-assisted extraction and automated hydrolysis and extraction (AHE). The extracted lipids are then analysed by use of High-Performance Liquid Chromatography (HPLC) due to its high efficiency of separation and highly efficient available detectors (Kinsey *et al.*, 2022). The extracted and derived plant extract can also be analysed using Gas Chromatography Mass Spectrophotometer (GCMS).

The goal of this study was to determine the influence of lipid fractions on sorghum's sensitivity to low temperature for purposes of isolating those conferring tolerance to be used in crop improvement.

CHAPTER THREE
CHARACTERIZATION OF SORGHUM GENOTYPES FOR REPRODUCTIVE COLD TOLERANCE

Abstract

Temperatures of below 15ºC at the reproductive stages of sorghum causes delayed panicle initiation and reduced grain yield thus limits its performance and promotion in the dry highlands of Kenya. Two hundred and fifty (250) sorghum genotypes were evaluated in 2020 and 2021 on a Randomized Complete Block Design (RCBD) with three replications in two sites: Egerton University (0°22'11.0"S, 35°55'58.0"E) a cool highland region (about 2265 m asl) and Marigat (0°28'15.0"S, 36°0'19 E) a warm lowland (1007 m asl.). The daily average day/night temperatures at Egerton University were 20/9 °C, and Marigat with average of 32/20 °C. The objective of the study was to characterize sorghum genotypes according to reproductive cold tolerance. Data was collected on plant height, number of tillers, days to 50% heading, panicle length, panicle weight, seed weight and panicle harvest index. Cluster analysis performed using R-software aggregated sorghum genotypes into three clusters at Egerton. The genotypes in cluster one remained vegetative; cluster two had productive panicles while cluster three, produced sterile panicles at Egerton site. The genotypes took between 125-213 days to head at Egerton while the same took 55-103 days to head at Marigat. The sorghum genotypes that headed (cluster two) at Egerton had a height range of 54 - 233 cm (mean of 125 cm) compared to (66 - 331, mean 197) cm at Marigat. Plants that delayed in heading developed sterile panicles and *Claviceps purpurea* (ergot) while those that headed earlier had productive panicles. BM genotypes series (5,6,29,31,32 and 32) and IESV LT series were cold tolerant as depicted by panicle index and seed weight. These genotypes can be cultivated in the highlands for use as feed and food upon more research on the grain and fodder quality. Most of the GBK series were cold sensitive as they remained vegetative and some with sterile panicles hence more research and breeding should be carried out to enhance cold tolerance. The physiological mechanisms of cold tolerance and cold sensitivity among these genotypes need to be evaluated for future breeding.

3.1 Introduction

The global temperatures are expected to rise by 2°C by the year 2050 (IPCC, 2018). Climate variability has created challenges in crop production which complicates food production. The world population is expected to rise and reach 11 billion by the year 2100 (United Nations, 2019) creating a demand for food and fodder for livestock. The demand could be met through

the adoption of climate-smart smart crops such as sorghum. Sorghum [*Sorghum bicolor* [L.) Moench] is the global 5th major cereal after maize, millet, wheat and rice (Dahlberg, *et al.,* 2012) and is grown for food, feed, biofuel and malting. Sorghum crop is grown in regions whose minimum temperatures are above 18ºC since it is sensitive to temperatures below 15 ºC (Emendack *et al*., 2021). The optimum mean temperature range in grain sorghum for germination is between 21-35 ºC, while the optimum temperatures for vegetative and reproductive growth stages are 26-34 ºC and 25-28 ºC, respectively (Senghor & Youm, 2020). Current trends have seen sorghum being introduced in the dry highlands, away from the lowlands where sorghum is highly adapted (Maulana & Tesso, 2013).

The effects of low temperatures include a reduction of photosynthetic efficiency as observed in wheat leading to a decline in dry matter accumulation and grain yields (Zhang *et al*., 2022). Low temperatures also impede chlorophyll function through modifications of chloroplast (Cui *et al*., 2019). The stage of transition from vegetative to flowering and reproductive of plants is the most sensitive to changes in temperatures and determines the productivity of plants (Hartfield & Prueger, 2015). Temperatures below 13°C during flowering and microsporogenesis exposes crop such as rice to male and/or pollen sterility leading to poor grain set (Souza *et al*., 2017). Research has shown that lower night temperatures during reproductive stages cause drastic changes in spikelet and flower fertility in rice (Pereira-Da Cruz *et al*., 2006). It has also been observed that the decline in yield of rice is a result of indehiscent anthers and low pollen receptivity on the stigma under low temperatures (Zeng *et al*., 2017).

There is a need to adopt climate-smart technologies to counter the effects of climate change scenarios. A critical factor in climate change in the arid and semi-arid lands of Kenya is the erratic and frequent decline in soil moisture. Crops tolerant to soil moisture deficit include sorghum and hence the need to promote them for food, feed and improved livelihood for communities in those regions. However, few sorghum genotypes are productive under low temperatures which are experienced in some of the dry highlands. The objective of the current study was to evaluate sorghum genotypes for reproductive cold tolerance, characterize them based on the time taken to heading, grain yield, and identify the genotypes associated with cold tolerance.

3.2 Materials and methods

3.2.1 Site description

The characterization of sorghum genotypes for reproductive cold tolerance was carried out in two locations: Egerton University research field in Nakuru County and Marigat in Baringo County. Egerton University research field (0°22'11.0"S, 35°55'58.0"E) is located at an altitude of 2265 m above sea level (a.s.l). The site is classified as Agro-ecological zone III, Lower Highland 2 to 5 (LH2 – LH5) and has a sub-humid modified tropical climate (Jaetzold *et al*., 2012). The area receives annual average precipitation of 908 mm with a daily mean temperature range of 16°C-17°C. The soils are classified as *mollic Andosols*, well-drained and dark reddish in colour. Daily wind speed at Egerton university is 7-11 km/h, with a relative humidity of over 70%. Marigat (0°28'15.0"S, 36°0'19 E) on the other hand is in agro-ecological zone IV, lower midland (LM) and is at an altitude of 1007 m asl. Marigat receives about 230 mm of rainfall annually and its temperature range is 30ºC-35ºC. The soils are poorly drained *Cambisols* with slightly acidic to near neutral pH (Jaetzold *et al*., 2012). The area experiences a wind velocity of 6.27 km/h at a relative humidity of 30 %.

3.2.2 Sorghum genotypes

The sorghum genotypes used in this experiment were obtained from the Kenya Gene Bank (GBK) (entry number 1-98), International Crops Research Institute for Semi-Arid Tropics (ICRISAT, Nairobi – Kenya) (entry 99-226) and farmer's collection (227-250). A total of two hundred and fifty genotypes were evaluated in the two sites described above. The list of the genotypes is provided in Table 3.1.

Table 3.1:

List of sorghum genotypes characterized for cold tolerance

N	GN	N	GN	N	GN	N	GN	N	GN	N	GN
1	GBK-000002	41	GBK-000060	81	GBK-000118	121	IS11167	161	IESV91018LT	201	ICSR93034
2	GBK-000010	42	GBK-000062	82	GBK-000119	122	IS21158	162	IESV91054LT	202	ICSV112
3	GBK-000013	43	GBK-000064	83	GBK-000120	123	IS21282	163	IESV91069LT	203	IESV23008DL
4	GBK-000014	44	GBK-000065	84	GBK-000121	124	Khalid	164	IESV91071LT	204	IESV91048DL
5	GBK-000015	45	GBK-000066	85	GBK-000125	125	LABA(MW5003)	165	IESV91073LT	205	IESV91049DL
6	GBK-000016	46	GBK-000067	86	GBK-000128	126	MACIA	166	IESV91075LT	206	IESV91063DL
7	GBK-000018	47	GBK-000068	87	GBK-000129	127	MiwaleniLocal	167	IESV91105LT	207	IESV94025SH
8	GBK-000020	48	GBK-000069	88	GBK-000130	128	SEREDO	168	IKINYARUKA	208	IS11167
9	GBK-000021	49	GBK-000070	89	GBK-000132	129	SERENA	169	IS11076	209	IS8193

10	GBK-000022	50	GBK-000071	90	GBK-000365	130	BUSIA_28-1	170	IS11141	210	PLOT_142SUDAN
11	GBK-000023	51	GBK-000073	91	GBK-000366	131	IESV91131DL	171	IS11159	211	R8602
12	GBK-000024	52	GBK-000075	92	GBK-000367	132	IESV92022/1SH	172	IS11162	212	SIAYA_42
13	GBK-000025	53	GBK-000077	93	GBK-000376	133	IESV92055/3SH	173	IS11187	213	SIAYA_46-1
14	GBK-000027	54	GBK-000079	94	GBK-000377	134	IESV92136DL	174	IS11228	214	SIAYA_81-2
15	GBK-000028	55	GBK-000080	95	GBK-000381	135	IESV92172DL	175	IS11257	215	SIAYA_97-1
16	GBK-000029	56	GBK-000081	96	GBK-000382	136	IS8884	176	IS11338	216	AIHR91075
17	GBK-000030	57	GBK-000082	97	GBK-000387	137	KARIMTAMA1	177	IS11350	217	ICSR160
18	GBK-000031	58	GBK-000083	98	GBK-000389	138	KibokoLocal-2	178	IS11353	218	ICSR172
19	GBK-000032	59	GBK-000084	99	ASARECA15-2-1	139	KSV12	179	IS11579	219	ICSR24008

20	GBK-000033	60	GBK-000085	100	ASARECA15-3-1	140	LARSVYT-58-85	180	IS11608	220	ICSR92003
21	GBK-000034	61	GBK-000086	101	ASARECA24-4-1	141	MAKUENILOCAL	181	IS1161	221	WM89/90#1615
22	GBK-000035	62	GBK-000088	102	BUSHUKA(ICSV210)	142	MexicoR-line#5	182	IS11612	222	ZSV3
23	GBK-000037	63	GBK-000089	103	CR:35:5	143	PP290	183	ICSH152002	223	ICSR43
24	GBK-000038	64	GBK-000090	104	GADAM	144	S35	184	ICSH152005	224	IESV93042SH
25	GBK-000039	65	GBK-000091	105	GAMBELLA1107	145	SIAYA_27-3	185	ICSH152011	225	MB23
26	GBK-000040	66	GBK-000092	106	HAKIKA	146	SIAYA_50-3	186	ICSH152016	226	MB27
27	GBK-000043	67	GBK-000093	107	ICSH152010	147	WAHI	187	IS24484	227	LITH
28	GBK-000044	68	GBK-000095	108	ICSH152014	148	BM29	188	IS26794	228	IBUNDU
29	GBK-000046	69	GBK-000096	109	ICSV111IN	149	BM31	189	IS2853	229	CYURE

30	GBK-000047	70	GBK-000097	110	IESH22002	150	BM32	190	SHAMBUKO(PP290)	230	IBUNDI
31	GBK-000048	71	GBK-000098	111	IESH22007	151	BM39	191	ABALESHYA	231	AINAMOI
32	GBK-000049	72	GBK-000099	112	IESH22017	152	BM5	192	AMASUGI	232	NYUNDO
33	GBK-000050	73	GBK-000100	113	IESH22020	153	BM6	193	AMATEGA	233	MUHIMPUNDU
34	GBK-000051	74	GBK-000102	114	IESH28002	154	Cyatanombe	194	BAYISHINYIKE	234	DHET
35	GBK-000052	75	GBK-000103	115	IESV23006DL	155	E1291	195	BM16	235	NAHADAVA
36	GBK-000055	76	GBK-000105	116	IESV23007DL	156	GATARAGA	196	BM17	236	IS25557
37	GBK-000056	77	GBK-000106	117	IESV24030SH	157	GICAMUNKONI	197	BM21	237	IS558
38	GBK-000057	78	GBK-000107	118	IESV92029DL	158	IESV90015LT	198	BM27	238	IS2558
39	GBK-000058	79	GBK-000111	119	IESV92170DL	159	IESV90042LT	199	F6YQ212	239	IS9201

40	GBK-000059	80	GBK-000116	120	IESV94076DL	160	IESV91003LT	200	Gambella1107	240	ARAKUCHOT
241	RWANDA	242	EST 15	243	EST 3	244	EST 26	245	EST 27	246	EST 20
247	EST 25	248	EST 7	249	EST 41	250	EST 48				

Key: N- Genotype Number; GN- Genotype Name

3.2.3 Experimental procedure

The experiment was conducted during July 2020 – March 2021 and June 2021 – February 2022 growing seasons in Egerton and from August – December (2020 and 2021) crop growing seasons in Marigat. Sorghum at Egerton University was grown under rain-fed while those in Marigat were furrow irrigated three times a week because it an extremely dry environment. The experiment in Marigat was set along river Perkerra irrigation scheme where majority of farmers use furrow irrigation. Two hundred and fifty genotypes were laid down using a Randomized Complete Block Design (RCBD) and replicated three times. Each experimental unit measured 1m × 0.60m with a block measuring 32m × 15m. Seeds were sown at a spacing of 60cm × drill in a depth of 2cm and later thinned to 10cm apart within a row giving a total of ten plants per genotype in each plot. During planting, nitrogen, phosphorus and potassium (NPK) (20:10:10) fertilizer was applied at a rate of 13.1 kg P ha^{-1} and top dressed with calcium ammonium nitrate (CAN) (26% N) at a rate of 60kg N /ha. The experiment was maintained weed-free through manual weeding, while sorghum shoot-fly, aphids and whiteflies were managed by use of Bulldock® (beta-cyfluthrin) at 25g $ha^{-1}L^{-1}$ before the pest incidences reached threshold levels. Diseases were not common though isolated cases of anthracnose (*Colletotrichum sublineola)* were managed using Daconil® (chlorothalonil) at $2.5L^{-1}$**.** During the reproductive phase, emerged sorghum panicles were bagged before anthesis using *Mafuco* bags to prevent cross-pollination and to minimize bird damage at seed set. The covered panicles were harvested individually upon physiological maturity and taken to the laboratory for additional data analysis.

3.2.4 Data collection

Data was collected and recorded as follows; days to 50% heading, plant height, number of tillers, panicle length, panicle weight, seed weight, and Panicle Harvest Index (PHI).

i. Days to 50% heading was taken as the mean number of days from planting until 50% of the plants in the experimental unit had emerged panicles.
ii. Plant height was recorded from the crown of each sorghum plant to the collar of the flag leaf. This parameter was taken at the reproductive growth phase when majority of the plants had fully exerted flag leaf.
iii. The numbers of tillers were recorded as the number of side-shoots emerging from the main plant. This was done at the growth point differentiation stage 3.
iv. Panicle length was taken as the length from the emergence of the panicle to the tip of the panicle using a tape measure.

v. Panicle weight was recorded as the weight of the panicle with seed before threshing using Ramtons kitchen (RM 299) scale. Seed weight of the individual panicles was weighed and recorded after threshing.

vi. Panicle harvest index was calculated as a ratio of the weight of dry grain over panicle dry weight as explained by (Krishnamurthy *et al*., 2014).

3.2.5 Statistical analysis

To aggregate the different grouping of the genotypes based on tolerance and sensitivity to cold, cluster analysis was conducted using R Software version 4.1.3 (R Development Core Team, 2020). The data was standardized using the Gower general coefficient of similarity since the data collected was of mixed types (qualitative and quantitative) (Gower, 1971) as shown below;

$$S_{Gower}(X,Y) = \frac{\sum_{i=1}^{m} s_i w_i}{\sum_{i=1}^{m} w_i}$$

Where; Si = 1 if xi = yi (binary or qualitative data); Si = 0 if xi ≠ yi (binary or qualitative data); Si = 1 - $\left|$ xi -yi $\right|$ /Ri (quantitative data); Wi = 1 if xi can be compared to yi and Wi = 0 if xi cannot be compared to yi. Therefore; D Gower (x,y)= 1

$$d_{Gower}(X,Y) = 1 - s_{Gower}(X,Y)$$

The unweighted pair-group method with Arithmetic mean (UPGMA) generated the dendrogram A cluster cut function was applied to draw a table of the three clusters. A silhouette plot was generated using the same software to test the goodness of the clustering technique as follows.

$$s_i = \frac{b_i - a_i}{max\{a_i\ , b_i\}}$$

Where Si = the silhouette width, ai = the average dissimilarity (di,k) between the ith accession and all others in its cluster; bi = the average dissimilarity (di,k) between the i th accession and its neighbour cluster.

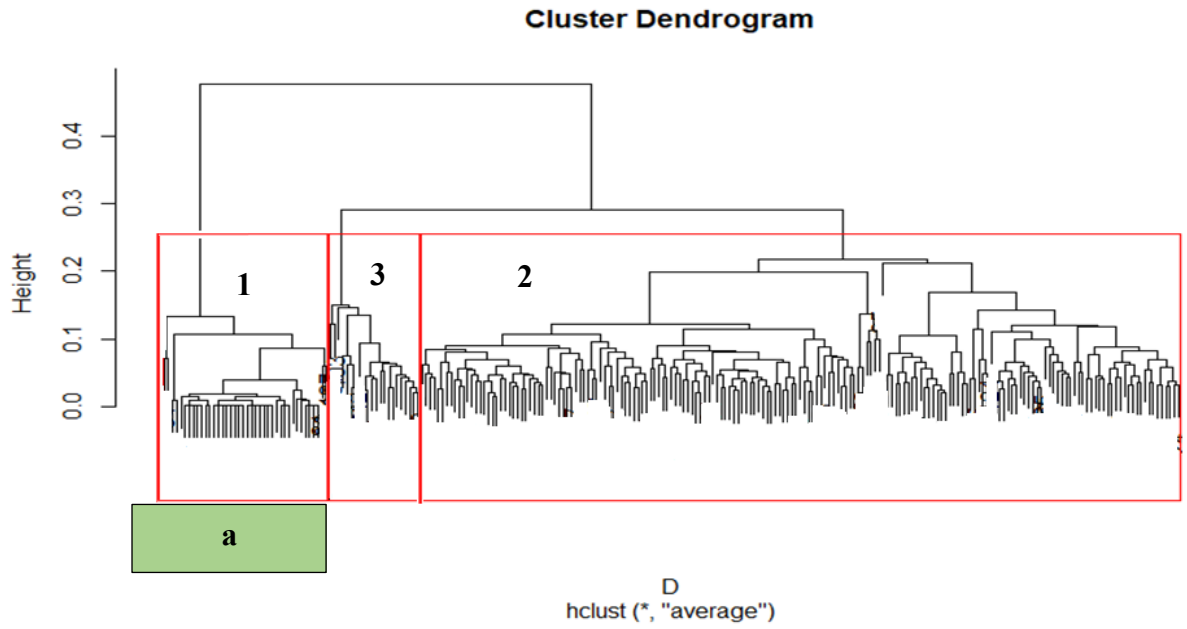
3.3 Results

3.3.1 Cluster analysis

Cluster analysis aggregated the Egerton-grown sorghum genotypes into three clusters (Figure 3.1a). Genotypes in cluster one remained vegetative; cluster two headed with productive panicles while cluster three had sterile panicles in Egerton site. In Marigat-grown sorghum, cluster analysis aggregated the genotypes into two clusters; cluster one where majority of the

genotypes attained physiological maturity early and cluster two with only seven genotypes that either headed late of did not germinate (Figure 3.1 b). The genotypes that were placed in cluster two include GBK 000010, GBK 000035, GBK 000043 IS 11167, Mexico r-line #5, IS 11257 and 1S 11579.

