PHYSIOLOGICAL, MORPHOLOGICAL AND BIOCHEMICAL RESPONSES OF SORGHUM VARIETIES TO PROGRESSIVE WATER STRESS AND REHYDRATION IN SIAYA AND BARINGO COUNTIES, KENYA

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DECLARATION

I declare that this thesis is my original work and has not been presented for award of a degree in any other University or any other award.

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DEDICATION

This thesis is a special dedication to my late beloved parents Josphat Njinju and Ann Kanini (RIP), who brought me up and provided for education so that I give back to the community. May the Almighty God rest their souls in eternal peace.

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ACRONYMS AND ABBREVIATIONS

AEZs	Agricultural Ecological Zones
ANOVA	Analysis of Variance
ASALs	Arid and Semi - Arid Lands
CDA	County Director of Agriculture
CSA	Climate Smart Agriculture
DAE	Days after Emergence
DAS	Days after Sowing
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistics
FGWt.	Filled Grain Weight
GS	Growth Stage
ICRC	Industrial Crops Research Centre
KALRO	Kenya Agricultural and Livestock Research Organization
KCSAP	Kenya Climate Smart Agricultural Project
LSD	Least significance difference
LWC	Leaf water content
MC	Moisture content
MOA	Ministry of Agriculture

MOALF	Ministry of Agriculture Livestock and Fisheries
RDW	Root Dry weight
SPAD	Soil-plant analysis development
SWD	Shoot Dry weight
TIMPs	Technology Innovations Management Practices
TGWt	Total Grain Weight
UGWt	Unfilled Grain Weight

ABSTRACT

Sorghum bicolor (L.) is an important food crop worldwide after maize, wheat, rice and barley. In Kenya, it is grown on an estimated 184,654 ha supporting over 25% as food and 26% as livestock feed. Despite having rich diversity in Arid and Semi-arid Lands (ASALs), its production has been below the optimum mainly because of the abiotic stresses, with drought being the major stress. Drought is an impediment to sorghum productivity because it leads to water stress in the plant lowering its productivity. Drought tolerance enhancement under arid environments is a process that involves a mechanism of maintaining plant water status in order to increase grain yield and quality. A study was conducted with the objective of evaluating critical growth and phenological stages of sorghum sensitive to progressive water stress and rehydration in Siaya and Baringo counties where: (1) A field survey to acquire sorghum accessions was conducted in Siaya and Baringo counties; (2) Field experiments conducted in Siaya and Baringo counties to evaluate sorghum accessions for drought tolerance and (3) Selected sorghum varieties evaluated for physiological and biochemical responses to progressive water stress and re-watering in a greenhouse at KALRO Mwea. The field experiments were Randomized Complete Block Design (RCBD) with 15 accessions, two moisture levels in three replications in Siaya and Baringo sites. A controlled greenhouse pot experiment was conducted at KALRO Mwea constituting five stress levels (well-watered; continuous stress, pre anthesis, anthesis and post anthesis), four varieties in three replicates in two soil types collected from Baringo and Siaya. Stress was induced after crop establishment and re-watering done after each stress episode. Growth and phenological data on proline, chlorophyll content, soil moisture, stomata conductance, yields and grain quality were collected appropriately. Survey data was analysed using Statistical Package for Social Scientists (SPSS V 25). Field data was analyzed using R software version 4.2. Treatment means were separated using Tukey Honestly significance difference (Tukey HSD) at 5%. Results indicated that farmers used local seed accessions (46.5%), preferred sorghum traits included drought tolerance, disease and pests resistance. Varieties response to re- watering after prolonged stress duration differed significantly. The grain weights, plant height, SPAD values, shoot dry weight and grain weight were highly significant in both Baringo and Siaya soil types. Results from the two sites indicated that there were significant differences on number of days to anthesis (IH and 50%H), total filled grains (TFGW), total grain weight (TGW), grain number panicle-1 (GPnW) and shoot dry weight (SDW) in varieties within sites at $p<0$. The results showed that stress for a short duration up to Vegetative stage followed by re-watering recorded significantly low proline and other amino acids accumulation under both Baringo and Siaya soil. Gadam and Nyagem varieties recorded higher yields under stress conditions in Siaya site ($p<0.05$). The Nyagem and Gadam varieties are recommended to farmers in ASALs for increased food security, income and better livelihoods. The physiological and biochemical properties were elevated in case of drought tolerant varieties and this can be used as rapid indicators for sorghum selection for growth in ASALs.

CHAPTER ONE: INTRODUCTION

1.1 Background Information

Sorghum bicolor (L) is quantitatively ranked as the world's fifth most important cereal grain after wheat, maize, rice and barley (FAO, 2018; Batista *et al.*, 2019; Rashwan *et al*, 2021). The crop is ranked second after maize in Sub-Saharan Africa (Hadebe *et al.*, 2017). Sorghum, an important cereal grain and fodder crop of the world, popularly known as 'King of Millet" due to its large grain size and vast area amongst all the millets (Biradar *et al.*, 2024). Its cultivation is mainly practiced in developing countries with 90 percent of the cultivated area found in African and Asian countries. Africa is the largest producer of sorghum accounting for one-third of global production. The suitability and adaptability to tropical conditions prevalent in Africa explain the crop's dominance (Munda *et al.*, 2019). It is able to perform relatively well under unfavorable, harsh and unpredictable weather conditions which are predominant in Sub Saharan Africa (SSA).

The crop is resilient to water scarcity, which is a major problem of the Arid and Semi-Arid areas and is often referred to as the camel of the plant kingdom (Fetene *et al.*, 2011). However, despite being able to withstand such environmental challenges, the crop is significantly affected by the water stress, which hinders its performance in terms of yield production (Kaaria *et al.*, 2021). Therefore, understanding the physiological responses of the crop to water scarcity is very vital for increased productions.

In SSA, the crop prominently serves as a viable cereal crop in the water scarce impoverished regions with the most food insecure household (Power *et al.*, 2019). It is an integral ingredient in the production of sorghum beer as malt and adjunct.

Sorghum is also an essential food security crop for food security in semi-arid areas of Africa (Munda *et al*., 2019). Often, sorghum has been used in the formulation of animal feeds such as the dairy meals (Aruna and Visarada, 2019; Wu *et al.*, 2016). Alternative uses of sorghum encompass the utilization of grain and sweet stalk in food and non-food sectors for the production of commercially valued products, such as alcohol, syrups, glucose, modified starches, maltodextrins, jaggery, sorbitol, and citric acid (Ratnavathi *et al*., 2016). Other by-products derived from sorghum plant include construction materials and fuel (Prażak, 2016). Statistics shows that sorghum provided livelihoods to more than three million people in Kenya by 2014 (MoALF, 2014). Its demand is increasing at 275,000T per year against production of 150,000T (FAOSTAT, 2019).

Due to the rising food shortage and use in the brewing industry, sorghum has been identified as one of their priority value chains for upgrading by eight Kenya Climate Smart Agriculture Project (KCSAP) counties among them Baringo and Siaya. The effects of climate change that leads to erratic rainfall, drought and salinity stress in Arid and Semi-Arid regions (ASALs) have resulted to low yields of sorghum, making it difficult to meet the rising demand. Hence the need to exploit drought tolerant crops such as sorghum that would reduce the impact of these associated climate changes. Production of sorghum has also stagnated due to lack of suitable drought tolerant varieties adaptable to the changing climate (Njinju *et al*., 2022a). Sorghum can withstand high temperatures, is drought resistant and can endure periods of exposure to waterlogging hence under rising trends in global warming and climate change, it is a promising alternative for enhanced food and income security, compared to other staple foods.

Sorghum is well-known for its strong resistance and wide adaptability to multiple biotic and abiotic stresses (Huang, 2018; Zhang *et al.*, 2019). The wide popularity of this crop is due to its (i) diverse end-uses as human. food, livestock feed, biofuel, and forage, (ii) high returns, (iii) more resilience to adverse environmental conditions compared to many other cereal crops, and (iv) well-performance under conditions of water and temperature constraints, especially in marginal lands (Zhang *et al.*, 2019; Hao *et al.*, 2021). Selection for drought tolerance is difficult due to complexity of genotype x environment (GXE) interactions.

In ASALs environments there is a tendency of intermittent rainfall that interferes with the plant's biophysical processes resulting to stress. It is important to have higher drought resistance during drought periods, but drought stress in plants is usually transient, and the capacity to recover from drought is also very important. An understanding of water requirement and distribution among growth stages of the crop is therefore important in ameliorating impact of water stress (Assefa *et al*., 2010: Kaaria *et al*., 2023).

Furthermore research on drought influence on traits like photosynthetic performance, conductance, physiological and biochemical effects provides important information on selection of suitable varieties for arid regions. Studies on production of compatible solutes and root elongation qualities of sorghum in regards to their capacities to recover from different stress durations and the relationship with drought-tolerance are important as they would provide a better understanding to drought and re-watering.

1.2 Problem Statement

Sorghum is an important crop grown in drought-prone areas of Siaya and Baringo counties of Kenya. In the recent past, the productivity of this crop has been in a declining trend due to the effects of climate change that has affected the amount of rainfall and its distribution in the country. The two counties have showed variation in terms of the yield. The differences could be due to local conditions, slope, soil depth and management practices. In both counties Sorghum is mainly grown under rainfed conditions with residual soil moisture, however, drought has been considered a major problem impeding its production. The permanent or temporary water deficit severely hampers the plant growth and development more than any other environmental factors (Anjum *et al.,* 2011). Although Sorghum has excellent drought resistance compared to most other major crops, it generally suffers from severe water stress during the growth and development phase.

The plant exhibits resilience to the effects of water stress, however, particular growth and developmental stages in its lifecycle are susceptible to drought stress with water demand being greatest during the early vegetative stage and reproductive stages (pre- and post-flowering) of the crop being more vulnerable to the effects of water deficit (Anjum, 2011: Gerik *et al*., 2003; Kebede *et al*., 2001). Drought is one of the major challenges in ASALs that reduces growth and productivity of sorghum (Cicek, and Cakirlar, 2002). This situation leads to a complete disruption of its production, especially on light and medium soils where grain and fodder yields decrease significantly. During the onset of stress, all important physiological processes such as photosynthesis, protein synthesis and energy metabolism undergo significant changes. Several enzymes have been shown affected by water stress in different crops.

Research has been conducted to understand plant responses to water deficit only; however, work describing the effects of water stress and re-watering on plants are limited (Takele, 2010). Besides, there are reports that show compensatory effects of crops under stress. The current study explored the possibility of plant recovery after exposure to drought to stimulate the erratic rains and also assess possible compensatory effects of re-hydration in different sorghum genotypes.

1.3 Research Objectives

1.3.1 Overall Objective

To contribute to the understanding of the critical growth and phenological stages of sorghum most sensitive to progressive water stress and re-watering in ASALs regions of Kenya

1.3.2 Specific objectives

The specific objectives;

(i) To establish the existing status of production of different sorghum accessions in Siaya and Baringo counties

(ii) To determine drought tolerant sorghum varieties for Siaya and Baringo counties

(iii) To evaluate physiological responses to progressive water stress and re-watering on selected sorghum varieties

(iv) To assess the effect of water stress on sorghum grain biochemical responses of the selected varieties

1.4 Research hypotheses

i) The performance of sorghum varieties differs in Siaya and Baringo counties.

ii) Drought tolerance in sorghum significantly varies in both Siaya and Baringo counties.

iii) There are significant physiological responses to progressive water stress and re-hydration among sorghum varieties

iv) There are significant effects of water stress on sorghum biochemical responses of the selected varieties

1.5 Justification of the study

The demand for sorghum in Kenya, is increasing hence there is need to focus on strategies that enhance production and productivity. The production-import gap is widening and therefore there is need for increased production to achieve self-sufficiency in food, industrial, market and foreign exchange (Kazungu *et al.*, 2023). Sorghum is a drought resilient crop that can strive well in Semi- Arid regions. In such areas lives the most vulnerable population with high risk of food insecurity associated with climate change as Njagi *et al*. (2019) reported. It is vital to build resilience among the poor rural households and help them to cope with this additional threat to food security by introducing to them drought-tolerant sorghum varieties suitable to their regions (Timu *et al.*, 2014).

Subsequently there is increased use of sorghum by industrial processors that has led to high demand for sorghum grain, in the food, baking, confectionary and brewing industry. According to the reports by Njagi *et al.* (2019), brewing industry has highest demand for sorghum of more than 30,000MT. Despite this the production is still low at (0.8 t/ha in 184,654 ha in 2016) (M'Ragwa *et al.*, 2001; Timu *et al.*, 2014); National Sorghum Production Statistics, 2016).

The current mean yield of sorghum in the semi-arid regions is 1.0 ton/ha that falls below the global average of 2.5 ton/ha is caused by recurrent droughts and heat stress (Yahaya *et al.,* 2022). Sorghum can be grown from 0 - 2200 m above sea level and has a greater potential for increasing production in the ASALs characterized by high temperatures, low, intermittent and unreliable rainfall. The challenge in such ecologies is low production due to use of inferior varieties, drought, soil salinity and fertility stresses. Sorghum has been known to withstand drought stress. Some varieties respond differently under progressive water stress and subsequent re-watering which is a strategy to enhance drought tolerance.

The plants prompt biochemical response to a re-hydration event is a good indicator of recovery and intensive research on the same has not been conducted. Alongside water stress, traits including modification of the root system, stomatal control, and leaf area, as well as matching plant phenology with the environment, could help in improving sorghum productivity under drought stress conditions. It is therefore important to identify crop varieties with enhanced drought tolerance and improve irrigation management for agricultural systems by understanding the physiological responses to drought.

This study investigated the effects of stress duration and re-watering on selected sorghum leaf functionality and assessed the capacity of different drought-resistant sorghum varieties to different stress duration and its recovery capacity beside their effect on grain and malting quality. The results provide a better understanding of the physiological responses to drought and re-watering as well. The information acquired will be integrated in sorghum cultivation management in areas under similar environments to develop a viable variety management system under climate smart agriculture for increased productivity, profitability and sustainability of sorghum farming in Kenya.

1.6 Significance of the study

Drought tolerance in sorghum is an economic and sustainable mitigation strategy under drought prone semi- arid regions of the country. The regions experience intermittent rainfall which recurs in short. Such cyclic occurrences may have adverse effects on sorghum crop growth that may lead to low grain yields of poor quality. The effect of such rains may lead to total death of the crop or possible recovery and produce harvestable yields. The sorghum varieties grown in the country may not have been fully evaluated for the recovery after periods of drought hence such information is lacking. Furthermore there is little information available on the effect of crop recovery following interrupted rains on sorghum grain yield and quality. Currently variety specific responses to drought and recovery have not been fully studied.

This study evaluated the commonly grown sorghum varieties selected from the recommended production list alongside selected from a collection of local drought tolerant varieties from the study regions of Siaya and Baringo counties in an attempt to find out the ones suitable for growing in the drought prone regions of Kenya. The physiological and morphological attributes of the selected varieties were studied under field and controlled (green house) conditions to gain the information relayed in the final results. The information therefore generated from this study will be useful in development of sorghum production package in arid and semi- arid lands. The breeders and farmers will gain the necessary knowledge on adaptable sorghum varieties for further cascading to the drought prone regions thereby increasing production and productivity. This will consequently increase food security and income for better livelihoods of the community.

1.7 Conceptual framework

The research model (Figure 1.1) uses the existing sorghum land races and hybrids acquired from different sources which include farmers, stockists and KALRO. It is assumed that though there is poor performance of sorghum under drought conditions, understanding the water requirement and applying at different growth stages will enhance stress tolerance leading to higher yields. Sorghum yields are influenced by soil nutrients, moisture, soil conditions, temperatures, spacing and varieties.

The model in the present study uses existing sorghum varieties for evaluation and selection in the study areas of Siaya and Baringo Counties for integration in other parts of the country with similar climatic conditions. The watering, mild and severe stressing then re-watering of the trial is supposed to have a significant effect on identifying prompt biochemical changes of sorghum varieties in response to water after a period of water deficit.

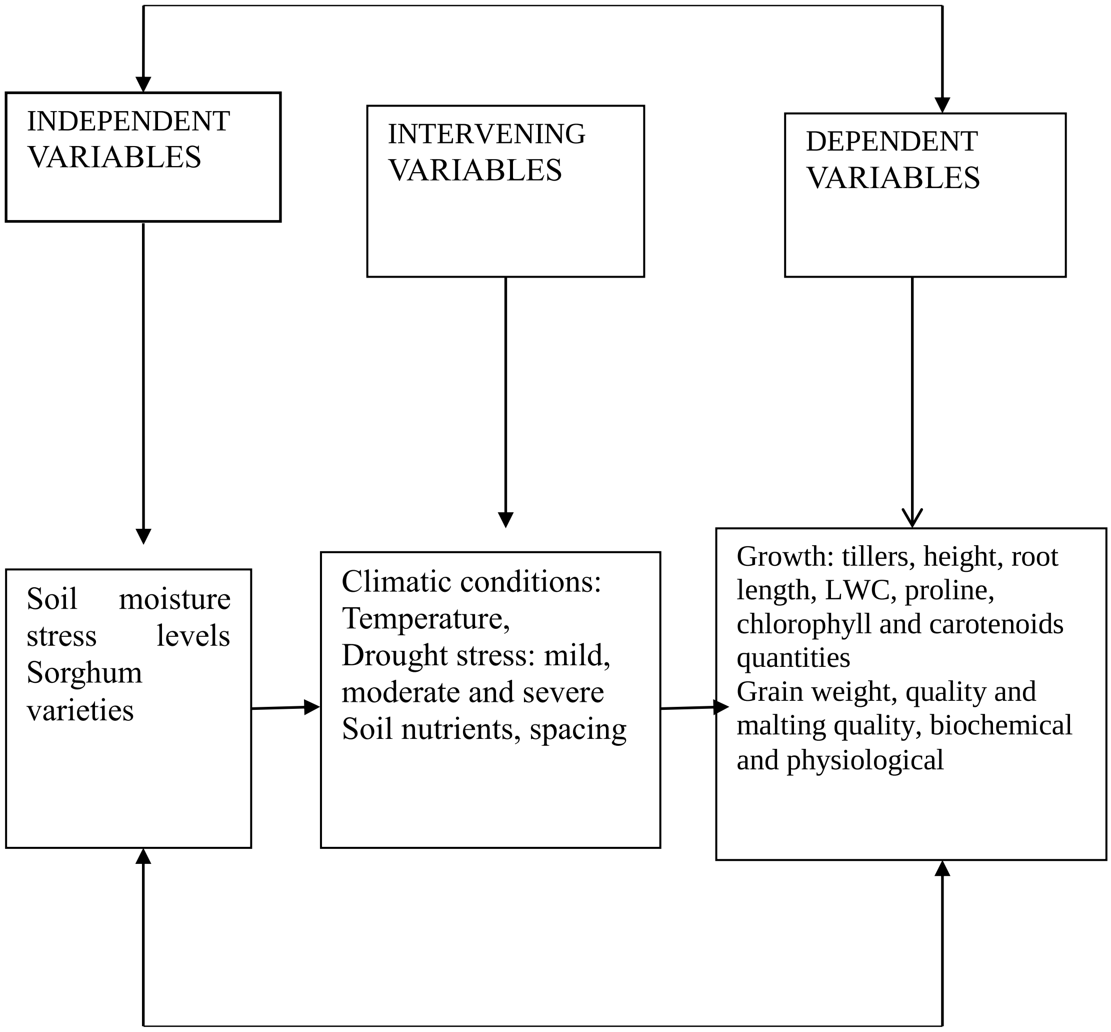


Figure 1.1 Conceptual Framework

CHAPTER TWO: LITERATURE REVIEW

2.1 Background information on sorghum production in marginal areas of Kenya

Sorghum [*Sorghum bicolor* (L.) Moench] is an important staple food crop, an industrial raw material and also a fodder crop (Rao *et al*., 2016). Worldwide, it is ranked fifth in importance as a food crop after maize, wheat, rice and barley (Batista *et al*., 2019, ICRISAT, 2015). It has been cultivated by millions of people in the Sub-Saharan Africa and parts of Asia as a source of livelihood. Its nutritional value is high with the grain constitution of 70 % starch and is gluten-free making it a healthy source of food to the consumers (Aruna and Visarada, 2019). Sorghum does well at altitudes ranging between 500 and 1700 meters above sea level with seasonal rainfall of 300mm and above. It is well suited to heavy clay soils (vertisols) commonly found in the western region of Kenya. Sorghum has a wide range of adaptability and lower risk of failure than other cereals being tolerant to drought, water logging, saline - alkaline, infertile soil and high temperatures, hence suitable to arid environments (Ejeta and Knoll, 2007; Meeske *et al*., 1993).

In Kenya, the crop is grown on an estimated 184,654 ha and it supports over 25% of Kenyans in food supply and more than 26% of livestock as source of feed. It is adapted to the arid and semi-arid lands which accounts for about 80% of Kenya’s total land mass and which receives less than 750 mm of annual rainfall. It is estimated that sorghum requires about 332 kg of water for 1 kg of dry matter compared to 368 kg and 514 kg of water for similar amount of dry matter in maize and wheat, respectively, making it a smart choice for climate smart agriculture

(CSA). Sorghum tolerates waterlogging and thrives in light sandy soils found in the lower eastern region of Kenya. Sorghum can endure a range of soil pH from 5.0 – 8.5 and is more tolerant to salinity than maize. It is adapted to poor soils and can produce grain on soils where many other crops would fail. There are approximately 240,000 small-scale sorghum farmers with farm sizes ranging from 0.4 to 0.6 ha in the country (KAVES, 2013). Although mono-cropping is highly recommended for sorghum, only a small proportion of farmers adhere to this recommendation due to the small land size (KAVES, 2013). The production of sorghum in the country has risen in the last ten years from 54,000 tonnes in 2008 to about 180,000 tonnes in 2018 (FAOSTAT, 2019). The rise has been occasioned by industrial demand, particularly the East African breweries limited (EABL) company. This demonstrates that market is the main driver for increasing sorghum production in Kenya.

Sorghum has the potential to withstand prolonged drought than any other cereal but this comes along with significant amount of yield loss (Barnabás *et al*., 2008). At vegetative stage, water stress is anticipated to reduce yield by more than 36% and at reproductive stage the yield loss is more than 55 % (Assefa *et al*., 2010; Blum 2004). Post-flowering drought stress reduces the number and size of the seeds per plant causing lower grain yield in sorghum (Assefa *et al*., 2010). This signifies the essence of having water supply to the crop during critical stages of growth to minimize the impact of yield reduction. At severe scenarios of water stress it can cause stomata closure which results in low stomatal conductance and low transpiration rates (Chaves *et al*., 2003). Water stress during reproductive stages can stop the development of pollen and ovules, prevent fertilization, and induce premature abortion of fertilized ovules (Raja *et al*., 2019). It is also believed that CO_2 assimilation by leaves is mainly reduced because of stomata closure under drought stress conditions (Flexas, 2016). For high productivity, approximately 450 to 650 mm of water is required during a growing season (Assefa *et al*., 2010). However, the daily requirement varies greatly depending on the growth stage and the genotype.

In drought cases, nutrient uptake by the roots is reduced; - a process that induces nutrient deficiency by lowering the diffusion rate of nutrients from soil to root, creating restricted transpiration rates and impairing of active transport and membrane permeability (Amouzou *et al*., 2019, Kunrath *et al*., 2018, Odhiambo *et al*., 2017).

Over the years, sorghum utilization as a source of food and industrial production of alcohol, biofuels and livestock feed has increased (Kazungu *et al*., 2023). It has also become an essential crop despite its unique viability. Kenya's sorghum productivity potential ranges between 2 and 5 t/ha against the current realized productivity levels of 0.7 t/ha (Njagi *et al*., 2019; Kazungu *et al*., 2023). At this productivity level, it is unlikely that the country can satisfy the demand in the domestic market that is growing with the increasing utilization of sorghum for industrial purposes.

Sorghum consumed as a food has the potential for value addition to manufacture alternative products, for example, gluten free flour (Ratnavathi *et al*., 2016). However, there is little utilization of sorghum in the production of gluten free flour. Availing gluten free products as a healthier alternative for people with gluten-related disorders can have a significant increase in demand for sorghum

(Ratnavathi *et al*., 2016). Sorghum has been preferred as an alternative to maize for livestock feed production because it is cheaper to produce/purchase (Njinju *et al.,* 2022). A reduction in costs of livestock feeds could have a great impact on the livestock industry because cost of feeds is one of the most significant costs of production for livestock value chains (Njinju *et al.,* 2022). Sorghum production is still low relative to the fact that it is among the few crops with rich diversity, which offer multiple possibilities for selection of genotypes adaptable to both CSA - TIMPs and a wide range of uses. Sorghum has potential to support a wide range of domestic food needs and industrial growth, particularly the feed processing, baking, brewing, agrochemical and bio-energy (Kazungu *et al*., 2023). These markets are yet to be tapped for economic gain and improved livelihoods of the players in the value chain. Otherwise increasing yields and quality of sorghum by management under drought conditions is of paramount importance in this study.

2.2 Factors contributing to low sorghum production and productivity in Kenya

Sorghum production is a flourishing enterprise in the Semi-Arid areas of Eastern, Western and Coastal regions of Kenya. Despite its importance, sorghum production is faced with challenges that include lack of suitable varieties to support the available market and where available, seed accessibility is limiting. There is also limited effort to deliberately avail and promote suitable sorghum material for different uses, such as; feed, baked food products, confectionaries, industrial alcohol, and as a blending ingredient in cereal food processing. Sorghum productivity in Kenya has stagnated over time leading to importation of more than one-third of the total consumption. It is estimated that more than half of the production is consumed as food, one percent as feed, about one fifth is processed and about 15 percent lost in the field after harvest (FAOSTAT, 2018).

Although utilization of sorghum for industrial processing is low, substantial growth has been recorded in the recent years. Over the last five years, in Kenya, the quantity of sorghum utilized for industrial purposes has increased by 25 percent (Tegemeo, 2018). This growth is mostly attributed to the emergence of sorghum beer. For industrial purposes, sorghum is used to manufacture wax, starch, syrup, dextrose agar and edible oils (Dicko *et al.*, 2006). Promotion of sorghum for beer production in Upper and Lower Eastern as well as Western regions has led to increased production and industrial use. Production of sorghum for beer is highly commercial and may out-compete other enterprises such as maize, millet and pigeon peas.

Based on its imperative use, sorghum is faced by a number of constraints that limit its maximum production potential including drought, pests and disease, low yields, weeds and marketing. Other constraints include lack of certified planting seeds, among others (Muui *et al*., 2013). Further studies by Muui *et al.* (2018) identified that low yields in sorghum was attributed to striga weeds, pests, diseases and lack of fertilizers. The major constraints leading to low yields in ASALS is water stress caused by inadequate and unevenly distributed rainfall. Farmers in these areas are poor and unable to afford irrigation facilities. The best strategy to increase yields in such areas is to identify drought tolerant varieties that have better biochemical responses to water after a period of water deficit.

2.3 Common Sorghum varieties Grown in Kenya

2.3.1 Released sorghum varieties

In most sorghum growing regions, most farmers obtain planting seeds from the Ministry of Agriculture and previous harvest whereas small amounts are sourced from Base titanium, Agro vets and East African Breweries Limited (Muui *et al.*, 2018). Among the thirty nine sorghum varieties recommended by the local research institutions in Kenya (Tegemeo, 2018) includes the ones listed in Table 2.1. These varieties are suitable in semi - arid areas (AEZs III and IV) receiving 250 -500mm of rainfall per season. The varieties bear desired attributes that attract the interest of the farmers in different ecological regions of the country such as adaptability, drought tolerance, pest and bird resistance, good taste, grain colour and dual purpose as indicated in Table 2.1.

Table 2.1 Sorghum varieties grown in Kenya.

Variety	**Grain colour**	**Maturity (months)**	**Eco zone and area**	**Special attributes**
Gadam	Grey/chalky white	2.5-3	Semi- arid lowlands of Machakos, Kitui, Kajiado, Embu Makueni, Mwingi, Parts of Rift Valley, North Eastern and Western Kenya	Tolerant to drought, birds, stem borers, shoot fly and foliar diseases
Seredo	Brown	3.5		Wide adaptability
Serena	Brown	3		Wide adaptability
KARI Mtama 1	White	3-3.5		Attractive to birds
KARI Mtama 2	White	3.5		Resistant to birds
E 1291	Brown	7	Baringo, Nakuru, Koibatek, Taita Taveta, Narok	Dual purpose, good beverage
E6518	Brown	8		Good beverage
IS76	White	3		Tolerant to stem borers
BJ28	Brown	7		Dual purpose

Source: Greenlife Crop Protection Africa (2019)

2.3.2 Attributes of the selected sorghum varieties

Gadam - is an open Pollinated variety that was released in 1994 by KARI. It is a highly drought, heat tolerant and early maturing (2.5-3 months) variety. It is recommended for altitude range of 250-1600 m .a.s.l and was originally targeted for coastal and semi-arid lowlands. It also grows in moderately dry to very dry areas of eastern Kenya. Gadam sorghum variety has chalky white grains with brown testa. The plant stature is short to medium. The grains yield ranges between 2-3.15 t/ha. It is well adapted to climate change, well accepted by farmers. Gadam has good brewing quality and is already commercialized. It is palatable and well digestible to human and suitable for animal consumption.