Cluster analysis was used to compare the performance of the genotypes grown in Egerton with the same ones grown in Marigat. The stability of the possible clusters from the data was tested using a silhouette plot. A test of possible clusters that could be generated from the data gave a silhouette width of 0.86, 0.39 and 0.62 for clusters 1, 2 and 3, respectively (Figure 3.2) at Egerton while it gave a silhouette plot width of 0.67 and 0.65 in Marigat, for cluster one and two, respectively (Figure 3.3). According to a silhouette plot, a stable cluster should be having a silhouette width close to 1, suggesting that cluster 1 with a value of 0.82 indicates the genotypes were placed in their right clusters, while cluster 2 valued at 0.39 suggests the cluster was weak and possibly some genotypes belonged to either of the two clusters. Cluster 3 with a value of 0.62 means a reasonable cluster had been found and the majority of the genotypes were correctly placed in that cluster. All the genotypes headed in Marigat.



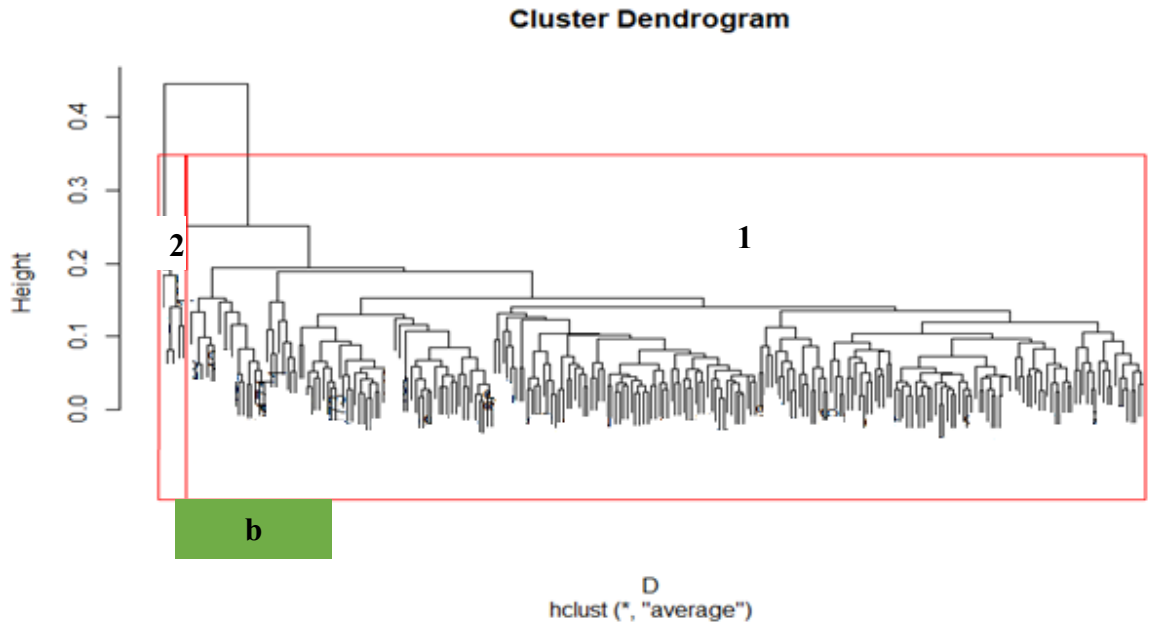


Figure 3.1: *Cluster dendrogram generated using the unweighted pair-group method with Arithmetic mean (UPGMA) calculated from the dataset of 245 sorghum genotypes Egerton (a) and 247 sorghum genotypes in Marigat (b)*

Table 3.2:

Sorghum genotypes categorized in cluster 1, 2 and 3 in the cluster analysis shown in figure 3.1a

Clusters	Genotype number								
1	1	2	8	17	19	22	23	26	27
	28	29	32	33	35	37	38	39	40
	43	44	46	50	51	58	73	74	81
	90	91	92	93	94	95	96	122	130
	136	171	181	215					
2	3	4	5	6	7	9	10	11	12
	13	14	15	16	18	20	21	24	25
	30	31	34	36	41	45	47	48	49
	52	53	54	55	56	57	59	60	61
	62	63	64	65	66	67	68	69	70
	71	72	75	76	77	78	79	80	82
	83	84	85	86	87	88	89	97	98
	100	101	102	104	105	106	107	108	110
	112	113	114	115	116	117	118	120	123
	124	125	128	129	131	133	135	137	138
	141	143	144	145	146	147	148	149	150
	151	152	153	154	155	156	157	158	159
	160	161	162	163	164	165	166	167	168
	169	170	173	174	175	176	177	178	179
	180	182	183	184	185	186	191	193	194
	195	196	197	198	199	200	201	206	209
	211	212	213	214	216	217	218	219	220
	221	222	223	224	225	226	227	228	229
	230	231	232	233	234	235	236	237	238
	239	240	241	242	243	244	245	246	247
	248	249	250						
3	42	99	103	109	111	119	126	127	134
	139	142	172	187	188	189	190	192	204
	205	207	208	210					

* Information regarding individual genotypes presented in cluster 1,2 and 3 is available in Table 3.1.

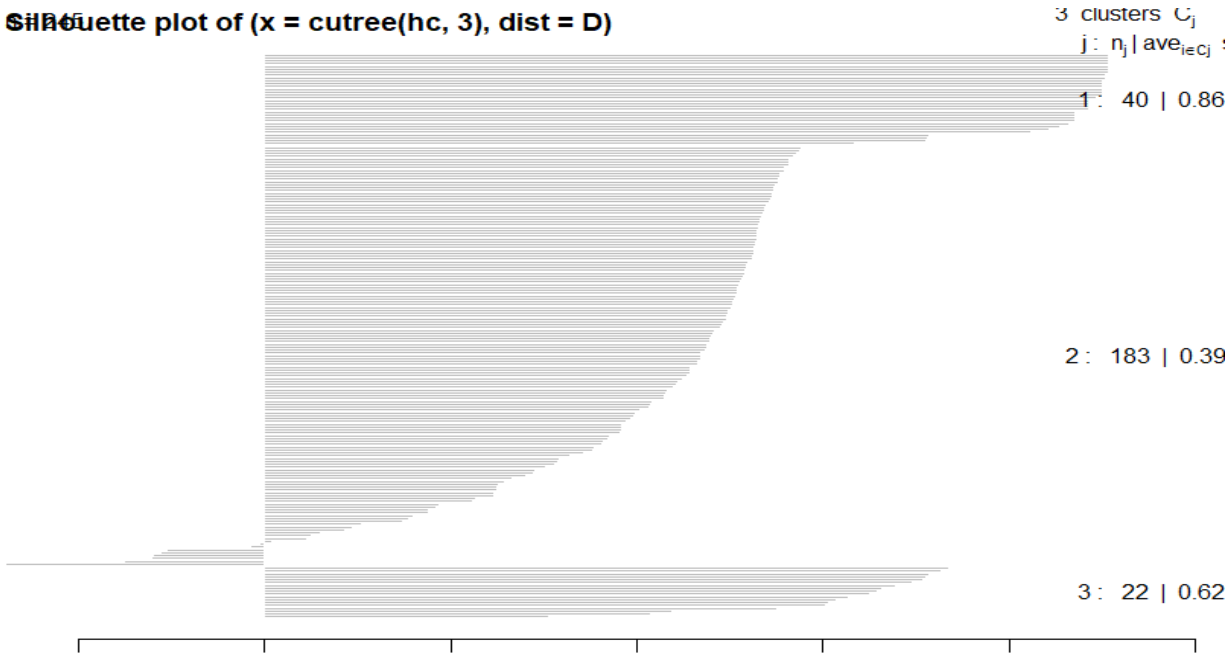


***Figure 3.1**.: Silhouette plot showing stability of clusters for the genotypes grown at Egerton as generated in Figure 1a*

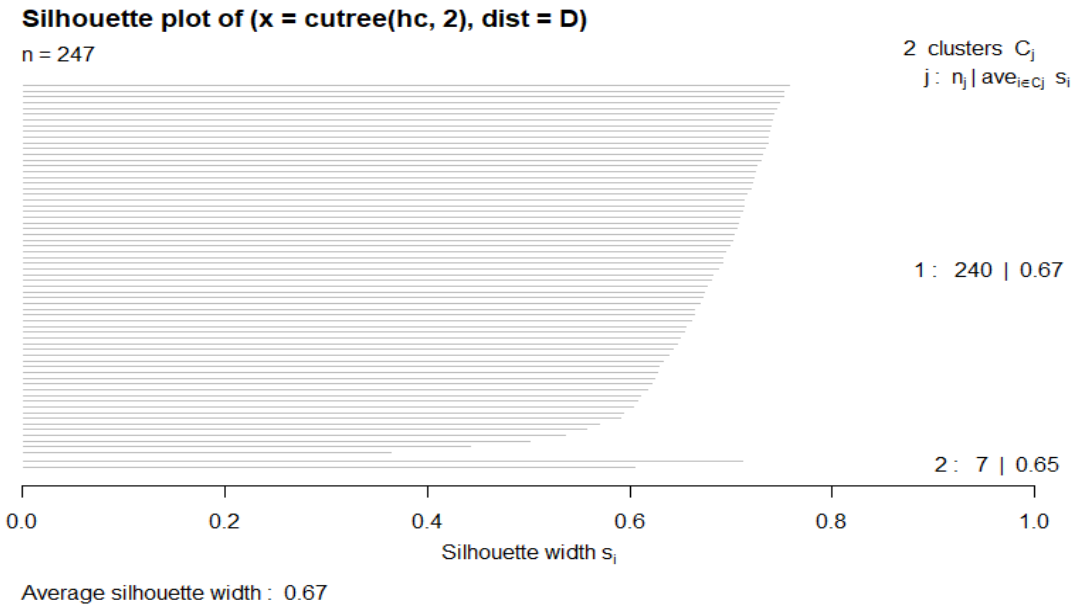


***Figure 3.2:** Silhouette plot showing stability of clusters for the genotypes grown at Marigat as generated in Figure 1b*

3.3.2 Tillers

There was a marked variation in the number of tillers within the clusters. Cluster one recorded the highest number of tillers averaging at 6, while cluster two and three had an average of 3

tillers each at Egerton. All the genotypes in Marigat in comparison to cluster one, two and three of Egerton had a mean of 2 tillers (Figure 3.4). The means for Cluster one, two and three were statistically different at $p<0.05$, $p<0.01$ and $p<0.0001$, respectively in the two locations.

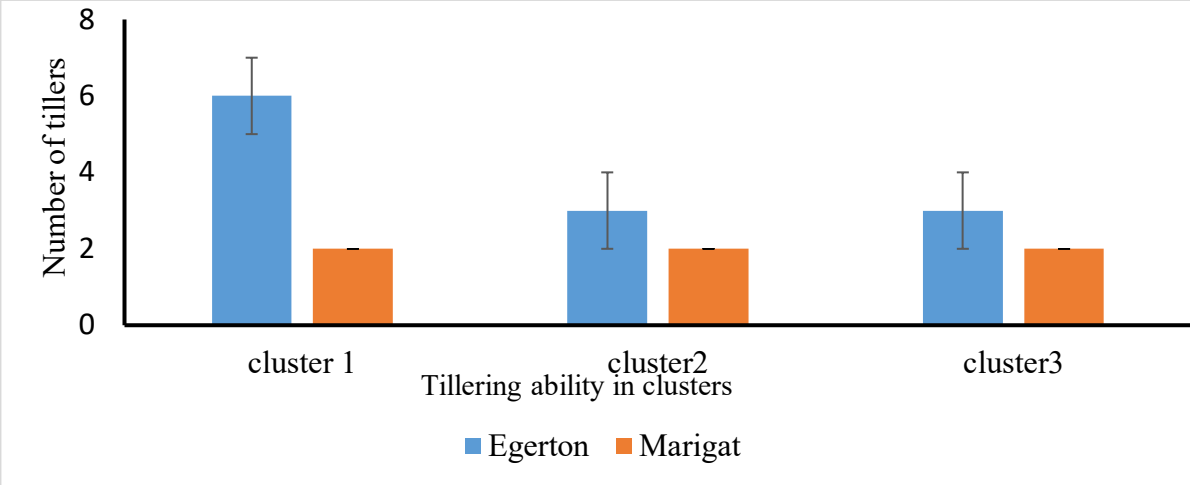


Figure 3.3: *Mean number of tillers of sorghum genotypes in cluster 1, 2 and 3 at Egerton and Marigat*

It was noted that the genotypes that remained vegetative tillered more than the genotypes which had productive panicles at Egerton. Cluster one genotypes which included GBK-000002, GBK-000010, GBK00100 and IS 8884 had a mean of 19, 20, 10 and 12 tillers, respectively at Egerton site while the same genotypes gave a mean of 5, 6, 2 and 1 tiller respectively, in Marigat. In cluster two, GBK000121, GBK000060 and IESH22020 produced 8, 7 and 7 tillers respectively in Egerton site with a majority of the genotypes having a mean of 4-5 tillers. Genotype CR: 35:5 had the highest number of tillers in cluster 3 (11) while KSV 12 had no tillers in Egerton site. There was no significant difference in the number of tillers in cluster three of Egerton and Marigat.

3.3.3 Days to 50% heading

The days to heading ranged from 125 - 203 for sorghum grown at Egerton and 55 - 103 days for the same genotypes in Marigat (Annex 1). It emerged that the mean number of days for the genotypes to heading were 167 ± 1.4 and 72 ± 6.7 for Egerton and Marigat, respectively at $P<0.0001$. It was also observed that all the genotypes headed and attained physiological maturity in Marigat while 40 of the genotypes remained vegetative and failed to produce panicles in Egerton (Table 2). All the genotypes which remained vegetative were categorized in cluster 1. The earliest genotypes to head in cluster 2 at Egerton were GBK 000125, IESH22020, IESV90015LT, IESV90015LT and Ikinyaruka at 125, 127, 129, 125 and 125 days, respectively. The same genotypes took 64, 68, 68, 60 and 76 days, respectively, in

Marigat. Those that took a medium number of days at Egerton were; GBK 000128, ASARECA15-3-1, HAKIKA, PP 290, SIAYA 50-3 and BM 29 at 147, 153, 151, 146, 144 and 153, respectively, and 59, 63, 72, 64, 70 and 74 days in Marigat, respectively. The genotypes that took the longest to head in cluster two of Egerton site include; GBK 000023, GBK 000024, GBK 000039, GBK 000080, GBK 000070, Makueni Local, IESV 91018 LT, IS 11187 and EST 3 at 186, 177, 207, 180, 180, 191, 181 and 206 days, respectively.

Majority of the genotypes in cluster three showed delay in days to 50% heading giving a mean of 177 days to heading. GBK 000062, CR:35:5, IESV92170 DL, IS 11162, AMASUGI, IS 24484 and IS 2853 had a mean of 200, 204, 200, 206, 214, 188 and 220 days respectively. The same genotypes achieved 50% to heading at Marigat in 77, 64, 72, 72, 99, 77 and 81 days, respectively. It is important to note that majority of the genotypes that remained vegetative at Egerton showed delayed days to heading at Marigat. Variety GBK 00002, GBK 000010, GBK 000100, GBK 000365, GBK 000367, IS 8884 and IS 11159 took 90, 81, 80, 82, 80, 93 and 107 number of days to heading, respectively.

3.3.4 Plant height

There was observed variation in plant height across the two environments. Sorghum genotypes were taller at Marigat than at Egerton. The sorghum genotypes that headed (cluster two) at Egerton had a height range of 54 - 233 cm and a mean of 125 cm compared to (66 - 331, mean 197±6.7) cm at Marigat (Figure 3.5). Sorghum genotypes in cluster 3 at Egerton had a height range of 41-182 cm with a mean of 94±3.4 cm (Figure 3.6) while the same genotypes at Marigat site had a mean of 158 cm at a range of 55-336.5 cm. The short genotypes were CR: 35:5, IESH22007, IESV91049 DL, PLOT_ 142 SUDAN and IESV92136 DL with 50, 70, 55, 69 and 40 cm, respectively, in Egerton and 240, 155, 155, 65 and 78 cm in Marigat, respectively. Those that grew tall were BM 39, BM 5, Cyatanombe, IS 11187 and BM 32 at 205, 217, 210, 207 and 233 cm, respectively in Egerton compared to Marigat where the heights were 258, 185, 259, 307 and 287 cm in that order (Figure 3.6).

The tallest genotypes in Marigat were GBK0002, IS 11159, GBK 000043, GBK 00037, GBK 000032, and GBK 000010 at the height of 321, 334, 380, 326, 353, and 329 cm, respectively. Plant height of genotypes in cluster one was not recorded since they remained vegetative and did not exert a flag-leaf at Egerton site. The same genotypes had a mean of 251.77 cm with a range of 145-380 cm in Marigat.

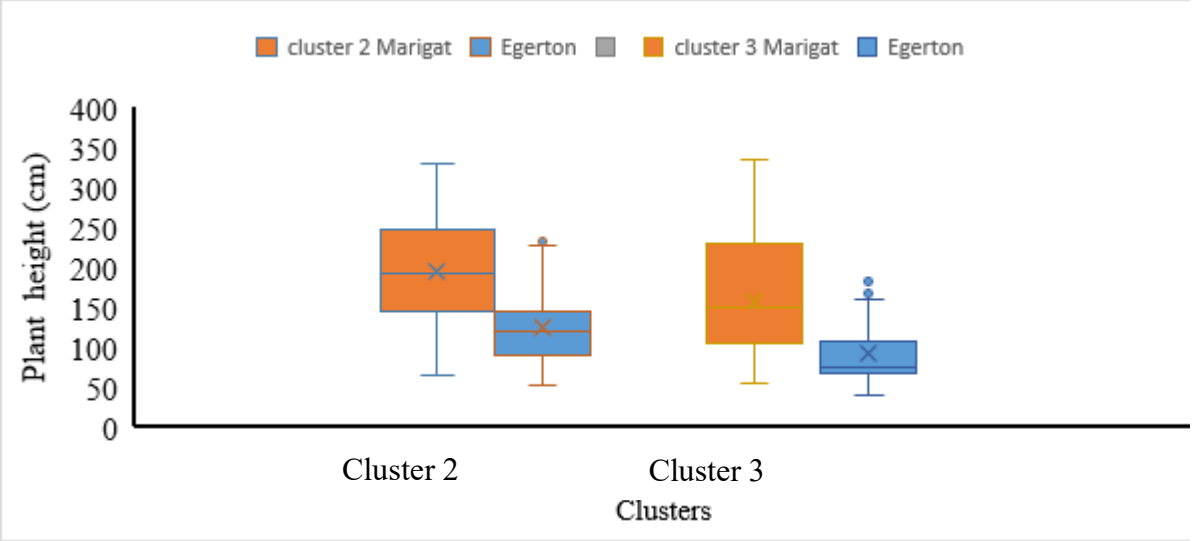


Figure 3.4: *Box and whisker plot of plant height (cm) at Egerton and Marigat in cluster 3 and 2, respectively*

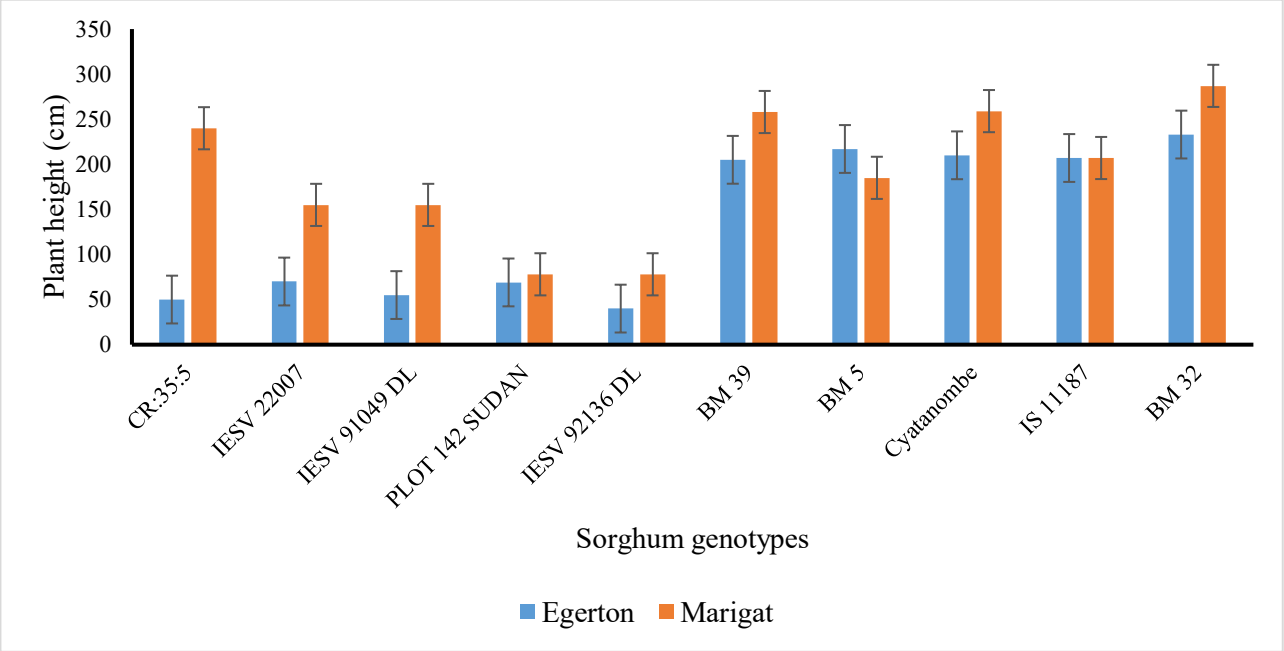


Figure 3.5: *Plant height (cm) of selected genotypes in Egerton and their performance in Marigat*

3.3.5 Panicle length

Panicle length was significantly different at $p<0.0001$ within the genotypes and across the two locations. Marigat had the longest mean panicle length of 26.17 ±0 .7 cm with a range of 18 cm to 41 cm. Cluster two of Egerton site had a mean panicle length of 18.91 ± 0.4 cm ranging from 12.58 to 26.83 cm. The genotypes with the longest panicles in cluster two of Egerton site include; GBK 000060, GBK 000068, GBK 000080, ICSH 152010, IESH 22002, IESV 92172 DL, IS 11076, IS 11612 and EST 7 scoring 30, 29, 30, 32, 33, 35, 37, 40 and 43 cm, respectively in Marigat and 17, 16, 18, 22, 23, 24, 25, 26 and 16 cm at Egerton. Cluster 3genotypes scored

a mean of 19 and 27 cm in Egerton and Marigat, respectively. Variety IS 11162, SP 74744, IS 24484, AMASUGI, and MACIA had lengths of 30, 25, 25, 14 and 18 cm, respectively while in Marigat, they measured 39, 27, 31, 22 and 27 cm in length (Figure3.7). It was also noted that some genotypes at Egerton site had challenges in panicle-emergence and the same had their panicles fused in the collar of the flag leaf.

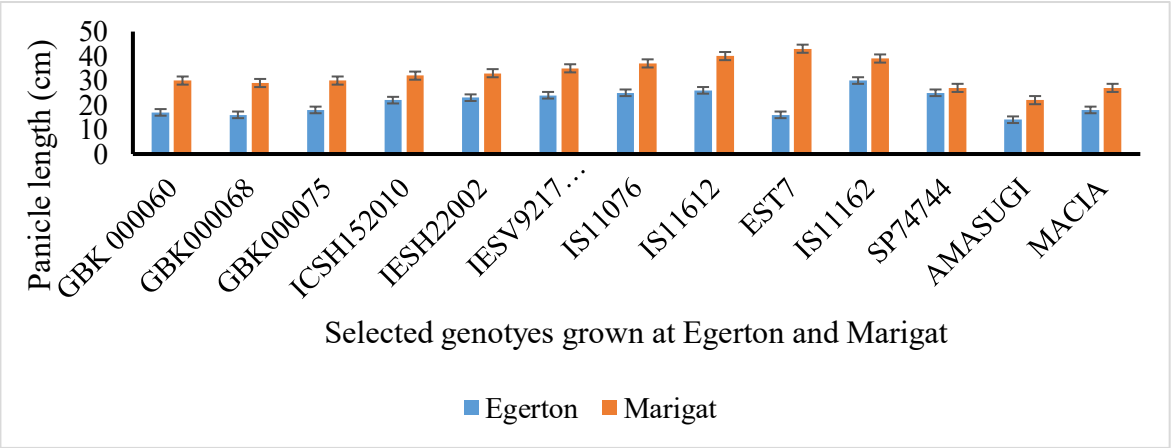


Figure 3.6: *Panicle length (cm) of selected sorghum genotypes performance at Marigat and Egerton sites*

3.3.6 Panicle weight

The mean panicle weight for Egerton site was 39.29 ± 2.9 g, with a range of 7 g to 107 g. Heavier panicles were observed in Marigat with a range of 15.84g to 135.6 g and a mean of 71.09 ± 5.8 g. GBK 00093, GBK 000096, GBK 000097, GBK 000099, GBK 00106, GBK 000130 recorded the heaviest panicles at 106, 129, 104, 126, 111,130 g respectively in Marigat and the same genotypes weighed 44, 38,53, 30, 25, and 28 g in Egerton site. Majority of the GBK genotypes had heavier panicle weights in Marigat than in Egerton. GBK 00119, IESV 92172 DL, BM 31, BM 16, IBUNDU had the lowest panicle weight as they weighed 30, 24, 31, 20 and 21 g in Marigat whilst in Egerton the average weight was 79, 15, 36, 59 and 81g.

Moreover, genotypes grouped in cluster 3, produced sterile panicles hence the weight was of no consequence. Some panicles were attacked by ergot (*Claviceps africana*) at Egerton site and showed exudation of sugary substances, such panicles could not be harvested. The genotypes that were attacked by ergot include IS 24484, ICSH 152011, GBK 000055 and IESH 28002. Sorghum genotypes such as IESV91048DL, IESV91049DL, SHAMBUKO (PP 290), KSV 12, MACIA and IESV91049DL had their panicles dry before seed formation.

3.3.7 Panicle harvest index (PHI)

Panicle harvest index (PHI) is used as a measure of seed set and efficacy in seed filling. It ranges from 0-1, where PHI of 0 indicates absence of seed set, and PHI of 1 shows a higher

efficiency in grain filling. However, no genotype can attain a PHI of 1 (Krishnamurthy et al., 2014). There was a significant difference in PHI at $p< 0.0001$ among the genotypes in cluster two at Egerton and Marigat. Sorghum grown at Egerton had a mean PHI of 0.688 and with a range from 0.322-0.916 (Figure 3.8) while the mean in Marigat was 0.750 with a range from 0 to 0.907. The genotypes with a higher PHI included; IESV 90042 LT with PHI >0.9, GBK 000021, GBK 000023, GBK 000024, GBK 000027, GBK 000031 and IESV 91003 LT had a PHI > 0.8. Sorghum genotypes that had a PHI >0.5 include Ibundu, MB 23, ICSR 43, ICSR 240008, SIAYA 46-1, R8602, Gambella 1107 and Serena. While F6YQ212, IS 11187, WAHI, Kari Mtama 1, IESV 91131 DL, IESH 28002, IESH 22002, ICSH 152010, Hakika, GBK 000022, GBK 000038, GBK 000092 and GBK 000128 among others recorded a PHI of < 0.4. However, IS 24484 and IESV94025SH, grouped in cluster 3 yet they had a panicle harvest index of 0.19 and 0.21 respectively.

One hundred and fifty-four sorghum genotypes in Marigat site attained a PHI of > 0.7 such are GBK 00047, SIAYA 50-3, IESV 90015LT, KARI MTAMA 1, IESV 92022/1SH, IESH 22017, GBK 000389 and ASARECA15-3-1 among the many genotypes, BM 29, EST 15 and EST 25 registered the lowest PHI of <0.6 in Marigat. Sorghum genotypes such as IS 11257, IS 11579 were not harvested due to delayed heading.

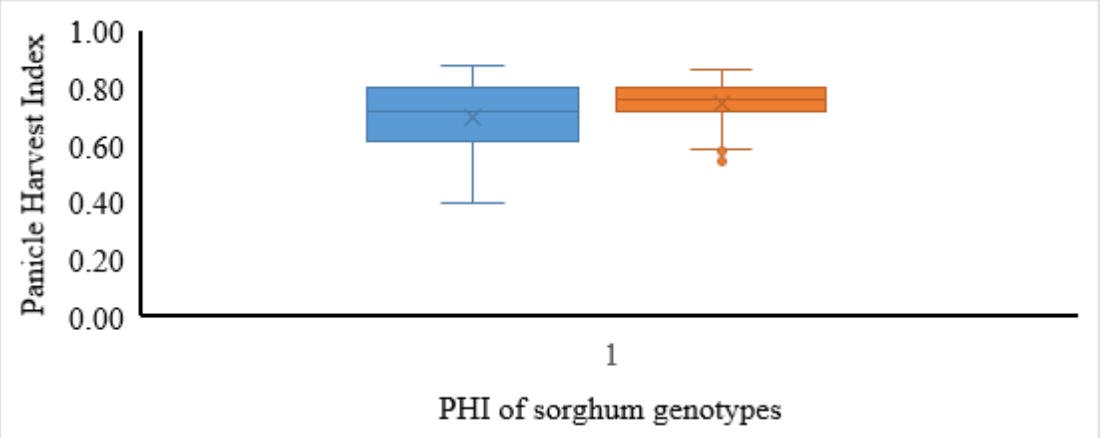


Figure 3.7: *Box and whisker plot of Panicle Harvest Index of Sorghum grown at Egerton (blue) and Marigat (orange)*

3.3.8 Correlation analysis of phenotypic indicators

Correlation analysis of phenotypic indicators are shown in Table 3.3 and 3.4. For sorghum genotypes grown at Egerton site showed very strong positive significant correlation (r=0.970) between panicle weight and seed weight indicating that these two traits are highly interdependent i.e., genotypes with heavier panicles have greater seed weight. Both panicle

weight (r=0.651) and seed weight (r=0.628) were strongly and positively correlated with plant height, suggesting that taller plants are associated with higher yield components in the highland environment. Furthermore, tillers showed a strong negative correlation with both panicle weight (r=−0.506) and seed weight (r=−0.519). This suggests an inverse relationship, where an increase in the number of tillers is associated with a decrease in individual panicle and seed weight, indicating a potential competition effect for resources. Days to 50% heading showed only a weak positive correlation with plant height (r=0.181) and a moderate positive correlation with tillers (r=0.356), but was not significantly correlated with either panicle weight (r=0.003) or seed weight (r=−0.063). This indicates that the time to flowering does not directly predict final yield component weight in highlands.

Table 3.3:

Correlation analysis of phenotypic indicators –Egerton site

	Plant height(cm)	**Tillers**	**Days to 50 % heading**	**Panicle length (cm)**	**Panicle weight(g)**	**Seed weight(g)**	**PHI**
Plant height(cm)	1	-.410**	.181*	.247**	.651**	.628**	.436**
Tillers	-.410**	1	.356**	-.300**	-.506**	-.519**	-.222**
Days to 50 % heading	.181*	.356**	1	.011	.003	-.063	.074
Panicle length (cm)	.247**	-.300**	.011	1	.388**	.333**	.164*
Panicle weight(g)	.651**	-.506**	.003	.388**	1	.970**	.569**
Seed weight(g)	.628**	-.519**	-.063	.333**	.970**	1	.699**
PHI	.436**	-.222**	.074	.164*	.569**	.699**	1

Correlation is significant at the 0.05 level (2-tailed).*

Correlation is significant at the 0.01 level (2-tailed).**

Similarly, correlation analysis of sorghum genotypes in dryland areas (Table 3.4) showed strong significant positive correlation (r=0.968) between panicle weight and Seed weight. In sharp contrast to Egerton, plant height showed no significant correlation with either panicle weight (r=0.107) or seed weight (r=0.104). This suggests that plant height is not a reliable predictor of yield components under dryland conditions. Tillers also showed no significant correlation with any other measured trait, including panicle weight (r=0.052) and seed weight (r=0.081). Days to 50% heading had a moderate significant positive correlation with plant height (r=0.519), indicating that taller plants take longer to head, which is typical. However, it showed a moderate negative correlation with PHI (r=−0.282).

Table 3.4:

Correlation analysis of phenotypic indicators –Marigat site

	Plant height(cm)	**Days to 50 % heading**	**Panicle length (cm)**	**Panicle weight(g)**	**Seed weight(g)**	**PHI**	**Tillers**
Plant height(cm)	1	.519**	-.085	.107	.104	-.041	.105
Days to 50 % heading	.519**	1	-.178*	-.113	-.124	-.282**	.049
Panicle length (cm)	-.085	-.178*	1	.367**	.286**	.305**	-.005
Panicle weight(g)	.107	-.113	.367**	1	.968**	.412**	.052
Seed weight(g)	.104	-.124	.286**	.968**	1	.535**	.081
PHI	-.041	-.282**	.305**	.412**	.535**	1	.081
Tillers	.105	.049	-.005	.052	.081	.081	1

Correlation is significant at the 0.05 level (2-tailed).*

Correlation is significant at the 0.01 level (2-tailed).**

3.4 Discussion

Results in this study show a significant effect of temperature on growth and development of sorghum. Though the temperature in the tropics is expected to be warmer with less variation throughout the year, there are variations occasioned by altitude. The localized temperature variations as a result of altitude influence the distribution of crops, sorghum among them. The

average temperatures for Marigat were recorded to be 29/18^{o}C (max/min) and 24/9.4^{o}C for Egerton. Earlier studies by Downes and Marshall (1971) revealed that low temperatures causes reduced pollen production leading to male sterility, reduced panicle weight and delay in panicle initiation, the results are consistent with the current study. Low temperature decreases the chlorophyll content through abnormal development of the chloroplast (Cui *et al*., 2019), reduction in transpiration, stomatal conductance and CO_2 assimilation (Ortiz *et al*. 2017), which could be attributed to the reduction in growth of the genotypes at Egerton site as observed by short panicles and delays in panicle initiation. Previous studies revealed that low temperature resulted in decreased photosynthesis affecting dry matter accumulation thus a decline in yield in wheat (Zhang *et al*., 2022).

The results of this study showed that temperature (environment) has a greater effect on plant height. In the warm environment, plants were taller as compared to the cooler environment. Despite plant height being genotype specific, about 30-50% can be attributed to environment in our current study. The reduction in dry matter accumulation as a result of reduced net photosynthesis can explain the reduction in plant height in the cooler region. A decrease in photosynthesis is majorly attributed to reduced gaseous exchange, decline in enzymatic activities of ribulose bis-phosphate carboxylase oxygenase and reduced stomatal opening (Bhattacharya, 2022). In the warmer environment, taller plants can be attributed to the role of Indole-acetic acid (IAA) concentrations which could have stimulated the opening of the stomates for gaseous exchange, increased transpiration rates thus increased net photosynthesis and assimilates for dry matter accumulation (Rao *et al* 2019).

The number of tillers for Egerton grown genotypes was higher than those of Marigat. Alam *et al*. (2014) observed that the temperature effect on the expansive growth on the main shoot height is greater at high temperature due to the amount of assimilate availability for tiller production in young shoots where the supply of assimilate is low. Rotili *et al*. (2021) also found that high temperature and low radiation reduce carbohydrate availability at the start of the tiller emergence period, which results in low appearance frequency of early tillers. Fewer tillers in Marigat site could be attributed to faster growth rate and the shift of assimilate to reproductive phase. For the Egerton grown genotypes the increased number of tillers could be a survival mechanism against cold injury.

Panicle initiation was delayed even for the cold tolerant sorghum genotypes at Egerton where forty genotypes remained vegetative. These results conform to earlier studies that panicle initiation and flowering were affected and delayed by low temperature (Amandin *et al*., 2020;

Emendack *et al*., 2021). The delay in flowering of the genotypes at Egerton can be attributed to the reduction in expression of genes that activate flowering; which include the florigen-related genes $SbCN_8$, $SbCN_{12}$ and SbEHD1 and possibly their up-stream regulators SbCo and SbEHD. Diminished florigen expressions by Sbg1-EMS1 allele was found to delay the vegetative to reproductive transition and caused slower stem growth and panicle development after the transition (Abdul-Awal *et al*., 2020). In another study by Murphy *et al*. (2014), it was revealed that SbPRR37 and SbGhd7 genes encode genes that inhibit flowering in long day plants. From the results we noticed the genotypes that delayed heading at Marigat remained vegetative at Egerton site, a phenomenon that could be attributed to the over expression of this flowering repressor genes (Murphy *et al*., 2011). Murphy *et al*. (2011) observed that genotypes with ghd7-1 null alleles were able to flower earlier, probably the reason for early headers of the sorghum genotypes at Egerton.

The results of this study have revealed that temperature has a great effect on the yield parameters of sorghum. Sorghum genotypes planted in Marigat showed a significant difference $p<0.05$ in terms of panicle length, panicle weight and panicle harvest index. The significant difference observed among the genotypes in Marigat depicts better performance of sorghum in the hot environment as opposed to the cooler environment at Egerton site. The genotypes in cluster two at Egerton showed cold tolerance as shown by heavy panicle weight, seed weight and consequently higher panicle harvest index (PHI). Panicle harvest index proved to be a reliable parameter to determine cold tolerance in sorghum genotypes and an efficient proxy for spikelet fertility (Charkrabarty *et al*., 2021). At Egerton the genotypes that scored a PHI of >0.7 were classified as cold tolerant. Previous studies linked decline in yield parameters to male sterility and infertile pollen formation (Booking 1976, 1979). Pereira-Da Cruz *et al*. (2006) reported that lower night temperatures during flowering cause major reductions in spikelet and flower fertility in rice.