E1291- Is a hybrid sorghum variety with brown grains, tall and late maturing (7months). The variety is tolerant to stem borers, dual purpose and suitable for beverage. It does well in Baringo, Koibatek, Nakuru, Narok, and Taita Taveta areas.

KARI Mtama1 –Is a hybrid sorghum variety with white grains. It has a wide adaptability, susceptible to bird damage and matures early (3 - 3.5months). It does well in Semi- arid lowlands of Machakos, Kitui, Kajiado, Embu, Makueni, Mwingi, parts of Rift Valley and North Eastern Province.

2.3.3 Land races

The land races (local varieties) seeds are usually retained from the previous harvest whose quality is not guaranteed (Muui *et al*., 2013). Farmer saved seed system accounts for 87 % of the sorghum seeds sources with the formal seed system accounting for the remaining 13 %. Through research, efforts have been made to help farmers access quality seeds by developing new varieties of sorghum though the adoption of these varieties is still low due to lack of awareness and access-related constraints. Based on a nationally representative sample, only 15 % of farmers were using improved sorghum seeds while the cultivated land area under improved sorghum varieties was only 15 % (Tegemeo, 2018). There has been slow growth in the development of improved sorghum varieties in the past until 2016 when 17 improved varieties were released to the market with the private sector accounting for only 10 percent (Tegemeo, 2018). However, the sorghum varieties released by the Kenya Plant Health Inspectorate Service (KEPHIS) had increased from 3 to 40 by 2017(Tegemeo, 2018).Reports from previous studies indicate that farmers planted diversity of sorghum landraces based on their preferred traits while the local germplasm could be used to improve sorghum production, (Muui *et al*., 2013; 2019).

2.3.4 Commercial sorghum varieties

Sorghum production in Kenya has largely remained non-commercialized with the majority of farmers producing land races only for home consumption. This is however changing gradually with the emergence of the sorghum malting industry. In 2008, the government through the Ministry of Agriculture initiated Gadam sorghum production on a commercial scale in a Public Private-Partnership (PPP) with other relevant stakeholders. Farmers were sensitized on how to commercialize sorghum and its potential benefits coupled with attractive market prices (Tegemeo, 2018).

The Kenya Breweries Limited (KBL) provides a ready market opportunity for sorghum with an estimated annual requirement of 60,000 tonnes and expected to rise with a projected increase in beer consumption. To get decent returns for investment, a requirement of drought tolerant sorghum varieties with quality grains and low cost of production is necessary in order to guarantee profitability at the prevailing market prices. In this regard, farmers must be advised on the best varieties and the cultivation management practices to undertake in the ASAL regions for better income and food security.

2.4 Drought tolerance as an important trait in sorghum production

2.4.1 Mechanisms of plant responses to drought

Drought can be defined as below normal precipitation that limits plant productivity (Du Plessis, 2008). It can be terminal where progressive decrease in soil water may lead to severe stress at a later period of crop development or intermittent with finite periods of inadequate rain/irrigation occurring at one or more intervals in a growing season(Schweiger *et al*., 2023). The most critical periods with the greatest demand for water are vegetative and reproductive stages that negatively impact on progressive decrease in CO_2 assimilation rate due to reduced stomatal conductance (Du Plessis, 2008).

Water stress reduces leaf size, stems extension and root proliferation disturbing plant water relations leading to reduced water-use efficiency (Anjum *et al*., 2011). Drought stress delays formation of sugar, lowers energy exchange and destroys the entire biochemical processes (Crosser, *et al.,* 2003*;* Rehman, *et al.,* 2011).

Plants may respond to soil-water deficit by exhibiting either drought escape or drought resistance mechanisms. To maintain adequate productivity, plants must reflect among others:- escape, avoidance and tolerance. Drought escape is the ability to complete the life cycle before severe stress arrives, in tolerance the plant performs despite low plant- water status while avoidance maintains plant water status despite a shortage of moisture in the environment (Shashidar, *et al*., 2000). In drought tolerance, the plant balances between maintenance of turgor and reduction of water loss helping it to survive drought stress (Shashidar, *et al*., 2000). Rain falling below the long term average (LTA) is termed as drought. Similarly, agricultural drought is the availability of insufficient soil moisture to meet crop water requirements leading to yield losses (Kazungu *et al*., 2023). Drought induced stress affects physiological processes and limits plant growth causing yields reduction more than other environmental factors (Abreha *et al*., 2022).

Plant response to water stress has corresponding negative effect particularly at sink sites causing reduction in the growth rate along with a suite of metabolic changes (Horst, 1993; Munns, 2002). Drought resistance is defined in terms of the degree of growth inhibition by water stress and major relative yield which can be done by comparing a genotype with others subjected to the same drought stress (Blum and Sullivan, 1986; Hall, 1993). It is an ecological adaptation attributed to originality as observed by Blum and Sullivan (1986) who reported that races of sorghum and millet that had evolved from dry regions tended to be more drought resistant than races that evolved from humid regions.

Faster transpiration under stress is associated with drought resistance, in terms of sustained growth under stress (McDowell, 2011). The association partly accounts for significant correlation between inhibition by stress and transpiration ratio under stress. A high transpiration ratio is often assumed to represent a physiological parallel to high-water-use efficiency, nevertheless other factors for drought tolerance include drought escape, phenotypic plasticity, root characteristics (Bidinger *et al*., 1982) or heat tolerance. Sorghum with such characteristics as deep root system, ability to reduce transpiration through leaf rolling, stomatal closure and reduced metabolic processes under drought makes it adapted for growing in environments with water deficit (Blum, 2004; Reddy *et al*., 2009).

2.4.2 Physiological and Biochemical effects of drought

Upon drought stress, some of the main mechanisms, genes and proteins are activated that affect many processes in the plants causing a variety of physiological and biochemical changes (McDowell, 2011). Some of these changes include loss of cellular turgor, changes in membrane fluidity and composition, changes in osmotic potential, and protein-protein interaction. Cell turgor is the most evident indicator of water stress and critical for survival and growth of the plant (McDowell, 2011). Loss of cell turgor may cause stomata closure limiting gas exchange which in turn decreases CO_2 supply for RuBisCo thereby decreasing photosynthesis by reducing the power and its rate of use by the Calvin cycle (Chaves, 2009).

Consequently, overproduction and accumulation of reactive oxygen species (ROS) alters the redox status damaging all major cell biomolecules and impairing their functions (Demidchick, 2015). In response to these effects plants activate several defense mechanisms that involve participation of numerous proteins such as late embryogenesis abundant (LEA) proteins, osmoprotectants, chaperons, detoxifying enzymes, and various proteases (Calzada *et al.*, 2019). Among the detoxifying enzymes, oxidative stress causes lipid peroxidation that impairs membranes and induces loss of their barrier function and consequently a breakdown of organelles occurs (Farmer *et al*., 2013). Plants have evolved defense response systems against excess of ROS which include: (i) non–enzymatic components containing cysteine, reduced glutathione and ascorbic acid and (ii) enzymatic responses systems such as superoxide dimutase (SOD), catalyse (CAT, ascorbate peroxidase (APX), glutathione reductase (GR) and others that eliminate or scavenge ROS (Calzada *et al*, 2019). Plants native to ASALs may have antioxidant defense systems to limit the deleterious effect of ROS (Gong *et al*., 2005; Calzada *et al.*, 2019).

2.5 Water stress and rehydration in plant physiology

Water stress causes stomatal closure resulting to low stomatal conductance and low transpiration rates. As a result carbon dioxide assimilation in the leaves is reduced as well photochemical efficiency of photosynthesis II (PS II). Stress effect on roots reduces the rate of cell expansion, cell size and growth rate, stem elongation and leaf expansion (Jia *et al.*, 2020). Reduction in plant height, leaf area and leaf appearance is also observed. Root: shoot ratio increases due to decrease in shoot growth rather than increase in roots growth under stress. Root weight increases under stress due to diversion of assimilate which could have been used to produce grains under non stressed conditions (Assefa *et al.*, 2010). Effect of water stress on reproductive stage is greater than vegetative stage where the former reduces by >36% while the latter reduces with > 55% (Assefa *et al.*, 2010).

However, reducing stomatal opening also restricts CO_2 entry in the leaf, which may lead to the decrease in photosynthesis rate a decrease in primary photochemical processes which will inhibit plant growth reducing dry matter accumulation and yield ((Jia *et al.*, 2020) Therefore, the measurement of photosynthetic performance is considered as a standard technique for studies on drought stress where the photosynthesis rate, stomata conductance and transpiration have been determined to be the most used techniques to discriminate the plant responses to drought (Jia *et al.,* 2020).

Tolerant cultivars recover all photosynthetic parameters faster than sensitive cultivars after rehydration (Rivathi *et al.*, 2015) hence tolerant cultivars are able to maintain higher photochemical activities and leaf gas exchange during water deficit for a longer period alleviating the stress effect to the photosynthetic machinery improving recovery ability (Rivathi *et al.*, 2015) As a result, re-watering drought tolerant cultivars enhances growth and eventually increasing yields.

2.6 Sorghum grain and malting quality

2.6.1 Grain quality

The sorghum grain quality largely depends on the grain type and its end use. It includes a range of properties that can be defined in terms of physical (moisture content, kernel size), sanitary (fungi and mycotoxin count), and intrinsic (fat content, protein content, hardness, starch content) characteristics (Ratnavathi *et al.*, 2015). The quality properties of a grain are affected by its genetic traits, the growing period, timing of harvest, grain harvesting and handling equipment, drying system, storage management practices, and transportation procedures (Ratnavathi *et al.*, 2015).

Moisture stress has significant effect on chemical composition of sorghum varieties. Increased drought leads to decreased protein and starch content in sorghum grains (Khaton *et al.,* 2016). Grain protein and starch contents also tend to differ with varieties where drought tolerant varieties have higher quality grains than less tolerant varieties (Khaton *et al.,* 2016). Alternative uses of sorghum include non-food sectors for the production of commercially valued products and brewing industry. Sorghum is a rich source of various phytochemicals including tannins, phenolic acids, anthocyanins, phytosterols and policosanols which have significant impact on human health promoting cardiovascular disease, cancer, and obesity (Awika and Rooney, 2004).

Primary processing includes grading, cleaning, destoning, dehulling and polishing of the grain to improve its appearance and market price while secondary processing involves preparation of different food products readily available for the urban consumer. The standard grain quality indicators include hectoliter weight(kg hL^{-1} at 12.5% moisture, kernel size, germination energy(%),, endosperm texture, grain protein content(%) and starch amylose content (%), diastatic power(DP, ^{o}L) and free amino nitrogen(Bekele *et al.*, 2012).

The alpha amylase and diastatic activity of different cultivars have been reported by Ratnavathi *et al*. (2016). The malting, adjunct quality and phenolic contents of sorghum offer it a great potential as a raw material for beer production (Embashu *et al*., 2019; Owuyama, 1997). Sorghum beer has 4% alcohol and higher free alpha amino nitrogen (FAN) content than sole sorghum malt beer requiring an adjunct (up to 810%) to achieve the required FAN content (140 mg/L) (Ratnavathi *et al.*, 2016).

2.6.2 Malting quality

Malting involves controlled grain steeping in water, germination in moist air and drying (Bekele *et al.*, 2012). It helps to mobilize endogenous hydrolytic enzymes (α- and β- amylases in the grain) to modify the structure of the grain so that it is readily solubilized during the brewing process to produce fermentable worts of desirable characteristics, flavours and colour with a minimum loss of dry weight.

Malting quality in sorghum is influenced by the malting processes particularly steeping and germination, brewing conditions and variety production (Embashu *et al.*, 2019; Owuyama, 1997). Dilute formaldehyde and NaOH improves the malt quality in varieties with high levels of condensed tannin by suppressing inhibitory effects on the malt enzyme while sodium hydroxide enhances water uptake by the sorghum grain (Bekele *et al.*, 2012). Waxy and hetero-waxy varieties have better malting potential hence more suitable for brewing because their soft endosperm texture allows hydrolytic enzymes access to starch granules that already have enhanced gelatinization compared with normal non waxy sorghums (Ratnavathi *et al.*, 2016).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Survey of sorghum production status in Siaya and Baringo counties

3.1.1 Survey area

A cross-sectional survey was conducted to establish the existing status of production of different sorghum accessions in Siaya and Baringo counties of Kenya (Figure 3.1). Survey is an efficient and cost-effective method for collecting data at a single point in time, allowing for the assessment of the current status, relationships, and prevalence of variables of interest within the population. There were three major reasons that informed the decisions of the two counties:

First, both counties have diverse agroecological zones suitable for sorghum cultivation, including semi-arid and sub-humid conditions, which allow for studying sorghum performance across different environments. Secondly, sorghum is a drought-tolerant crop, and both counties experience periods of water scarcity. Studying sorghum in these regions can help develop climate-smart agricultural practices. Lastly, Siaya and Baringo have varying soil types, including sandy, loamy, and clay soils, which can influence sorghum growth and yield which allowed for a comparative study on soil suitability.

Siaya lies on latitude 0.0626^{o}N and longitude 34.2878^{o}E at an elevation of 1180-1310 meters above sea level while Baringo lies on latitude 0.8555^{o}N and longitude 36. 0893^{o}E and elevation of 1090-2200 m asl (Google map). The climate for these sites is semi – arid which is typical for drought tolerant experiments -AEZ UM5 (Jaetzold *et al*., 2006). The region experiences long rains from March to May and short rains from October to December with a well-defined dry season between May and July (Jaetzold *et al*., 2006).

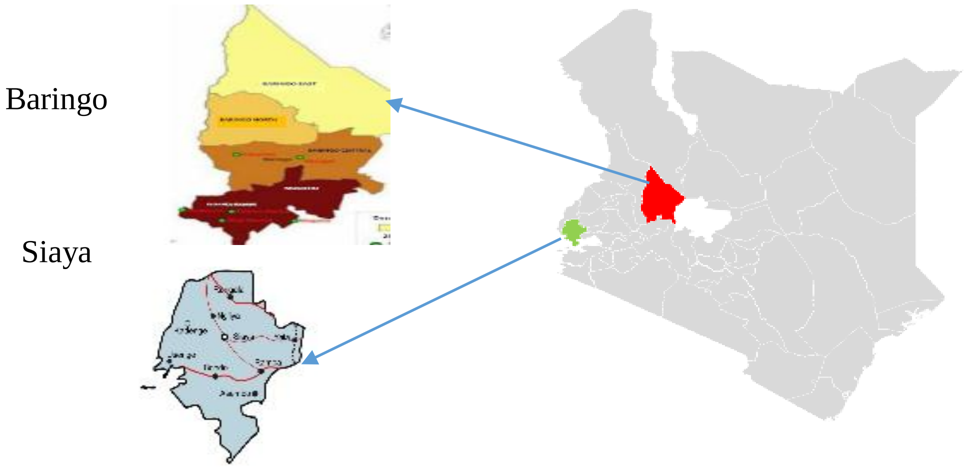


Figure 3.1 Baringo and Siaya study sites

The survey was conducted between 12th and 21st January, 2021. The sampling sites covered the sorghum growing areas of Siaya and Baringo counties.

There were two activities carried out during the survey including interview and sorghum sample collection.

3.1.2 Sampling frame and Interviews

Farmers were interviewed using a semi - structured questionnaire (Annex III) to determine the sorghum production status in the regions (Plate 3.1). The sample was drawn from the total sorghum farmers in each county with the assistance of the County Agricultural Officers. The respective county agricultural offices provided the information leading to sample selection and guided on gave a lead to farmers' fields. The sample size for the house holds to be interviewed was determined using the formula (Nassiuma, 2000)

$$n = Nc^2/(c^2+(N-1)\,e^2)$$

Where: n= Sample Size

N= Population Size

C= Coefficient of Variation (0.5)

e= Error Margin (0.05)

$n = 75 \text{x } 0.5^2/0.5^2 + (75-1)\ 0.05^2 = 43.103$

A total of 43 respondents comprising of sorghum farmers were interviewed from both counties including 23 from Siaya and 20 from Baringo Counties. One farmer group in Siaya was also interviewed during the survey. The information gathered using the questionnaire included: GPS location, demographic data, crops grown, sorghum varieties grown, area under sorghum, purpose of growing, preferred traits within varieties, production constraints, yields per unit area and constraints encountered in the course of production. For ease of communication the farmers were interviewed on their farms with the aid of the agricultural staff (Plate 3.1)

Plate 3.1 Farmer interview session in Baringo County

3.1.3 Sorghum seed sample collection

During the survey, Sorghum seed samples from the local germplasm were collected from the farmers interviewed during for evaluation in the field experiments. At least 2 kgs of seed was obtained from each site. The samples entailed the local landraces (farmer owned seeds) and the hybrid varieties grown by the farmers from both Siaya and Baringo Counties. Within the area covered 45 accessions were collected during the survey. The varieties already released and available in the seed system were obtained from the seed stockists and KALRO Katumani seed unit.

3.1.4 Data processing and analysis

Captured data was translated from the questionnaires to an excel spreadsheet. The data was collated for consistency and accuracy, cleaned, validated and saved. Finally, the data was exported to SPSS software version 25 for analysis. A report was prepared based on the analysed data. Some responses were entered in multiple response format (i.e. more than one response from a respondent- e.g. varietal traits).

Descriptive statistics were carried out which included measure of central tendency (i.e. means), measure of dispersion (i.e. standard deviation), percentage of respondents and number of occurrences. The analysis was partitioned into the two counties (i.e. Siaya and Baringo) and the overall sample (i.e. both counties-overall). The output of the analysis was summarised into tables and bar graphs showing percentage of respondents.

3.2 Determining drought tolerant sorghum varieties for Siaya and Baringo counties

3.2.1 Location and description of the trial sites

There were two field experiments conducted in Siaya and Baringo counties to evaluate and determine drought tolerant sorghum accessions (land races) among those acquired from farmers during the field survey. The varieties were evaluated in the two sites, and the superior ones selected and recommended for cultivation in similar agro ecological zones in Kenya.

3.2.1.1 Siaya Site

Siaya site was located at the Siaya Institute of Technology farm in Siaya County approximately 3kilometers north -west of Siaya town. The site lies at Longitude 34^{o} 17’ E; Latitude 0^{o} 4’ N at an elevation of 1299 meters above sea level. It lies within the Agro Ecological zone Lower Medium 2 (AEZ LM2) and experiences a bimodal rainfall pattern in February to July and August to December. The average annual rainfall ranges between 1400-1500mm (450-700mm per season). The mean temperatures range between 21.5^{o}C and 22.3^{o}C. The soils are classified as a complexity of perfectly drained very shallow to moderately deep dark red to strong brown, friable, gravely clay to clay. They comprise of Acrisols, Cambisols and Lithosols phases (Jaetzold *et al*., 2006).

3.2.1.2 Baringo Site

The site was located at KALRO Perkerra research farm about 5 kms south east of Marigat town in Baringo County. It lies on latitude 35^{o} 59’E and longitude 0^{o} 28’N at an elevation of 1030 meters above sea level. The site is within the Agro Ecological zone Lower Medium 5 (AEZ LM5) and experiences annual average rainfall of 652mm with a bimodal rainfall pattern in January to March and July to

September. The mean monthly rainfall ranges from 23-86mm while mean annual low temperature of 16.8^{o}C and mean annual high temperature of 32.3 ^{o} C are experienced in the area. The soils are poorly drained, very deep, greyish brown to light olive brown, friable, strongly calcareous, strongly saline and slightly to moderately sodic. Silt loam to clay (Solon Chaks) undifferentiated; with Fluvisols, saline sodic phase (Jaetzold *et al.,* 2006).

3.2.2 Soil physical and chemical properties in Siaya and Baringo Sites

The chemical characteristics of the soil samples collected from Siaya and Baringo trial sites were as indicated in Table 3.1. The Baringo soil was slightly alkaline (pH 7.3), while the Siaya soil was strongly acidic (pH 4.9). The results conformed to the preferable pH range of 4-8 which is suitable for most productive soils including sorghum (FAO, 2000; Esilaba *et al.,* 2021). The soils from both sites had low total nitrogen of less than 0.17% that required amendment to sufficient levels. In Siaya both phosphorous and potassium were low at 6ppm and 0.2 me% respectively. In Baringo both phosphorous and potassium were high at 48% and 2.02 me% respectively (Table 3.1). The soil organic carbon at Siaya and Baringo was 1.81% and 1.51%, respectively, which was moderate according to Bruce and Rayment, (1982). In Siaya, Copper (2.82ppm), Magnesium (1.9 me %) and Manganese (0.89 me %) were adequate while at Baringo they were low, high and adequate with concentrations of 0.5 6 ppm, 3.35 me% and 0.78 me% respectively. The micro elements present in both soils were sufficient for sorghum cultivation. The results obtained indicated that the soils from both sites were suitable for sorghum production having conformed to the reports by FAO, (2000) and Esilaba *et al.* (2021).

Table 3.1 Chemical characteristics of soils at Siaya and Baringo trial sites (0-15cm depth

	Siaya		**Baringo**	
Soil chemical property	Value	Class	Value	Class
Soil pH	4.87	Strong acid	7.32	Slight alkaline
Exchangeable Acidity me%	0.6	adequate		
Total Nitrogen %	0.17	low	0.14	Low
Total Organic Carbon %	1.81	moderate	1.45	moderate
Phosphorus (olisen) ppm			48	High
Phosphorus (Mehlich) ppm	6	low		
potassium me %	0.2	low	2.02	high
Calcium me %	1.6	low	11.6	adequate
Magnesium me %	1.9	adequate	3.35	high
Manganese me %	0.89	adequate	0.78	adequate
Copper ppm	2.82	adequate	0.56	low

Soil structure and texture

The soil texture in Siaya site was clay, while that of Baringo site was clay loam (Table 3.2). The results conform to the recommendations by Esilaba *et al.* (2021) that clay to loamy soil texture was suitable for production of most crops. The clay textural class of the soil in Baringo is an important parameter due to its ability to conserve moisture for a longer period and retention of nutrients against leaching. The soil chemical characteristics combined with the clay loam texture observed in Baringo site soils made it more suitable more suitable for sorghum production than Siaya soils that may had led to the significance difference in performance of the sorghum varieties under study.

Table 3.2 Results of soil texture analysis from Siaya and Baringo trial sites

Sample Description: site	**Sand %**	**Clay %**	**Silt %**	**Texture Grade**
Siaya	24	64	12	C
Baringo	44	30	26	CL

KEY:

C - Clay

CL –Clay Loam

3.2.3 Experimental Design

The experiment was a split-plot arrangement in completely randomized design with two water regimes (well - watered and stressed treatments) in three replicates. The main plots comprised of the two watering regimes, while the sub plots were sorghum varieties. A similar trial design was applied at both Baringo and Siaya County trial sites. The trials were planted off-season (towards the end of the rainy season) so that the drought period coincided with growth stages when plant water demand was highest. Siaya trial was planted on 30th April, 2021 while Baringo trial was planted on 3rd May, 2021.

3.2.4 Land preparation, plots layout and randomization

The field was prepared conventionally as practiced by the sorghum farmers. The tractor was used to plough and harrow in both sites while human labour was used to open furrows. Each block had 15 plots measuring 2.4 m x 3 m (7.2 m^2) with 4 planting rows each 3 m long. The experiment comprised of 90 plots (Table 3.3 below)

Table 3.3 Experimental layout for Siaya and Baringo sites

Regime 1: No stress (control)		
Rep I	**Rep II**	**Rep III**
V1	V11	V15
V2	V1	V6
V3	V15	V1
V4	V2	V14
V5	V7	V7
V6	V3	V2
V7	V14	V13
V8	V5	V5
V9	V13	V10
V10	V4	V3
V11	V10	V12
V12	V6	V8
V13	V12	V4
V14	V8	V9
V15	V9	V11

Regime 2: Stressed (>50 kPa)		
Rep I	**Rep II**	**Rep III**
V1	V11	V15
V2	V1	V6
V3	V15	V1
V4	V2	V14
V5	V7	V7
V6	V3	V2
V7	V14	V13
V8	V5	V5
V9	V13	V10
V10	V4	V3
V11	V10	V12
V12	V6	V8
V13	V12	V4
V14	V8	V9
V15	V9	V11

Furrows were opened at Baringo site for ease of flood irrigation. Siaya site had no furrows hence overhead irrigation was applied using hose pipes.

3.2.5 Soil sampling and analysis

The soil samples were collected from each site at 0 - 15cm depth using a soil auger. They were drawn from different points in a zig zag movement. The samples from each farm were air-dried, mixed homogeneously and a sample of one kilogramme was picked, placed in a well labelled khaki paper bag for laboratory analysis.

During the laboratory analysis, soil pH and solution were determined using a glass calomel electrode system as described by Crockford and Nowell (1956) while organic matter was determined by wet oxidation chromic acid digestion method adopted from Walkey and Black (1934). The soil N was determined by the micro-Kjedahl method (AOAC, 1995). The soil K, Ca, Mg and Na was extracted with a 1M NH_4OAC pH 7 solution and analysed with a flame photometer. The soil Mg was determined with an atomic absorption spectrophotometer (Ogunwale and Undo., 1978). The exchangeable acidity (H^+ and Al^{3+}) was measured from 0.1M HCL extractant by titrating with 0.1 M Na OH (Mclean, 1965). The micronutrients Cu, Zn, Mn and Fe were extracted with 0.1 M HCL (Ogunwale and Undo, 1978) and read on a Perkins Klimer atomic absorption spectrophotometer.

3.2.6 Selection of sorghum varieties

Fifteen sorghum varieties evaluated were selected from a total of 45 accessions collected from both counties during the survey. Their attributes were analyzed using the SPSS software to identify those that were superior and could be considered in the study. The random effect model was applied to select the varieties, and the attributes of yields and consumer preference traits were prioritized, giving a total of fifteen candidates for evaluation in the field trials at Siaya and Baringo sites. Farmers preferences in each site was considered in coming up with the 15 selections. The details of the varieties are indicated in Table 3.4.

Table 3.4 Variety coding, name and source

Variety	Variety code	Variety Name	Source
1	001	Ochuti	Siaya- Bondo- Yimbo East
2	003	Nyakitosi	Siaya Bondo Yimbo east
3	004	Nyakabala	Siaya Bondo Y E Pala
4	007	Hela	Siaya W Sakwa Nyawita
5	008	Nyagem	Siaya W Sakwa Nyawita
6	020a	IESV-24029-54	Siaya Ugunja-Sindindi
7	023	Nyaurang	Siaya Ugunja Sigomore
8	024	Local brown	Baringo Kapropita
9	030	Local white	Baringo- Barwesa
10	031	Local red	Baringo-Lawan
11	043	Serena	Stockist
12	045	E1291	Eastcom
13	046	Gadam	Stockist- Cert. KALRO Seed Unit
14	047	Seredo	Stockist
15	048	Kari Mtama 1	KALRO Seed Unit

3.2.7 Field management practices

The planting holes with a depth of 5cm were dug in rows spacing of 75 cm apart and 20cm between plants. Sorghum seeds were sown in excess that were later thinned 30 days after emergence to maintain one plant per hill (200,000 plants ha^{-1}.) Initial irrigation was applied before sowing.

At sowing, basal fertilizer was applied following the results obtained from the baseline soil analysis and the nutrients requirement of sorghum plant using NPK 17:17:17 at 100 Kgha^{-1} (72 g/plot), basal fertilizer was thoroughly mixed with the soil to avoid contact with the seeds. The seeds were thereafter placed and covered with light soil. Weeds were controlled manually by hand weeding. The frequency of weeding depended on the weed density in particular trial site. Top dressing was applied 30 days after sowing with CAN at 100Kg ha^{-1} (72 g/plot) based on the soil analysis results and nutrient requirements for sorghum plants. The computed amount of fertilizer was applied after irrigation as a ring surrounding the plant. When they appeared, Escort Ec® was used to control lepidopteran pests including the fall army worm, while Confidor Ec® was used to control aphids and other sucking pests at recommended rates. The rainfall data during the growing period was obtained from the respective County meteorological offices.