Genotypes in cluster three at Egerton presented sterile panicles and were also associated with Ergot (*Claviceps africana*) attack. The panicle sterility observed in cluster three genotypes could be attributed to decline of pollen grains occasioned by anther indehiscence on the stigma as low temperatures was found to reduce stigma receptivity, and pollen germination rate (Rao *et al* 2019). In cold sensitive cultivars of rice, it was noted that spikelet infertility was caused by poor pollen grains and pollen germination on the stigma under cold temperatures (Matsui *et al*., 2001; Prasad *et al*., 2006; Zeng *et al*., 2017). Zeng *et al*. (2017) also noticed that low temperatures led to small pollen counts which result in fewer germinated pollen grains on the

stigma in rice. The primary source for pollen germination and pollen tube growth in rice is starch, which was reduced by low temperatures thus reduced pollen viability (Gunawardena *et al.,* 2003; Rao *et al* 2019). A similar scenario could have occurred in sorghum as observed in the current study.

At both sorghum growing sites, the strong positive correlation between panicle weight and seed weight is an instance of phenotypic interdependence, where the primary yield components are directly linked. The differential results between the two sites confirm the principle of genotype and environment Interaction, where the genetic expression of traits and their utility for yield are not stable across environments, as seen by plant height's correlation with yield changing from strong-positive in the highland to non-significant in the dryland (Yan & Tinker, 2006). The inverse relationship between tillering and main panicle yield in the favorable environment is explained by the source-sink concept and resource competition, where tillers compete with the main reproductive structure for limited photosynthates, a phenomenon known to reduce final yield in high-input cereals (Lafarge & Hammer, 2002). Conversely, the dryland results indicate that resource limitation (likely water stress) is the overriding environmental factor, which decouples vegetative traits like height and tillering from final grain yield, rendering them poor selection criteria in stress-prone environments (Blum, 2011)

3.5 Conclusion and recommendations

Lower temperatures limit successful production and promotion of sorghum grain crop in the dry highlands. Most of the cold sensitive sorghum genotypes remain vegetative while others produce sterile panicles. Some genotypes are able to produce panicles with grains despite the low temperature limitations, clearly indicating the presence of genetic diversity with regards to low temperature response. Cold tolerant genotypes included BM 5-BM 39, IESV 90015, 90042, 90054, 90069, 90071, 90073, 90075 LT, and IESV 91105, IKINYARUKA, IS 11228, ICSH 152005, ISCH 152016, ABALESHYA, AMATEGA, BAYISHINYIKE, MB 23, and MB 27 among others. They can be cultivated productively in the highlands and cooler regions for grain production (supplementary files). The diverse response to growing temperature provides an opportunity to identify, isolate and use genetic traits associated with cold tolerance to widen environmental adaptability of sorghum. The mechanism of cold tolerance needs to be determined and the associated gene loci identified for use in sorghum improvement programme.

CHAPTER FOUR
LIPID FRACTIONS ASSOCIATED WITH COLD SENSITIVE AND COLD TOLERANCE IN SORGHUM GENOTYPES

Abstract

Sorghum is an important crop for the arid and semi-arid and is produced globally for food, feed, biofuel and malting. Growing sorghum in the highland is mainly limited by low temperatures. Fatty acid molecules have been observed to confer tolerance against abiotic stresses especially those caused by low temperatures in several crops. Field and a growth chamber experiment were conducted to determine the fatty acid molecules associated with cold tolerant and cold sensitive sorghum genotypes. The field experiment consisted of nine genotypes clustered into cold tolerant, cold sensitive and partially cold sensitive. The genotypes were grown from July to December 2021 at two locations with contrasting temperature regimes: Egerton University, with average day/night temperatures of 20/9 °C, and Marigat, with averages of 32/20 °C. The field experiments were arranged in a Randomized Complete Block Design (RCBD) while growth chamber experiment arranged in Completely Randomized Design (CRD) with three replications. The growth chamber study simulated these two field conditions with the same sorghum genotypes. Leaf samples were harvested from each individual genotype at the boot stage for the cold tolerant and the last leaf for the cold sensitive. Lipid profiles were extracted using n-hexane and methylated into individual Fatty Acid Methyl Esters (FAMES) by Boron trichloride (BCl_3) and subjected to GC-MS analysis. Data acquisition was conducted using Mass hunter (B.07.00) software while fatty acid profiles were searched using NIST library (NIST 08, Wiley 7, Christie: Palmitic acid, 1-Methylbutyl hexa decanoate (C21:0), Linolenic acid, Methyl linolenate, Arachidonic acid, n-Butyl laurate (C16:0) and Tetra decanoic acid/Pentadecanoic acid fatty acids were profiled under filed conditions. Growth chamber grown genotypes had Oleic acid, Cis Vaccenic acid, Linoleic acid, Linolenic acid, Benzyl linoleate and benzyl oleate as unsaturated fatty acids (USFAs). Methyl linolenate was responsible for the plant's response towards temperature changes in sorghum genotypes grown under field conditions. Understanding the roles of Methyl linolenate and other unsaturated fatty acids can provide valuable insights in plant resilience and aid in crop productivity under temperature stress.

4.1 Introduction

Sorghum bicolor L. Moench is an important C_4 crop grown in the arid and semi-arid lands of Kenya and is adapted to the hot and dry environment (Yali, 2011). It is the fifth most important

cereal after wheat, rice, maize and barley (Dahlberg *et al.,* 2012; Mullet *et al.,* 2014). Sorghum is important for fodder, grain, biofuel and, malting and brewing. These benefits of sorghum have led to increased interest of growing it in most areas including low temperature environments (Maulana & Teso, 2013).

Temperature is a critical abiotic factor that affects crop productivity especially that of sorghum (Sikora *et al*., 2019). Crops of tropical origin such as sorghum are greatly affected by cold stress which is manifested by necrosis, chlorosis and growth retardation (Moradtalab *et al*., 2018). Low temperatures have been shown to impair grain development in sorghum by lengthening days to heading, increasing pollen sterility and indehiscent anthers, reducing the ability of the crop to exert a panicle and hence reducing panicle harvest index (Amandin *et al*., 2019; Zeng *et al*., 2017). However, not all genotypes of sorghum succumb to cold stress. Some genotypes have the capacity to adapt and perform under low temperatures.

Low temperatures lead to accumulation of Reactive Oxygen Species (ROS) as measured by malondialdehyde (MDA) which leads to the destruction of plant biomolecules resulting in cell death (Wang *et al*., 2013). Low temperatures can disrupt the integrity of plant cell membranes, resulting in increased electrolyte leakage and the loss of intracellular contents (Zhou *et al*., 2005). The thylakoid membrane is the most sensitive to changes in the environment and is greatly affected by heat and cold stress (Ali *et al*., 2022). Plant bio membrane is mainly the first site of injury upon cold exposure and the fluidity and stability of the said membranes is closely related to cold tolerance (Tian *et al*., 2022). Exposure of crops to low temperatures triggers a series of cold acclimation mechanisms that is modulated by osmotic regulators, cell membrane structural substances and antioxidant enzyme system (Zhu, 2016). Many physiological and molecular changes for cold adaptation reveal that the process is complex and involves more than one pathway (Sanghera *et al*., 2011). These physiological processes are regulated by a complex series of genes upon reception of the cold and these genes are quantitatively controlled by minor polygenes (Ma *et al*., 2015). Under low temperatures, plants employ a series of physiological responses which include but not limited to accumulation of soluble sugars to lower the level of electrolyte leakage (Zhou *et al*., 2005), and to accumulate anthocyanin content for protection against photooxidation scavenger thus inhibiting membrane peroxidation (Chu *et al*., 2010).

Fatty acids are essential in defending the plants against biotic and abiotic stress (Okazaki & Saito, 2014). Phospholipids are the main fatty acid molecules found in the components of various organelles such as plasma membrane, cell membranes and microsomes and are

responsible for membrane fluidity and stability (Yu *et al*., 2022). Under chilling stress, the fluidity of plant cell membrane changes from the lipid-crystalline shape to a solid state as determined by the ratio of saturated to un-saturated fatty acids (Quinn, 1988). Thus, to increase membrane fluidity under low temperatures, unsaturated fatty acids should be increased by upregulation of the fatty acid desaturase enzyme (Berestovoy *et al*., 2020; Cheng *et al*., 2015; Holthuis & Menon 2014). In order for the gaseous exchange and smooth photosynthesis process in plants, there's need for a membrane lipid remodelling. Previous studies suggest that lipid membrane unsaturation for cold tolerance is associated with linolenic acid (C18:3) (Upchurch, 2008). For cold tolerant crops, the degree of unsaturated fatty acids increases in both cell membranes and thylakoid membranes to enhances the degree of cold tolerance (Xue *et al*., 2019). Palmitic acid and linolenic acid constitute the major lipids in the thylakoid membranes (Li *et al*., 2021), and the ratio between the saturated and un-saturated fatty acid determines the level of membrane stability. For example, rice genotypes accumulated more of unsaturated fatty acids than the saturated fatty acids when exposed to chilling stress for the cold tolerant genotypes and the vice versa. The same author also observed the levels of linolenic acid increasing for the cold tolerant genotypes (Cruz *et al*., 2010). Guo *et al*. (2016) found out that when sweet sorghum genotypes were exposed to cold, the unsaturated fatty acid and the double bond of major lipids increased significantly upon cold treatment. Flax (*Linum usitatissimum*) is an annual crop grown for its oil content was found to have poly unsaturated fatty acids such as linoleic and linolenic acid controlled by FAD2A gene and their upregulation enhances cold tolerance in this crop (Wang *et al.,* 2021).

Increasing unsaturated fatty acid protects the photosynthetic apparatus and stabilizes the photosynthesis process. The unsaturation of phosphatidylglycerol (PG) in the chloroplast enhances protection of photosystem II (PSII) against cold induced photo-inhibition which leads to cold tolerance (Moon *et al*., 1995). There exist several literatures on the effect of lipids on cold tolerance in higher plants (Tian *et al*., 2022; Wang *et al*., 2021; Yu *et al*., 2021). Cruz *et al*. (2010) noted that, lipid saturation among the cold tolerant genotypes was through substitution of linoleic with linolenic acid when the genotypes were exposed to 10ºC.

Few studies have evaluated the role of fatty acid in sorghum plant and its response to grain formation and transition into reproductive phases. This experiment was designed to determine the fatty acid profiles associated with cold sensitive and cold tolerant sorghum genotypes under field conditions and under controlled conditions. The findings are aimed at identifying key fatty

acids associated with cold tolerance in sorghum genotypes for future linking with responsible gene loci for ease in crop improvement.

4.2. Materials and methods

A field experiment in two locations and a laboratory experiment involving a growth chamber were used to determine the association of fatty acids in cold tolerance of sorghum as described below.

4.2.1 Field experiment

a) Materials selection

Nine sorghum genotypes with varying tolerance to cold temperatures were randomly selected from experiment one after characterization of sorghum genotypes to cold tolerance. The genotypes were selected within the three clusters as detailed in section 3.3.1 above. Genotypes included three cold tolerant (GBK 000075, Ikinyaruka and BM 29), three cold sensitive (GBK 000065, GBK 000046 and GBK 000365 and three partially cold tolerant genotypes (IS 2853, ICSH 152011 AND IS 24484). The cold tolerant genotypes were selected on the basis that they headed earlier, had productive panicles with a panicle harvest index (PHI) index of more than 0.7 while the cold sensitive genotypes included those that remained vegetative and did not attain panicle initiation at Egerton site. The partially cold tolerant genotypes produced a panicle which was sterile at the low temperature site of Egerton.

b) Site description

The nine genotypes were grown in the two sites described previously under section 3.2.1.

c) Experimental procedure

The field experiment was set up in a 4m × 3m plots in a Randomized Complete Block Design (RCBD) and replicated three times. Seeds were sown at a spacing of 60cm × drill in a depth of 2cm and later thinned to 10cm apart within a row. During planting, NPK (20:10:10) fertilizer was applied at a rate of 13.1 kg P ha^{-1}, and top-dressed later with CAN (26% N) at a rate of 60kg N ha^{-1}. The crop grown at Marigat was irrigated three times a week through furrow irrigation since it's a dry environment while the crop at Egerton it was rain fed.

4.2.2 Growth chamber experiment

a) Plant materials

Three cold tolerant sorghum genotypes (GBK 000075, Ikinyaruka and BM 29) and three cold sensitive sorghum genotypes (GBK 000046, GBK 000365 AND GBK 000065) were used in this experiment. The materials were selected based on the clustering criteria detailed under

section 3.3.1 however, fatty acid data from the intermediate genotypes showed no significance difference with the cold tolerant genotypes. Therefore, to clearly evaluate the fatty acid response, two distinct categories of cold tolerant and cold sensitive were used for this experiment.

b) Experimental procedure

Ten seeds from each of the six genotypes were planted in two-litre pots filled with vermiculite and arranged in a Completely Randomized Design (CRD). The pots with seeds were put in the greenhouse (25–28^{o}C, 12/12 h day/night) until the seedling stage was achieved at Egerton Research field 7 before the cold treatment. Each of the pots was watered daily with nutrient water containing Hydro A with an N-P-K ratio of 5-0-2, is rich in nitrogen, promoting vibrant leaf growth and chlorophyll production and Hydro B, with a 0-4-4 ratio, supplies essential phosphorus and potassium, crucial for robust root development and prolific flowering. Both formulations are enriched with vital micronutrients such as magnesium, copper, iron, manganese, and zinc, ensuring comprehensive nourishment. Thereafter, the plants were moved into two growth chambers in Molecular lab at Egerton University which were set at different temperature regimes to simulate natural conditions. The first growth chamber simulated field conditions at Marigat site which is a lowland coupled with high day and night temperatures of 32/20^{o}C. The second growth chamber simulated lower temperatures 18/9^{o}C at Egerton site. The light conditions were 12h of darkness and 12h of daylight. Relative humidity was set at 70%. The plants were watered with nutrient water and allowed to adapt to the conditions for three days thereafter, they were removed, cut into small sizes for drying and milling in readiness for fatty acid analysis.

4.2.3 Sample preparation

Leaf samples were harvested from individual sorghum genotype before boot stage for the cold tolerant genotypes. While, for the cold sensitive, the second last leaf of each individual genotypes were harvested since they did not reach boot stage. Leaves of individual sorghum genotypes were put into zip lock polythene bags and fixed in a cooler box loaded with ice blocks for transportation to the laboratory. At the laboratory, the leaf samples were cut into small pieces and put in the oven for drying at 65^{o}C for three days. Dried leaves were ground into a course powder using laboratory mill (OHMS OBP-K600G). Sample contamination was prevented by thoroughly blowing the hopper, blade and sieve. The ground powder was put in zip-lock bags and clearly labelled awaiting fatty-acid extraction. Fatty acid extractions were

done both at Chemistry Department and Safe Foods Laboratory (SAF-LAB) at Egerton University.

Chemicals and reagents

Boron tri-fluoride (≥99.5%), n-hexane GC grade (≥99.5%) and anhydrous sodium sulphate were obtained from Sigma Aldrich, Germany while Helium gas (≥99.5%) was sourced from British Oxygen Company (BOC) in Kenya.

4.2.4 Fatty acid extraction and derivation

Fatty acid extraction for each sample was carried out using the Soxhlet method (A.O.A.C, 1984) by use of n-hexane. Where, seven grams of the ground powder was put in a thimble and 250 ml of n-hexane poured in the round bottom flask via the condenser. The temperature of the heating mantle was set at 60ºC, for two hours. Thereafter, the content in the round bottomed flask was fitted into the rotary evaporator to recover n-hexane as well as concentrate the extract.

Fatty Acid Methyl Esters (FAMES) were determined by esterification catalysed by boron tri-chloride (Murthy & Nathan, 2013). Fatty acid derivation was conducted by addition of 2ml of boron tri-chloride (BCL_3) together with the extract into a reaction vessel. The vessel was heated for ten minutes at 60ºC. The mixture was then cooled to 30ºC then 1 ml of n-hexane and 1 ml of de-ionized water was added. The mixture was put into a separating funnel and shaken thoroughly to allow phase separation. The lower part was decanted and the upper organic part transferred into a glass vial. Anhydrous sodium sulphate was added into the glass vial to absorb any moisture.

4.2.5 Gas chromatography and mass spectrophotometry (GC-MS) analysis

Samples were subjected to Gas Chromatography and Mass Spectrophotometry (GC-MS) analysis to profile and quantify for the Fatty acids. The derived sample was filtered through a 0.22µm filter into a clean GC vial for GC-MS analysis, 1µL of the sample was injected into a 7890B GC (Agilent, Palo Alto, CA, USA), equipped with a 5977B single quadrupole mass detector (Agilent, Palo Alto, CA, USA). Chromatographic separation was performed on an Agilent HP-5MS-UI capillary column (30m × 0.25mm × 0.25µm, Agilent, Palo Alto, CA, USA). The injection port temperature was set at 250 ºC, while the oven was programmed at 50ºC at 0.50 min. Temperature was ramped to 194ºC at 30 ºC/min for 3.50 min, and then to 240ºC at 5ºC/min, where it was held for 10min. Total run time was 28 minutes. The 1 µl injection was done using a split mode at 10:1, An 870µL universal low pressure drop, an ultra-inert liner with glass wool was used (Agilent 5190-2295, Palo Alto, CA, USA). Helium was

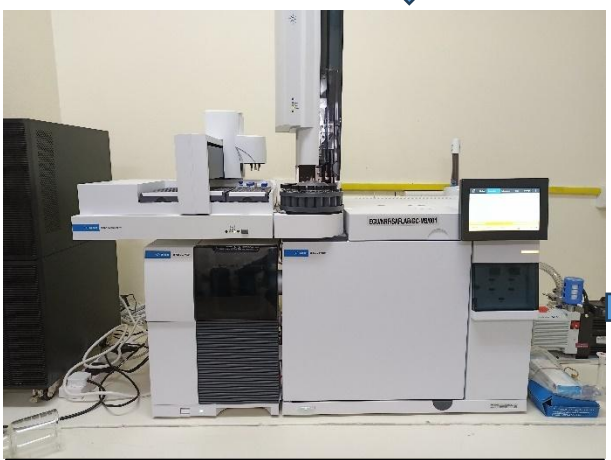
the carrier gas and the flow rate was set to 1.0mL/min (constant flow). MS source and quad temperatures were maintained at 230^{o}C and 150^{o}C, respectively. Solvent delay time was set at 3 min. Data acquisition type was by SCAN mode with a mass range between 50-400m/z. Data analysis was performed using Mass Hunter qualitative (B.07.00) software. Fatty acid profiles were identified through searching in the NIST library (NIST 08, Wiley 7, Christie). A brief procedure of the method described above has been illustrated under Figure 4.1.

Plants in Greenhouse

Growth Chamber

Soxhlet extraction

Grinding



GCMS Instrument

GCMS Method

Figure 4.1: *Step-wise description of methodology for the selected genotype treatment and laboratory GC-MS image used in the fatty acid profiling*

4.3 Results

4.3.1 Fatty acid profiles associated with cold sensitive and cold tolerant sorghum genotypes under field conditions

Seven fatty acids were identified in selected sorghum genotypes. These were: n-hexadecanoic acid (Palmitic acid), 1-Methylbutyl hexa decanoate (C21:0), 9,12,15-octadecatrienoic acid (Linolenic acid), 11,14,17-Eicosatrinoic acid (Arachidonic acid), 9,12,15-octadecatrionic acid (α-Linolenic acid) n-Butyl laurate (C16:0) and Tetra decanoic acid/Pentadecanoic acid (Table 4.1).