In the early growth stages (0-30 days after sowing) crop management and irrigation was managed uniformly until full establishment. Thereafter regulated irrigation commenced until crop maturity. After flowering (grain filling stage), the plants with panicles were covered with khaki bags to protect them from bird damage. Bird scarers were also engaged to keep them off. Harvesting was done when most varieties had attained hard dough stage. The harvest sample plots consisted of 10 plants/hills in a plot representing one square meter selected from the three inner rows. The harvested samples were sun-dried for two days to reduce the moisture content to a safe level for storage (about 14%). Threshing and winnowing of the samples was done manually and the processed seeds were put in khaki paper bags, weighed using digital weighing balance and recorded in grams (gm). Obtained yield was calculated and recorded in tones/ha. The samples were then stored in the cold room for further measurements.

3.2.8 Water stress treatment application

Determination of the soil water potential was done using the soil tensiometers (Daiki soil and moisture, Daiki Rika Kogyo Co.) installed at 25cm depth diagonally in the blocks one in each replicate. Plate 3.2 below

Plate 3.2 Soil moisture monitoring using soil tensiometers

The crop was maintained uniformly as per the farmers practice from sowing until full establishment (about 30 days after emergency) when the stress treatment was initiated.

Irrigation in the well -watered or non - stressed block (control plots) was done after every 7 days. Soil moisture was maintained at field capacity. Otherwise, the soil moisture in the well-watered treatments was maintained by monitoring using the soil tensiometer readings and maintaining an average reading of < -20kPa.

In the water stress treatment block irrigation was done when the average reading of the tensiometers for the three replicates was below –45kPa.

At Baringo site gravity or furrow irrigation was applied while overhead irrigation using a three quarter – inch hosepipe was used to irrigate at Siaya site.

Water in the stress treatment was withheld until when the grains had reached full maturity a period of (30 – 60days after emergence).

3.2.9 Soil water potential

In the well-watered plots, plants were irrigated regularly to maintain the soil water potential near 0 kPa throughout the growth period. In the fluctuating soil moisture plots, drought stress was imposed at 30 DAS by withholding irrigation, and the soil was allowed to gradually dry to −80 kPa. This was followed by irrigation in the same manner as the well-watered plots for a period of 9–13 days. This cycle was repeated until plants reached maturity in the sites. The characterization of the cycles of fluctuating soil moisture stress was based on the growth stage that coincided with in the course sorghum growth.

3.2.10 Data collection and procedure

Meteorological data

The meteorological data was obtained from the records taken daily using instruments placed at the weather station near the trial sites throughout the experiment growth period (April-July, 2021). The data included daily rainfall and other relevant weather data. The mean monthly rainfall data for the two study sites is shown in Appendix VI

3.2.11 Sampling Procedure

The plots consisted of four rows Plants earmarked for data collection were selected from the inner two rows excluding the two outer plants in each row serving as border plants. Systematic random sampling method was used where the first plant was selected and pegged and thereafter every other fifth plant in a zig zag movement. Five plants were selected for data collection in each plot. Data collection commenced one month after germination and continued at seven-day intervals until full anthesis. The parameters measured included plant height, number of tillers and days to anthesis.

Plant height

Plant height involved measuring the distance of the plant from the ground level (base) to the tip of the tallest fully formed leaf. The measurements were repeated after every seven days until the plants attained full anthesis.

Tillering

Involved counting of the side shoots emerging from the main plant. The counts were done at seven day intervals and were terminated at maturity.

Days to anthesis

The duration to flowering was obtained by estimating and counting the number of days from sowing to when the plot attained more than 50% anthesis. Earliness in anthesis was observed in Gadam sorghum variety (Plate 3.3)

Scoring for drought

It was done using a scale of 1-3 where: score 1 was **no** rolled leaves at all (no stress); score 2 was **V-rolled** leaves (moderate stress) and score 3 were **fully rolled** or withered leaves (severe stress). This was done on the previously 10 tagged plants.

Plate 3.3 Earliness observed in Gadam sorghum variety (front) - a strategy to escape drought

Relative growth weight

One plant from each plot in the three reps was cut at the base and the initial fresh weight (FW) was immediately measured. The samples were obtained from treatments in both regimes. The plants were sundried and thereafter placed in brown khaki paper bags and oven dried at 70^{o}C for more than 48 hours until they were moisture free. The dried plants were removed from the oven and the final dry weight recorded (DW). After 14 days of stress (maximum stress) the exercise was repeated from both well-watered and stressed treatments. Watering in both treatments followed immediately after sampling. The exercise was repeated for three cycles at 14-day intervals where fresh and dry weights were recorded until maturity. The relative growth weight (RGW) was computed using the equation:

$$RGW (\%) = [(FW-DW)/FW] \times 100$$

Stomatal Conductance

At maximum stress level, sorghum plants from each treatment in both water regimes were selected for measurement of stomatal conductance. The readings were taken from the topmost fully opened healthy leaf using a leaf porometer - Decagon SC-1 Leaf Porometer (Decagon Devices, Inc.) between 09:00 and 11:00 hr on clear sunny days (Plate 3.4). The measurements were conducted at Baringo site and were terminated at grain filling stage.

Plate 3.4 Recording of stomatal conductance at Baringo site

3.2.12 Yields and yield components

The parameters to determine yield and yield components measured included shoot and root biomass, shoot and root dry weight, grain yield, 1000 grain weight and filled grain ratio.

Shoot biomass

The shoot of one plant in each treatment was randomly chosen cut at the base before harvest. The plants were sun dried to reduce the moisture. They were placed in khaki bags, oven dried at 80^{o}C for 72hrs and the dry weights (SDW) recorded. Shoot biomass was determined by computing the mean weights of the dried plant samples for each variety.

Root biomass

After the removal of the shoot (above ground) the roots were sampled using a stainless-steel cylinder-monolith, of 15 cm diameter (Kang *et al.,* 1994) by inscribing it into the soil to a depth of 20 cm (Plate 3.5a below). The root extracts were washed with running water and sieved through a 1mm gauze (Plate 3.5b). The sampled root system was then cut into approximately 1-cm segments and spread in a thin film of water on transparent plastic sheets with minimal overlapping.

Plate 3.5 Root sampling (a) Root excavation zone (b) Root preparation for scans

To the spread roots, digital tiff files were then taken using an EPSON scanner (ES-10,000G, Seiko Epson Corporation) with a transparency adapter of 300 dpi resolution. Total root length per cylinder for each plant (henceforth referred to as total root length) was determined using WinRhizo software v. 2007 d (Regent Instruments Inc.; Bouma, Nielsen, and Koutstaal, 2000).

Root and shoot dry weight

After length measurements, the roots were oven dried at 80°C for 72hrs and the dry weights (RDW) taken. The root biomass was determined by computing the mean weights of the dried root samples of each sorghum variety. To determine the shoot dry weights, plants were cut at the ground surface and weighed. They were sun dried to reduce moisture and later oven dried at 80°C until a constant dry weight (SDW) was recorded. The dry weights of both shoots and roots were determined using an electronic balance. The shoot and root biomass were used to determine the root to shoot ratio.

Grain yield

Harvesting was done when grains in most plots were mature (hard dough stage). The harvest sample plots consisted of 10 plants/hills in a plot representing one square meter selected from the three inner rows. The harvested samples were sun dried for two days to reduce the moisture content to a safe level for storage (about 14%). After threshing and winnowing of the samples the seeds were placed in khaki paper bags, weighed using digital weighing balance and recorded in grams (gm). Obtained yield was calculated and recorded in tones/ha.

The yield from harvested plots was determined by collecting, sun drying, cleaning and weighing the grains from one meter square (10 plants/ plot). The moisture content and weights of both filled grains (FGWt) and unfilled grains (UGWt) were both recorded adding up to total grain weight (TGWt). The weight was corrected to 13% moisture content then extrapolated to Kg ha^{-1}.

1000 grain weight

Sorghum grain samples from each plot were picked randomly from the treatment sample plots. The grains were counted using a counting machine adding up to one thousand. Three samples were picked from each treatment. The grains were weighed using an electronic balance and the mean of the 1000- grain weight for each variety was determined and recorded.

Filled grain ratio

The grains harvested from the plots were threshed and cleaned manually. The samples were put in buckets of water where the poorly filled and empty grains floated while the well filled grains settled at the bottom. The filled and unfilled grains separated by floating method were then dried. The two categories of grains (filled and unfilled) were dried and later weighed using an electronic balance. The percentage of filled grains was calculated according to MoA, (2011).

3.2.13 Data analysis

The data was captured in an excel spread sheet where charts and graphs were generated. Further ANOVA was done using the R software. The general linear model procedure of the R program was applied in consideration of two factors: sorghum variety and water regime. Significant differences were compared by the Tukey Honestly Significant Difference at 5% probability level ($p \leq 0.05$). The stress ratio was determined by computing the grain filling ratio of control to stressed treatments.

Correlation analysis was done using R software to determine the linkages between the traits and varieties under drought stress conditions. Based on grain yields and the degree of stress, the highest, moderate and least drought tolerant sorghum varieties were selected.

3.3 Evaluation of physiological and biochemical responses to progressive water stress and re-watering on selected sorghum varieties

3.3.1 Location and description of the trial site

A controlled greenhouse pot experiment was conducted at KALRO, ICRC, Mwea Research Center using selected sorghum varieties to evaluate the responses to progressive stress and re-watering. The trial was sown on January 21, 2022.

The study site was located in Kirinyaga County about 112 km North east of Nairobi. It lies on latitude 0^{o} 37’S and longitude 37^{o}20’E at an elevation of 1218.3 meters above sea level.

The site is within the Agro Ecological zone Lower Medium 3 (AEZ LM3) and experiences a bimodal rainfall pattern in April– June and October - December. The rainfall ranges between 500-750mm, where temperatures range between 21^{o}C and 30^{o}C. The soils are Pellic vertisols, which are classified as imperfectly drained, dark grey to black with slightly saline deeper sub soil (Jaetzold *et al.,* 2006).

3.3.2 Experimental design

The Greenhouse trial was a split split-plot arrangement in a completely randomized design with four replicates. The main plot comprised of two soil types i.e., from Siaya and Baringo counties, the sub-plots had water stress treatment duration, while sub-sub-plot comprised of the sorghum varieties. There were four sorghum varieties: Nyagem, E1291, Kari Mtama1and Gadam which had been selected from the field evaluations conducted in Siaya and Baringo sites in the previous season.

The sorghum varieties were selected based on their field performance in terms of grain yields and drought tolerance while Gadam sorghum variety was used as a local check. The experiment was arranged in five blocks (well-watered, vegetative, reproductive, grain filling and continuous) each representing water stress treatment duration. The varieties were laid in four rows each with four pots (replications) planted with similar sorghum variety (Table 3.5)

The soil chemical analysis was done to establish the fertility status and take remedial measures before sowing.

Table 3.5 Plots layout and randomization

REP 1	REP 2	REP 3	REP4		REP 1	REP 2	REP 3	REP4
BT1 V1	BT1 V1	BT1 V1	BT1 V1		ST4 V1	ST4 V1	ST4 V1	ST4 V1
BT1 V2	BT1 V2	BT1 V2	BT1 V2		ST4 V2	ST4 V2	ST4 V2	ST4 V2
BT1 V3	BT1 V3	BT1 V3	BT1 V3		ST4 V3	ST4 V3	ST4 V3	ST4 V3
BT1 V4	BT1 V4	BT1 V4	BT1 V4		ST4 V4	ST4 V4	ST4 V4	ST4 V4
ST2 V1	ST2 V1	ST2 V1	ST2 V1		BT3 V1	BT3 V1	BT3 V1	BT3 V1
ST2 V2	ST2 V2	ST2 V2	ST2 V2		BT3 V2	BT3 V2	BT3 V2	BT3 V2
ST2 V3	ST2 V3	ST2 V3	ST2 V3		BT3 V3	BT3 V3	BT3 V3	BT3 V3
ST2 V4	ST2 V4	ST2 V4	ST2 V4		BT3 V4	BT3 V4	BT3 V4	BT3 V4
ST3 V1	ST3 V1	ST3 V1	ST3 V1		BT5V1	BT5V1	BT5V1	BT5V1
ST3 V2	ST3 V2	ST3 V2	ST3 V2		BT5V2	BT5V2	BT5V2	BT5V2
ST3 V3	ST3 V3	ST3 V3	ST3 V3		BT5V3	BT5V3	BT5V3	BT5V3
ST3 V4	ST3 V4	ST3 V4	ST3 V4		BT5V4	BT5V4	BT5V4	BT5V4
BT4 V1	BT4 V1	BT4 V1	BT4 V1		ST1 V1	ST1 V1	ST1 V1	ST1 V1
BT4 V2	BT4 V2	BT4 V2	BT4 V2		ST1 V2	ST1 V2	ST1 V2	ST1 V2
BT4 V3	BT4 V3	BT4 V3	BT4 V3		ST1 V3	ST1 V3	ST1 V3	ST1 V3
BT4 V4	BT4 V4	BT4 V4	BT4 V4		ST1 V4	ST1 V4	ST1 V4	ST1 V4
ST5V1	ST5V1	ST5V1	ST5V1		BT2V1	BT2V1	BT2V1	BT2V1
ST5V2	ST5V2	ST5V2	ST5V2		BT2V2	BT2V2	BT2V2	BT2V2
ST5V3	ST5V3	ST5V3	ST5V3		BT2V3	BT2V3	BT2V3	BT2V3
ST5V4	ST5V4	ST5V4	ST5V4		BT2V4	BT2V4	BT2V4	BT2V4

Varieties coding and attributes

The selected sorghum varieties selected from the field trials in Baringo and Siaya sites and their attributes included:

Table 3.6 Varieties coding and attributes

Variety code	**Variety name**	**Plant height**	**Grain colour**	**Flowering**	**Maturity**	**Attributes**
V1	Nyagem	150-190cm		61-64days	95-120days	Local variety susceptible to drought
V2	E1291	120-170cm		63-68days	90-115days	Moderately drought tolerant and late maturing
V3	Gadam	100-130cm	Grey	45-52days	85-95days	drought tolerant variety –standard
V4	KARI Mtama 1	50-170cm	White	58-65days	95-100days	Low drought tolerant standard

Source: Mwadau *et al.* 2013

Treatments

The water stress treatments were applied at five durations followed by re-watering as indicated below:

T1 – No stress (NS)/well - watered (Control)– regular watering
T2 – Continuous stress (CS) with controlled watering
T3 – Vegetative stress (Pre anthesis) –Low stress + re-watering
T4 – Reproductive stress (Anthesis) – Moderate stress + re-watering
T5 – Grain filling stress (Post anthesis)- Severe stress + re-watering

3.3.3 Trial management

The trial was conducted in a greenhouse structure covered with a translucent PVC sheet all round. The experiment was set in plastic pots that measured 25cm diameter and 26cm depth (Plate 3.6). The pots were perforated with holes at the base for ease drainage of excess water. Each pot was filled with 7Kgs of dry soil - weight taken using an electronic balance. The pot soil was thoroughly mixed with 100 Kg/ha (38.8g/pot) of N P K-17:17:17 as basal fertilizer. The fertilizer rate was arrived at based on the recommendations given from the results of the analysed soil samples. The pot soil was watered to field capacity (saturation) observed when drips of excess water started emerging through the holes perforated at the base of the pots.

Plate 3.6 Layout of pot plants (30days after emergence)

The saturated soil pot was re-weighed with a digital balance and the final mean weights (weight of Pot + moist soil) recorded. Sowing was done with excess seeds per hole and later thinned gradually to maintain three plants per pot (30 days after emergence). Top dressing was applied after thinning with CAN at 60Kg ha^{-1} (23.1

g/pot). Weeds were kept off appropriately. Sprays were applied to control pests and diseases when need arose. Normal blanket routine irrigation to all pots was maintained to field capacity until crop establishment by watering after every three days where each pot received one liter of water.

3.3.4 Treatment application

The treatment application commenced after crop establishment at tillering stage (30 days after seedling emergence). The plants were stressed by withholding irrigation for specified number of days depending on the treatment. A pre-determined quantity of water enough for survival was applied during the water withholding duration. At the end of each stress duration, re-watering was resumed until the crop maturity. The stress durations imposed (treatments) were as follows:

(i) **Control (no stress/well - watered)** - Pot plants were weighed to determine the amount of water lost. The water lost was replenished to field capacity daily throughout the growth period (until grain maturity).

(ii) **Continuous stress** – The withdrawal of water commenced after crop establishment and continued until maturity. Daily weights of the potted plants were taken and predetermined amount of water added to provide at least enough moisture necessary for survival.

(iii) **Vegetative stress (pre - anthesis)**: The stress was administered 30 days after sowing (DAS) until the varieties started booting (panicle initiation). The stress was administered by taking daily weights and providing just enough moisture necessary for survival by replenishing the water lost over time. The length of stress duration depended on the candidate variety since panicle initiation for each variety differed.

(iv) **Reproductive stress (anthesis)**: The stress condition commenced at 30 DAS until >50% of the treatment plots attained flowering depending on the sorghum variety. The stress was administered daily by taking weights of the potted plant and replenishing the water lost.

(v) **Grain filling stress (post anthesis stages of development):** The stress was administered by taking daily weights and replenishing the amount of water lost. The condition commenced at 30 DAS until the varieties completed grain filling stage depending on the variety.

Plate 3.7: Well - watered versus stressed plants at grain filling stage under Baringo soil

At the end of each stress duration, moderate re-watering was applied by exposing the plants to water three hours before harvesting the samples to identify prompt biochemical changes in response to water after a period of water deficit. At the end of each stress treatment regime, watering resumed and continued normally until grain maturity.

3.3.5 Sampling Procedure

Plants for data collection were selected randomly and tagged from the four pots with twelve plants of the same sorghum variety. The sampling and data collection was commenced from the beginning of each stress episode and terminated at the end of that period. The parameters measured included the following:

i) Tiller number

This comprised of all emerging shoots in each hill commencing at 26 DAS (pre-treatment) at two-week intervals until the end of tillering and after self- thinning stage (when uniform counts were reached).

ii) Plant height

The measuring was taken from the base to the shoot apex of the plant using a meter rule. The measurements commenced from 26 days after sowing and continued at weekly intervals until maturity.

iii) Stomatal conductance

This is a physiological trait indicating stomata opening in avoidance of excessive water loss. The measurement was recorded between 9.00hr – 11.00hr using a Decagon SC-1 Leaf Porometer (Decagon Devices, Inc.) The readings were taken on plants selected from each stress duration treatment on clear sunny days. The data was collected at three occasions: at the beginning, in the mid and at the end of the treatment duration at seven-day intervals (28/2/2022; 5/3/2022 and 12/3/2022).

iv) SPAD measurements

The soil-plant analysis development (SPAD) is a value for chlorophyll concentration that was determined by recording the mean readings taken at three points of the top most fully opened leaf blade of the main tiller in each hill using a chlorophyll meter device (SPAD-502 plus; Konica Minolta Inc. Tokyo, Japan). The reading was obtained from three plants (replications) in each treatment. The initial data was recorded at 25 DAS (16/2/2022) and subsequent readings taken after every seven days respectively. The SPAD measurements were terminated at the point of grains maturity

v) Days to anthesis

The number of days from emergence to when flowering started was noted on daily bases for each plot. The observations for the number of days to fifty percent and full anthesis were also recorded.

vi) Root length

The Sorghum roots were extracted from each pot at harvest. They were washed regularly while passing through a 1mm mesh sieve with running water until they were clean. The debris and foreign materials were also removed from the cleaned roots. The root samples were spread in small volumes avoiding overlapping. Digital tiff files were taken using a root scanner model EPSON scanner (ES-10,000G, Seiko Epson Corporation) with a transparency adapter of 300 dpi resolution and images obtained. The root measurements for each plant sample was determined by subjecting the scanned images WinRhizo software v. 2007 d

(Regent Instruments Inc.; Bouma, Nielsen, and Koutstaal, 2000) where the total length, diameter size etc. per cylinder for each plant were obtained.

vii) Root and shoot dry weight

After scanning, the roots were placed in khaki bags, oven dried at 80^{o}C for 72hrs and the dry weights (RDW) taken. The root biomass was determined by computing the mean weights of the dried root samples of each variety. Shoots of plants grown under fluctuating soil moisture conditions were sampled at the beginning and end of each drought or re-watering episode. The timing of root and shoot samplings for plants grown under well-watered conditions matched that of the soil moisture fluctuation plants They were oven dried at 80^{o}C for 72hrs and shoot dry weight (SDW) recorded.

viii) Soil moisture content

Soil water potential was regularly recorded using a soil tensiometer (Daiki soil and moisture, Daiki Rika Kogyo Co.) installed at 20 cm soil depth in each treatment. In the well-watered plots, plants were irrigated daily (at 18:00) to maintain the soil water potential near 0 kPa throughout the growth period. In the fluctuating soil moisture plots, drought stress was imposed at 30 days after sowing (DAS) by withholding irrigation. The soil was allowed to gradually dry to −80 kPa. The watering was restricted to the growth stage where only enough water was applied for plant survival. In well - watered, maximum watering was maintained while in continuous stress watering was restricted throughout the growth period. This was followed by resumption of irrigation in the same manner as the well-watered plots for each stress duration until grain maturity.

xi) Biomass and yield measurements

(a) Shoot and root biomass

The below and above ground biomass weight data was collected at harvest. Shoots were cut at ground level in each plot. The parts were sun dried to reduce moisture and later dried at 80^{o}C in an oven until constant dry weight was obtained.

Roots were extracted by removing them from the pots washing regularly with running water and passing them through a 0.1mm sieve. The root length was determined using a computer software (Section 3.2 above). The roots were later oven dried and dry weight recorded. The dry weights of both shoots and roots were determined using an electronic balance while shoot and root biomass was used to determine the root to shoot ratio. The shoot dry weights and total root lengths of each drought and re-watering duration were sampled at harvest.

(b) Grain yield ha^{-1}

Harvesting was done when grains in most plots were mature. Panicles of plants from each treatment were harvested and processed. They were threshed, sun dried, cleaned and weighed. Both filled (FGWt) and unfilled (UGWt) grain weights were recorded adding up to total grain weight (TGWt). The grain weights from sampled plants per treatment were corrected to 13% moisture content then converted to yields in Kg ha^{-1}.

3.4 Evaluation of biochemical responses to progressive water stress and re-watering on selected sorghum varieties

3.4.1 Determination of Amino Acids

Samples were collected at the end of each stress duration by excising the top fully opened leaves. They were weighed, put in khaki bags then oven dried at 80°C for 72hrs. The leaves were dried and analysed for amino acids concentration. The method used was that described by AOAC (2000) with minor modifications. The samples were processed and analyzed at Coffee Research Institute (CRI), Ruiru laboratories. Primary amino acids from the sorghum leave samples were determined using the Ultra High Performance Liquid Chromatography (UHPLC) method. Test samples were prepared by milling as described in the SOP: NUT000.00. Sample milling procedures for nutritional composition in foods, feeds and plant materials were followed.

The amino acids from the test samples undergo performic acid oxidation-acid hydrolysis. The Sulphur-containing amino acids cystein and methionine, was oxidised with performic acid into cysteic acid and methionine sulfone, respectively. Sodium metabisulfite was added to decompose excess performic acid. The amino acids were liberated from proteins by hydrolysis with 6 N HCl. The amino acid hydrosylates analysed by pre-column derivatization with *o*-phthalaldehyde (OPA) and, separated on a reverse-phase UPLC with fluorescent detection. The concentration of amino acids in test solution was determined by relating the peak area of the sample to respective individual calibration curves.

The area of the sample and standard peaks was measured for each individual amino acid and the amount (X), in mg of amino acid per 100 grams sample, was calculated as follows:

$$X = \frac{C \text{ x M x V X } 100}{m \text{ x } 1000}$$

M = molecular weight of the amino acid being determined in g/mol,

C = concentration of sample determined from the calibration curve in pmol/ml,

m = sample weight (mg),

V = total dilution factor (200)

100 = conversion factor to mg/100g

1000000000 = conversion factor from picogram/ml to mg/ml

Cystine and cysteine were both determined as cysteic acid in hydrolysates of oxidised sample, but calculated as cystine ($C_6H_{12}N_2O_4S_2$, MW=240.30 g/mol) by using M=120.15 g/mol (= 0.5 x 240.30 g/mol). Methionine was determined as methionine sulphone in hydrolysates of oxidised sample, but calculated as methionine by using MW of methionine: 149.21 g/mol. The concentration of amino acids in test solution was determined by relating the peak area of the sample to respective individual calibration curves. The results were recorded in mg per 100 g sample, as whole number.

3.4.2 Proline quantification

The protocol for the quantification of proline was adopted from Bates *et al*. (1973). Acid ninhydrin reagent was prepared by dissolving 1.2 g ninhydrin in 20 ml 6 N orthophosphoric acid by warming and gently swirling. The reagent was kept ice-cold and used within 24 hours. The third fully expanded sorghum dry leaf (0.5 g

FW) was ground to a fine powder in liquid nitrogen and homogenized in 5 ml 3%(wv) sulphosalicylic acid. The homogenate was centrifuged at 10000 rpm for 5 min. Equal parts (2 mL) of the supernatant, acid-ninhydrin reagent and glacial acetate acid was combined in a test-tube and placed in a warm water bath at 100^oC for 1 hr.

The reaction was terminated and placed on ice. Toluene (4 ml) was added to the reaction mixture, vigorously mixed and left at ambient room temperature for 15 min until separation of layers was observed. The optical density of the chromophore-containing toluene (upper phase) was measured at 520 nm using a quartz cuvette and toluene as a blank with Beckman Coutler™, Inc.(USA), DU®800 spectrophotometer. Proline concentration was determined from a standard curve and calculated on a fresh weight basis using the formula Bates *et al*. (1973) Proline ($mgg^{-1}FW$) = [(µg proline/ml x 4 ml troluene) / (0.5 g sample/2.5)]/1000.

3.4.3 Data analysis

To examine the effects of soil moisture, variety, and their interaction on total root length and shoot dry weight at different sampling times and upon termination of experiment, an analysis of variance (ANOVA) was performed using the general linear model (GLM) procedure of the R (version 4.4.2.), where soil moisture content was the main plot factor, different nitrogen levels were the split-plot factor, and the varieties were the split-split-plot factor.

Differences in means of shoot dry weights and total root lengths across soil moisture treatments for each soil type at different sampling times and at treatment termination were evaluated based on the honest significant difference (HSD) of Tukey's test at $p \leq .05$. Correlation analysis was done using R software to determine the linkages between the traits and varieties under drought stress conditions.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Survey results

4.1.1 Gender, education level and choice of crop

Results showed that there were more males than females. The total number of respondents from the counties included 25 males and 18 females, representing an average of 62.8% and 37.2% respectively (Table 4.1)

Table 4.1 Gender of the sampled households in Siaya and Baringo counties

Gender	County		Overall sample (n=43)
	Siaya (n=23) %	Baringo (n=20) %	Percent (%)
Male	52.2	75.0	62.8
Female	47.8	25.0	37.2
Total	100.0	100.0	100.0

The total number of respondents from Siaya county were 23 comprising of 12 males (52.2%) and 11 females (47.8%): while Baringo county had 20 respondents comprising of 13 males (65%) and 7 females (35%) respectively.

Regarding the level of education, majority of the respondents from the two counties had attained secondary school education (39.5%). Results showed that the respondents who had attained secondary education in Siaya County were 43.5% while Baringo County had 35% (Table 4.2). The results indicated that in both Counties, 16% of the respondents had achieved above secondary school education, 39.5% secondary, 39.5% primary, while 4.7% had not attended any school (Table 4.2).

Table 4.2 Education level of respondents

Education	Siaya%	Baringo%	Overall%
Secondary	43.5	35.0	39.5
Primary	39.1	40.0	39.5
Above	8.7	25.0	16.3
None	8.7	0.0	4.7

This study revealed that sorghum production was dominated by males in both Siaya and Baringo Counties. During the survey, men being the heads of the households in most families, presented in larger numbers than women for the interview resulting to male dominated results. Meanwhile, women were involved in most farming activities, but when it came to decision making men played the key role. The study is in agreement with that by Muui (2019), Robert *et al.* (2013) and Patrick, (2013) who identified women as key players in sorghum production in Coast and Nyanza regions by providing labour in sorghum production activities.

Regarding the level of education, majority of the respondents had progressed up to primary school and above. With such literacy level the farmers in these regions could be able to understand and apply good agricultural practices in their farming activities. Literacy helps one to understand the application of advanced technologies and skills involved in farming such as use of good quality seeds and good agronomic practices among others that are necessary for realization of high and quality yields. This corroborates with the findings of Bernard *et al.* (2020), who argued that the literacy level influences the understanding of farmers in performing different farming activities.