The genotypes grown at Marigat profiled fatty acid as follows: GBK 000075 had n-hexadecanoic acid, pentadecanoic acid and Linolenic acid. Ikinyaruka had, Tetradecanoic acid, n-hexadecanoic acid, Linolenic acid, α-Linolenic acid, and n-butyl laurate. BM 29 had, n-hexadecanoic acid, linolenic acid and α-Linolenic acid. GBK 000065 had only 11,14,17-Eicosatrinoic acid. GBK 000046 did not have any fatty acid. GBK 000365 had, n-hexadecanoic acid and α-Linolenic acid acid. IS 2853 had Tetradecanoic acid and linolenic fatty acid. IS 152011 had, Pentadecanoic acid, n-hexadecanoic acid, Linolenic acid and α-Linolenic acid. IS 24484 had Linolenic acid, Pentadecanoic acid, α-Linolenic acid acid and n-hexadecanoic acid (Table 4.1).

Egerton grown genotypes had their fatty acid as follows: GBK 000075 had Tetradecanoic acid and Methyl linolenate. Ikinyaruka had no fatty acid. BM 29 had Tetradecanoic acid and Methyl linolenate. GBK 000065 had Tetradecanoic acid and Methyl linolenate acid. GBK 000046, GBK 000365 and IS 152011 had no fatty acids. IS 2853 had Tetradecanoic acid and Methyl linolenate. IS 24484 had Tetradecanoic acid, n-hexadecanoic acid, Methyl linolenate acid and Pentadecanoic acid (Table 4.1).

The most frequent fatty acids were palmitic acid C16:0, followed by Linolenic acid C18:3. Palmitic acid was the most abundant saturated fatty acid (SFA) at Marigat site while tetradecanoic acid most abundant saturated fatty acid (SFA) at Egerton site. α-Linolenic acid was the dominant un-saturated fatty acid (USFA) at Egerton site.

The data shows that sorghum grown under low temperatures responded by increasing unsaturated fatty acids while the genotypes exposed to warm temperatures respond by increasing saturated fatty acid profiles in their membranes.

Table 4.1:

Fatty acid profiles associated with cold sensitive and cold tolerant sorghum genotypes under field conditions

Site	**Genotype**	**Major Fatty acid**					
		Palmitic acid	Methyl palmitate	Myristic acid	Methyl linolenate	Linolenic acid	Arachidonic acid
Marigat	Cold tolerant						
	GBK 000075	✓	✓	✓	✓	✓	*
	Ikinyaruka	✓	*	✓	✓	✓	*
	BM 29	✓	*	*	✓	✓	*
	Cold sensitive						
	GBK 000065	*	*	*	*	*	✓
	GBK 000046	*	*	*	*	*	*
	GBK 000365	✓	*	*	✓	*	*
	Intermediate						
	IS 2853	✓	*	✓	✓	✓	*
	ICSH 152011		*	✓		✓	*
	IS 24484	✓	*	✓	✓	✓	*
Egerton	Cold tolerant						
	GBK 000075	*	*	✓	✓	*	*

Ikinyaruka	*	*	*	*	*	*
BM 29	*	*	✓	✓	*	*
Cold sensitive						
GBK 000065	*	*	✓	✓	*	*
GBK 000046	*	*	✓	*	*	*
GBK 000365	*	*	*	*	*	*
Intermediate						
IS 2853	*	*	✓	✓	*	*
ICSH 152011	*	*	*	*	*	*
IS 24484	✓	*	✓	✓	*	*

Key: *-Fatty acid not detected; ✓-Fatty acid detected

4.3.2 Fatty acid associated with sorghum genotypes under controlled temperature

The fatty acid profiles from the growth chamber grown sorghum genotypes, retention times, chemical formulae and mass were noted in (Table 4.2). Total fatty acids profiles obtained from the sorghum genotypes under controlled conditions were classified as saturated or unsaturated. In this research, a total of seven unsaturated fatty acids were profiled and they included; Oleic acid, monolinolenin, Cis Vaccenic acid, Linoleic acid, Linolenic acid, Benzyl linoleate and benzyl oleate while Palmitic acid and Methyl palmitate were the only saturated fatty acids profiled. Unsaturated fatty acids were the majority of the profiles in this study as opposed to saturated fatty acid.

GBK 000075 accumulated Palmitic acid and oleic acid, Ikinyaruka Oleic acid only while BM 29 had Methyl palmitate, Palmitic acid, Methyl linolenate, Linoleic acid and Oleic acid among the cold tolerant genotypes. GBK 000065 had Methyl palmitate, palmitic acid and Cis-Vaccenic acid; GBK 000046 profiled Methyl palmitate, Palmitic acid, Methyl linolenate, Cis-Vaccenic acid, Linoleic acid and oleic acid while GBK 000365 had Methyl palmitate, palmitic acid and Benzyl linoleate (Table 4.2).

There was reduction in fatty acid profiles in sorghum genotypes exposed to high temperatures in this study. Variety GBK 000075 and Ikinyaruka had no fatty acid in their profiles. Variety BM 29, GBK000055 and GBK 000365 had Benzyl oleate. Variety GBK 000046 was the only genotype in this treatment that recorded Benzyl oleate as well as Oleic acid (Table 4.2).

Table 4.2:

Fatty acid associated with sorghum genotypes under controlled temperature

Genotype	Fatty acid name	Temperature Condition		Mass	Retention time	Saturated/ unsaturated
		Cold **(18/9 ºC)**	Warm **(30/20 ºC)**			
GBK 000075	n-hexadecanoic acid (Palmitic acid)	✓	*	256	12.407	Saturated
	Oleic Acid	✓	*	282	16.867	Unsaturated
Ikinyaruka	Oleic acid	✓	*	282	12.403	Unsaturated
BM 29	Hexadecanoic acid, methyl ester (Methyl palmitate)	✓	*	270	11.830	Saturated
	n-hexadecanoic acid (Palmitic acid)	✓	*	256	12.419	Saturated
	9,12,15-Octadecatrienoic acid,Methyl ester (Z,Z,Z)(Linolenic acid)	✓	*	292	14.802	Unsaturated
	9,12-Octadecadienoic acid(z,z)(Linoleic acid)	✓	*	280	15.528	Unsaturated
	Oleic acid	✓	*	282	15.861	Unsaturated
	9-Octadecenoic acid (z,)-Phenylmethyl ester (Benzyl oleate)	*	✓	372	17.737	Unsaturated
GBK 000065	Hexadecanoic acid, methyl ester (Methyl palmitate)	✓	*	270	11.805	Saturated

	9-Octadecenoic acid (z,)-Phenylmethyl ester (Benzyl oleate)	*	✓	372	17.737	Unsaturated
	n-hexadecanoic acid (Palmitic acid)	✓	*	256	12.382	Saturated
	Cis- Vaccenic acid	✓	*	282	15. 449	Unsaturated
GBK 000046	Hexadecanoic acid, methyl ester (Methyl palmitate)	✓	*	270	11.797	Saturated
	n-hexadecanoic acid (Palmitic acid)	✓	*	256	12.391	Saturated
	9,12,15-Octadecatrienoic acid,Methyl ester (Z,Z,Z)	✓	*	292	14.802	Unsaturated
	Cis- Vaccenic acid	✓	*	282	15. 449	Unsaturated
	9,12-Octadecadienoic acid (z,) (Linoleic acid)	✓	*	280	15.833	Unsaturated
	Oleic acid	✓	✓	256	18.405	Unsaturated
	9-Octadecenoic acid (z,)-Phenylmethyl ester (Benzyl oleate)	*	✓	372	17.737	Unsaturated
GBK 000365	Hexadecanoic acid, methyl ester (Methyl palmitate)	✓	*	270	11.789	Saturated
	n-hexadecanoic acid (Palmitic acid)	✓	*	256	12.366	Saturated
	9,12-Octadecadienoic acid (Z, Z)-, phenymethyl Ester (benyzyl linoleate)	✓	*	370	13.532	Unsaturated
	9-Octadecenoic acid (z,)-Phenylmethyl ester (Benzyl oleate)	*	✓	372	17.737	Unsaturated

Key: ✓-Fatty acid detected, *- Fatty acid not detected.

4.3.4 Fatty acid markers associated with cold tolerance in sorghum genotypes

Cold tolerant genotypes GBK 000075, BM 29 and Ikinyaruka grown in the two locations expressed 9,12,15-Octadecatrienoic acid commonly known as Methyl linolenate. The expression of Methyl linolenate fatty acid profiled from all the genotypes under field conditions and controlled conditions in the Egerton site can be used to conclude that Methyl linolenate fatty acid is a preferable fatty acid marker associated with cold tolerance. Oleic acid, linoleic and Cis-Vaccenic acid unsaturated fatty acid would also be possible fatty acid markers for crops grown under controlled cold temperatures.

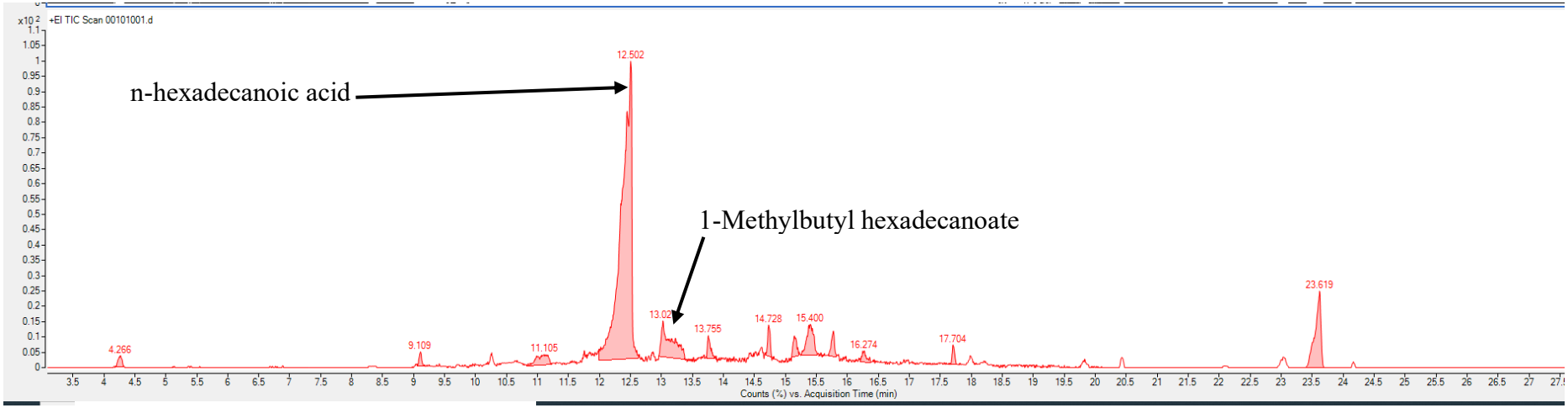
4.3.5 Common name, physical properties of fatty acids identified under field and controlled conditions

A total of fifteen fatty acids were profiled in the two experiments, out of this; six fatty acids were saturated while nine were unsaturated. The physical properties such as melting point of the fatty acids have been described under Table 4.3. The melting points of saturated fatty acid is higher for example, palmitic acid has a higher melting point of 63. Melting points of unsaturated fatty acids is lower as demonstrated by Methyl linolenate. Some of the unsaturated fatty acids are reported to have a higher melting for example, Oleic acid and Cis-Vaccenic acid. Chromatograms, mass spectrum and chemical structure of selected fatty acids are outlined in Figures 4.2-4.6.

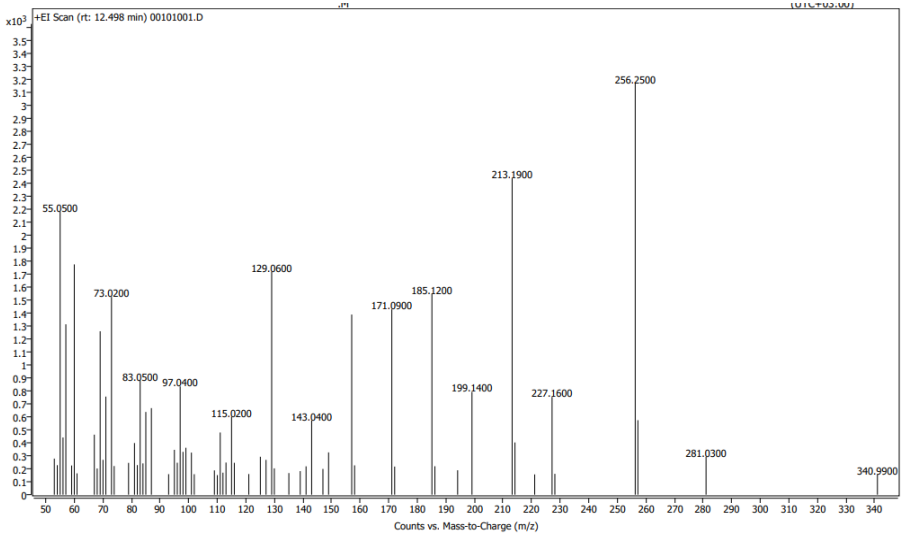
Table 4.3:

Physical properties of saturated and unsaturated fatty acids

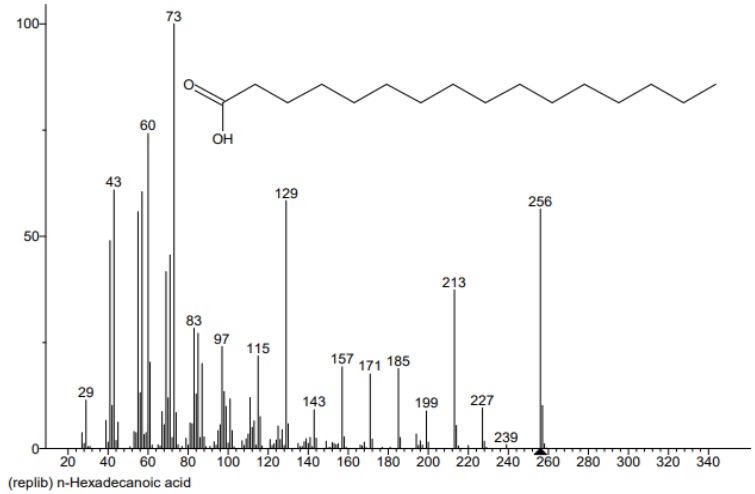
Saturated					
NO	**IUPAC Name**	**Common name**	**Molecular mass**	**Structural formulae**	**Melting point**
1	n-Hexadecanoic acid	Palmitic acid	256	$C_{16}H_{32}O_2$	63
2	1-Methylbutyl hexadecanoate	s-amyl palmitate	326	$C_{21}H_{42}O_2$	10-13
3	Tetradecanoic acid	Myristic Acid	270	$C_{17}H_{34}O_2$	53.9
4	Pentadecanoic acid	Pentadecanoic acid	270	$C_{17}H_{34}O_2$	61.3
5	n-Butyl laurate	Lauric acid	256	$C_{16}H_{32}O_2$	-7
6	Hexadecanoic acid, methyl ester	Methyl palmitate	270	$C_{17}H_{34}O_2$	30
	Unsaturated				
1	9,12,15-octadecatrionic acid.	Methy linolenate	292	$C_{19}H_{32}O_2$	-57
2	11,14,17-Eicosatrinoic acid	Arachidonic acid	320	$C_{21}H_{36}O_2$	-50
3	9,12,15-octadecatrienoic acid,(Z Z Z)-	Linolenic acid	278	$C_{18}H_{30}O_2$	-11
4	9-Octadecenoic acid (Z)	Oleic acid	282	$C_{18}H_{34}0_2$	16
5	9,12,15-Octadecatrienoic Acid, 2,3-Dihydroxypropyl Ester,(Z,Z,Z)	monolinolenin	352	$C_{21}H_{36}O_4$	-
6	9,12-Octadecadienoic acid	Linoleic acid	280	$C_{18}H_{32}O_2$	-5
7	Cis- Vaccenic acid	Cis-vaccenic ac id	282	$C_{18}H_{34}O_2$	44
8	9,12-Octadecadienoic acid (Z,Z)-,phenymethyl Ester	Benyzyl Linoleate	370	$C_{25}H_{38}O_2$	-
9	9-Octadecenoic acid (z,)-Phenylmethyl ester	Benzyl oleate	372	$C_{25}H_{40}O_2$	-



Chromatogram

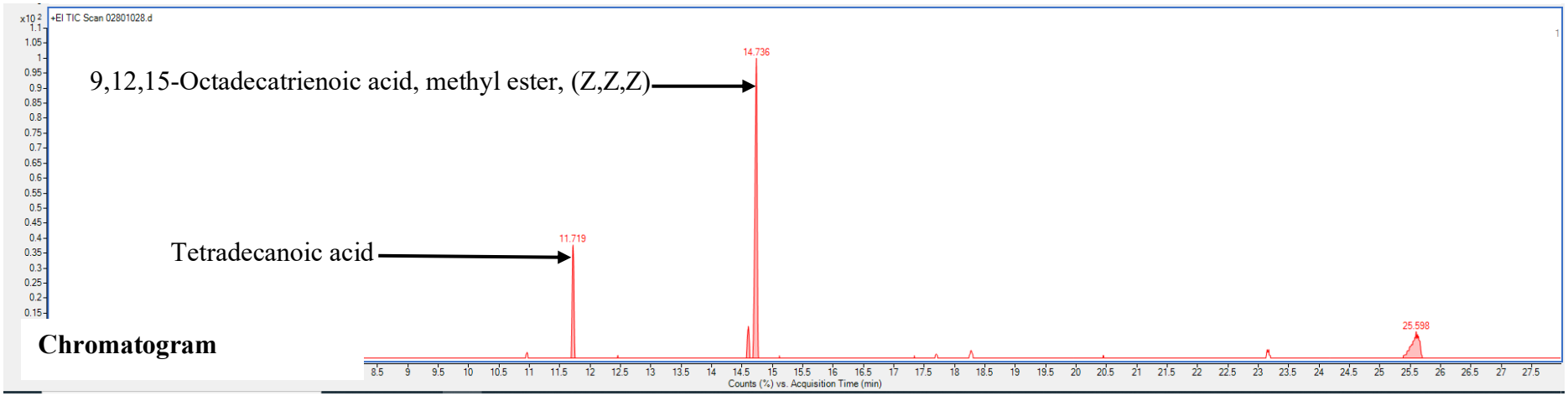


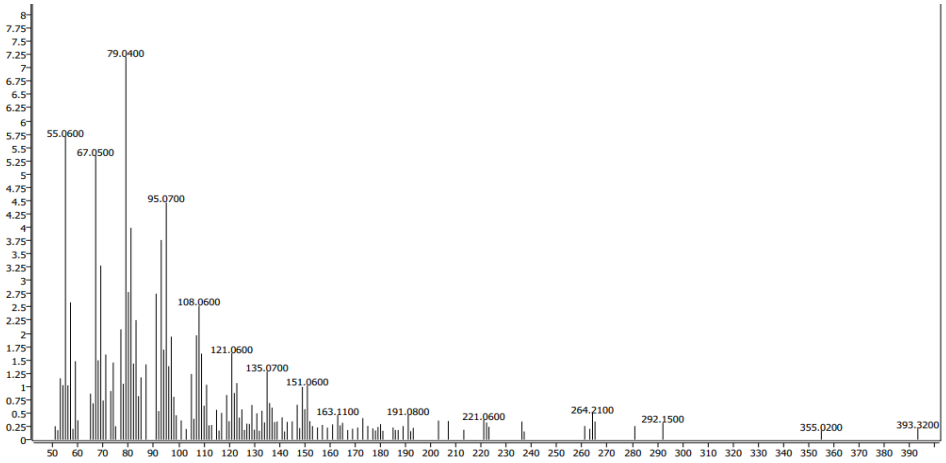
n-hexadecanoic acid mass spectrum



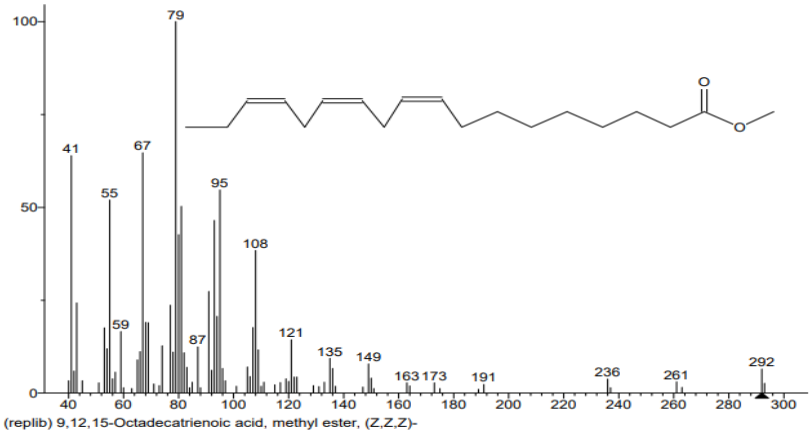
n-hexadecanoic acid structure

Figure 4.2: *Chromatogram, mass spectrum and structure of fatty acids observed in GBK 000075 grown at Marigat site*