4.1.2 Sorghum Production in Siaya and Baringo Counties

The results from the survey indicated that farmers from Siaya and Baringo counties cultivated sorghum as a first priority crop due to its ability to tolerate drought as indicated by 61% and 100% respondents, respectively (Table 4.3). Other reasons for sorghum cultivation included resistance to insect and birds attack (40%), required less labour (33%), ability to recycle seeds (33%), required less fertilizer (30%) and its ability to ratoon (26%). From the study area, the main reasons for producing sorghum were for provision of food, sale and fodder (Table 4.3).

Table 4.3 Reasons for sorghum being chosen as a first priority

Reason for prioritizing sorghum	Siaya	Baringo	Overall
Drought tolerance	60.9	100.0	79.1
Resistance to insects and birds attack	30.4	50.0	39.5
Require less labour	13.0	55.0	32.6
No need of hybrid seeds	17.4	50.0	32.6
Require less fertilizers	8.7	55.0	30.2
Ratooning	0.0	55.0	25.6
Other reasons	30.4	0.0	16.3

Sorghum's versatility and resilience make it a priority crop for subsistence and commercial purposes, ensuring its role in enhancing livelihoods and supporting sustainable agriculture. With its ability to withstand high temperatures and water stress, sorghum plays a critical role in enhancing food security in the face of climate change. Sorghum is highly adaptable to arid and semi-arid regions due to its efficient water-use system and tolerance to prolonged drought conditions. This makes it a reliable crop in regions prone to erratic rainfall and climate variability.

Research by Hadebe *et al.* (2017) and Ndlovu *et al.* (2021) highlights several morphological, physiological, and phenological adaptations that enable sorghum to cope with drought and heat stress. Notably, sorghum possesses deep, dense root systems that enhance its ability to absorb water during dry periods (Chen *et al.*, 2020). Its drought tolerance is strongly associated with specific root characteristics, such as root length density, the number of nodal roots, and the development of late metaxylem vessels in nodal roots under water-limited conditions (Tsuji *et al.*, 2005).

4.1.3 Purpose for sorghum production in Siaya and Baringo Counties

Of the farmers interviewed in Baringo County, most of them (100%) produced sorghum for food while 75% produced for sale and 50% for fodder. From Siaya County 82.6% of the farmers produced sorghum for food and 69.6% for sale with none producing it for fodder (Figure 4.1).

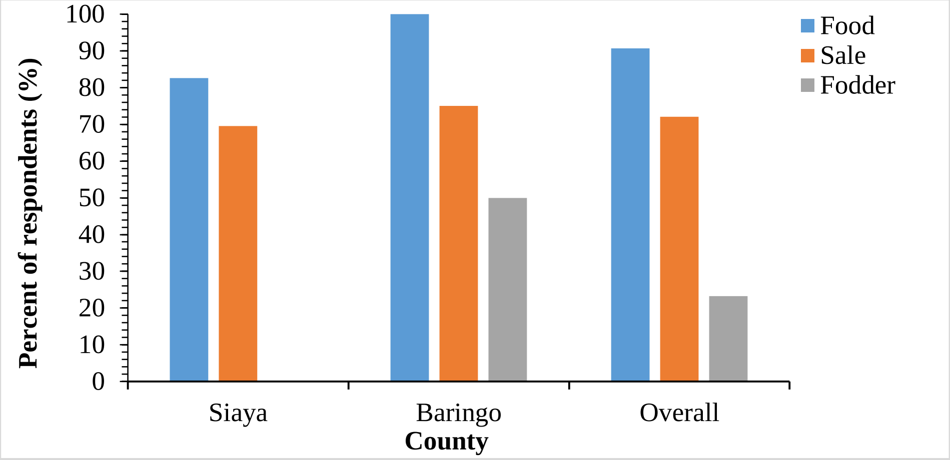


Figure 4.1 Purpose for sorghum production in Siaya and Baringo Counties

As a suitable crop for areas with low rainfall its attribution to drought tolerance makes sorghum a suitable cereal crop in such areas. The crop is key to food security due its ability to thrive in such environmental conditions where other cereal crops cannot perform better. In Siaya County, farmers also grow sorghum for food, but the slightly lower proportion may indicate greater crop diversity or the availability of other staple crops, such as maize or cassava, which might compete with sorghum as a dietary preference.

Similarly Muui *et al.* (2019) identified sorghum as a priority food crop for human consumption and fodder in the drought prone areas of Kenya. The absence of sorghum utilization for livestock in Siaya suggests either a lack of reliance on livestock farming or the availability of alternative fodder sources in the county. The variation in sorghum utilization highlights its adaptability to different farming systems and regional needs. In Baringo, sorghum serves as a multifunctional crop essential for food security, income, and livestock production. In Siaya, it primarily supports food and income needs, reflecting diverse farming systems and alternative resources for fodder. These differences underscore the importance of tailoring agricultural interventions to local contexts to enhance productivity and livelihoods effectively. In their reports, Leder, (2004); Ministry of Agriculture, (2010) and Muui *et al.* (2013) established that the sorghum varieties cultivated by farmers were those suitable for food such as preparing ugali, porridge and other traditional dishes

4.1.4 Sorghum varieties grown in Siaya and Baringo Counties

During the survey, 43 farmers from the two counties were interviewed where 48 sorghum accessions were collected. The results indicated that most of the accessions from the farmers (29/48) in Siaya and Baringo counties were land races (Table 4.4). In Siaya County the respondents identified the sorghum accessions by their local names. Whereas majority of the respondents in Baringo County had no specific names for the accessions but referred to them generally as “Mosong’i”- the local name for sorghum in the region. In this respect, the accessions were described using the name of the location of origin or the colour of the seed, hence local white, local red or local mix (Table 4.4). Apart from the local land races of sorghum seeds for planting, a few who obtained certified seeds from the seed stockists.

Table 4.4 List of sorghum accessions collected from Siaya and Baringo Counties

Accession name	No of entries	Accession name	No of entries(*f*)
Adventor	1	Local red	8
Ex KALRO	1	Local white	1
Ex Kitui	2	Nyagem	1
Ex ugunja	1	Nyakabala	1
Hela	2	Nyakitosi	1
Gadam	5	Nyaurang'	1
H293	1	Ochuti	3
IESV-24029-54	3	Ochuti-Nyakabala mix	1
IS 9183	1	Ofunji	1
IS 9184	1	Orimba joleje	1
KARI Mtama	1	Saitoti	1
local gus nook	2	Seredo	2
local mix	1	Serena	1
IS 9184	1	Sila	1
IS 9183	1	**Total**	**48**

A total of 48 entries spanning diverse accessions underscore the rich genetic diversity of sorghum in the two counties. This diversity is crucial for maintaining resilient agricultural systems, especially in the face of climate change and evolving market demands. The dominance of certain accessions, such as Gadam and Local red, reflects farmer preferences that should be factored into agricultural development and extension services. An earlier study by Muui *et al.* (2019) revealed that farmers planted diversity of land races. From the wide range of local accessions, it is easier for the farmers to select the accession with traits that satisfies their preferences.

4.1.5 Farmers' preferred traits

The survey established that the choice of the sorghum variety produced by the farmers was based on their preferred traits. From the wide range of local accessions, it is easier for the farmers to select the accession with traits that satisfies their preferences. The common traits included drought tolerance, high yields, resistance to diseases and pests (birds), early maturity, nutritional value, marketability and the grain colour (Appendix V). Among the sorghum varieties with the preferred traits were Local red (21 counts), Nyakabala (8counts) and Gadam (8counts) (Appendix V).

The accessions were selected due to their adaptability to those traits and more important was tolerance to drought. The trait aspect is key to production since it's the best way the farmers use to select the variety that would satisfy the need for production. As reported by Kholová *et al.* (2013) and Kebede *et al.* (2001), drought tolerance is an important trait in the arid regions where production is often limited by water availability, resulting in a high risk of crop failure and loss of yields.

Similar to the current studies, Shashidar, *et al.* (2000) reported that sorghum varieties which matured early were mostly preferred by the farmers due to drought tolerance and escape mechanisms owing to the fact that the study regions experienced low and unpredictable rains. Bird damage was a great menace in the region as most labour was spent on bird scaring. In this respect the varieties resistant to birds' damage gained more popularity in the regions.

4.1.6 Source of planting seeds

Among the farmers interviewed from the two Counties, majority planted their own saved seeds (46.5%). The rest obtained planting seeds from the market (20.9%), stockists (16.3%), friends (9.3%), neighbors (7.0%), KFA (2.3%) and Agricultural office (2.3%). The highest number of farmers who planted own saved seeds was recorded from Siaya County (60.9%) while Baringo County recorded a lower number (30%). The rest of the farmers obtained planting seeds from the local market, seed stockists, friends, KFA and neighbors (Fig. 4.2).

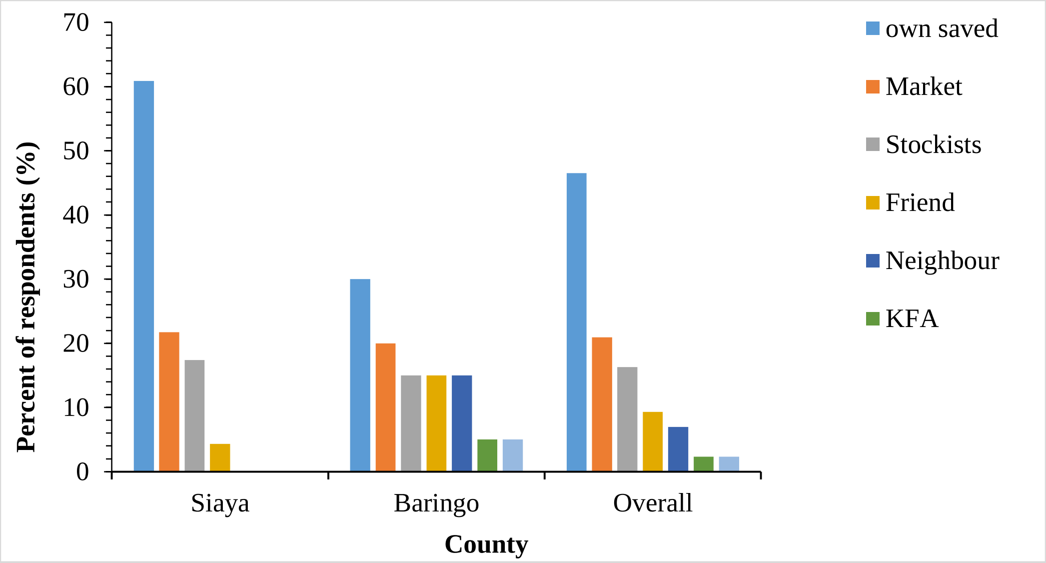


Figure 4.2 Farmers' sources of planting seeds in Siaya and Baringo Counties

The use of landraces from own saved seeds, neighbours or seeds purchased from the local market, was due to the high cost of certified seeds. Lack of finances to buy quality seeds largely contributed to the practice. This preference is higher in Siaya indicating strong traditional practices and possibly limited access to external seed sources. Farmers in Siaya may also prefer saved seeds due to trust in their adaptability to local conditions and familiarity with performance.

In Baringo County, a comparatively lower reliance on saved seeds suggests a greater openness to external sources, likely driven by access to stockists, markets, or agricultural services. Farmers in Siaya County primarily depend on saved seeds, potentially due to limited access to formal seed systems or economic constraints. The county's higher rainfall and better soil fertility may encourage traditional seed recycling as a viable option. Baringo County's lower reliance on saved seeds may be attributed to its more arid climate, which can negatively impact seed quality over multiple planting seasons, prompting farmers to seek external seed sources.

Similar findings were reported by Timu *et al*. (2014). For traceability there is need to characterize the collected germplasm for seed conservation. This practice might have possibly been contributed by lack of technical knowledge from agricultural extension staff to promote the use of certified seeds. Previous studies in these regions show that farmers used own saved seeds that translated to low yields (Muui *et al.*, 2020). For better yields, use of certified seeds is of paramount importance since it translates to vigorous crop capable of utilizing available resources for conversion to yields.

4.1.7 Use of fertilizer in sorghum production

The study revealed that most farmers used inorganic fertilizers in sorghum production in both counties (Fig 4.3). They used DAP fertilizer for sowing where Baringo (34.1%) had more farmers than Siaya county (30.4%). Other fertilizers used by famers for sowing included SSP, NPK and Yala Mila though by few respondents. During top dressing farmers used CAN with 38.9% of Baringo and 30.4% in Siaya reported to adhere to the practice. It was also observed that a good number of farmers did not use any fertilizer in farming where Siaya (34.4%) had bigger population than Baringo (16.7%) (Fig 4.3).

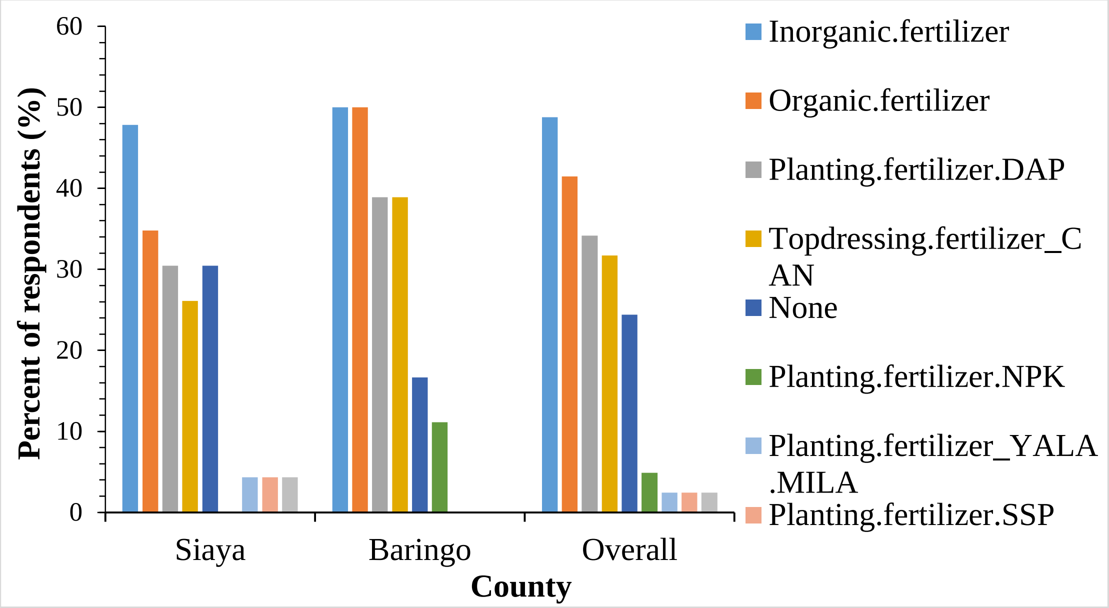


Figure 4.3 Farmer fertilizer use practices in sorghum farming in Siaya and Baringo counties

Use of fertilizers in crop production is key to realization of high yields, however due to meagre resources in the study region, a substantial number of farmers could not afford to apply. While the use of inorganic fertilizers is widespread in both counties, the variations in practices between Siaya and Baringo point to different soil fertility conditions, economic factors, and access to fertilizer inputs. Siaya generally has better soil conditions, which may reduce the need for extensive use of fertilizers. The favorable agroecological environment might provide sufficient nutrients for sorghum cultivation without much external input.

Farmers in Baringo may prioritize fertilizers to address the challenging growing conditions despite potential cost barriers. Farmers in Siaya may rely more on traditional practices, including the use of organic fertilizers or no fertilizers at all.

Farmers in Baringo may view fertilizer use as a critical strategy to counter the effects of poor rainfall and degraded soils. In Siaya, reliance on better natural fertility might reduce the perceived need for fertilizers, especially among subsistence farmers.

In contrast, Baringo's more arid and less fertile soils likely require more fertilizer use, especially phosphorus and nitrogen, to achieve optimal yields. Tailoring interventions to local needs can enhance fertilizer efficiency and support sustainable sorghum production. The results would be low yields of poor quality. Previous studies associated low grain yields to attack by pests and diseases, drought, lack of market incentives, use of poor-quality seeds, poor crop production practices as well as lack of adequate capital to acquire farm inputs such as fertilizers for crop production (Ogecha, 1995; Chepng'etich, 2013).

4.1.8 Producers' sorghum Preferred traits

Most of the producers from the study counties preferred sorghum varieties due to various traits. Among the traits included good taste (46.7%), germinability (30%), early maturity (20%), non- red colour (13.3%) and bird tolerance (10%) (Figure 4.4). In Siaya majority of producers preferred sorghum due to good state (62.5%). Others preferred it because of early maturity non red colour, resistance to birds and storability. Majority of producers in Baringo County preferred sorghum due to good germinability (57.1%) while the rest preferred it due good taste (28.6%) and storability (7.1%) among others (Fig. 4.4)

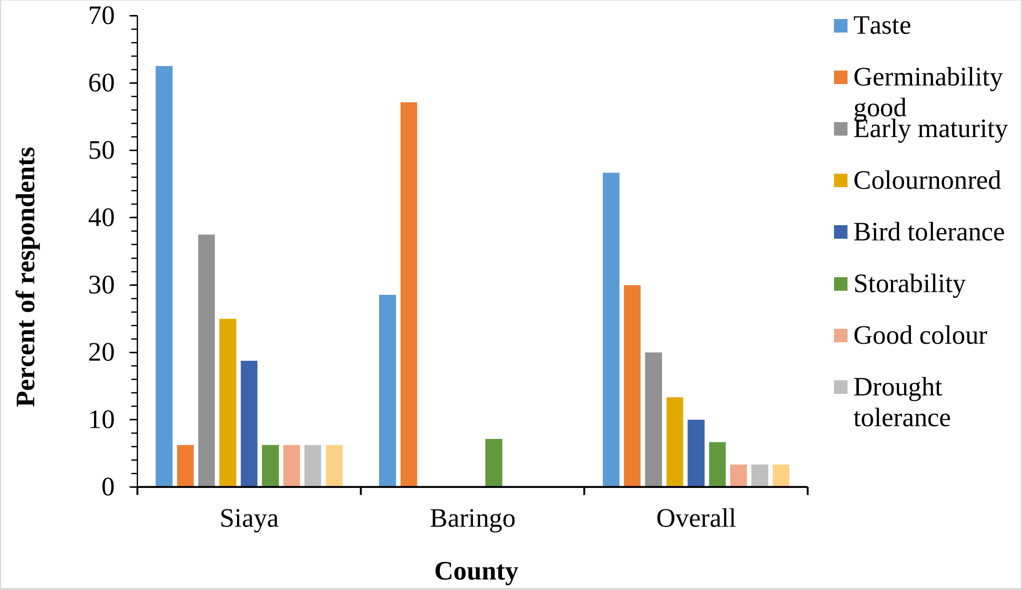


Figure 4.4 Producers preferred sorghum traits

The semi-arid conditions of Baringo make germinability and early maturity critical for ensuring crop success under short or erratic growing seasons while Siaya, with relatively better growing conditions, allows farmers to focus on traits like taste and bird tolerance. Farmers in Siaya might prioritize specific colors or taste profiles for cultural or culinary reasons, as sorghum is a staple food. In Baringo, where sorghum might also be stored for fodder or future use, traits like storability become more important.

In their report, Muui *et al.* (2011), Muui *et al.* (2020) and Timu *et al*. (2014) established that farmers planted a diversity of sorghum landraces to satisfy consumers preferred traits. To achieve this purpose, it is crucial for the sorghum farmer to choose the sorghum variety with the traits that would satisfy the consumers' needs. In their reports, Leder. (2004); Ministry of Agriculture. (2010), Munda *et al.* (2019) and Muui *et al.* (2013) established that farmers cultivated sorghum for preparing foods such as ugali, porridge and other traditional dishes hence accessions with such traits were highly preferred.

4.1.9 Challenges facing sorghum farmers

The main production challenges encountered by the respondents were pests and diseases (93%), weeds (striga) (88.4%), drought (86%) and market (81%) and low yields (71%) in both counties (Figure 4.5). In Siaya County drought was the major challenge (100%) while in Baringo County it was rated at 70% (Figure 4.5). The farmers interviewed relied on rainfall for sorghum production hence their biggest challenge was drought since the counties lie within the marginal ecological areas that experience low erratic rainfall.

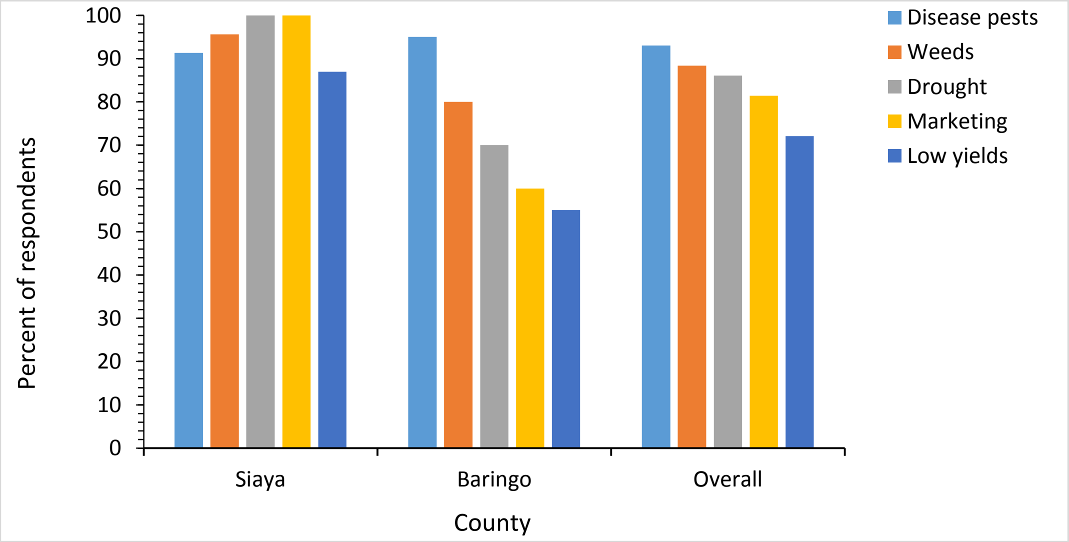


Figure 4.5 challenges faced by sorghum growing farmers in Siaya and Baringo counties

The widespread pest/disease issues could be due to inadequate pest control practices, the lack of resistant varieties, or changing climatic conditions fostering pest outbreaks. Farmers' reliance on manual weeding due to limited access to herbicides or mechanization increases the prevalence of weeds. The labor-intensive nature of weed management may also reduce its effectiveness. Baringo's semi-arid conditions make drought a recurrent challenge. Poor water-harvesting techniques and limited access to drought-tolerant varieties exacerbate the issue. Poor market linkages, low market prices, and high transportation costs are likely contributors to this challenge. The lack of organized producer groups or cooperatives to negotiate better prices could also play a role. Pests, weeds, and drought combine to lower productivity, especially in Baringo. Limited access to inputs such as improved seeds and fertilizers further reduces yields.

Report by Muui *et al*. (2019) identified lack of market incentives, use of poor-quality seeds, poor crop production practices as well as lack of adequate capital to acquire farm inputs such as fertilizers for crop production as major constraints to sorghum production. Studies by Mwadalu and Mwangi (2013) established that only 18 hybrid varieties of sorghum compared to 164 improved maize varieties had been released up to the year 2011 contributing to low yields of sorghum.

4.2 Determining drought tolerant sorghum varieties for Siaya and Baringo counties

4.2.1 Interaction of water regimes on different parameters in Baringo and Siaya sites

Results from the two sites indicated that there were significant differences on number of days to anthesis (IH and 50%H), total filled grains (TFGW), total grain weight (TGW), grain number panicle^{-1} (GPnW) and shoot dry weight (SDW) in varieties within sites at $p<0.001$ (Table 4.5). However there was no significance difference on interactions between regimes, site versus regime, regimes versus varieties and Site versus regimes versus varieties on days to anthesis, total grain weight (TGW), total filled grain weight (TFGW), weight of grains panicle^{-1} (GPnW) and shoot dry weights (SDW) at $p<0.001$ (Table 4.5).

Table 4.5 Interaction of water regimes on anthesis, panicle, shoots and grain weights in Baringo and Siaya sites

Source	IH	50%H	TFGW	TGW	GPnW	SDW
Site	***	***	***	***	***	***
Regime	ns	ns	ns	ns	ns	***
Variety	***	***	***	***	***	***
Site x Regime	ns	ns	Ns	ns	ns	*
Site x Variety	***	***	***	**	***	ns
Regime x Variety	ns	ns	ns	ns	ns	ns
Site x Regime x Variety	ns	ns	ns	ns	ns	ns

Table 4.6 Interactions of water regimes on sorghum shoot dry weight in Baringo and Siaya sites

Source	SDW 1	SDW 2	SDW 3	SDW 4
Site	***	***	***	***
Regime	ns	***	***	***
Variety	ns	***	***	***
Site x Regime	**	**	***	*
Site x Variety	ns	***	ns	ns
Regime x Variety	ns	ns	ns	ns
Site x Regime x Variety	ns	*	ns	ns

Total grain weight (TGW), total filled grain weight (TFGW), weight of grains panicle^{-1} (GPnW) and shoot dry weights (SDW). IH (anthesis) IH (50% fifty percent anthesis). ns; not significant; **Significant at p$\leqslant$0.01; *** significant at p $\leqslant$0.001

4.2.2 Effect of water stress on plant height of sorghum varieties

The plant heights between well- watered and stressed regimes did not significantly differ at $P \leq 0.01$ (Table 4.7). However, the plant height under water stress significantly differed within sorghum varieties in both Siaya and Baringo sites at $P \leq 0.01$ (Table 4.7). The highest mean plant height in Siaya site was recorded in Nyakitosi variety (160 cm) followed by Nyakabala (114cm). Sorghum varieties Ochuti, IESV 24029-54 and Local Serena recorded significantly low mean plant heights under stress at 99.2cm, 96.9cm and 96.5cm respectively (Table 4.7).

Table 4.7 Effect of water stress on plant height at Siaya site

	Height 1		Height 2		Height 3		Height 4	
Variety	**Stressed**	**Non stressed**	**Stressed**	**Non stressed**	**Stressed**	**Non stressed**	**Stressed**	**Non stressed**
E1291	57.70a	38.00e	63.10bcd	54.80bcde	77.20ab	77.30bcde	114.00fg	112.00def
Local white Barwesa	56.90a	57.30ab	70.20ab	69.50ab	65.10b	91.20abc	126.00cde	127.00abcd
Nyakitosi	55.80ab	42.80de	72.70a	52.40cde	105.00a	76.50bcde	160.00a	114.00cde
Nyakabala	53.90abc	54.00abc	69.00ab	64.20abc	98.70ab	94.00ab	144.00b	142.00ab
KariMtama1	51.90abc	44.00cde	63.80bcd	55.20bcde	88.00ab	72.40def	118.00ef	119.00cde
Seredo	51.60abcd	49.70bcd	67.60abc	60.20abc	92.40ab	88.80abcd	130.00cd	124.00bcd
Hela	49.90bcde	35.70e	64.50bcd	43.40e	93.40ab	68.60ef	135.00bc	93.10fgh
Gadam	49.00cdef	39.00e	60.50cd	53.60cde	94.00ab	75.20cdef	94.80i	86.30gh
Nyagem	47.70cdef	40.30de	59.60de	51.50cde	70.70ab	67.20ef	106.00gh	103.00efg

Local red Lawan	45.30def	38.00e	57.40def	49.20cde	84.10ab	68.40ef	130.00cd	103.00efg
Nyaurang	44.50efg	40.70de	57.20def	51.40cde	81.29ab	71.10ef	122.00def	113.00cde
Local brown Kapropita	43.70efg	63.70a	58.20def	75.70a	72.30ab	98.70a	105.00gh	144.00a
Ochuti	43.60efg	50.00bcd	52.30efg	59.60bcd	74.70ab	98.00a	99.20hi	131.00abc
IESV-24029-54	42.70fg	34.30e	51.10fg	43.30e	63.90b	58.70f	96.90hi	82.30h
Local Serena	38.50g	35.00e	47.70g	44.20de	69.80ab	68.80ef	96.50hi	103.00efg
p value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

In the same column, means with the same letter(s) are not significantly different at $p \leqslant 0.05$.

The observed results can be attributed to the genetic variability among sorghum varieties, which determines their physiological and morphological responses to water stress. Varieties like Nyakitosi and Nyakabala, which maintained higher heights under stress, likely possess traits such as deeper root systems, efficient water use, and greater osmotic adjustment, enabling them to withstand drought conditions. In contrast, sensitive varieties like Local Serena and IESV-24029-54 may lack these adaptive traits, resulting in reduced growth under stress. Stable performance in varieties such as Local brown Kapropita suggests a balance of genetic resilience and adaptability to both stressed and non-stressed conditions. Additionally, the greater impact of water stress at mid-to-late growth stages highlights the critical role of water availability during these periods for biomass accumulation and overall plant growth.