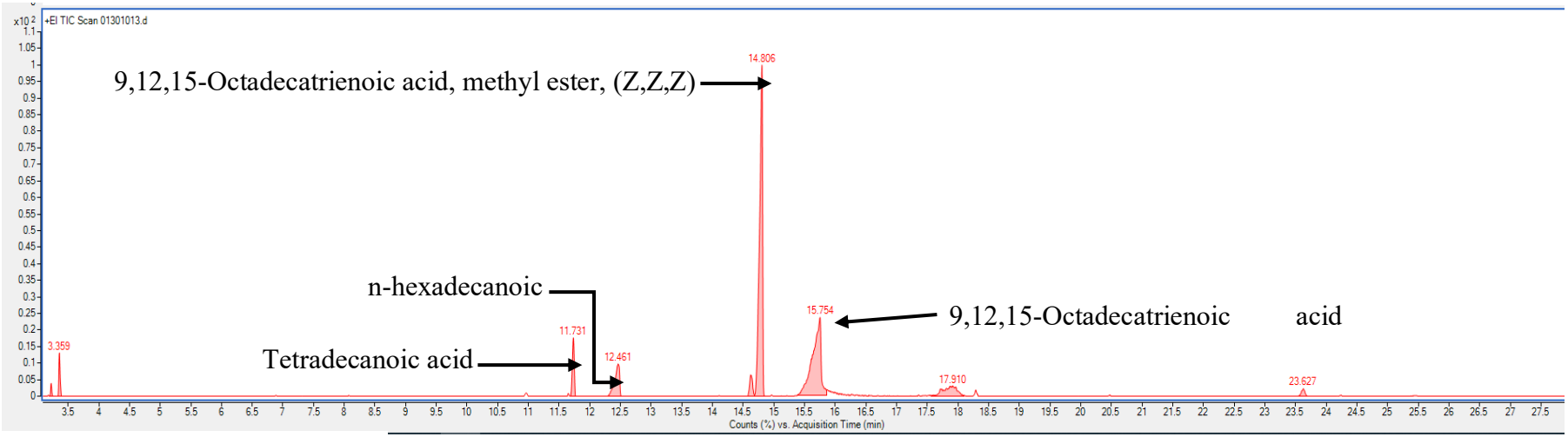


9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) mass

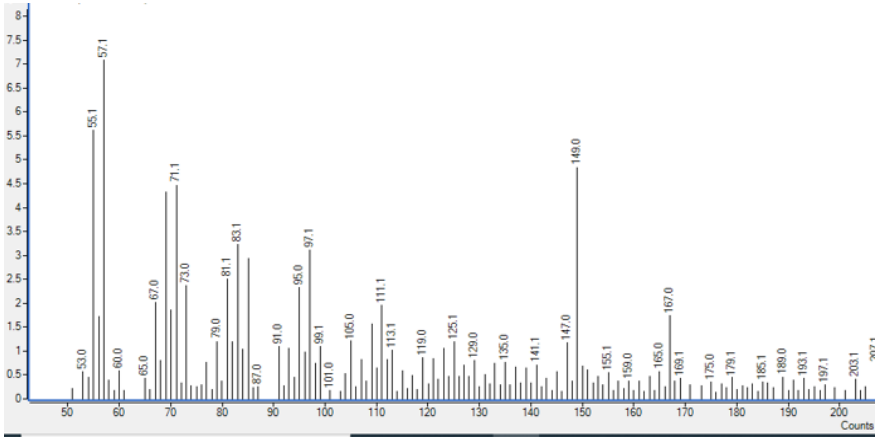


9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)

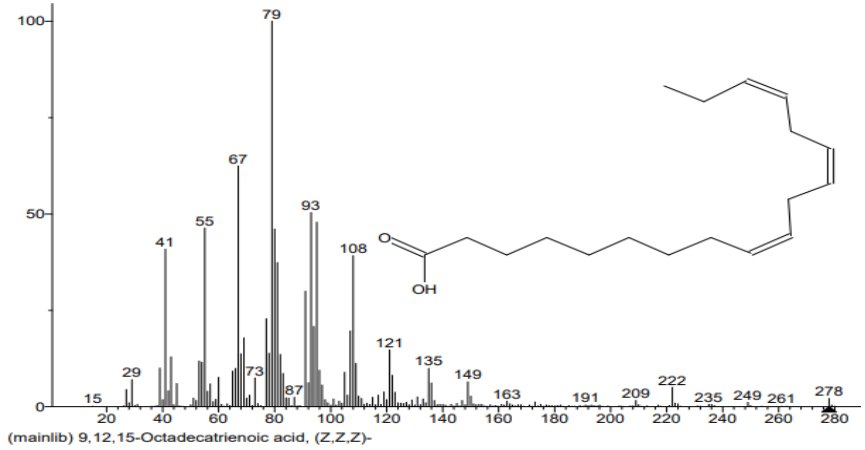
Figure 4.3: *Chromatogram, mass spectrum and structure of fatty acids observed in GBK 000075 grown at Egerton site*



Chromatogram



9,12,15-Octadecatrienoic acid, (Z, Z, Z) mass spectrum



9,12,15-Octadecatrienoic acid, (Z, Z, Z) structure

Figure 4.4: *Chromatogram, mass spectrum and structure of fatty acids observed in BM 29 grown at Marigat site*

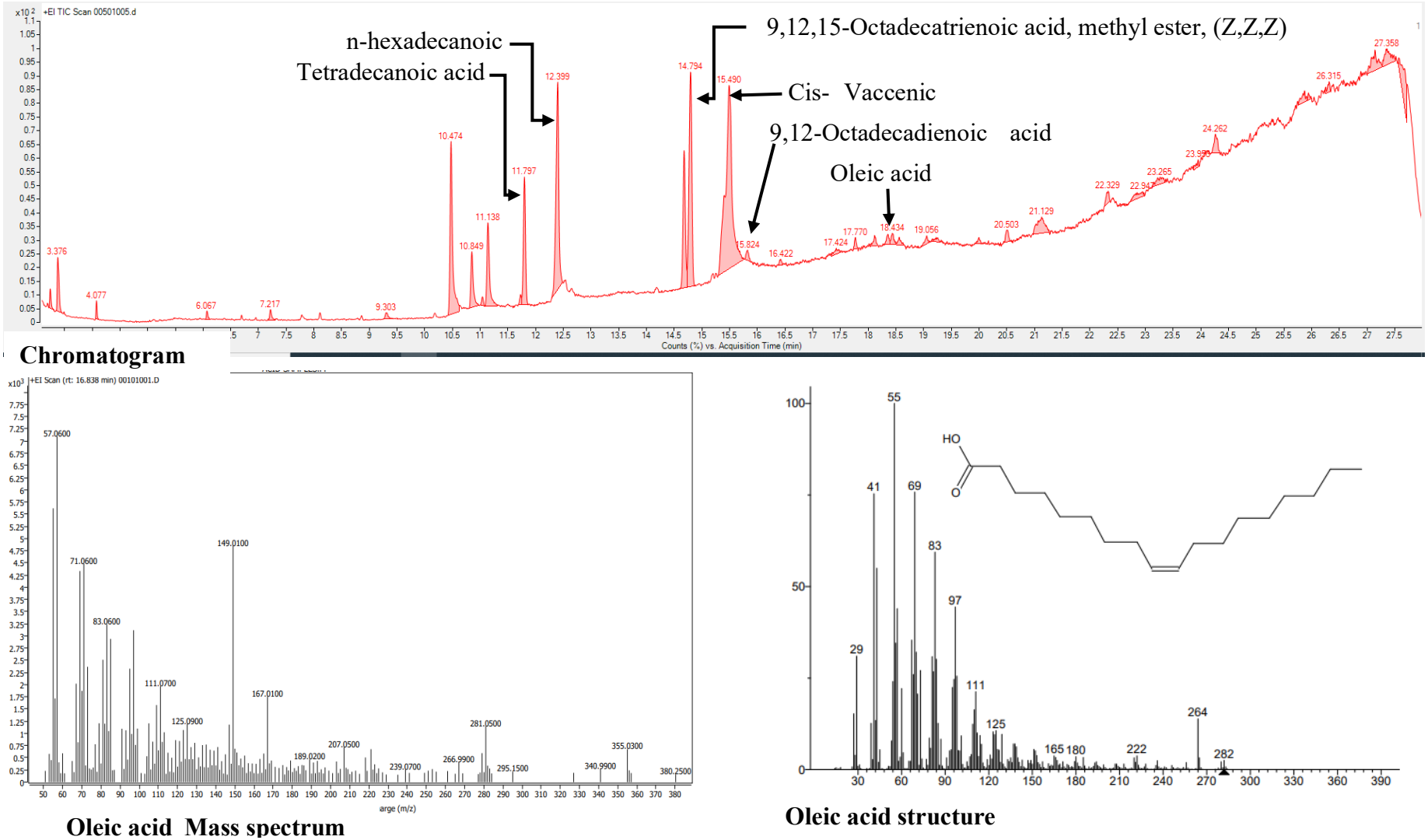
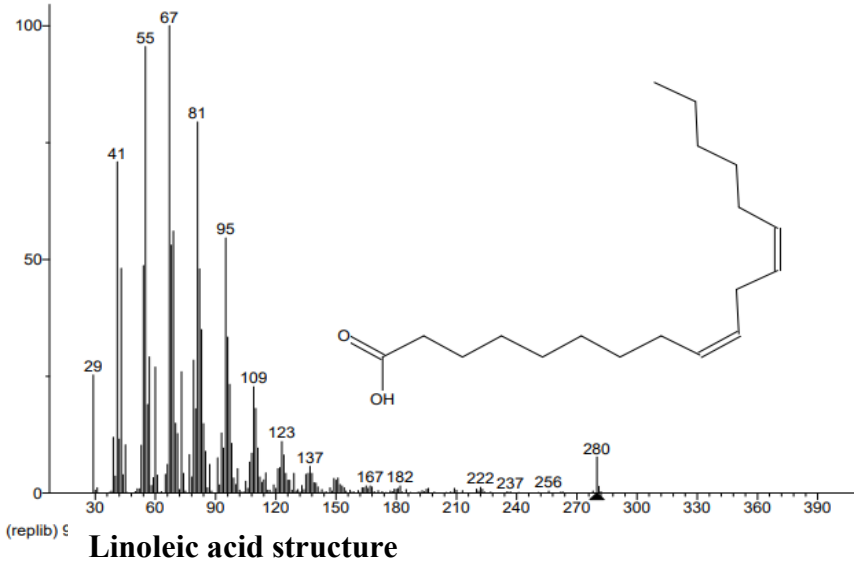
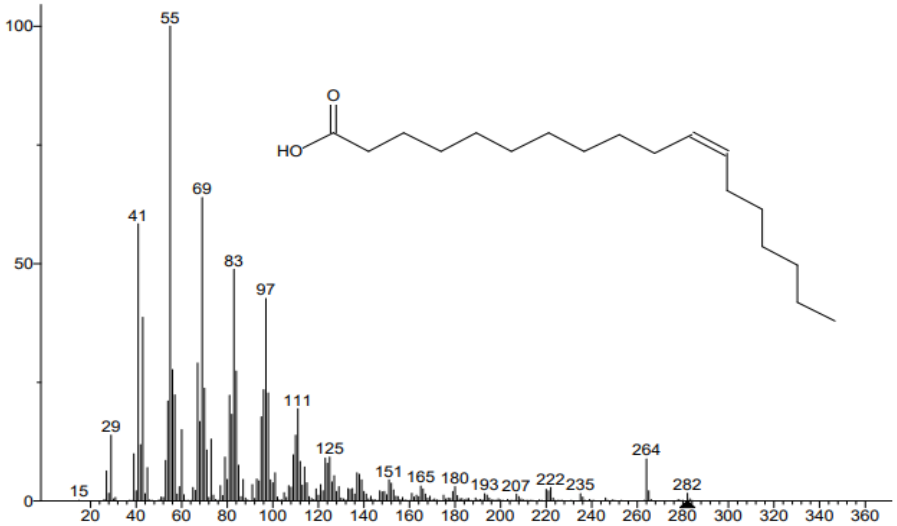


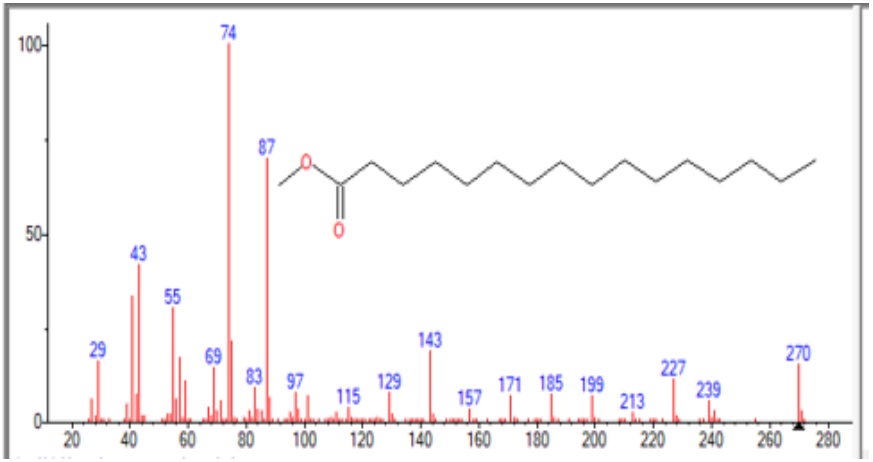
Figure 4.5: *Chromatogram, mass spectrum and structure of fatty acids observed in BM 29 grown in cold temperature- growth chamber*



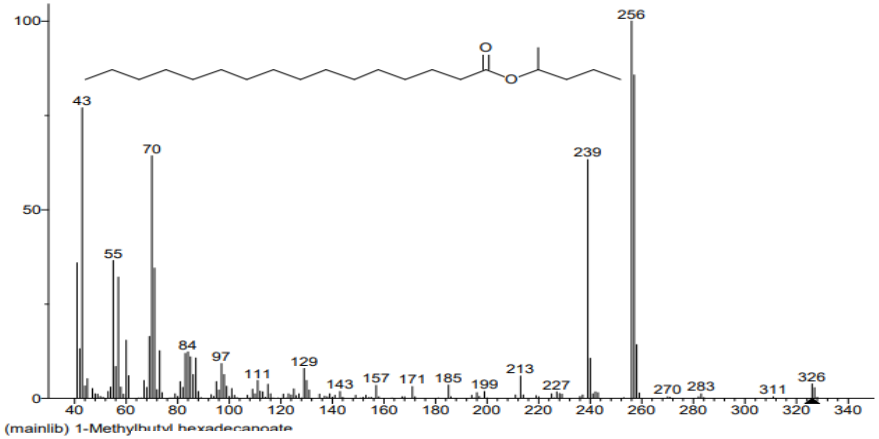
Linoleic acid structure



cis- Vaccenic acid structure



Tetradecanoic acid structure



1-Methylbutyl hexadecanoate structure

Figure 4.6: *Structures of selected fatty acids detected in GC-MS outputs*

4.4 Discussion

The analysis of the sorghum genotypes in the two field locations and under the three clusters of cold tolerance, revealed a wide range of saturated and un-saturated fatty acids. The analysis revealed the presence of n-hexadecanoic acid (Palmitic acid), 1-Methylbutyl hexa decanoate (C21:0), 9,12,15-octadecatrienoic acid (Linolenic acid), 11,14,17-Eicosatrinoic acid (Arachidonic acid), 9,12,15-octadecatrionic acid (Methyl linolenate) n-Butyl laurate (Lauric acid), Tetra decanoic acid (Myristic acid) and Pentadecanoic acid fatty acids in sorghum genotypes. Palmitic acid C16:0 and Linolenic acid fatty acids were the dominant fatty acids in the hot environment while tetra-decanoic acid and methyl linolenate were dominant SFA and USFA, respectively at Egerton site. In Marigat palmitic acid, 1-Methylbutyl hexadecanoate, n-Butyl laurate (C16:0), tetradecanoic acid and pentadecanoic acid function the same way in plants in enhancing membrane stability and high efficiency of photosystem II (Prasertthai *et al*., 2022). Saturated fatty acid has a higher melting point due to the uniform rod-like packing of molecules (Libretexts chemistry), this enable the phosphomembranes to remain in solid state at high temperatures thus reducing membrane fluidity (Narayanan *et al*., 2016). Plants require more of saturated fatty acid under high temperatures than unsaturated fatty acids, to reduce membrane lipid peroxidation, membrane fluidity and electrolyte leakage (Narayanan *et al*., 2016; Prasertthai *et al*., 2022). The fatty acid 11,14,17-Eicosatrinoic acid (C21:3) (Arachidonic acid) which is a long chain fatty acid helps in formation of wax in cuticles for reduced water loss and facilitate light dispersion for reduced heat load under high temperatures as well as raise basal levels of jasmonic acid (Jetter *et al*., 2006; Prasertthai *et al*., 2022; Savchenko *et al*., 2010). Saturated fatty acids; Palmitic acid, 1-Methylbutyl hexa decanoate, n-butyl laurate, tetradecanoic acid and pentadecanoic acid function the same way in plants in enhancing membrane stability and high efficiency of photosystem II under high temperatures.