The reduction in height and other growth-related parameters was associated with decline in the cell enlargement in the plant under water stress resulting reduces plant size. Report by Khan *et al.* (2001) established substantial impairment of growth-related traits in maize where plant height, stem diameter, number of leaves/plant and leaf area decreased significantly with increasing water stress.

4.2.3 Effect of Stress on sorghum leaf rolling in Baringo site

There were differences in leaf rolling on a 1-3 scale (1 least rolled and 3 most rolled) scores between sorghum varieties in Baringo site. The varieties that recorded low mean scores included Nyakabala, Hela, Nyagem, Nyaurang, Local brown, E1291, Gadam, Seredo and Kari Mtama1 (Fig 4.6) The most affected varieties were Nyakitosi. Local white and Ochuti, IESV24029-54, Local red and Serena with mean scores of 2.3 and 1.7 respectively.

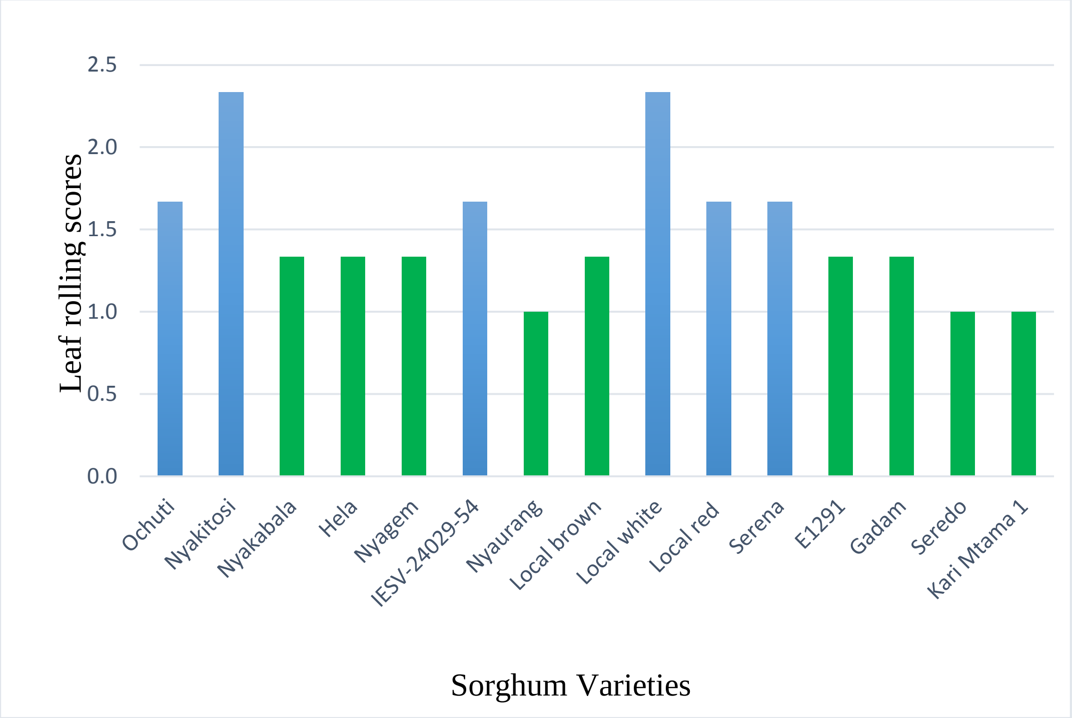


Figure 4.6 Effect of moisture stress on sorghum leaf rolling in Baringo site

The sorghum varieties that exhibited significantly low means in leaf rolling scores may have been subjected to drought stress due to high surface exposure leading to poor performance. The observed differences in leaf rolling among sorghum varieties at the Baringo site highlight the genetic variability in drought tolerance. Varieties such as Nyakabala, Hela, Nyagem, Gadam, E1291 and Kari Mtama1 showed low mean scores, indicating better drought resilience through mechanisms like superior leaf turgor maintenance and higher osmotic adjustment, which reduce stress-induced rolling. In contrast, the high mean scores for varieties like Nyakitosi, Local white, Ochuti, and Serena suggest sensitivity to moisture stress, likely due to less effective water retention or stomatal control. Leaf rolling, a common plant response to water stress, minimizes water loss through transpiration; however, excessive rolling in sensitive varieties can impede photosynthesis and reduce productivity. These results underline the importance of selecting drought-tolerant varieties for arid regions like Baringo. The ability and degree of the sorghum plants to exhibit leaf rolling enables it to reduce water loss through the surface which is a mechanism for drought avoidance as reported by Shashidar, *et al.* (2000).

4.2.4 Effect of moisture stress on days to anthesis

The number of days to 50% anthesis differed in sorghum varieties under stressed conditions from both experimental sites. The stressed treatment took more days (55-78) than the well – watered (54-74days) in Baringo site while the stressed took fewer days in Siaya (str 63-75: ww 66-77days) (Table 4.8). The Gadam and KARI Mtama 1 varieties in Baringo flowered earlier taking less than 60 days in Baringo County while Nyagem and Local red - Lawan flowered late with more than 70 days (Table 4.8). The differences in days to anthesis of sorghum varieties in different sites could have been contributed to environmental variations including temperatures and soils. Local adaptability of the varieties might have also led to the differences in time anthesis.

Table 4.8: Days to anthesis in Baringo and Siaya under two water regimes

	Baringo				Siaya			
Variety	ww 50%	str 50%	Initial ww	Initial str	ww 50%	str 50%	Initial ww	Initial str
Ochuti	62	63	56	58	70	71	60	61
Nyakitosi	68	69	58	63	74	71	64	66
Nyakabala	69	69	64	64	74	75	68	66
Hela	68	67	61	60	73	74	68	66
Nyagem	78	71	64	64	72	71	62	65
IESV-24029-54	64	71	59	62	72	74	65	66
Nyaurang	69	69	64	64	74	69	65	66
Local brown-Kapropita	70	72	67	69	74	75	66	66
Local white - Barwesa	68	70	64	66	74	73	63	66
Local red-Lawan	78	78	74	75	75	74	67	65
Serena	60	63	55	58	76	74	62	66
E1291	65	67	60	62	77	74	68	66
Gadam	54	55	51	51	66	63	56	53
Seredo	63	62	56	58	72	70	63	64
Kari Mtama 1	58	58	54	54	73	71	62	63

Generally, varieties in Siaya showed higher performance under both water-stressed and well-watered conditions compared to Baringo, which could be attributed to regional differences in climate, soil properties, and management practices.

Notably, varieties like Local red-Lawan and Local brown-Kapropita performed consistently well in both counties, possibly due to their inherent genetic resilience and adaptation to varying water conditions. Conversely, Gadam displayed the lowest values under both conditions in both counties, suggesting it is more susceptible to water stress. The smaller differences between initial and final values for well-watered and stressed conditions for varieties such as Nyakabala and Hela indicate greater stability and tolerance to water stress. This underscores the importance of selecting drought-resilient varieties like these for regions prone to water scarcity.

4.2.5 Effect of water stress on grain filling of sorghum varieties

Water stress significantly affected grain filling in both experimental sites. High grain filling ratios were recorded at Baringo site Nyagem and Local red sorghum varieties with 1.6 and 1.8 stress ratios, respectively. Sorghum varieties Nyaurang and IESV- 24029-54 had the least stress ratios of 0.9 and 0.8 respectively (Fig. 4.7).

At Baringo site Nyagem and Local red (B) varieties recorded significantly higher grain filling ratios under stress (1.42) (Fig 4.7). Most of the local varieties recorded positive increase in yields under stress conditions including Hela (1.08), Local brown-B (1.09), Nyaurang (1.13) and Local white-B (1.13). The conventional sorghum varieties Gadam and Kari Mtama1 and the other cultivated sorghum varieties recorded a negative yield ratio under stress conditions indicating their sensitivity to moisture stress.

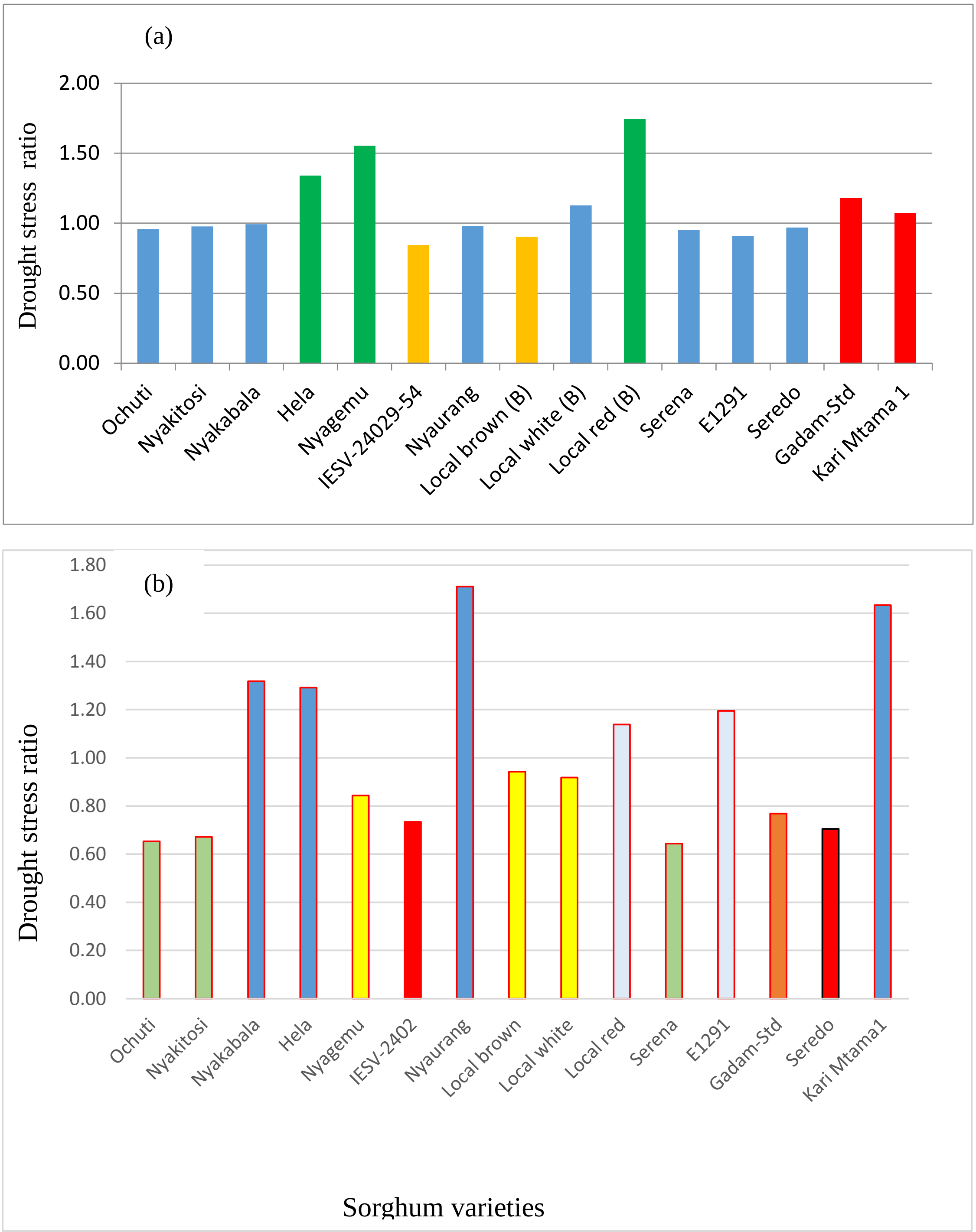


Figure 4.7 effect of moisture stress on total grain filling in (a) Baringo and (b) Siaya counties. Bars with similar colours are not significantly different at p<0.05

Interestingly, several local varieties, including Hela, Local brown-B, and Local white-B, exhibited positive yield ratios under stress, indicating their adaptability and genetic potential to thrive in water-scarce environments. In contrast, conventional varieties such as Gadam and Kari Mtama1 showed negative yield ratios, demonstrating sensitivity to drought conditions. These results emphasize the suitability of locally adapted sorghum varieties for regions prone to water stress and highlight the need to prioritize these resilient genotypes in breeding programs and cultivation in arid and semi-arid areas. As expected, the drought stress treatment increased R:S and WUE. This indicates a greater proportion of DM allocation to the roots and a more efficient utilization of available water (i.e., more DM produced per unit of water used) under drought stress conditions. Similar increases in R:S and WUE under drought stress have been reported previously (Gebre and Earl, 2020; Gebre and Earl, 2021).

4.2.6 Effect of moisture stress on grain weight of sorghum varieties

There was no significance difference in grain weights within sorghum varieties in Baringo site under stress conditions $p<0.05$ whereas under well-watered, grain weights significantly differed at $p<0.05$. However, Sorghum varieties Kari Mtama1and Ochuti recorded the highest total grain weights at 1326.77g and 1276.6g plot^{-1} respectively. In Siaya site Sorghum varieties Gadam and Nyagem recorded significantly higher total grain weights under water stress than other varieties at 416.97g and 224.8g plot^{-1} respectively ($p<0.05$). (Table 4.9)

Table 4.9 Ranked total grain weights (g) in Siaya and Baringo sites

	Baringo		**Siaya**	
Sorghum Variety	**ww**	**str**	**ww**	**str**
Ochuti	1330.57 a	1276.6	98.83ab	128.03bc
Serena	1249.83a	1192.1	111..87b	91.57bc
Kari Mtama1 std	1241.23a	1326.77	309.67b	28.93c
Nyakabala	1124.83ab	1117.1	48.07b	76.57bc
Seredo	1088.93abc	1056.43	221.77ab	216.2bc
Local brown	1002.2abc	903.7	138.6ab	102.97bc
IESV-24029-54	950.23abc	800.8	79.13b	105.6bc
Nyakitosi	874.33abc	853.93	81.13b	25.1c
Gadam-std.	864.83abc	1018.8	418.9a	416.97a
E1291	845.73abc	767.53	23.77b	30.63c
Local white	845..57abc	954.4	227.83ab	144.53bc
Hela	805.57abc	1077.13	48.27b	51.13bc
Nyaurang	797.83abc	782.73	46.67b	178.33bc
Local red	506.77bc	885.93	127.67b	187.9bc
Nyagem	467.37c	725.53	217.7ab	224.8ab
LSD	639.38	891.42	281.99	193.36
	**	NS	**	**

In the same column, means with the same letter(s) are not significantly different at $p \leqslant 0.05$. ww, str means well-watered and stressed respectively.

Higher total grain weights plot^{-1} indicated higher tolerance of the sorghum variety to water stress. Among the local accessions under study Nyagem variety in Siaya site performed similar to the drought tolerant check Gadam variety. In terms of yields, Gadam variety had significantly higher grain weights under drought stress compared to other varieties. The low grain weights under water stress were attributed to pollen shedding leading to poor grain filling. Additionally, low tillering, small panicle sizes, incomplete grain filling, biomass portioning and reduced grain sizes may have led to the low grain weights in water stressed plants. Similar results were reported by Gernik *et al.* (2003) where drought at developmental growth stage (GSII) resulted to decreased yields due to poor grain filling.

4.3 Evaluating physiological and morphological responses to progressive water stress and re-watering on selected sorghum varieties

4.3.1 Effect of stress duration on days to anthesis of sorghum varieties under Baringo and Siaya soil types

The study revealed that drought stress duration and re-watering had significant effect on performance of the sorghum varieties (Figure 4.8). Results from the greenhouse experiment at Mwea indicated that anthesis of sorghum varieties differed within the two soil types (Figure 4.8 a and b). Under both Baringo and Siaya soil types, Nyagem sorghum variety took the longest duration to attain full anthesis (73 and 97 days respectively) at continuous stress. Under continuous stress in Siaya soil, Nyagem and KARI Mtama1 took significantly longer period of 97 and 73 days to head as compared to Gadam and E1291 at 59 and 61 days respectively.

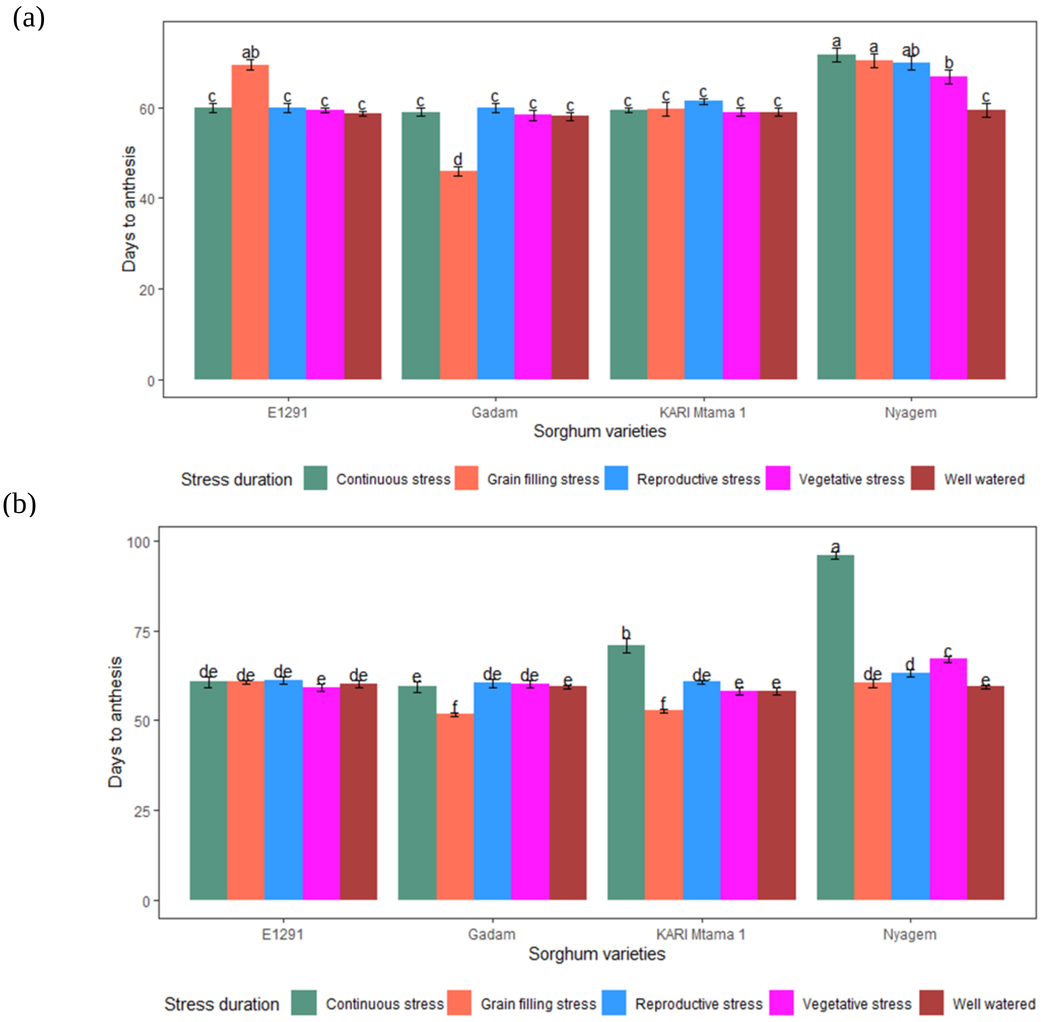


Figure 4.8 effect of stress duration on days to Anthesis; (a) Baringo and (b) Siaya soils

The observed differences in anthesis duration among sorghum varieties under drought stress and re-watering conditions can be attributed to genetic variability and adaptive traits. Varieties like Nyagem and KARI Mtama1 exhibited prolonged anthesis under continuous stress, particularly in Siaya soil, likely due to their drought tolerance mechanisms, such as delayed reproductive development to conserve resources during stress. The longer anthesis period in Siaya soil compared to Baringo soil may be linked to differences in soil properties, such as nutrient availability, water-holding capacity, and organic matter content, which influence plant stress responses. In contrast, faster-maturing varieties like Gadam and E1291 may employ escape strategies by completing their life cycle rapidly, allowing them to mitigate the adverse effects of prolonged stress. These results highlight the interaction between soil type, variety-specific genetic traits, and stress adaptation strategies, emphasizing the need to consider these factors when selecting sorghum varieties for drought-prone environments.

Under well-watered the varieties took 59 days to anthesis. However under both soil types, the days to anthesis of E1291, Gadam and KARI Mtama1 sorghum varieties did not differ in number of days to anthesis with well- watered treatment (Figure 4.11). This study concurs with Schweiger *et al*. (2023) where changes in precipitation patterns had negative influence on growth and yields.

4.3.2 Drought effect on sorghum growth

Results on drought tolerance showed differences in stress tolerance between varieties at different growth stages. Stress at anthesis and grain filling affected yields as plants failed to recover after re-watering. The study established that stress after anthesis affected yields due to pollen shedding and poor grain filling. The increase in growth differed in stress levels and sorghum varieties. The varieties with higher relative growth acquiring higher growth and yield parameters.

4.3.2.1 Interactive effects varieties and water stress on the root biomass

There was significance difference in interactions of root dry weight with varieties, soil types and different stress durations at $p<0.05$ (Figure 4.9). Kari Mtama1 and Nyagem sorghum varieties recorded high root dry weights across stress durations (Figure 4.9).

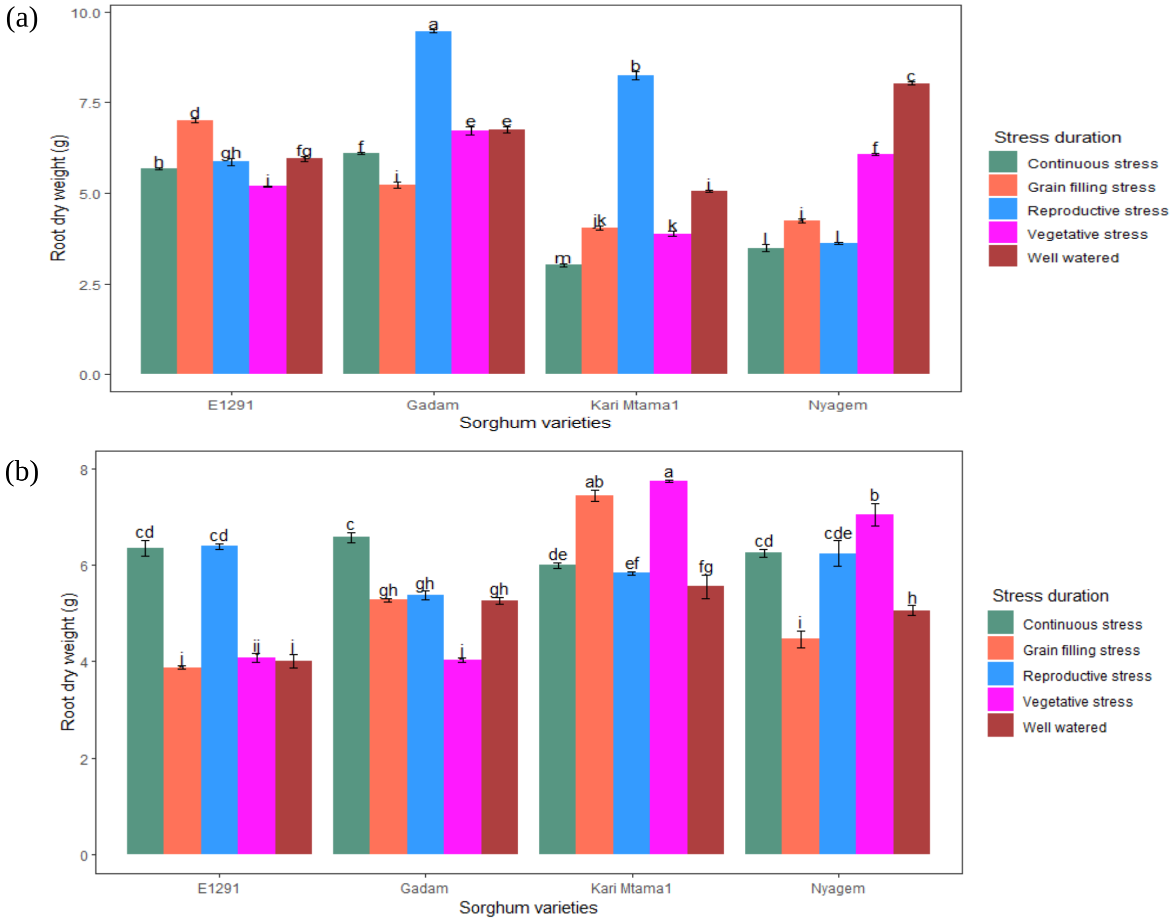


Figure 4.9 Root Dry Weight Under Different Stress Durations In (A) Siaya And (B) Baringo Soils

The higher root weights may have contributed to better accessibility to resources (water and nutrients) resulting to higher biomass production under actual cultivation environments, soil moisture content fluctuates from wet (immediately after rain) to dry several days later. Sorghum recovered after re-watering at early growth stages of vegetative and reproductive stages. Evidence in the literature suggests that lateral roots respond differently to various soil moisture regimes under fluctuating drought and wet soil conditions (Menge *et al.,* 2020). For instance, Bañoc, *et al.* (2000a) showed that lateral root development was promoted during drought for some rice genotypes, while in others it was halted, but then significantly increased in response to re-watering. There are some studies that demonstrated genotypic variation in the ability to enhance root branching under fluctuating soil moisture conditions (Niones *et al.,* 2012; Suralta *et al.,* 2008b), tillering to the anthesis growth stage.

Past studies by Badigannavar, *et al*. (2018) have reported several mechanisms that contributed to drought tolerance including osmotic adjustments, stay green, leaf rolling, waxyness on stem, root morphology and its architecture, transpiration efficiency and secretion of soluble solutes among others. Roots may regenerate after re-watering following drought stress as reported by Menge *et al.* (2020) who compared root elongation and shoot growth with fluctuating soil moisture. The study revealed that an enhanced root system during drought could contribute to shoot growth when sufficient water becomes available, specifically around the maximum

Moreover, the Quantitative Trait Loci (QTL) involved in these responses was detected at the seeding and vegetative growth stages; but no QTL were detected at anthesis stage (Niones *et al.,* 2015).

In this study, it is noted that drought tolerant sorghum varieties increased its total root length in response to constant moderate drought under deep-root-restricted field conditions because of enhanced lateral root growth while variety non tolerant varieties did not (Menge *et al.,* 2016). Such increased root system volumes under moderate drought contributed to maintaining dry matter production through enhanced water uptake. Furthermore, the increased root system under drought conditions in the varieties was enhanced by re-watering. Similar results were observed by Menge *et al.* (2019). However, no thorough evaluation has been performed to clarify the root response characteristics under soil moisture fluctuation, or the relationship between root development and shoot growth during each episodic drought and re-watering period.

4.3.2.2 Effect of stress duration on shoot dry weight of sorghum varieties

There was significant difference in shoot dry weights in sorghum varieties in both Siaya and Baringo sites under varying stress durations ($p<0.05$). The overall mean shoot dry weights for Nyagem variety and E1291 variety 59.99g and 52.98g plant^{-1} respectively which were significantly higher than Kari Mtama1 and Gadam varieties with 47.63g and 45.8g plant^{-1} respectively (Figure 4.10). Sorghum Varieties were significantly affected by stress durations with Nyagem performing better (Figure 4.10). The variety accumulation of biomass may be a good choice for dual purpose, food and livestock feed.

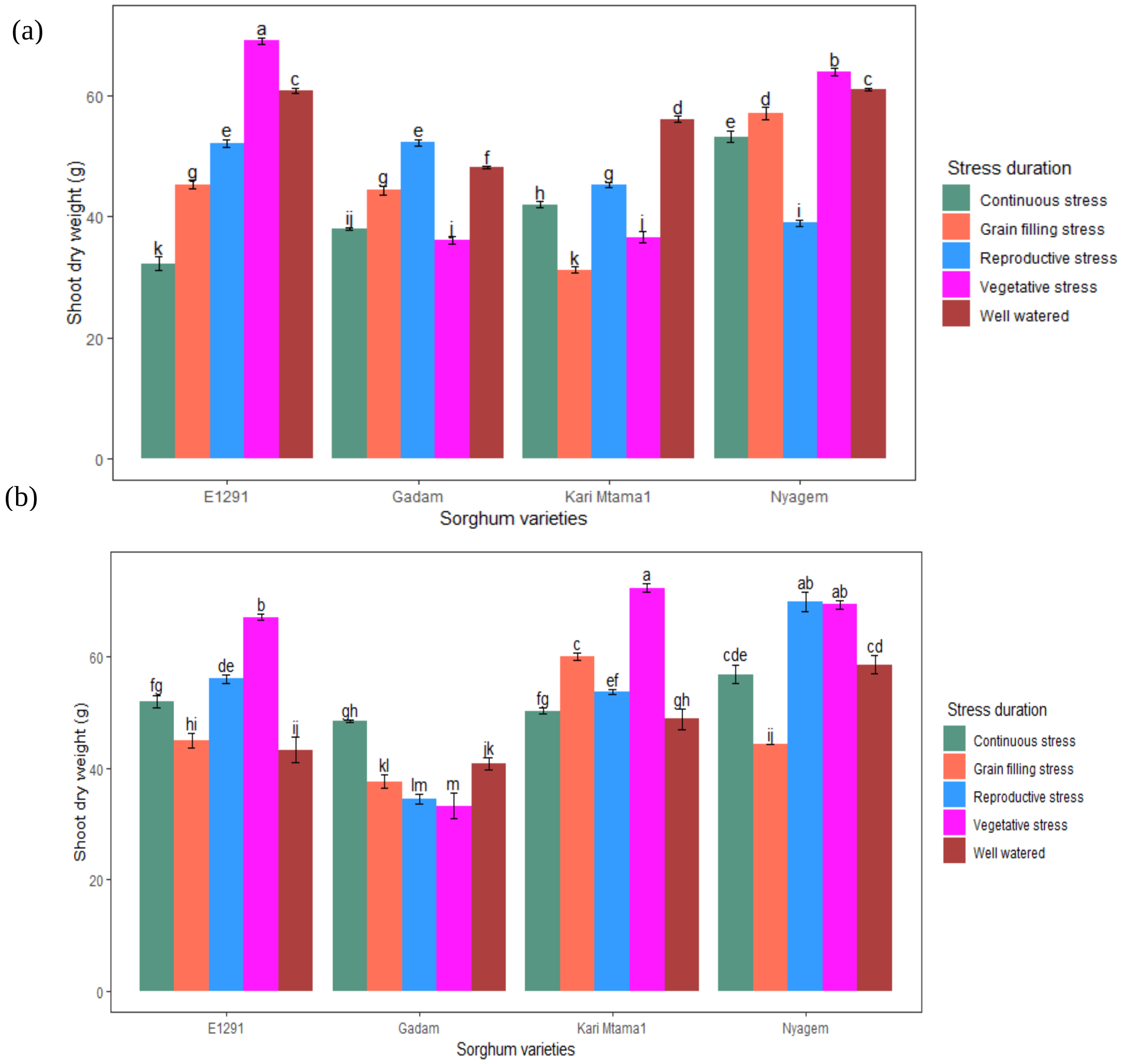


Figure 4.10 Effect of stress durations on shoot dry weight of sorghum varieties in (a) Siaya and (b) Baringo soils

The local variety, Nyagem performance was contributed by its adaptability to the environment. The landraces may perform better compared to the newly introduced varieties. Sonobe *et al.* (2010) reported that races of sorghum that have evolved in dry regions tended to be more drought resistant than races evolved in humid regions, when drought resistance is defined in terms of the degree of growth inhibition by stress.

It has been noted that water-deficit stress terminates dry matter accumulation before physiological maturity (Borrell *et al.,* 2014). Similar reductions in shoot dry weight of sorghum plants due to water-deficit stress, as observed in this study, have been reported (Nxele *et al.,* 2017; Perrier *et al.,* 2017).

4.3.3 Stress duration effect on stomatal conductance

Prolonged drought stress durations progressively decreased stomatal conductance significantly in all the sorghum varieties (Table 4.10). As the stress duration increased the varieties decreased in stomatal conductance with well-watered exhibiting highest readings while grain filling and continuous stress durations recording significantly low readings (Table 4.10).

Table 4.10 Interactive effects of variety and stress duration on stomatal conductance of sorghum leaves

Variety	**Stress duration**	**Baringo**	**Siaya**
Gadam	Grain filling stress	239±20.8 a	149±15.2 cdef
Gadam	Well-watered	235±21.2 a	162±20.7 cde
Kari Mtama1	Well-watered	231±11.3 a	259±20.5 a
Nyagem	Well-watered	147±18.6 b	162±1.9 cde
E1291	Grain filling stress	141±14.5 b	142±11.7 defg
Nyagem	Reproductive stress	136±25.4 b	120±9.2 efg
E1291	Well-watered	136±21.9 b	141±13.5 defg
Gadam	Vegetative stress	130±22.4 b	168±21.5 cd
Gadam	Reproductive stress	129±10.7 b	170±22.1 cd
Nyagem	Vegetative stress	124±13.5 b	100±6.29 g
Nyagem	Grain filling stress	121±16.7 b	111±14.9 fg
E1291	Reproductive stress	115±32.4 b	194±12.5 bc
Kari Mtama1	Continuous stress	114±10.6 b	226±19 ab
Gadam	Continuous stress	111±19.7 b	192±17.0 bc
Kari Mtama1	Grain filling stress	110±9.8 bc	128±9.87 defg
Kari Mtama1	Reproductive stress	109±12.4 bc	137±11.1 defg
E1291	Vegetative stress	104±18.2 bc	158±2.90 cde
Nyagem	Continuous stress	103±10.6 bc	247±10.8 a
Kari Mtama1	Vegetative stress	98±3.8 bc	126±12.5 defg
E1291	Continuous stress	55±21.4 c	223±17.2 ab
p value		**<0.001**	**<0.001**

In the same column, means with the same letter(s) are not significantly different at p⩽0.05.

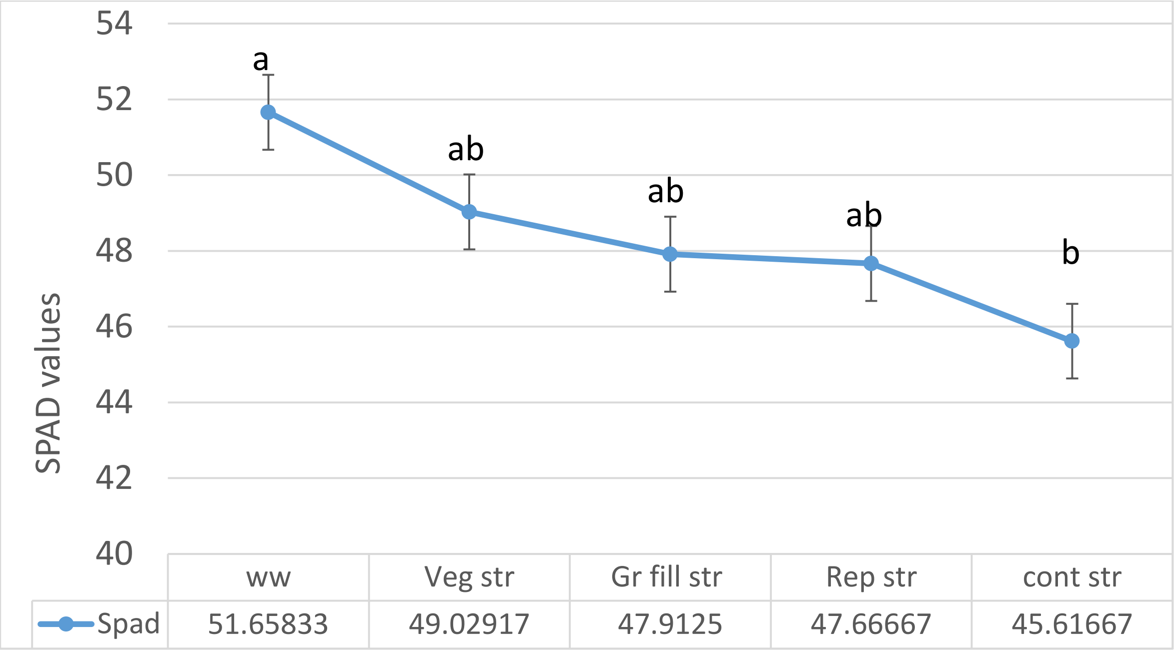
Prolonged drought stress progressively decreased stomatal conductance (gs) in all sorghum varieties, with well-watered plants showing the highest gs and plants under grain filling and continuous stress displaying significantly lower readings. This decrease in gs is a common adaptive response to drought, as the plant closes its stomata to conserve water and prevent dehydration. The longer the drought stress, the more the plant reduces gs to minimize water loss, which also limits CO_2 uptake and photosynthesis. The variation among varieties suggests differences in their drought tolerance mechanisms, with some maintaining higher gs for longer periods, while others restrict it more quickly to conserve water, especially during critical stages like grain filling.

The stomata conductance response was important for the plants to maintain water potential in avoiding desiccation. Similar findings were reported by Tsuji *et al.* (2003) where cultivars drought tolerant cultivars maintained the water potential while the desiccation-tolerant one, reduced the water potential under drought stress. Furthermore, drought-tolerant genotypes showed a significantly higher WUE than drought sensitive genotypes during drought stress period (Fracasso *et al.*, 2016). Transpiration efficiency and water extraction were reported to be significantly associated with grain yield in sorghum (Vadez *et al.*, 2011b).

Genotypes with reduced stomatal conductance and reduced transpiration rate (E) throughout the vegetative phase conserve water that may be used during grain filling stage in water-limited environments (Lopez *et al.*, 2017), and hence can be categorized as drought tolerant. The effect of drought stress in sorghum a C4 crop plant can be slightly ameliorated at elevated CO_2 levels caused by stomata opening.

4.3.4 Effect of water stress on SPAD Values

The SPAD values decreased significantly with increase in water stress in both sites (Fig. 4.11). The highest mean SPAD value under well-watered (51.65) significantly differed with the lowest under continuous stress (45.61).



Stress Durations

Figure 4.11: SPAD values under varying stress durations

The significant decrease in SPAD values with increased water stress at both sites is likely due to the reduction in chlorophyll content as the plant experiences water limitation. SPAD values, which estimate chlorophyll concentration, typically decrease under drought stress because water scarcity impacts photosynthetic activity and the overall health of the plant. Under well-watered conditions, plants maintain optimal chlorophyll levels for efficient photosynthesis, reflected by the highest SPAD value (51.65). However, under continuous water stress, plants exhibit reduced chlorophyll content, resulting in the lowest SPAD value (45.61). This decline is a typical response to drought, as reduced water availability leads to reduced leaf turgor, impaired photosynthesis, and chlorophyll degradation.

Drought increases senescence by accelerating chlorophyll degradation leading to a decrease in leaf area and photosynthesis. There is evidence that stay-green phenotypes with delayed leaf senescence can improve their performance under drought conditions (Lopes and Reynolds, 2012). In wheat and sorghum, genotypic variability has been detected in chlorophyll content as well as in the rate of leaf senescence (measured with a portable leaf chlorophyll meter) during grain-filling (Harris *et al.,* 2007; Lopes and Reynolds, 2012). In durum wheat (*Triticum turgidum* ssp. durum) stay-green mutants growing under glasshouse conditions remained green for longer and had higher rates of leaf photosynthesis and seed weight (Spano *et al.*, 2003). Therefore, a delay in leaf senescence would increase the amount of fixed carbon available for grain filling.

4.3.5 Effect of drought on number of grains per panicle

Stress duration significantly affected the number of grains per panicle in sorghum varieties in Baringo soil (Fig 4.12). Gadam sorghum variety recorded significantly higher mean grain number per panicle than other sorghum varieties under different stress durations (Fig 4.12). Under continuous stress it recorded significantly low mean grain number per panicle. The earliness in Gadam resulted to escape mechanism which was enhanced by re-watering however under continuous stress duration, Gadam variety did not recover hence lower grain number. Kari Mtama1 recorded significantly higher number of grains per panicle under well- watered stress durations, vegetative and reproductive stress while under grain filling and continuous stress durations low counts were recorded. Sorghum varieties Nyagem and E1291 were the most affected by drought in grain hence least number of mean grain numbers per panicle (Fig 4.12).

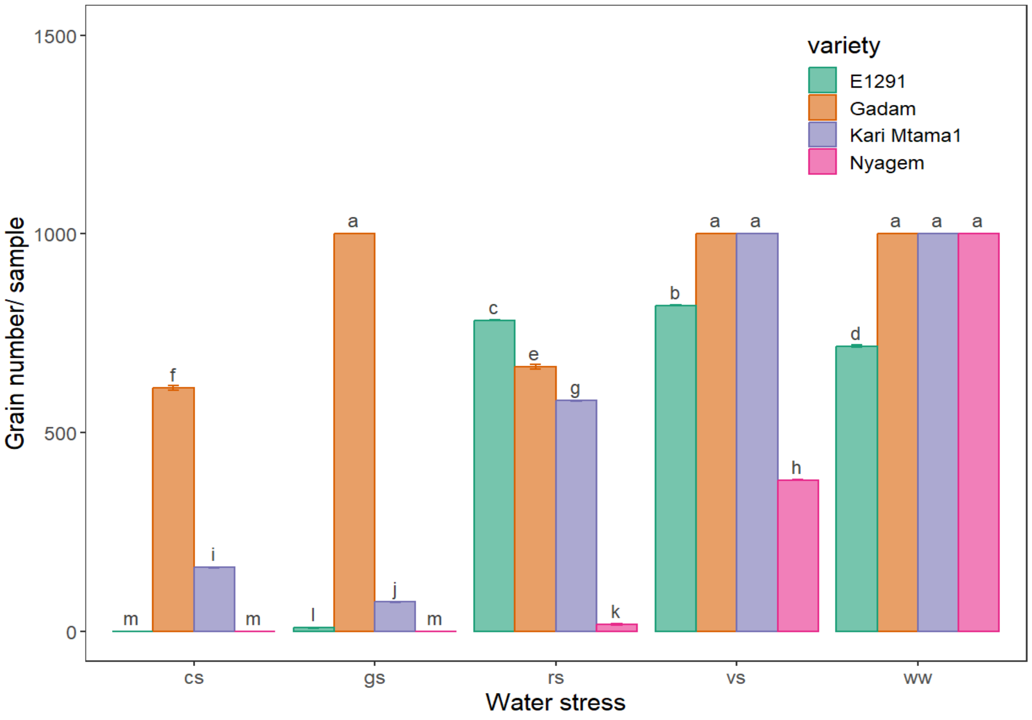


Figure 4.12 Impact of stress duration on grain number per panicle of sorghum varieties under Baringo soils. cs- continuous stress; gs- grain filling stress; rs- reproductive stress; vs- vegetative stress; ww- well watered.

This study indicates how stress duration affected the grain number per panicle in sorghum varieties with least effect on Gadam variety. Among the varieties under study, Gadam yielded the highest number of grains panicle^{-1} across the water stress durations. Further to this, stress duration induced at early growth stages (vegetative to reproductive) did not have impact on number of grains panicle^{-1} in Gadam, E1219 and Kari Mtama1 sorghum varieties but had negative effect on Nyagem variety. This indicates an early susceptibility to drought of Nyagem variety in comparison to the other three sorghum varieties. The sorghum plants re-watered after prolonged stress duration (continuous stress) did not recover which might have attributed to the decreased number of grains per panicle. Grain yield is the result of the expression and association of several plant growth components (Abreha *et al.*, 2021). Prolonged exposure to drought stress affects the flowering and grain filling stages by decreasing grain filling and flower production, leading to drastic losses in crop yield (Farooq *et al.*, 2017; Yang *et al.*, 2019). This was indeed manifested in all varieties, though some were more drastically affected.

Most of the parameters measured in this study were significantly affected in the sorghum varieties under controlled watering in the two study sites. It is imperative that the deficiency of water leads to severe decline in yield traits e.g. grain per panicle of crop plants probably by disrupting leaf gas exchange properties which not only limits the size of the source and sink tissues but also impairs the phloem loading, assimilate translocation and dry matter portioning (Farooq *et al.*, 2009). Drought stress inhibits the dry matter production largely through its inhibitory effects on leaf expansion, leaf development and consequently reduced light interception impacting on grain development (Nam *et al.*, 1998). The grain number per panicle was significantly reduced in most varieties on stress durations up to reproductive stress and beyond. Drought at flowering commonly results in barrenness as observed by Yadav *et al*., (2004) where reduction in assimilate flux to the developing ear in maize below some threshold could not sustain optimal grain growth.

4.3.6 Effect of stress durations and re-watering on grain filling of sorghum varieties

4.3.6.1 Interactive effects of varieties and stress duration on filled grain of sorghum varieties

Stress duration significantly affected the grain filling of sorghum varieties (Figure 4.13). Under well - watered and vegetative stress high number of filled grains was recorded while under reproductive stress, grain filling and continuous stress durations low filled grains were recorded (Figure 4.13).

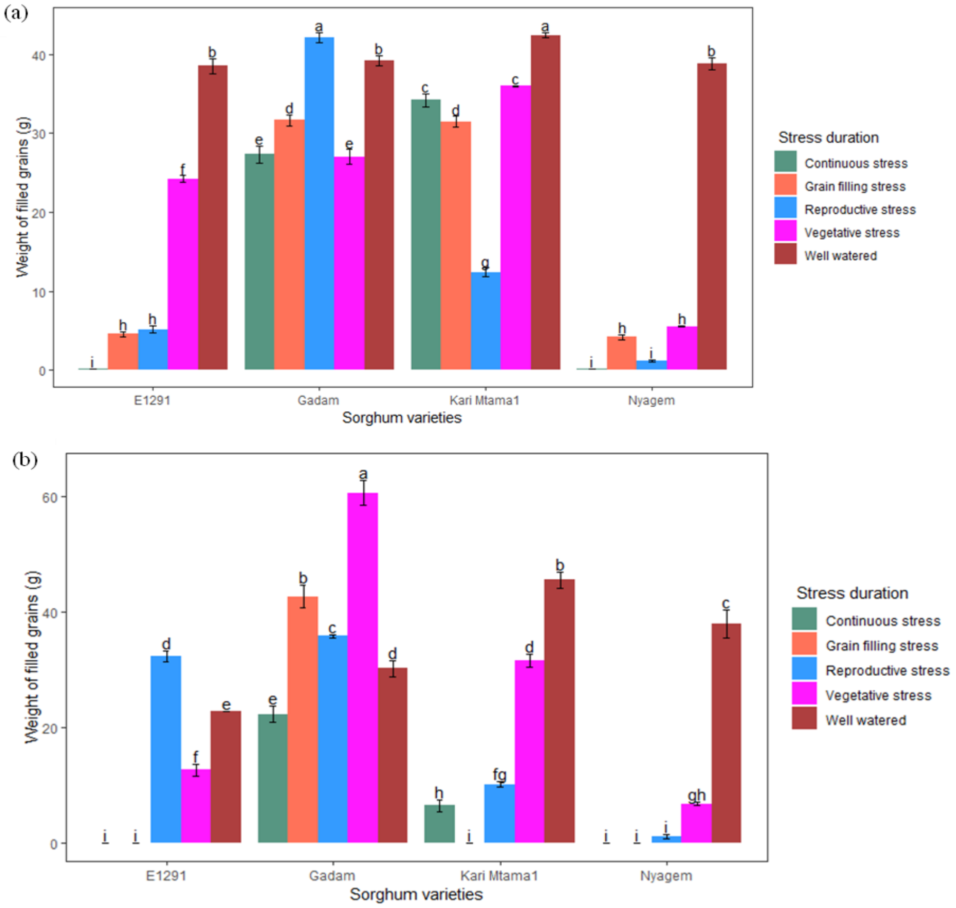


Figure 4.13 Interactive effects of varieties and stress durations on weight of filled grains of sorghum varieties in (a) Siaya and (b) Baringo soils

The reduction of grain quality and quantity under drought stress even in sorghum occurs at both pre- and post-anthesis growth stages (Blum, 2011). Water stress during seed filling influences various metabolic processes in the leaves which are involved in the biosynthesis of functional constituents such as seed reserves and minerals (Sehgal *et al.*, 2018). Drought is extremely detrimental for seed production by reducing seed size and number and seed quality (Sehgal *et al.*, 2018).

4.3.6.2 Effects of stress duration on filled grain of sorghum varieties

Based on the results, Siaya county indicated less pronounced differences, while Baringo county showed a more significant stratification of treatments with greater clarity in the trends (Figure 4.14). Well-watered consistently outperforms all other treatments, while Continuous stress and Grain-filling stress exhibit the lowest filled grain weights. Significant differences between treatments are evident, as indicated by non-overlapping letter groupings.

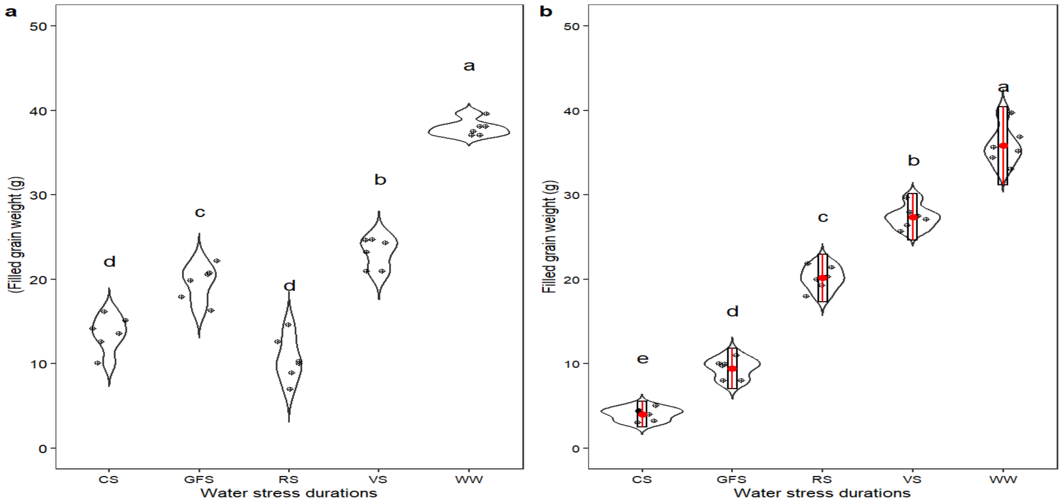


Figure 4.14 Effect of stress duration on filled grain weight (a) Siaya and (b) Baringo; cs- continuous stress; gs- grain filling stress; rs- reproductive stress; vs- vegetative stress; ww- well watered.

The observed differences in sorghum grain weight between Siaya County and Baringo County under various water stress conditions can be attributed to differences in environmental conditions, soil characteristics, and possibly genetic adaptation of sorghum varieties in the two counties. Baringo generally shows higher grain weights across all treatments, which could be due to better soil water retention, higher nutrient availability, or a more favorable microclimate that reduces the impact of water stress. Sorghum grown in Siaya may experience harsher conditions, such as lighter soils with lower water-holding capacity or higher temperatures that exacerbate evapotranspiration, reducing grain filling potential.

Under continuous stress and grain-filling stress, both counties exhibit reduced grain weights due to limited water availability during critical growth phases, which hampers photosynthesis, assimilate transport, and grain filling. However, reproductive stress and vegetative stress allow for partial recovery depending on the timing and severity of the stress. Well-watered conditions enable optimal physiological functioning, maximizing photosynthesis, nutrient uptake, and grain filling, which explain the highest grain weights in both counties, with a marked advantage for Baringo. The differences underscore the need for site-specific management practices to mitigate water stress impacts and enhance sorghum productivity.

During these stages, the potential number of grains is determined, such that grain yield potential after this phase is based on successful grain-set and grain filling (Roozeboom and Prasad, 2016). Water-deficit stress during anthesis results in reduced seed-set, and in the post anthesis stage it mainly results in decreased individual grain weight (Kadam *et al.,* 2014; Maman *et al.,* 2004).

4.3.6.3 Interactive effects of varieties and stress duration on unfilled grain of sorghum varieties

The unfilled grain weights of sorghum varieties significantly increased when drought stress duration was prolonged in both sites (Figure 4.15). This was indicated by the increased mean weights (gm plant $^{-1}$) of unfilled grains (Figure 4.15). Gadam sorghum variety was the most affected with 7.97, 27.12 and 3.85 gm of unfilled grain weights plant $^{-1}$ at reproductive, grain filling and continuous stress durations respectively.

Kari Mtama1 and E1291 followed while Nyagem sorghum variety was the least affected with 1.71, 27.12 and 3.85 gm plant $^{-1}$ at reproductive stress however there were no filled grains at grain filling and continuous stress. This indicates that Nyagem responded positively to re-watering improving on grain filling. The results on unfilled grains indicated that sorghum varieties responded differently to re-watering on stress durations where Gadam and Kari Mtama1 which are short duration varieties were more affected than longer duration Nyagem and E1291 varieties.

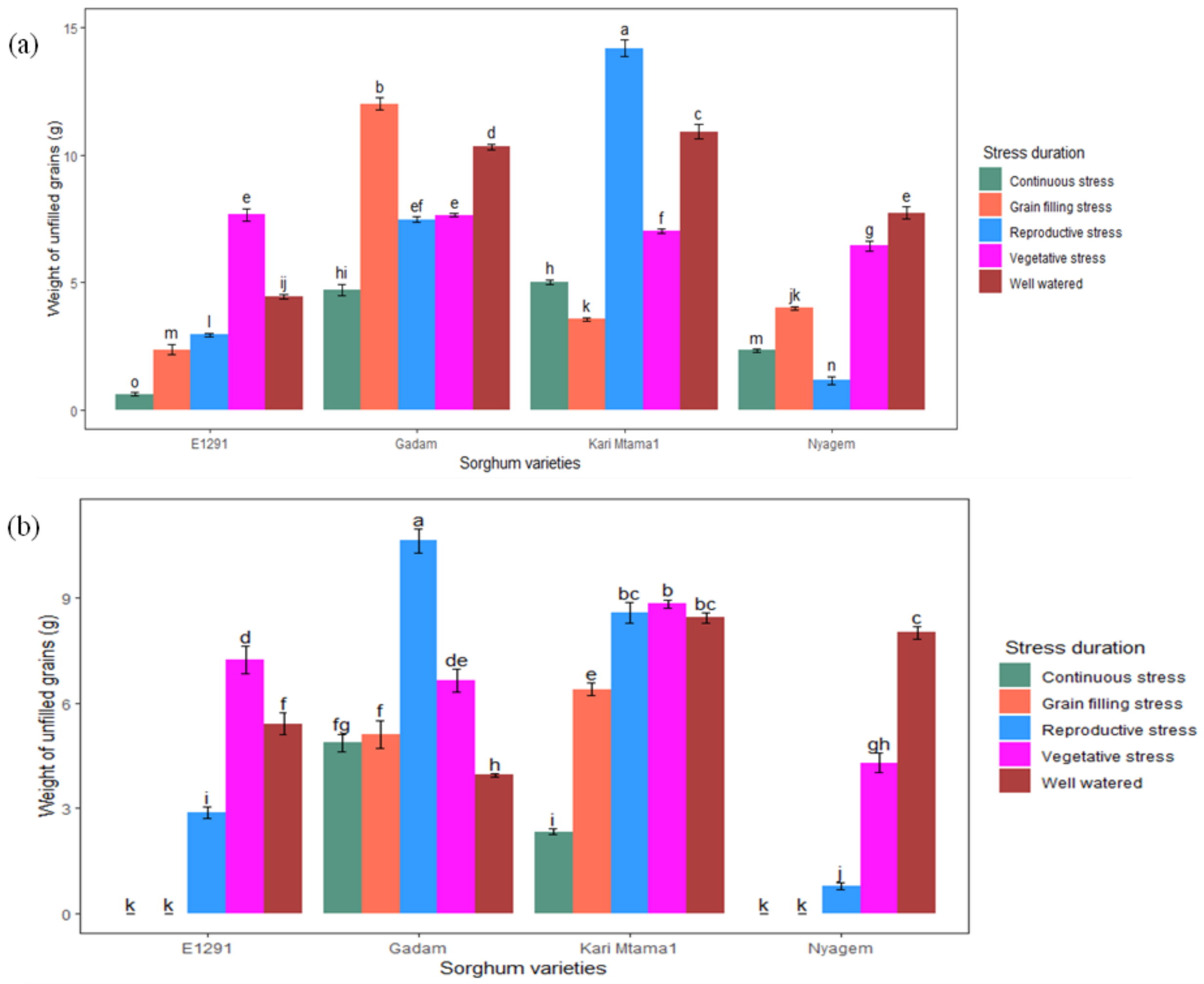


Figure 4.15 Effect of stress durations and re-watering on unfilled grains of sorghum varieties

The production of sorghum is affected by drought stress during both pre-flowering (panicle development) and post flowering stage (between flowering and grain development). Reproductive stages such as flowering, pollination, microsporogenesis, and seed filling (Sarshad *et al.*, 2021) were shown to be critical that can adversely affect grain yield. Particularly, seed filling, which involves a number of metabolic processes, diverse enzymes, and transporters located in the leaves and seeds, is considered the most sensitive stage to drought stress (De Souza *et al.*, 2015; Sehgal *et al.*, 2018).