In cold environments, saturated fatty acids form a “kink” leading to less membrane fluidity and thus hindering the cell physiological processes such as photosynthesis and respiration (Wang *et al*., 2023). The thylakoid membranes are the most sensitive to abiotic stresses such as cold stress and the survival of plants is dependent on the extent of injury to these membranes (Hu *et al*., 2020). The major types of lipids in the thylakoid membranes are phospholipids, galactolipids and sulfolipids (Mongrand *et al*., 1998; Yamamoto, 2016). Linolenic and Palmitic acid are known to be the primary derivative of phospholipids a framework of lipid bilayer (He *et al* 2020). This indicates that in the process of cold response, sorghum genotypes supplemented the structural components of cell membrane by having tetradecanoic

acid and linolenic acid, and maintained the membrane fluidity by synthesizing more unsaturated fatty acids (Tian *et al*., 2022). Methyl linolenate, linoleic and oleic acid are the major fatty acids in the thylakoid membranes which helps in maintaining membrane fluidity in the said membranes (Van Eerden *et al*., 2015). The low melting points of unsaturated fatty acids indicated that the membranes remain fluid at low temperatures thus preventing a kink (Harayama & Antonny, 2023).

Methyl linolenate poly unsaturated fatty acid has the same function as α-Linolenic acid and is reported to prevent oxidation or peroxidation of unsaturated fatty acids (Okuda *et al*., 2005). It is a special fatty acid that enhances chilling tolerance in crops such as cucurbits by enhancing the synthesis of Linolenic acid for jasmonic acid (Liu *et al*., 2019; Liu *et al*., 2021; Shi *et al*., 2019). Linolenic acid is a pre-cursor in jasmonic acid formation. Jasmonic acid is a Phyto-hormone involved in modulating the expression of genes involved in abiotic stresses especially those mediated by reactive oxygen species (ROS) signalling (Aslam *et al*., 2021). In cold adaptable genotypes, enough content of linolenic acid triggers accumulation of jasmonic acid levels, and when the level of linolenic acid is insufficient the plants accumulate anthocyanins to adapt to cold (Wang *et al*., 2021). Jasmonic acid mediates important plant processes including but not limited to male and female organ formation, movement of leaves as well as leaf senescence. The phytohormone also mediates responses of plants to light and cold among others (Ruan *et al*., 2019). The availability of $C_{19}H_{34}O_2$ (Methyl linolenate) could be attributed to heading in cold tolerant at Egerton site.

Oleic acid, cis-vaccenic acid, linoleic were observed under controlled conditions, these unsaturated fatty acids aids in membrane fluidity thus reducing electrolyte leakage. The end gain of this unsaturation is protecting the photosynthetic apparatus through enhanced photosystem II photochemistry (Prasertthai *et al*., 2022). De Palma *et al*. (2008) postulated that fatty acid unsaturation is first by addition of a first double bond to C:18, which yields a C18:1 (Oleic acid) by a Delta-9 stearoyl-ACP desaturase enzyme localized in the chloroplast. Further additional bonds yield C18:2 (Linoleic) and C18:3 (Linolenic acid) acids by microsomal desaturase, Delta-6 and Delta-3 desaturases. This step-wise process could explain why oleic and linoleic acid were only profiled at the growth chamber and not at in the field as the genotypes in the growth chamber took less time compared to those in the field.

Cis-Vaccenic (C18:1 Δ11) acid is an unusual mono unsaturated fatty acid in plants which was profiled in growth chamber-grown GK 000065 and GBK 000365 genotypes. The fatty acid is believed to be formed by conversion of palmitic acid to palmitoleic acid by endogenous

elongases enzyme. Cis-Vaccenic acid, the double bond positioning at position C_{11} instead of C_9 on the same side of the chain forms a 'kink' and results to a more flexible, stable and fluid membranes at low temperatures compared to palmitic acid (De Palma *et al*., 2008). Whereas these two genotypes remained vegetative under the field conditions the contribution of Cis-Vaccenic acid need to be evaluated to understand the molecular basis of its presence in the growth chamber grown genotypes.

4.5 Conclusion

Low temperatures at Egerton site significantly affected the growth and development of sorghum genotypes. Sorghum genotypes grown at Egerton site expressed tetradecanoic acid and Methyl linolenate. Fatty acid alterations appear to be acclimation mechanisms to maintain optimal membrane fluidity under low-temperature conditions. The remodelling of leaf fatty acids was primarily manifested in terms of increase in unsaturation levels of membrane lipids. The possible mechanism of cold tolerance was through increase in unsaturated fatty acids at Egerton site. In this study, the cold tolerant genotypes responded by maintaining their lipid unsaturation through oleic, linoleic and linolenic acid molecules. Under controlled environment, the cold tolerant genotypes that were exposed to low temperature growth chamber profiled oleic, linoleic fatty acids which were not present at the field. Methyl linolenate could have enabled GBK 0000075 and BM 29 to withstand cold and hence remain productive at Egerton site. These two genotypes can be evaluated further and used as breeding materials to aid in development of cold tolerant genotypes that can withstand low temperatures in the highlands. Further studies should be conducted to quantify these fatty acids to determine the changes in response to cold temperature.

CHAPTER FIVE

GENERAL DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

5.1 General discussions

5.1.1 Characterization of sorghum genotypes for reproductive cold tolerance

The ability of sorghum genotypes to produce a panicle with grains is regarded as reproductive cold tolerance while without a panicle is an indication of cold sensitivity. Characterization of sorghum genotypes for cold tolerance is the best way to evaluate and inform which genotypes to grow in the right agro ecological zone. Cluster analysis is an efficient way to classify genotypes as either cold tolerant, sensitive or intermediate. The information on cold tolerance or sensitivity is important for crop improvement. Cluster one genotypes namely GBK 0000 (2, 10, 20, 30,32,35, 37, 40, 43, 44, 46, 49, 50, 52, 56, 57, 58, 59, 64, 65, 67, 67, 72, 73, 83, 100, 102, 118, 365, 366, 367, 377, 381, 382), Busia_28-1, IS 8884, IS 11159, IS 1161 and Siaya 93-1 are not suitable for growing in the highlands. The genotypes in cluster three which included GBK 000062, Asareca 15-2-1, CR 35:5, ICSV 111 IN, IESH 22007, IESV 92170 DL, Macia, Miwaleni local, IESV 92136 DL, KSV12 Mexico R-line #5, IS 11162, IS 26794, IS 2853, Shambuko (PP290), Amasugi, IESV 91048 DL, IESV 91049 DL, Plot 142 Sudan, IESV 92025 SH, IS 24484 and SP 74744 are also not suitable for highlands. Cluster two genotypes such as BM (29, 31, 32, 39, 5, 6, 16, 17, 21, 27), Cyatanombe, E1291, Gataraga, Gicamunkoni, all IESV….LT series, Ikinyaruka, IS (11076, 11141, 11187, 11228, 11257, 11338,11350, 11579), Amatega, Bayishinyike, MB 23 & MB 27, Ibundu, Ainamoi, Nahadava and IS 255557, can be cultivated satisfactorily since they headed early and had grains of good quality.

Lower temperatures reduce phenological development of sorghum. In this research, plant height, panicle length, panicle weight and seed weight were drastically reduced in sorghum genotypes. Panicle harvest index regarded as measure of cold tolerance by Krishnamurthy *et al*. (2014) could be a good tool to dissect for cold tolerance. In this research, the genotypes that had harvest index of more than 0.7 showed cold tolerance and had earlier heading at 125 days, and relatively heavier panicles which translated to higher seed weights. Genotypes that had a panicle harvest index of below 0.2 were cold sensitive despite heading since they had sterile panicles.

Plant height is affected by genotype by environment. In the current study about 30% reduction in height was noted in Egerton grown genotypes. However, GBK 00002, and GBK 000010 had a plant height of more than 350 centimetres in Marigat but were bushy at Egerton since they remained vegetative. This was an indication that high temperature accelerates growth while

low temperatures repress growth. Heading was contrasted in the two sites with diverse temperatures. The genotypes at Egerton site took longer days to head and the first genotype to head was observed at 125 days after sowing. The same genotypes took 57 days to head in Marigat. This is a clear indication that temperatures affect the development of crops.

Tillering ability was affected by temperatures. Sorghum had fewer number of tillers in Marigat compared to increased tillering at Egerton site. Tillering was found to be more in the cold sensitive genotypes under cluster one compared to the other two clusters.

5.1.2 Fatty acid associated with cold sensitive and cold tolerant sorghum genotypes

The results have revealed that plants require both unsaturated and saturated fatty acids in their membranes. Palmitic, tetradecanoic acid, pentadecanoic saturated fatty acid were found in Ikinyaruka when grown at Marigat site while the same genotype showed no fatty acid at Egerton site but profiled Oleic acid in the growth chamber. Methyl linolenate acid was the only unsaturated fatty acid profiled in Egerton grown genotypes and this fatty acid has been found to increase a phytohormone that modulates against cold stress. The cold sensitive genotypes grown at Egerton site had no fatty acid in their membranes and only GBK 000065 had tetradecanoic acid and Methyl linolenate. The absence of fatty acid in these genotypes makes them sensitive to cold and thus photosynthesis is reduced due to photosystem II destruction (Tian *et al*., 2022). Oleic acid, linoleic, linolenic and cis-Vaccenic acid unsaturated fatty acids profiled in growth chamber grown genotypes have been reported to ease chilling stress and restore the proper functioning of the cell membranes (Guo *et al*., 2016). Oleic, linoleic and cis-Vaccenic acid were not profiled in the field perhaps because the genotypes took a shorter time to adjust and more research should be conducted to ascertain their contribution to cold tolerance. In the current study, cold tolerant genotypes such as GBK 000075, BM 29 accumulated Methyl linolenate acid both under field condition and growth chamber conditions hence can be targeted for enhancing cold tolerance in crop improvement. Understanding the association of fatty acid in sorghum genotypes under warm and cold environments is important in development of markers for cold tolerance.

5.2 Conclusions

i. Sorghum is sensitive to temperature. Three clusters of sorghum genotypes emerged as a result of exposure to low temperature; Cluster one containing forty genotypes that did not produce a panicle; Cluster two with one hundred and eighty-five genotypes that produced grain despite delayed heading and Cluster three with twenty-two genotypes that had sterile panicles in low temperature environment.

ii. The Genotypes grown at Marigat showed an increase in the number of saturated fatty acid possibly to reduce membrane fluidity and peroxidation while, the genotypes that were productive at Egerton had predominantly unsaturated fatty acids.

iii. Linolenic acid, Methyl linolenate, oleic acid and linoleic acid were responsive to cold tolerance in sorghum grown under low temperatures. Methyl linolenate was found in GBK 000075 and BM 29 genotypes grown under field conditions and these two genotypes upon further research can be used as breeding materials for the low temperature highlands.

5.3. Recommendations

i. Sorghum genotypes should be grown in their right environment based on temperature regime. Cold tolerant genotypes such as cold tolerant genotypes listed in (Annex 2) for example, BM (29, 31, 32, 39, 5, 6, 16, 17, 21, 27), Cyatanombe, E1291, Gataraga, Gicamunkoni, all IESV….LT series, Ikinyaruka, IS (11076, 11141, 11187, 11228, 11257, 11338,11350, 11579), Amatega, Bayishinyike, MB 23 & MB 27, Ibundu, Ainamoi, Nahadava and IS 255557 can be grown in the highland where temperatures are low. More research and breeding should be done on genotypes in cluster one and three for growth in the highlands.

ii. Fatty acids such as Oleic acid, Methyl linolenate, linolenic, Linoleic acid and Cis-Vaccenic acid are associated with cold tolerance. There is need to confirm their plasticity response by incorporating their standards in the analysis and subsequently linking the production with responsible gene loci. The information will hasten sorghum improvement for cold tolerance.

iii. The sorghum genotypes GBK 000075 and BM 29 demonstrate strong potential as breeding materials for the low-temperature highlands. At the Egerton site, they maintained good productivity under cooler conditions and profiled *Methyl linolenate*, a fatty acid marker associated with cold tolerance. These attributes make them valuable for developing varieties suited to highland environments, where farmers could benefit from adopting such genotypes to ensure more reliable yields.

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APPENDICES

Appendix I: Table of means for cluster 2

Entry	Genotype name	Plant height(cm)		Tillers		Days to 50 % heading		Panicle length (cm)		Panicle weight(g)		Seed weight(g)		PHI	
		Egerton	Marigat	Egerton	Marigat	Egerton	Marigat	Egerton	Marigat	Egerton	Marigat	Egerton	Marigat	Egerton	Marigat
3	GBK-000013	118	163	5	2	163	79	16	24	11	41	7	28	0.56	0.67
4	GBK-000014	81	130	5	1	170	70	16	24	21	75	15	55	0.61	0.70
5	GBK-000015	99	128	6	1	161	74	20	23	40	40	31	32	0.72	0.71
6	GBK-000016	91	148	2	2	155	56	16	21	17	42	12	32	0.60	0.77
7	GBK-000018	81	155	4	2	159	60	17	27	15	126	10	89	0.58	0.71
9	GBK-000021	128	210	4	1	173	74	18	26	62	77	56	65	0.90	0.85
10	GBK-000022	122	239	4	1	204	74	17	22	23	114	12	88	0.46	0.79
11	GBK-000023	98	285	5	3	186	81	18	24	41	98	35	86	0.82	0.87
12	GBK-000024	138	193	4	2	177	74	21	24	64	67	55	55	0.87	0.82
13	GBK-000025	119	224	4	4	174	83	18	24	51	58	42	45	0.81	0.76
14	GBK-000027	131	218	3	3	178	80	19	23	74	83	63	73	0.84	0.86
15	GBK-000028	147	194	3	2	166	74	20	24	53	89	46	76	0.88	0.85
16	GBK-000029	128	184	4	2	175	64	20	22	43	54	35	46	0.81	0.84
18	GBK-000031	129	179	5	2	162	80	18	23	62	84	53	70	0.85	0.83
20	GBK-000033	129	226	4	2	184	80	17	24	43	63	33	52	0.79	0.73

21	GBK-000034	138	220	3	2	166	79	19	23	62	77	54	60	0.86	0.79
24	GBK-000038	80	228	3	2	128	58	16	24	12	83	7	67	0.50	0.81
25	GBK-000039	167	233	4	2	207	82	23	29	107	64	58	40	0.52	0.69
30	GBK-000047	123	230	5	1	201	74	13	17	13	87	10	80	0.73	0.91
31	GBK-000048	107	260	5	4	207	71	17	29	26	93	22	81	0.82	0.87
34	GBK-000051	142	195	5	2	180	75	15	24	31	50	20	38	0.64	0.65
36	GBK-000055	99	174	5	2	172	73	17	23	23	85	16	59	0.64	0.69
41	GBK-000060	110	161	7	4	176	75	17	23	32	78	24	61	0.74	0.77
45	GBK-000066	66	108	3	3	184	76	23	30	52	78	33	54	0.63	0.69
47	GBK-000068	138	260	3	2	163	75	16	29	39	107	18	82	0.57	0.77
48	GBK-000069	149	247	5	1	189	88	17	26	52	75	44	51	0.84	0.66
49	GBK-000070	132	283	3	3	180	77	15	24	33	107	28	93	0.84	0.87
52	GBK-000075	126	209	4	2	170	76	16	25	32	43	23	32	0.65	0.63
53	GBK-000077	117	166	4	2	181	67	18	24	41	78	36	64	0.85	0.80
54	GBK-000079	121	241	5	2	200	74	18	29	53	100	43	76	0.77	0.75
55	GBK-000080	132	259	4	3	201	83	18	30	22	85	14	67	0.53	0.78
56	GBK-000081	139	238	4	1	167	63	17	25	28	116	21	94	0.69	0.81
57	GBK-000082	135	290	5	1	182	84	14	25	24	78	20	64	0.78	0.82
59	GBK-000084	144	275	4	2	190	78	15	28	31	94	24	71	0.76	0.75
60	GBK-000085	134	255	4	1	174	77	16	26	29	68	22	51	0.73	0.75
61	GBK-000086	136	303	6	3	163	70	18	22	22	96	16	82	0.72	0.86

62	GBK-000088	116	294	4	4	185	75	15	27	25	67	19	47	0.73	0.62
63	GBK-000089	118	278	5	2	178	75	15	24	30	98	21	65	0.70	0.68
64	GBK-000090	129	275	5	2	182	77	17	29	53	93	40	48	0.77	0.53
65	GBK-000091	147	237	5	2	174	78	15	26	32	88	26	65	0.76	0.74
66	GBK-000092	164	315	5	3	190	92	22	28	44	50	15	28	0.34	0.56
67	GBK-000093	161	278	4	1	162	76	16	27	32	136	24	99	0.74	0.72
68	GBK-000095	164	158	4	2	160	78	17	26	36	77	26	59	0.71	0.75
69	GBK-000096	137	266	4	4	180	83	17	27	39	129	29	106	0.69	0.83
70	GBK-000097	113	243	2	5	180	75	16	29	53	104	44	91	0.76	0.87
71	GBK-000098	131	278	4	4	187	81	17	27	23	73	17	56	0.76	0.77
72	GBK-000099	172	287	3	1	167	76	17	26	30	126	24	106	0.80	0.81
75	GBK-000103	132	209	4	4	213	76	17	23	47	108	39	91	0.82	0.84
76	GBK-000105	129	243	5	2	165	65	13	27	33	96	22	77	0.66	0.79
77	GBK-000106	130	249	4	2	177	67	15	25	26	111	16	88	0.54	0.80
78	GBK-000107	145	304	3	2	183	80	17	25	41	79	32	64	0.77	0.81
79	GBK-000111	119	212	3	3	154	67	14	19	26	67	16	51	0.63	0.75
80	GBK-000116	151	193	5	2	157	70	17	27	29	130	22	106	0.69	0.81
82	GBK-000119	145	288	3	2	198	79	16	23	79	31	64	25	0.81	0.75
83	GBK-000120	119	168	5	2	171	71	18	28	19	89	11	73	0.57	0.82
84	GBK-000121	120	283	8	3	174	77	17	25	19	46	16	37	0.81	0.81
85	GBK-000125	79	113	4	2	125	64	15	23	18	67	13	52	0.63	0.78

86	GBK-000128	94	126	5	2	147	59	18	22	14	51	7	41	0.48	0.80
87	GBK-000129	86	131	6	2	170	62	15	24	22	86	18	69	0.80	0.80
88	GBK-000130	82	132	4	2	130	65	16	23	11	80	7	63	0.57	0.79
89	GBK-000132	101	229	4	2	194	74	16	29	12	87	9	66	0.69	0.77
97	GBK-000387	101	148	4	2	173	78	16	21	21	61	15	47	0.62	0.77
98	GBK-000389	126	257	4	3	171	69	15	23	23	87	11	73	0.50	0.84
100	ASARECA 15-3-1	84	104	4	1	153	63	15	27	17	118	12	107	0.64	0.91
101	ASARECA 24-4-1	74	124	4	1	167	70	18	20	13	80	8	68	0.50	0.86
102	BUSHUKA (ICSV 210)	128	217	5	1	133	67	20	32	25	83	17	59	0.62	0.72
104	GADAM	93	123	5	2	161	67	17	23	18	53	14	41	0.76	0.77
105	GAMBELLA 1107	92	148	4	1	175	80	15	25	16	104	9	82	0.56	0.79
106	HAKIKA	58	99	4	1	150	72	21	29	23	56	12	39	0.46	0.70
107	ICSH 152010	78	141	3	2	151	62	22	32	23	71	13	48	0.49	0.65
108	ICSH 152014	131	208	4	2	158	68	21	26	30	117	23	101	0.75	0.87
110	IESH 22002	81	158	5	2	134	58	23	33	32	92	15	71	0.45	0.70
112	IESH 22017	112	128	7	2	159	62	17	27	35	79	22	70	0.57	0.88
113	IESH 22020	76	128	3	2	127	68	20	32	38	107	30	80	0.75	0.75

114	IESH 28002	91	139	6	3	130	68	21	28	7	85	3	66	0.35	0.79
115	IESV 23006 DL	96	148	3	1	130	58	18	27	22	73	13	59	0.55	0.80
116	IESV 23007 DL	98	143	3	2	151	59	19	25	24	78	14	60	0.56	0.78
117	IESV 24030 SH	85	123	5	4	180	68	18	27	22	69	14	55	0.54	0.81
118	IESV 92029 DL	69	118	5	2	160	75	22	31	25	77	16	46	0.65	0.61
120	IESV 94076 DL	88	153	4	1	159	72	14	31	11	123	5	82	0.45	0.66
123	IS 21282	127	272	4	2	188	77	15	27	9	106	6	79	0.63	0.73
124	Khalid	149	181	4	3	160	74	17	26	54	101	37	79	0.50	0.79
125	LABA (MW 5003)	81	149	5	1	164	71	14	22	18	46	12	30	0.65	0.62
128	SEREDO	93	129	5	2	178	64	20	28	27	62	17	47	0.60	0.68
129	SERENA	135	124	5	2	187	68	18	28	27	78	17	54	0.56	0.73
131	IESV91131DL	56	95	3	3	174	77	19	33	18	95	8	75	0.42	0.79
133	IESV92055/3SH	75	105	5	2	168	71	19	27	51	75	36	59	0.69	0.78
135	IESV92172DL	60	66	0	1	159	68	24	35	15	24	7	13	0.47	0.51
137	KARIMTAMA1	95	205	2	1	153	70	20	27	23	125	9	98	0.38	0.79
138	Kiboko Local-2	116	219	1	1	168	64	16	28	21	69	14	50	0.68	0.75
141	MAKUENILOCAL	118	210	5	3	191	67	16	29	27	81	20	63	0.72	0.79
143	PP290	86	128	5	0	146	64	21	29	15	72	6	49	0.40	0.68

144	S35	79	173	4	2	182	70	18	24	38	83	21	58	0.55	0.75
145	SIAYA_27-3	84	164	8	3	174	71	16	25	29	67	22	52	0.66	0.78
146	SIAYA_50-3	98	141	5	2	144	70	16	29	32	86	24	69	0.74	0.81
147	WAHI	60	95	2	0	160	70	24	30	19	75	8	61	0.42	0.82
148	BM 29	137	331	1	1	153	74	19	26	64	70	52	50	0.80	0.72
149	BM 31	196	162	0	2	139	67	19	21	37	14	30	6	0.78	0.43
150	BM 32	233	287	1	2	140	75	20	25	68	41	56	34	0.83	0.81
151	BM 39	205	258	1	2	153	69	22	27	72	74	60	59	0.82	0.79
152	BM 5	217	185	1	2	144	68	21	30	87	61	74	48	0.85	0.77
153	BM 6	156	263	2	1	149	80	17	24	30	40	25	33	0.79	0.82
154	Cyatanombe	210	259	1	2	149	74	25	32	71	73	61	57	0.86	0.77
155	E 1291	110	148	1	1	135	72	19	23	55	39	46	31	0.82	0.76
156	GATARAGA	186	268	1	1	151	80	19	23	89	59	73	46	0.83	0.80
157	GICAMUNKONI	160	171	2	2	144	100	22	29	49	48	42	38	0.86	0.79
158	IESV 90015 LT	98	83	3	1	129	68	20	24	29	38	24	32	0.81	0.84
159	IESV 90042 LT	89	123	3	2	125	60	20	25	30	36	22	30	0.92	0.75
160	IESV 91003 LT	131	147	3	2	141	71	20	22	55	62	47	50	0.84	0.79
161	IESV 91018 LT	120	179	2	2	181	68	20	30	38	71	28	60	0.66	0.86
162	IESV 91054 LT	90	111	2	3	161	77	23	24	60	40	43	30	0.71	0.76
163	IESV 91069 LT	82	155	1	4	130	68	20	24	63	32	53	25	0.85	0.75
164	IESV 91071 LT	94	140	3	2	130	71	20	22	56	37	47	30	0.85	0.82

165	IESV 91073 LT	117	167	3	2	127	69	22	31	49	62	37	45	0.68	0.72
166	IESV 91075 LT	98	154	4	2	125	59	19	24	27	34	23	24	0.81	0.71
167	IESV 91105 LT	209	241	1	3	151	77	21	25	61	44	51	31	0.78	0.72
168	IKINYARUKA	137	242	1	1	136	76	22	29	54	43	44	35	0.83	0.78
169	IS 11076	139	264	2	3	173	71	25	37	38	74	26	55	0.67	0.74
170	IS 11141	228	305	2	2	192	103	18	28	43	60	23	48	0.45	0.80
173	IS 11187	207	307	4	3	206	76	17	19	62	49	17	35	0.32	0.72
174	IS 11228	179	236	0	2	136	78	21	24	75	37	63	31	0.84	0.82
175	IS 11257	184	265	2	1	193	101	26	0	38	0	24	0	0.68	0.00
176	IS 11338	148	259	3	3	170	74	20	26	60	39	47	25	0.79	0.49
177	IS 11350	216	237	2	2	164	75	19	25	60	40	51	30	0.84	0.74
178	IS 11353	191	329	2	2	199	84	22	28	29	89	16	67	0.55	0.77
179	IS 11579	136	247	3	3	183	96	23	0	78	0	59	0	0.73	0.00
180	IS 11608	219	300	4	3	206	72	27	37	45	73	27	58	0.58	0.78
182	IS 11612	227	309	5	3	204	98	26	40	51	86	35	63	0.69	0.73
183	ICSH 152002	74	227	4	2	140	76	23	28	38	98	22	73	0.54	0.71
184	ICSH 152005	137	148	3	1	132	60	22	30	22	73	14	57	0.65	0.77
185	ICSH 152011	82	127	5	3	128	59	26	35	15	94	6	68	0.40	0.72
186	ICSH 152016	72	128	4	2	140	61	22	26	26	53	17	33	0.64	0.63
191	ABALESHYA	143	188	0	2	147	71	23	21	58	27	47	20	0.79	0.73
193	AMATEGA	198	185	1	1	135	77	20	26	80	56	67	41	0.84	0.76

194	BAYISHINYIKE	194	149	1	1	149	72	23	27	73	41	59	32	0.80	0.77
195	BM 16	136	182	2	2	143	76	19	19	59	20	52	15	0.88	0.76
196	BM 17	197	235	1	2	147	90	20	27	73	60	60	39	0.80	0.66
197	BM 21	224	258	2	2	142	78	22	25	85	49	70	36	0.80	0.74
198	BM 27	218	248	0	2	152	76	20	24	98	74	84	60	0.86	0.82
199	F6YQ212	59	99	6	3	161	56	15	21	9	58	4	50	0.48	0.85
200	Gambella 1107	104	164	4	2	174	75	17	21	24	75	16	62	0.63	0.82
201	ICSR93034	108	190	4	1	160	77	20	24	19	90	10	74	0.54	0.82
206	IESV91063DL	62	94	3	2	159	65	20	26	36	60	23	48	0.63	0.80
209	IS8193	98	232	4	3	166	72	18	24	34	58	25	47	0.69	0.83
211	R8602	71	118	4	3	128	67	20	29	22	52	13	39	0.56	0.75
213	SIAYA_46-1	88	142	5	2	149	68	16	28	16	62	10	46	0.55	0.77
214	SIAYA_81-2	93	165	5	4	166	63	16	24	39	71	31	57	0.77	0.81
216	AIHR 91075	79	153	5	3	163	72	19	29	30	101	23	81	0.71	0.80
217	ICSR 160	77	146	2	2	159	67	21	31	17	82	13	58	0.73	0.74
218	ICSR 172	70	114	5	4	127	70	17	27	18	43	15	27	0.74	0.69
219	ICSR 24008	102	128	3	1	164	74	25	29	27	95	18	79	0.62	0.81
220	ICSR 92003	78	118	5	2	170	73	21	30	44	87	35	71	0.79	0.81
221	WM89/90#1615	96	155	5	2	164	69	19	30	12	119	6	88	0.52	0.74
222	ZSV3	103	188	6	3	137	71	15	22	25	87	20	75	0.72	0.86
223	ICSR 43	83	127	7	1	149	72	23	32	33	73	24	55	0.68	0.74

224	IESV 93042 SH	54	90	3	1	136	66	24	33	20	68	11	49	0.51	0.72
225	MB 23	151	186	1	2	154	80	19	23	90	42	76	30	0.84	0.70
226	MB 27	171	181	2	1	154	78	20	28	69	43	48	24	0.69	0.58
227	LITH	108	225	3	2	185	68	18	31	10	55	6	42	0.56	0.76
228	IBUNDU	216	208	1	2	137	70	19	19	81	21	63	14	0.79	0.59
229	CYURE	172	244	1	2	142	70	19	27	70	98	56	66	0.79	0.67
230	IBUNDI	207	303	2	1	143	74	21	26	103	56	87	45	0.85	0.79
231	AINAMOI	117	193	2	3	134	71	18	24	51	62	43	50	0.84	0.79
232	NYUNDO	148	168	2	3	130	68	21	20	72	37	63	32	0.87	0.86
233	MUHIMPUNDU	179	306	2	1	128	74	18	25	37	44	29	33	0.79	0.76
234	DHET	81	170	6	4	179	62	16	24	16	108	11	89	0.66	0.83
235	NAHADAVA	98	147	5	2	175	66	18	28	33	92	24	71	0.70	0.76
236	IS 25557	164	198	1	2	157	77	21	24	99	48	78	32	0.79	0.64
237	IS 558	209	265	0	2	143	74	24	27	69	78	59	61	0.83	0.78
238	IS 2558	200	255	2	2	138	71	21	28	51	62	43	54	0.84	0.87
239	IS 9201	172	271	3	1	139	78	21	23	56	37	45	28	0.80	0.75
240	ARAKUCHOT	130	213	4	2	157	62	19	32	26	61	22	56	0.85	0.83
241	RWANDA	119	184	2	2	128	64	24	25	59	30	50	20	0.83	0.64
242	EST 15	87	249	4	2	175	72	23	35	50	106	40	55	0.76	0.54
243	EST 3	83	137	3	1	182	65	20	25	47	40	34	32	0.72	0.73
244	EST 26	88	229	3	2	171	66	19	25	30	50	16	37	0.47	0.72

245	EST 27	83	91	2	3	140	67	24	30	27	48	16	38	0.62	0.79
246	EST 20	107	201	2	2	153	70	24	29	59	94	47	79	0.80	0.84
247	EST 25	67	128	3	2	125	66	19	32	18	68	9	40	0.50	0.59
248	EST 7	101	195	6	2	164	75	16	43	26	70	17	57	0.60	0.81
250	EST 48	201	266	2	1	151	74	23	29	73	63	56	50	0.78	0.79
	Means	**125**	**197**	**3**	**2**	**162**	**72**	**19**	**26**	**11**	**41**	**7**	**28**	**0.56**	**0.67**
	s.e.d.	3.4	6.7	0.1	0.2	1.4	2.8	0.4	0.7	2.9	5.8	2.5	4.5	0.012 52	0.024 70

Appendix II: Table of means for cluster 3

	Marigat							Egerton						
Entry	Genotype name	Plant height(cm)	Tillers	Days to 50% heading	Panicle length (cm)	Panicle weight(g)	Seed weight(g)	PHI	Plant height	Tillers	Days to 50% heading	Panicle length	Panicle weight	Seed weight	PHI
42	GBK-000062	243	2	77	22	170	138	0.82	116	6	200	16	-	-	-
99	ASAREC A 15-2-1	103	3	73	25	59	47	0.81	62	4	171	13	-	-	-
103	CR: 35:5	240	2	64	25	68	56	0.82	50	11	204	12	-	-	-
109	ICSV 111 IN	128	1	62	26	96	77	0.81	73	2	194	21	-	-	-
111	IESH 22007	155	1	56	35	60	49	0.76	70	3	133	24	-	-	-

119	IESV 92170 DL	155	1	72	27	62	44	0.71	75	1	133	18	-	-	-
126	MACIA	113	2	67	28	86	60	0.72	89	4	184	16	-	-	-
127	Miwaleni Local	156	1	73	27	59	44	0.60	93	2	200	19	-	-	-
134	IESV92136DL	78	2	79	20	29	19	0.56	42	1	159	17	-	-	-
139	KSV 12	138	2	72	27	83	61	0.70	105	0	159	18	-	-	-
142	Mexico R-line # 5	105	0	72	0	0	0	0.00	74	3	157	21	-	-	-
172	IS 11162	337	2	90	39	91	72	0.79	183	4	206	30	-	-	-

188	IS 26794	235	3	77	34	93	71	0.76	160	5	222	23	-	-	-
189	IS 2853	232	2	81	37	139	99	0.72	169	3	220	24	-	-	-
190	SHAMBUKO(PP290)	133	2	72	25	34	26	0.70	70	4	143	21	-	-	-
192	AMASUGI	267	2	99	22	75	62	0.81	173	3	214	14	-	-	-
204	IESV91048DL	150	1	83	23	124	86	0.78	92	4	159	17	-	-	-
205	IESV91049DL	107	2	71	27	96	76	0.80	55	5	165	18	-	-	-
210	PLOT_142 SUDAN	65	1	70	25	64	50	0.76	73	1	161	25	-	-	-

207	IESV94025SH	55	2	79	18	28	21	0.71	69	2	152	19	-	-	-
187	IS 24484	171	2	77	31	69	47	0.66	109	2	188	25	27	5	0.18
208	SP 74744	118	2	70	27	75	58	0.78	58	2	170	14	13	5.5	0.21
	Mean	**158**	**2**	**74**	**26**	**75**	**57**	**0.71**	**94**	**3**	**177**	**19**	**2**	**-**	**0.018**

Appendix III: Cluster one genotypes performance at Marigat

Genotype	Plant height	tiller	heading	Panicle length	Panicle weight	Seed weight
GBK-000002	321	4	101	35	49	40
GBK-000010	329	6	91	.	.	.
GBK-000020	291	1	75	19	136	117
GBK-000030	236	2	106	18	68	66
GBK 000032	305	2	83	23	96	87
GBK-000035	335	2	83	22	64	39
GBK-000037	320	2	89	.	.	.
GBK-000040	161	3	117	24	96	87
GBK-000043	380	2	122	.	.	.
GBK-000044	298	1	106	24	88	79
GBK-000046	238	2	67	29	76	50
GBK-000049	293	3	88	31	137	98
GBK-000050	245	3	71	22	92	71
GBK-000052	256	2	81	22	85	66
GBK-000056	258	1	74	19	79	65

GBK-000057	193	2	73	25	49	39
GBK-000058	249	3	107	29	75	65
GBK-000059	274	3	77	31	49	39
GBK-000064	223	3	72	26	75	61
GBK-000065	191	2	74	30	70	64
GBK-000067	229	2	80	24	89	67
GBK-000071	246	2	79	20	74	34
GBK-000073	221	2	77	28	71	61
GBK-000083	260	2	78	31	69	57
GBK-000100	268	2	76	33	77	68
GBK-000102	285	2	80	31	111	80
GBK-000118	263	1	83	26	60	42
GBK-000365	238	2	92	27	59	43
GBK-000366	215	2	65	27	50	38
GBK-000367	145	2	80	22	109	88
GBK-000376	184	1	86	27	96	83

GBK-000377	203	4	85	19	88	66
GBK-000381	218	2	90	16	27	21
GBK-000382	233	2	87	21	84	67
BUSIA_28-1	208	3	66	18	74	58
IS8884	193	1	93	14	37	28
IS 11159	334	3	107	40	41	24
IS 1161	254	4	93	31	53	38
SIAYA 93-1	226	2	72	23	100	77
Average	**252**	**2**	**85**	**25**	**76**	**60**

Appendix IV: Pictorial presentation of cluster analysis; A= cluster one genotypes that remained vegetative, B=cluster three genotypes with sterile panicles and C=cluster two genotypes with productive panicles observed in Egerton site.

GBK00002

GBK 000365

IS 24484

ICSH 152011

BM 5

GBK 00093

Appendix V: Research permit

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Appendix VI: Publication

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Phenotypic expressions of diverse *Sorghum bicolor* [L.] Moench genotypes exposed to cold and warm temperatures

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Low temperatures at the reproductive stages of sorghum limit its performance and promotion in the dry highlands of Kenya. Two hundred and fifty sorghum genotypes were evaluated in 2020 and 2021 on a randomized complete block design (RCBD) with three replications in two sites. Egerton University a cool highland region and Marigat a warm lowland. The daily temperature range at Egerton was recorded as 20/9°C while that of Marigat was 32/20°C during the growing period. The objective of the study was to characterize sorghum genotypes according to their reproductive cold tolerance. Data were collected on plant height, number of tillers, days 0 to 50% heading, panicle length, panicle weight, seed weight and panicle harvest index. A cluster analysis aggregated sorghum genotypes into three clusters at Egerton. The genotypes in cluster one remained vegetative; cluster two had productive panicles, while cluster three produced sterile panicles at Egerton site. The genotypes took between 125 and 213 days to head at Egerton while the same took 55 to 103 days to head at Marigat. Plants were taller in Marigat site as compared to Egerton site. The effect of cold temperatures at the reproductive stage is detrimental and causes irreversible changes which could lead to absence of and/or sterile panicles.

Key words: Reproductive stage, cold tolerance, clusters analysis, vegetative, sterile.

INTRODUCTION

The global temperatures are expected to rise by 2°C by the year 2050 (IPCC, 2018). Climate variability has created challenges in crop production which complicates food production. The world population is expected to rise and reach 11 billion by the year 2100 (United Nations, 2019) creating a demand for food and fodder for livestock. The demand could be met through the adoption of climate-smart smart crops such as sorghum. Sorghum [*Sorghum bicolor* [L.) Moench] is the global 5th major cereal after maize, millet, wheat and rice (Dahlberg et al., 2012) and is grown for food, feed, biofuel and malting. Sorghum crop is grown in regions whose minimum temperatures are above 18°C since it is sensitive to temperatures below 15°C (Emendack et al., 2021). The

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