4.3.6.4 Effects of stress duration on filled grain of sorghum varieties

The unfilled grain weights under for grain filling and continuous stress durations significantly differed with those of well- watered, vegetative and reproductive stress in Baringo soil (Figure 4. 16). A slight difference in trend was obtained under Siaya soil where vegetative, reproductive, and grain filling stages did not significantly differ in unfilled grain weights (Figure 4. 16). Well –watered and vegetative stress durations did not affect grain filling Resource mobilization was not adequate under prolonged stress durations that led to high volumes of unfilled grains.

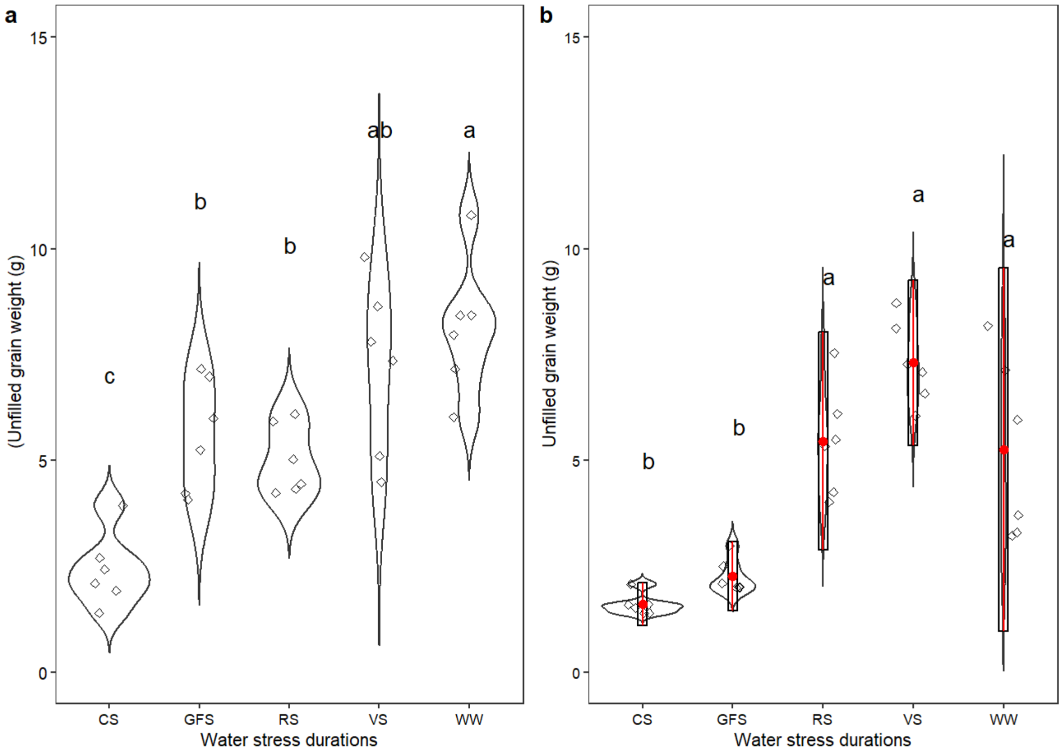


Fig 4.16 unfilled grain weights (a) Siaya and (b) Baringo: cs- continuous stress; gs- grain filling stress; rs- reproductive stress; vs- vegetative stress; ww- well watered.

The differences in unfilled grain weight between Siaya and Baringo counties under various water stress durations can be attributed to the varying impacts of water stress on pollination, grain development, and resource allocation. In Siaya, unfilled grains are higher under less severe stresses (vegetative stress and well-watered), likely due to suboptimal soil fertility or higher evapotranspiration rates reducing effective grain filling.

In contrast, Baringo shows consistently lower unfilled grains under grain filling stress and continuous stress, indicating better adaptation of sorghum to manage water deficits during critical stages like grain filling. The higher unfilled grain weights under well-watered and vegetative stress in both counties may result from an imbalance in water and nutrient uptake, leading to excessive vegetative growth at the expense of grain filling, particularly in less fertile soils. These results highlight the importance of timing and management of water availability in enhancing sorghum productivity.

A study on sorghum by (Kapanigowda *et al.,* 2013) showed that both pre- and post-flowering drought stress significantly reduces grain quantity and quality. The occurrence of drought stress during flowering stage can also cause a reduction in number of grains per panicle, which is a trait directly contributing to grain yield. However, a drought during post-flowering stages has a more severe impact on grain yield compared to a drought during pre-flowering stages.

For example, sorghum growers in Ethiopia and Burkina Faso indicated that severe drought during post-flowering stages is a major sorghum production constraint (Ouedraogo *et al.,* 2017; Derese *et al.*, 2018).

4.3.6.5 Interactive effects of varieties and stress duration on total grain of sorghum varieties

The stress durations significantly reduced total grain weight in both Siaya and Baringo Soils (Figure 4.17). The highest grain weights were recorded under well - watered and thereby reduced progressively to the least under continuous stress (Figure 4.17). The reduction in grain weights was significantly higher in Baringo than Siaya soils. The reduction in grain weight may have been contributed by moisture stress at critical growth stages.

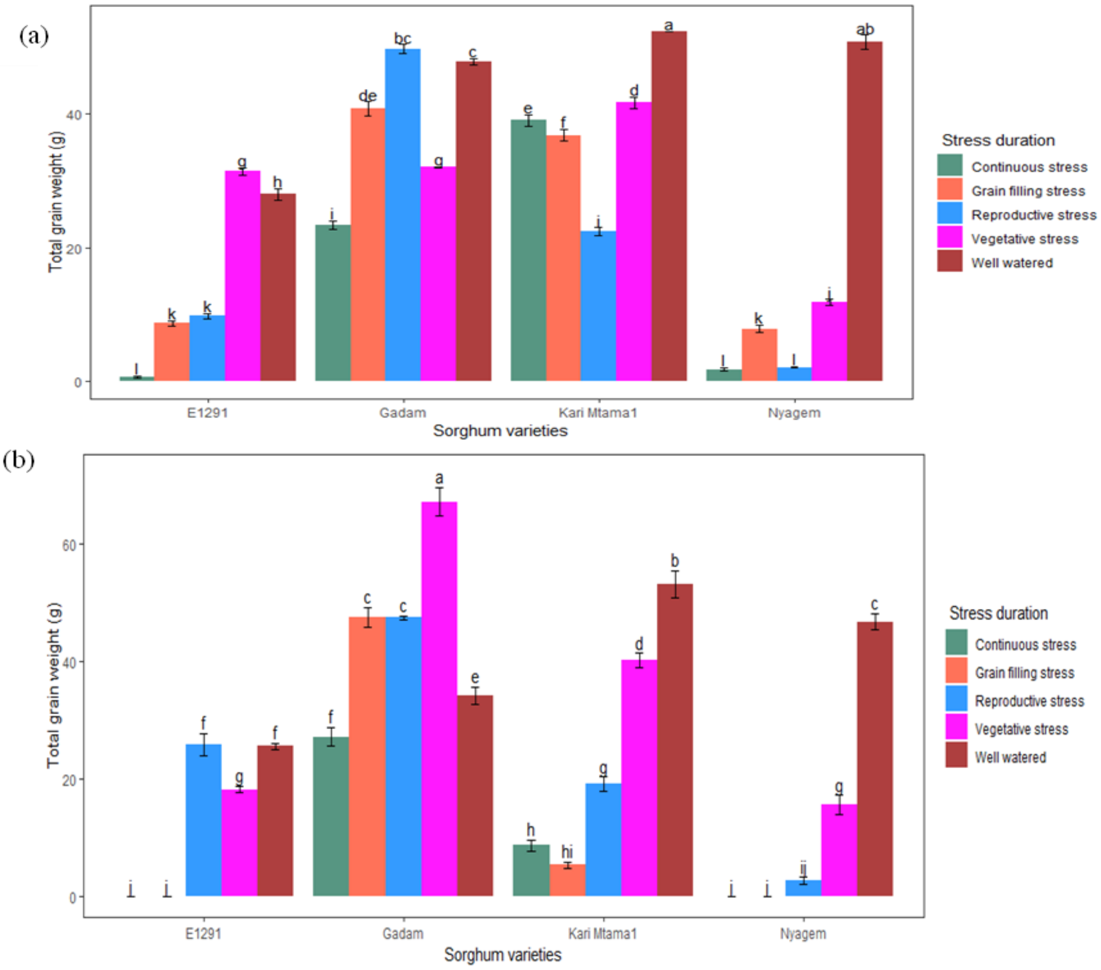


Figure 4.17 effect of stress durations on total grain weight of sorghum varieties in (a) siaya and (b) baringo soils

Report by Ndovu *et al.* (2021) established that booting and flowering stages in sorghum were the most sensitive growth stages affected by moisture stress that led to low grain yields.

A classical study involving 30 sorghum cultivars showed that drought stress during post-flowering stage reduced grain yield by about 50% (Batista *et al.,* 2019). However, the effect of drought stress on different sorghum genotypes may differ due to the variability in their response to the stress. For example, drought stress during terminal post-flowering stage, genotypes with a high growth rate and short duration of grain filling produced larger grains compared to genotypes with longer duration of grain development (Tuinstra *et al.,* 1997).

Drought stress at pollination stage can lead to significant decrease in grain yield because of the deficiency of insemination of eggs inside the ovary (Sarshad *et al.*, 2021). This is related to the fact that the transfer of pollen grains from male to female organs and contact with the eggs in the ovary require sufficient moisture, which is a limiting factor under drought stress conditions.

4.3.6.6 Effects of stress duration on total grain of sorghum varieties

Grain weights in Siaya county were significantly ($p<0.05$) affected by water stress duration. (Figure 4.18). The highest total grain weight (50 g) was recorded under well-watered conditions, while continuous stress resulted in the lowest grain weight (20 g). Grain weights under vegetative stress, reproductive stress, and grain filling stress followed an intermediate pattern, with grain filling stress outperforming reproductive stress and vegetative stress. Statistically, well watered and grain filling stress yielded comparable results, whereas continuous stress and vegetative stress were the most impacted.

In Baringo county, grain weights were highest under well watered conditions, but the magnitude of the difference across stress levels was more pronounced (Figure 4.18). Continuous stress yielded the lowest grain weight (below 10 g), significantly ($p<0.05$) lower than in Siaya County. Grain weight under grain filling stress and reproductive stress was significantly higher than continuous stress but still lower than vegetative stress and well watered.

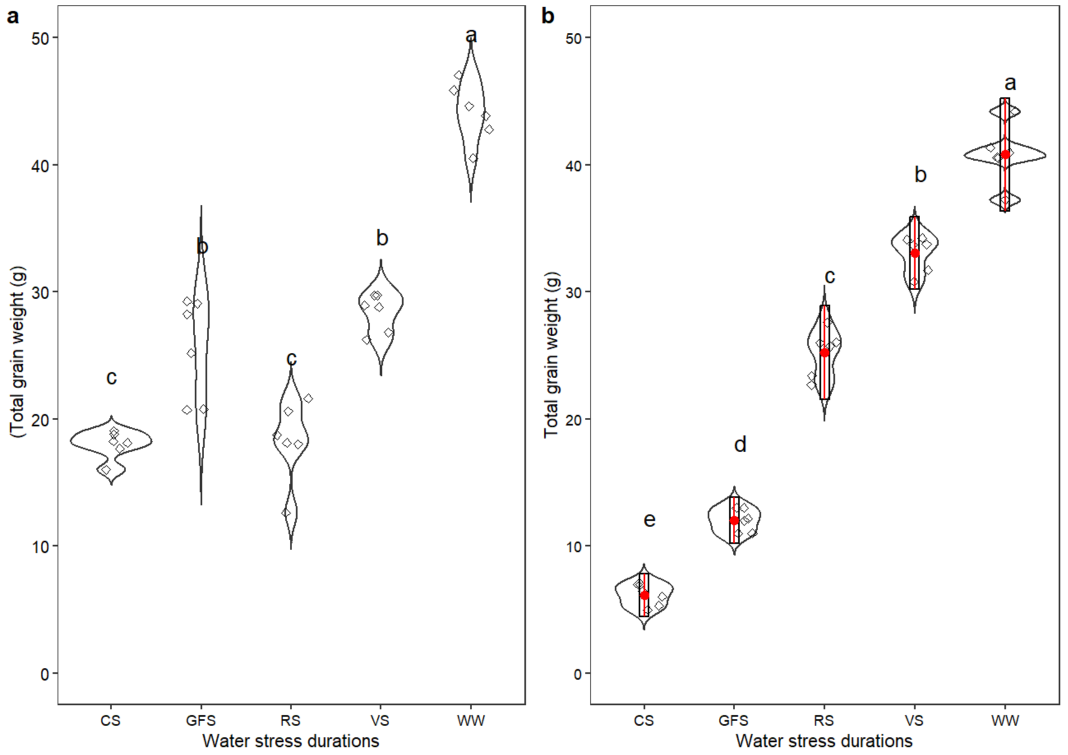


Figure 4.18 Total grain weight Siaya and Baringo: cs- continuous stress; gs- grain filling stress; rs- reproductive stress; vs- vegetative stress; ww- well watered

In Siaya, the moderate decline in grain weight under stress conditions compared to well-watered suggests some level of adaptation in the local varieties to water stress. However, grain filling stress may allow better recovery due to adequate moisture availability during critical grain-filling phases. In Baringo, the sharp decline under continuous stress indicates a greater sensitivity of the varieties to prolonged drought, possibly due to harsher climatic conditions or reduced soil water retention capacity. The pronounced difference in grain weights between well-watered and stress conditions in Baringo reflect a stronger dependency on optimal water availability for achieving high yields.

In line with this, Manjarrez-Sandoval *et al.* (1989) reported that severe drought stress prior to microsporogenesis caused a decrease in grain number per panicle (but with slight increase in grain size), which subsequently led to lower grain yield. On the other hand, a study by Sarshad *et al.* (2021) showed that drought stress after grain filling has no significant adverse effect on gain yield.

Typically, water-deficit stress during the grain-filling period induces early senescence, limits assimilate availability, shorten the grain-filling period, and ultimately reduces grain yield (Zhang *et al.*, 2018; Samarah, 2005; Yang and Zhang, 2005).

4.3.7 Effect of stress duration on 1000 grain weight

Stress durations had significant effect on one thousand grain weight under Siaya soil at p<0.05 (Figure 4.19). However the grain weight under Baringo soil significantly differed with stress duration at p<0.05 (Figure 4.19). The well-watered, vegetative and reproductive stress durations had significantly higher grain weights than grain filling and continuous stress durations (Figure 4.19).

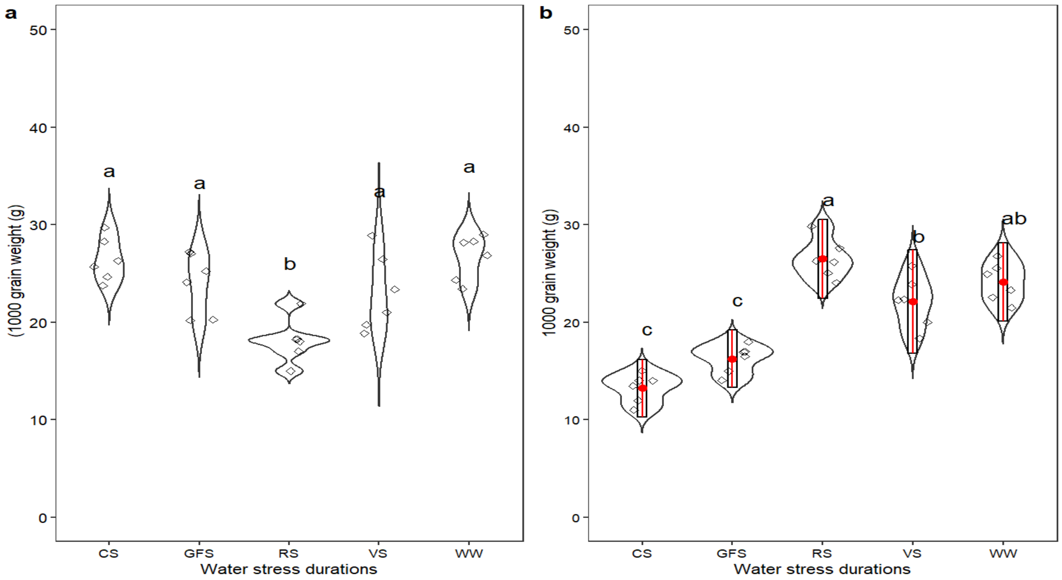


Figure 4.19 1000 grain weight in Siaya and Baringo samples (a) Siaya and (b) Baringo

The observed patterns can be attributed to the critical physiological processes affected by water stress during different growth stages. In Siaya County, the minimal impact of stress during grain filling stress and VS suggests that plants are more resilient during these stages, likely due to favorable environmental conditions and better soil moisture retention. However, the significant reduction under reproductive stress highlights the sensitivity of reproductive processes, such as flowering and grain filling, to water deficits. In contrast, the pronounced effects of stress in Baringo County may be due to harsher environmental conditions, such as lower baseline soil moisture and higher temperatures, which exacerbate stress impacts. The strong response to grain filling and continuous stress in Baringo highlights the importance of sustained water availability for photosynthesis and nutrient translocation, which are critical for grain development. The report conforms to Alikhani *et al*., (2012) on morphological and physiological growth indices affecting sorghum yields.

4.3.8 Correlation of water stress on measured parameters

Exposure of sorghum plants to drought had different impacts on the parameters measured (Table 4.11). Proline accumulation in sorghum leaves was positively correlated with increase in drought (r=0.413) (Table 4.11). The study results also indicated that as stress duration progressed, the amino acid accumulation in plant leaves increased in all the varieties. The highest proline accumulation was recorded from the longest stress duration that extended until grain filling stage, however the well - watered and vegetative growth stages had the least proline accumulation. These results are similar to those by Devanarian *et al*. (2016) on the studies on selected African sorghum land races to progressive water stress and re-watering where proline significantly increased under severe stress. Devanarian *et al.* (2016a) reported that drought stress at grain filling stage highly impacted on grain yields

Stress duration negatively impacted on shoot dry weight, root dry weight, plant height, SPAD values and grain yields (Table 4.11). Drought stress had the highest impact on Grain yields (r=-0.538) where increased drought resulted to significant reduction in grain yield. Drought stress duration had least negative impact on plant height (r=-0.023) (Table 4.11).

Table 4.11 Correlation of stress duration to various data parameters

Correlation parameter	**Spearman correlation coefficient**
Drought stress : SDWt	-0.3348614
Drought stress: RDWt	-0.04116739
Drought stress: Height	-0.02322646
Drought stress:Spad4	-0.328673
Drought stress: Yield	-0.5385808

The 1000grain weight is an important parameter in determining the weight of grainness and fullness of the grain and flour yield. It determines the quality of the endosperm which is the main source of the flour in the grain.

These varietal differences are similar to those reported by Abreha *et al.* (2021) in which drought stress during terminal post-flowering stage, affected genotypes with a high growth rate and short duration of grain filling which produced larger grains compared to those with longer duration of grain development.

Correlation analysis revealed that stress duration and re-watering had negative relationship with grain yield. This may be because of increased translocation of stored photosynthates from the stem reserves when the current photosynthesis ceases due to environmental stress factors, particularly during grain filling and grain development period after re-watering similar to report by Erick and Musick, (1979).

The reduction of grain weight reported in this study was similar to the study by Zhan *et al.* (2015) who reported a high correlation of 1000 grain weight with grain quality in rice. Similarly, the total grain weight significantly reduced as stress duration extended beyond reproductive stage in conformation to the finding by Bing *et al*. (2014). The underlying reason the effect being the reduction in starch synthesis enzyme activities resulting to starch accumulation with negative impact on starch components under drought stress (Bing *et al.*, 2014). Therefore the sorghum grains formed were light, partially filled and of low quality.

4.4 Effect of water stress on grain quality and biochemical properties of selected sorghum varieties

4.4.1 Effect of stress duration on proline accumulation in sorghum varieties

Proline concentration between control and treatments under different stress duration in sorghum varieties significantly differed at $p< 0.5$ (Figure 4.20). The results showed that stress for a short duration up to Vegetative stage followed by re-watering recorded significantly low proline accumulation under both Baringo and Siaya soil (Figure 4.20 a and b).

Proline accumulation in leaves ($g100mg^{-1}$) increased with increase in stress durations (Figure 4.20). In Siaya soils there was significantly higher concentration of proline under continuous than other stress duration (Figure 4.20). The grain filling, reproductive, vegetative and well-watered stress durations did not significantly differ in proline concentration (Fig 4.20). Under Baringo soil, continuous stressed plants recorded higher proline concentration followed by grain filling, reproductive which however were not significantly different, while well-watered plants had least proline concentration which was significantly different from the other treatments.

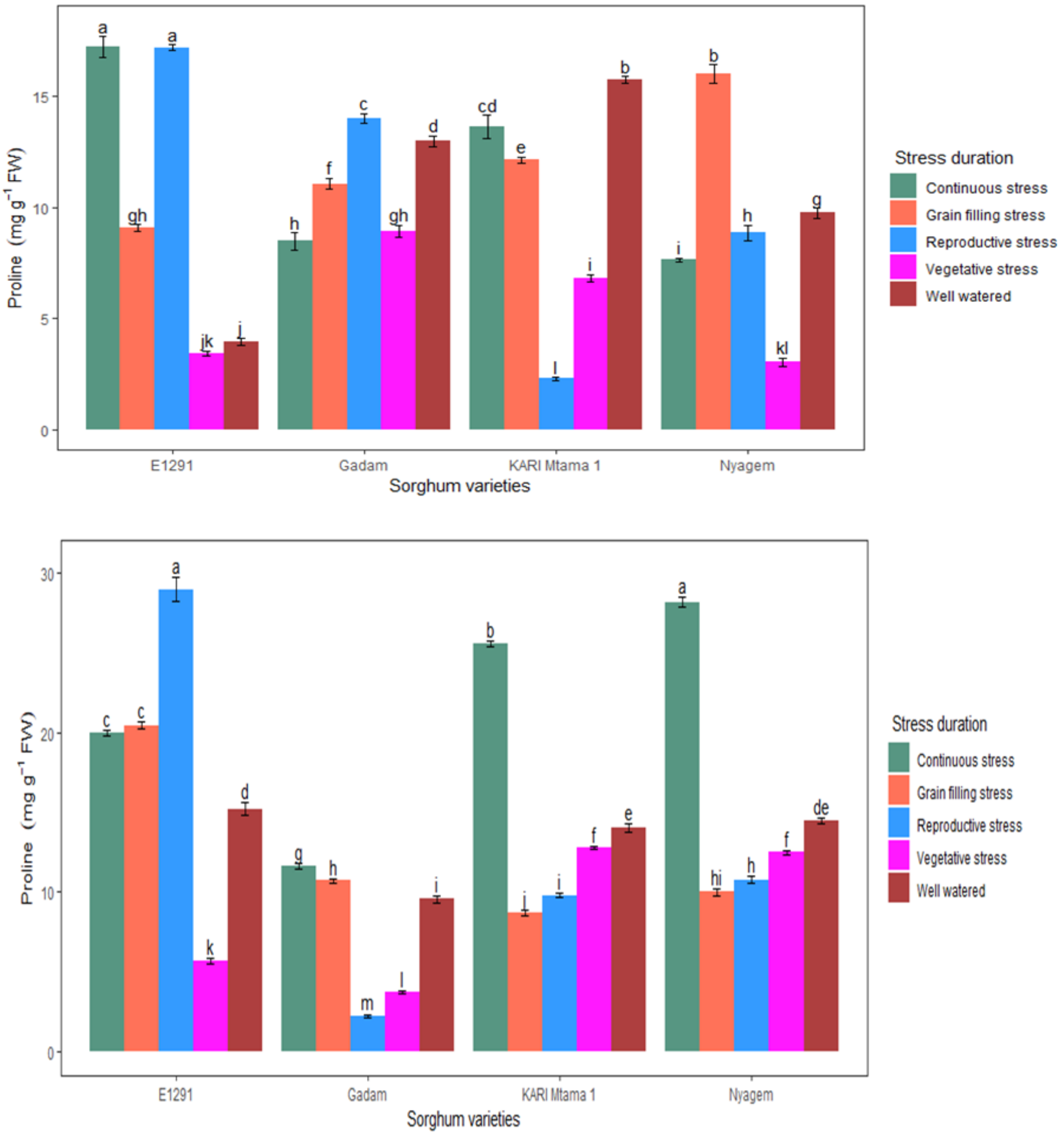


Figure 4.20 Effects of varieties and stress durations on total grain weight of sorghum varieties in (a) Siaya and (b) Baringo soils

Previous results by Skalska *et al.* (2021) revealed that proline levels increased in *Brachypodium distachyon* (L.) accessions using flow infusion electrospray high-resolution mass spectrometry under varying drought stress; in addition, sugar and starch, antioxidant synthesis, and polyphenolic metabolism changed, but these changes may have been normal physiological responses to drought including bioenergetic resource provision and adaptations to oxidative stresses. There are also documented reposts that show the contribution of proline (Pro) to be significant in improving drought tolerance as previously shown in barley, where greater ability to accumulate proline correlated with decreased membrane injury (Bandurska, 2000).

This seems to agree with the results in Fig 4.20. More addition, a large increase in the proline precursors; i.e. glutamate (Glu) and arginine (Arg) have been previously been observed in Brassica napus during drought (Good and Zaplachinski, 1994). Incidentally, drought also induced more than five-fold increase in isoleucine (Ile), leucine (Leu) and aspartate (Asp) contents, as well as an increase, albeit small, in the levels of all other amino acids (Good and Zaplachinski, 1994). Similar findings seem to be partly in agreement with the current results (Tables 4.12, 4.13) especially as stress level increased. This was particularly evident for more tolerant varieties like KARI Mtama1.

In the current work, plants exposed to prolonged stress durations followed by re-watering had lower chances of recovery leading to high levels of proline accumulation. Re-watering after short stress durations enabled recovery of the plants leading to decline in proline contents. The levels of proline and leucine varied significantly across stress conditions and crop varieties, reflecting their distinct roles in stress tolerance and metabolic functions. Proline levels were highest under continuous stress in E1291 and grain-filling stress in Nyagem, both exceeding 16 mg, suggesting that proline accumulation is a key adaptive response to osmotic stress. This aligns with its role in osmotic adjustment, scavenging reactive oxygen species, and stabilizing cellular structures during water deficits. Conversely, varieties under well-watered conditions, such as E1291, recorded the lowest proline levels (3.96 mg), consistent with the reduced need for stress mitigation.

The study established that Nyagem sorghum variety in Baringo soils had significantly higher proline concentration at grain filling followed by Kari Mtama1, Gadam and E1291. These results conform to those by Abdi *et al.* (2018) where proline accumulation differed with varieties of wheat and moisture stress levels. Further results showed that re-watering sorghum after short stress durations particularly at vegetative and reproductive stress durations had significant low proline concentrations. Abid *et al.* (2018) had similar findings where higher proline accumulation was associated with drought tolerance.

Proline is an important osmoprotectant amino acid, which acts as an osmolyte that stabilizes membranes and proteins protecting them against radicals (Hayat *et al.*, 2012). It is normally a mechanism of crop plants to tolerate drought stress (Hayat *et al.*, 2012) It this study established that proline accumulation was positively correlated to stress levels, where higher concentration was associated with drought tolerance. Proline accumulation in the cytosol might also be relevant for balancing the increased vacuolar osmolarity due to autophagic degradation of macromolecules during stress (Signorelli *et al.*, 2019).

Bruria, (2010) established that proline contents in plants differed with stress durations while re-watering the sorghum plants restored plants to optimal growth conditions resulting to a rapid decline in the proline content. The reasons underlying the results is that proline accumulation is a common physiological response to various stresses. It may affect solubility of proteins protecting them against denaturation under stressed conditions.

Looking and different mechanisms and pathways, proline provides protection to plant cell membranes and proteins and functions as a scavenger of reactive oxygen species (Delauney and Verma 1993; Hare *et al*., 1998). The similar significant increase in proline contents was reported by Marcin´ska *et al*. (2013) and Chorfi and Taı¨bi (2011) in their study with wheat. Proline accumulation is possibly caused by an increase in glutamate-mediated biosynthesis (Zhang *et al*., 2011).

4.4.2 Effect of stress duration on amino acids accumulation in sorghum varieties

In Baringo County, stress duration significantly influenced the amino acid content across the sorghum varieties. Gadam showed the highest serine content (4.13ml/mol) during grain filling stress, while Nyagem had the lowest (0.39ml/mol) under vegetative stress (Table 4.12). Methionine levels peaked in Gadam during reproductive stress (25.7ml/mol), whereas Nyagem recorded the lowest values (1.12ml/mol) under reproductive stress. Similarly, valine levels were highest in E1291 under grain filling stress (22.1ml/mol) and lowest in Gadam under continuous stress (5.07ml/mol).

Leucine content was notably highest in Gadam during reproductive stress (12.4ml/mol). In Siaya County, Gadam under grain filling stress recorded the highest arginine levels (492ml/mol), while E1291 under vegetative stress showed the lowest (88.8ml/mol). Methionine levels were highest in KARI Mtama 1 during continuous stress (19.5ml/mol) and lowest in Nyagem under grain filling stress (4.8ml/mol). Leucine, an essential branched-chain amino acid, showed peak levels in Gadam under reproductive stress (12.4 mg) and grain-filling stress in Nyagem (9.65 mg). In the data, serine levels peak during grain-filling stress in Gadam (4.13 mg) and are lowest during vegetative stress in Nyagem (0.39 mg).

Table 4.12: Interactive effects of varieties and stress duration on different amino acids accumulation in Baringo soils.

Variety	Stress duration	Serine	Arginine	Methionine	Valine	Iso leusine	Leucine
KARI Mtama 1	Vegetative stress	3.02±0.14b	149±0.74e	9.91±0.21i	5.45±0.44 f	17.9±0.22 e	8.96±0.15c
KARI Mtama 1	Grain filling stress	3.07±0.10b	314±2.84a	11.1±0.19 fg	1.37±0.15 k	5.74±0.26 j	6.49±0.34e
Gadam	Vegetative stress	2.46±0.19c	243±8.14c	19.8±0.36b	9.66±0.55 b	14.2±0.25 g	5.44±0.22 fg
Gadam	Grain filling stress	4.13±0.19a	109±1.00g	10.2±0.18 hi	1.10±0.11k	5.89±0.22 j	9.65±0.20 b
Nyagem	Grain filling stress	1.7±0.07 d	58.2±0.29jk	15.2±0.11d	4.42±0.15 g	18.4±0.17de	5.75±0.06 fg
Gadam	Continuous stress	1.58±0.10d	99.2±2.14h	6.67±0.28 l	1.16±0.08 k	5.07±0.25jk	3.62±0.16i
E1291	Reproductive stress	2.35±0.21c	157±1.71de	10.6±0.18 ghi	7.89±0.14 c	12.5±0.24 h	1.39±0.07k
Nyagem	Reproductive stress	2.48±0.15c	161±1.12d	1.12±0.09 o	6.41±0.26de	17.9±0.16 e	2.4±0.14 j
E1291	Grain filling stress	3.02±0.12b	276±4.43b	16.6±0.18 c	1.56±0.13jk	22.1±0.25 b	7.36±0.18 d
Nyagem	Well watered	1.47±0.09 de	122±2.14 f	4.33±0.08 m	3.02±0.15 h	14.7±0.21 g	5.18±0.24g
E1291	Continuous stress	1.21±0.13 ef	159±4.24 de	7.71±0.29 jk	5.87±0.18ef	26.4±1.08 a	4.38±0.38h
KARI Mtama 1	Reproductive stress	0.65±0.05 gh	96.6±1.03 h	10.9±0.48 gh	1.08±0.07 k	19.3±0.39 d	4.3±0.13 h
KARI	Continuous stress	1.13±0.12 ef	80.9±1.29 i	7.43±0.26 kl	4.02±0.23 g	12.6±0.13 h	3.64±0.1 i

Mtama 1							
E1291	Well watered	1.2±0.05 ef	164±8.01 d	2.40±0.08 n	2.14±0.04 ij	14.5±0.18 g	3.58±0.2i
Nyagem	Continuous stress	1.46±0.07 de	122±1.24 f	8.40±0.26 j	7.91±0.14 c	16.7±0.16 f	5.54±0.12 fg
Gadam	Reproductive stress	0.52±0.09 h	48.4±0.92 k	25.7±0.12 a	6.06±0.12 def	20.7±0.19 c	12.4±0.20a
Gadam	Well watered	0.58±0.05 gh	59±1.50 j	12.7±0.23 e	2.67±0.09 hi	4.56±0.27 k	6.00±0.21 ef
KARI Mtama1	Well watered	1.17±0.07 ef	119±3.58 fg	11.9±0.68 ef	6.74±0.05 d	10.9±0.13 i	2.25±0.13j
Nyagem	Vegetative stress	0.39±0.06 h	12.8±0.22 l	7.61±0.43 jk	12.8±0.46 a	4.57±0.34 k	6.52±0.35 e
E1291	Vegetative stress	0.91±0.10 fg	14.3±0.36 l	6.66±0.30 l	6.73±0.16 d	18.4±0.30 de	3.34±0.10 i
p value		**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

In the same column, means with the same letter(s) are not significantly different at $p \leqslant 0.05$.

Table 4.13: Interactive effects of varieties and stress duration on different amino acids accumulation in Siaya soils.

Variety	Stress duration	Arginine	Methionine	Valine	Leucine	Iso leusine	Serine
Gadam	Grain filling stress	492±2.70 a	4.8±0.11 m	10.6±0.20 c	6.55±0.22 e	16.8±0.15 i	4.13±0.11 a
KARI Mtama1	Reproductive stress	348±1.04 b	11.0±0.16 e	4.55±0.07 i	8.21±0.10 c	12.0±0.14 m	3.71±0.17 b
E1291	Grain filling stress	323±0.90 c	16.6±0.23 c	7.16±0.10 f	7.12±0.13 d	15.5±0.20 k	2.46±0.10 d
Nyagem	Grain filling stress	305±1.14 d	9.8±0.17 fg	4.02±0.12 jk	2.40±0.08 k	23.2±0.16 c	2.18±0.09 def
Nyagem	Reproductive stress	292±1.44 e	6.22±0.12 l	4.56±0.12 i	4.72±0.14 f	6.78±0.160 p	3.49±0.27 b
Nyagem	Continuous stress	251±0.88 f	8.78±0.09 ij	2.31±0.11 l	2.58±0.09 jk	21.0±0.19 e	3.11±0.15 c
Gadam	Continuous stress	233±1.10 g	11.8±0.16 d	2.53±0.12 l	3.28±0.11 i	18.0±0.12 gh	1.83±0.08 fgh
Gadam	Vegetative stress	232±0.63 g	6.56±0.17 l	6.96±0.10 fg	3.74±0.10 h	22.0±0.20 d	2.11±0.07 def
E1291	Continuous stress	219±1.21 h	9.91±0.13 f	4.80±0.09 i	4.55±0.07 f	12.9±0.13 l	2.33±0.08 de
Gadam	Well watered	216±1.23 h	8.74±0.13 j	3.73±0.13 k	2.3±0.08 k	19.5±0.24 f	2.27±0.08 de
KARI Mtama1	Vegetative stress	213±0.72 i	9.21±0.10 hi	4.74±0.13 i	6.3±0.11 e	7.83±0.14 o	2.38±0.09 de

KARI Mtama1	Grain filling stress	210±0.90 i	7.48±0.11 k	9.26±0.11 d	2.93±0.08 ij	17.9±0.09 h	1.61±0.05 h
Nyagem	Vegetative stress	204±2.01 j	6.30±0.165 l	11.2±0.18 b	1.75±0.06 l	26.1±0.18 b	1.02±0.08 i
Nyagem	Well watered	196±0.83 k	8.69±0.17 j	7.71±0.11 e	2.33±0.06 k	25.9±0.17 b	2.04±0.10 efg
KARI Mtama1	Continuous stress	193±0.66 k	19.5±0.11 a	15.8±0.16 a	14.6±0.18 a	21.9±0.25 d	1.84±0.09 fgh
KARI Mtama1	Well watered	188±0.78 l	9.45±0.22 gh	11.3±0.14 b	1.78±0.07 l	16.1±0.08 j	2.25±0.16 de
Gadam	Reproductive stress	152±1.14 m	17.5±0.11 b	10.5±0.13 c	4.11±0.10 g	16.6±0.15 ij	1.71±0.04 gh
E1291	Reproductive stress	143±0.37 n	4.84±0.13 m	6.58±0.14 gh	6.43±0.14 e	32.6±0.18 a	3.01±0.14 c
E1291	Well watered	133±0.85 o	8.37±0.09 j	6.43±0.11 h	3.75±0.13 h	10.9±0.20 n	1.71±0.12 gh
E1291	Vegetative stress	88.8±0.58 p	4.89±0.12 m	4.14±0.12 j	10.9±0.16 b	18.4±0.22 g	0.71±0.07 i
p value		**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

In the same column, means with the same letter(s) are not significantly different at p⩽0.05.

There were elevated levels of difference amino acids with leucine standing out as one of the amino acids produced during intense drought, especially for Gadam in both Baringo and Siaya (Tables 4.12 and 4.13). These elevated leucine concentrations may be linked to its role in protein biosynthesis, energy production, and signaling pathways critical for stress recovery and growth. As a branched-chain amino acid, it is integral to protein biosynthesis and energy generation, especially during stress recovery. Elevated leucine levels under reproductive and grain-filling stress, such as in Gadam and Nyagem suggest its involvement in stress-related metabolic shifts. Stress often triggers protein turnover, releasing amino acids like leucine for energy production through branched-chain amino acid catabolism.

This process supports ATP generation and maintains metabolic homeostasis under energy-demanding stress conditions. Lower leucine levels under well-watered conditions, such as in E1291, reflect a reduced need for protein turnover and energy-intensive repair processes, as optimal growth conditions do not induce the same metabolic strain. These findings are in line with those of (Duan *et al.*, 2016). The lowest leucine levels were observed under well-watered conditions, particularly in Nyagem and E1291, as stress-induced catabolism to down-regulate proteins synthesis and to release amino acids like leucine is minimized. These results underscore the metabolic shifts plants undergo to prioritize survival under adverse conditions, with proline accumulation marking osmotic stress adaptation and leucine contributing to stress recovery and energy regulation.

Serine plays a critical role in protein synthesis, cell membrane formation (via phospholipid synthesis), and one-carbon metabolism. Serine and arginine exhibit metabolic flexibility, responding dynamically to growth and stress phases. The elevated serine levels in Gadam during grain-filling stress likely support active protein synthesis needed for grain development. Its low concentration in Nyagem under vegetative stress may indicate reduced metabolic activity or prioritization of other metabolic pathways during this growth stage.

The spike in arginine during grain-filling stress aligns with its role in promoting cell division and growth during critical developmental phases, while the sharp decline in vegetative stress suggests reduced biosynthetic activity and a shift in resource allocation toward stress defense. Arginine metabolism is therefore thought to play a key role in nitrogen storage during embryogenesis and nitrogen mobilization during germination (Llebrés *et al.,* 2018).

Both methionine and S-adenosylmethionine are involved in the biosynthesis of ethylene, nicotianamine, and polyamines (Sauter *et al.,* 2013). Additionally, Arabidopsis with elevated methionine levels was found to enhance metabolic and transcriptomic responses related to desiccation stress and mitochondrial energy metabolism (Cohen *et al.,* 2014). A previous study also demonstrated that plants with higher methionine content exhibit increased tolerance to abiotic stress (Ma *et al.,* 2017).

In contrast, under well-watered conditions, the reduced environmental stress diminishes the need for such protective measures, explaining the significantly lower amino acid levels. Arginine, a precursor for polyamines and nitric oxide, shows marked variability, with high levels in KARI Mtama 1 during grain-filling stress. This can be attributed to its role in enhancing cell division, growth, and osmotic adjustment during critical developmental phases. The sharp reduction in arginine under vegetative stress in Nyagem might indicate limited demand for polyamine biosynthesis during early stress responses, reflecting a reprioritization of metabolic resources. Similarly, methionine, a precursor for ethylene and S-adenosylmethionine (SAM), peaks in Gadam under reproductive stress, likely due to its critical role in ethylene biosynthesis, which mediates stress signaling and developmental adjustments. The low methionine levels in Nyagem under well-watered conditions align with reduced stress signaling demands, as the plant does not need to activate stress-responsive pathways in optimal conditions.

Valine and isoleucine, branched-chain amino acids vital for energy production and osmoprotection, also show distinct patterns. Valine's highest concentration in Nyagem under vegetative stress suggests its role in maintaining metabolic activity by supplying energy through its catabolic pathways during stress. Meanwhile, its lower levels in KARI Mtama 1 during reproductive stress could indicate a metabolic shift toward other critical amino acids or pathways supporting reproductive development. Isoleucine's peak in E1291 during continuous stress highlights its role in prolonged stress adaptation, providing a steady source of energy and contributing to osmotic balance. Its lower levels in KARI Mtama 1 during reproductive stress might reflect reduced reliance on branched-chain amino acid catabolism as plants prioritize reproductive processes.

In this study, some amino acids were positively associated with the adaptation to drought stress; conforming to the report by Cui *et al.* (2024) where asparagine, phenylalanine, methionine, and serine increased in drought effect on bermudagrass. Significant increase in amino acids due to progressive drought duration has been also been reported in previous studies where proline, leucine, valine, and arginine, were associated with adaptation to water scarcity (Huang *et al.,* 2017). Their accumulations in stems and leaves significantly increased under osmotic stresses. This was attributed to the upregulated expressions of key genes involved in their biosynthesis pathways (Sun *et al.,* 2024).

Similarly, branched-chain amino acids (BCAAs) including leucine, isoleucine, and valine) have been reported to increase higher than proline under drought stresses in hybrid bermudagrass (Sun *et al.,* 2024; Du *et al.,* 2012). The BAAs act as compatible osmolytes and alternative energy resources to enhance the drought resistance of plants (Huang *et al.,* 2017).

Other members of amino acids that are also positively associated with the adaptation of plants to drought or osmotic stress; are asparagine, phenylalanine, methionine, and serine they overcome osmotic stress using transcriptome sequencing and the qRT-PCR method (Pan *et al,* 2016)

CHAPTER FIVE: SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary

Sorghum performs well in semi-arid areas because it is a drought-resistant crop. The ability to perform well in drought areas makes sorghum to be an important cereal crop which can be used to substitute maize in these areas in order to achieve food security. Farmers in the study area planted a diversity of sorghum landraces because of their preferred traits such as high yielding, resistance to drought, good taste, and early maturing. However, the survey report showed that sorghum production in these regions is poor since the majority of farmers usually got low yields from their farms. The low yields could be attributed to the constraints reported by farmers, including drought, low yielding local sorghum varieties, striga weeds, pests and diseases.

Moreover, many farmers do not use fertilizers in their farms because of financial constraints. This creates the need to identify sorghum varieties which can perform well under low fertility. The locally available germplasm could be used to improve sorghum production in these areas. The performance sorghum varieties were affected by drought inducement in both trial sites. Baringo site with more fertile soils recorded higher yields than the less fertile Siaya site. Among the landraces studied, Ochuti and Nyagem sorghum varieties performed better though they did not out compete the hybrids Gadam and Kari Mtama1.Stress durations affected the growth, yields and quality of sorghum grains. The most affected growth stages affected by drought were reproductive and grain filling stages. Re-watering following these stress durations led to non- recovery of sorghum varieties. The effect of drought stress was reflected in low number of grains per panicle, high number of unfilled grains, low total grain weights and reduced shoot biomass.

5.2 Conclusions

The cross-section survey conducted in this study established that drought, pests, diseases and marketing to be the major constraints in sorghum production from the study regions. Increasing production of sorghum in these regions will contribute significantly towards realizing food security. The number of days to anthesis, plant height, shoot dry weight, panicle and grain weight were highly significant in both sites. Grain yields in Baringo site were higher than Siaya site due to environmental effects. Gadam sorghum variety recorded higher yields while Nyagem and Ochuti local varieties followed under stressed conditions in Siaya site. Sorghum has been identified by the farmers as an important crop in ASALs due to its ability to tolerate drought, resistance to pests, less labour and fertilizer requirement, and its ability to ratoon.

The results identified drought-tolerant sorghum varieties capable of maintaining higher grain weights under severe water stress conditions. These varieties demonstrated superior physiological mechanisms, including efficient water use and carbohydrate allocation. Conversely, varieties that performed poorly under stress indicate a need for region-specific selection and breeding programs to enhance drought tolerance.

Drought stress has been identified to affect different sorghum varieties at different growth stages with the post reproductive stages being more critical leading to grain substantial yield loss than the early and vegetative growth stages. Gadam sorghum variety recorded higher yields while Nyagem and Ochuti local varieties followed under stress conditions in Siaya site. The grain weights of Nyagem and Ochuti local sorghum varieties did not significantly differ under stress in Baringo though Nyagem performed better than other local varieties in Siaya. This parameter made Nyagem as a suitable candidate for drought evaluation alongside other recommended sorghum varieties. Based on grain yields, sorghum plants did not recover when exposed to drought stress beyond reproductive growth stage. Among the sorghum varieties under study, Gadam sorghum variety was the most tolerant to drought in both Siaya and Baringo sites.

Drought stress had positive impact on biochemical accumulation. This was indicated by the high proline accumulation under high stress. Continuous and drought up to grain filling stage had significantly higher proline accumulation as compared to well- watered and short stress periods of vegetative and anthesis stages. Drought-tolerant varieties maintained better biochemical stability, such as higher carbohydrate content and efficient protein synthesis, under stress conditions. In response to several kinds of abiotic stress, plants greatly increase their accumulation of free amino acids. Although stress-induced proline increases have been studied the chain amino acids (BCAAs; leucine, isoleucine, and valine)

5.3 Recommendations

It is recommended that farmers select drought tolerant and pest tolerant varieties to enhance production of sorghum and this could be increased by providing extension services on the best agronomic practices and sensitizing people on the benefits of the crop. Increasing production of sorghum in these regions will contribute significantly toward realizing food security.

It is recommended that the varieties like Gadam and KARI Mtama1 based on sites (Siaya and Baringo) and E1291 that are already released be included for cultivation in drought prone areas. The drought tolerant sorghum varieties identified in the study could be used as possible candidates' for further evaluation under controlled environment. Varieties with short growth durations are recommended in drought prone areas to optimize the available moisture resulting from intermittent rainfall.

Physiological cues can be rapid indicators of drought tolerance. It is recommended to give emphasis on physiological characters such as stomatal conductance and SPAD values. The varieties that maintained higher stomatal conductance are the best candidates in drought prone areas.

The biochemical compounds such as proline and other amino acids can be used in choice of drought tolerant varieties. It is recommended to choose the varieties based on elevation of double chain amino acids and compatible solute compound like proline as guide for choosing drought-correct sorghum idiotypes.

More studies are necessary to verify the obtained results that led to difference in performance of varieties under different soil types. For traceability there is need to characterize the collected accessions for ease of identification. Nyagem sorghum variety was identified as a suitable candidate for ASALs of Kenya.

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APPENDICES

Appendix I: Questionnaire

Objectives

i) To assess the production status of sorghum crop as practiced by the farmers.

ii) To document sorghum variety management scenario for designing future strategies of production plan.

iii) To determine the constraints in sorghum production

1. Personal details
   a) Name of respondent……………………………………… b) Age ……..

   c) Gender ……………….. d) Education level ……….. e) Occupation ……………
2. Location details: County ……………. Sub-county………………….

   Location (ward)………………sub-location (Village) …………………..

   Nearest market or town ………………………… GPS--------------------
3. How big is your farm …………………………. (acres)
4. Do you grow sorghum ……………………….. Yes/No
5. If yes, which varieties (indicate landraces or hybrid) ………………………………………………………………………………

6. What traits makes you prefer the sorghum type you grow(list top 3)

(i)---

(ii)---

(iii)---

7. Area(acres) under sorghum---
8. Indicate sorghum yields in bags/unit area (acre)
   ………………………………………………………………..
9. Which other crops do you grow on your farm? List in order of priority

   (i)----------- (ii)------------(iii) ----------- (iv) ----------(v)------------------
10. Which cropping system do you use? a) intercropping b) pure cropping c) mixed cropping

11. What fertilizers do you use for planting? a) organic …… b) inorganic…… c) none………… Type………………………………..

12. What fertilizers do you use for top dressing? --------------------------------
13. Where do you get sorghum seeds for planting? ……………………………. and at what price/kg …………………………

14. Do you separate seed crop from grain crop? YES …………NO ……………
    If yes, how do you manage the seed crop? …………………………………….……………………………………………
    ………………………………………………………

15. Do you grow sorghum under (a) irrigation -------- (b) rainfed………………

16. Indicate intervals of irrigation in days--

17. How long does sorghum crop take to mature? ……………………………….……………………………………………

18. How do you harvest the seed crop? ……………………………………………………….

19. What type(s) of seed treatment or coating do you apply to the finished seed? ……………………………………………………………………………………

20. How do you store your sorghum seed after harvesting? ……………………………………………………………………………………
21. For how long do you store the seed before planting? …………………………………………

22. For what purpose do you plant sorghum crop? (a) Food …………
    (b) Fodder………… (c) Sale………

23. If sale who are your customers--

24. Why do (23 above) prefer your produce-------------------------------------

25. What constraints do you face in sorghum production? List the constraints in order of severity(i most - v least)

    (i)……………………………………………………………………………

    (ii)……………………………………………………………………………

    (iii)……………………………………………………………………………

    (iv) ……………………………………………………………………….

    (v) ………………………………………………………………………

Appendix II: Conference proceedings

Njinju Symon Mugo, Gweyi, J. and Mayoli, R. M. Sorghum Production Challenges in Arid and Semi-Arid Lands: A Case of Siaya and Baringo Counties, Kenya. Paper presented during the proceedings of the Kenya Climate Smart Agriculture Project (KCSAP) Scientific Conference: held at Lake Naivasha Resort. Naivasha, Kenya; 22nd – 26th, November, 2021

Appendix III: Publications

Njinju, S.M., Gweyi, J.O., Mayoli, R.N. (2022). Drought-Resilient Climate Smart Sorghum Varieties for Food and Industrial Use in Marginal Frontier Areas of Kenya. In: Kumar, A., Kumar, P., Singh, S.S., Trisasongko, B.H., Rani, M. (eds) Agriculture, Livestock Production and Aquaculture. Springer, Cham. https://doi.org/10.1007/978-3-030-93262-6_3

Njinju, S. M., Gweyi, J., and Mayoli, R. (2022). Sorghum Production Challenges in Drought Areas of Siaya and Baringo Counties, Kenya. *East African Agricultural and Forestry Journal*, *86*(1-2), 8. Retrieved from https://www.kalro.org/www.eaafj.or.ke/index.php/path/article/view/547

Appendix IV: Parameters

SOV	**Proline content**	**Shoot Dry weight**	**Root Dry Weight**	**Height harvest**	**1000 Grain weight**	**Yield** @13% M	**Spad 4**
Soil type	283.17**	1214.82 **	0.1613	653.3 **	1.08	180.8	478.40 ***
Treatment	409.72 ***	1025.80 ***	5.3516 ***	2442.7 ***	59.85	3742.3 ***	117.19 ***
Variety	66.54	1216.65 ***	2.9972 ***	5746.7 ***	1509.76 ***	6081.5 ***	381.76 ***
Soil type × Treatment	193.99 ***	621.72 **	9.4511 ***	765.6 ***	145.05 **	540.0 ***	6.92
Soil type × Variety	124.35*	319.62	16.4668 ***	385.9 **	100.10 *	173.2	25.30
Treatment × Variety	61.19.	194.77	4.6984 ***	149.0	65.99	496.0 ***	52.23 ***
Soil type × Treatment × Variety	81.63 **	229.32	6.4372 ***	348.7 ***	58.59	230.7 **	27.09 **
Residuals	32.78	148.22	0.0411	80.1	35.78	93.8	10.45

Appendix V: Sorghum Accessions grown in Siaya and Baringo Counties

Variety	Trait	Count	Percent (%)
Adventor	Nutritional value	1	100
Ex KALRO	Drought tolerant	1	100
Ex Kitui	Bird Resist	1	100
Ex ugunja	Bird Resist	1	33.3
	High yield	1	33.3
	Early maturity	1	33.3
Total		3	100
ExKitui	Early maturity	1	25.0
	Resist birds	2	50.0
	Nutritional value	1	25.0
Total		4	100.0
Gadam	Marketability	1	12.5
	Drought tolerant	1	12.5
	High yield	2	25.0
	Early maturing	3	37.5
	Other	1	12.5
Total		8	100.0
H293	Drought tolerant	1	33.3

	Resist birds	1	33.3
	Early maturity	1	33.3
Total		3	100.0
Hela	Early maturity	1	25.0
	Resist birds	1	25.0
	Good colour	1	25.0
	High yield	1	25.0
Total		4	100.0
IESV-24029-54	Early maturity	1	25.0
	High yield	2	50.0
	Nutritional value	1	25.0
Total		4	100.0
IESV-24029-55	Early maturity	1	100
IS 9183	Resist birds	1	100
IS 9184	Resist birds	1	100
KARI mtama	Drought tolerant	1	50
	Early maturing	1	50
Total			100
local	Early maturity	1	25.0

	Resist birds	2	50.0
	High yield	1	25.0
Total		4	100.0
local gus nook	Resist birds	1	25.0
	Drought tolerant	2	50.0
	Taste	1	25.0
Total		4	100.0
local mix	High yield	1	33.3
	Resist birds	1	33.3
	Pest disease resistant	1	33.3
Total		3	100.0
local red	Drought tolerant	3	14.3
	Early maturing	1	4.8
	High yield	1	4.8
	Marketable	3	14.3
	Nutritional value	2	9.5
	Pest disease resistant	3	14.3
	Ratooning	1	4.8

	Resist birds	4	19.0
	Taste	3	14.3
Total		21	100.0
local white	Taste	1	50
	Drought tolerant	1	50
Total		2	100
Nyagem	Resist birds	1	33.3
	Early maturity	1	33.3
	Taste	1	33.3
Total		3	100.0
Nyakabala	Drought tolerant	2	25.0
	Early maturing	2	25.0
	Good colour	1	12.5
	High yield	1	12.5
	Resist birds	1	12.5
	Taste	1	12.5
Total		8	100.0
Nyakitosi	Good colour	1	33.3
	Early maturing	1	33.3

	High yield	1	33.3
Total		3	100.0
Nyaurang'	High yield	1	33.3
	Early maturing	1	33.3
	Pest disease resistant	1	33.3
Total		3	100.0
Ochuti	Resist birds	1	33.3
	Early maturity	1	33.3
	Taste	1	33.3
Total		3	100.0
Ochuti Nyakabala mix	Big heads	1	33.3
	Marketable	1	33.3
	Taste	1	33.3
Total		3	100.0
Ofunji	Early maturity	1	100
Orimba joieje	Resist birds	2	66.7
	Early maturity	1	33.3
		3	100.0
Saitoti	Good colour	1	100

Seredo	Drought tolerant	1	33.3
	Early maturing	2	66.7
Total		3	100.0
Serena	Taste	1	25.0
	Resist birds	1	25.0
	Taste	1	25.0
	Drought tolerant	1	25.0
Total		4	100.0
Sila	Early maturity	1	33.3
	Good colour	1	33.3
	Drought tolerant	1	33.3
Total		3	100.0

Appendix VI: Rainfall Data for Siaya and Baringo

Daily Rainfall (mm) for Siaya site: May-July 2021

(a)

	Date	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
S	**May**	0.1	7	6	11.4	1.8	29.3	2.2	4	11.8	12	2.1	0.1	0	1.4	0	0
B																	1.5
S	**Jun**	0.1	6	0	0	1.5	3.4	0.1	0	0	0	1.5	1.9	0	0	0	0.9
B										10	5				15		
S	**July**	1.3	0	0	0	0	2.1	0.9	0	0	0	0.4	0.1	0	1	0	0
B		5	5														

(b)

	Date	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	**Total**	**Ave**
S	**May**	9.8	0.2	8	0	0	13.2	0.1	0.1	0	1	0	0	0	0	0	**122**	**8**
B		10	18	10			5.0	8.0									**53**	**5**
S	**Jun**	4.8	16	0	0	13	0	0	0	0	0	0	0	3	4	-	**56**	**5**
B												5	5				**30**	**2**
S	**July**	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	**9.1**	**2**
B																	**10**	

NB the records for **(a). Siaya** were obtained from Ngiya mixed Primary school met station Courtesy of Arodi Oginga CDMS Siaya County: **(b). Baringo** from KALRO Perkerra, courtesy of Daniel Biwot.