INFLUENCE OF SOIL MOISTURE LEVELS ON YIELD AND SEED QUALITY PARAMETERS OF BIOFORTIFIED COMMON BEAN VARIETIES

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DECLARATION

This thesis is my original work and it has not been presented for award of a degree in any other university.

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DEDICATION

I dedicate this work to my beloved parents Mr. Bonane Kalihira, Mrs. Baderha Shomberwa and my future fiance Mr. Noly for their endless love, support, encouragement, fervent prayers in my academic work and guidance in life.

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ACRONYMS AND ABBREVIATIONS

AAS:	Atomic Absorption Spectrophotometer
UM:	Upper Midland
PC:	Pot Capacity
ANOVA:	Analysis of Variance
AOAC:	Office Methods of Analysis
CIAT:	International Central for Tropical Agriculture
CTA:	Centre for Tropical Agriculture
CGIAR:	Consultative Group for International Agricultural Research
DRC:	Democratic Republic of Congo
FAO:	Food Agriculture Organization of the United Nations
Fe:	Iron
G:	Gram
Ha:	Hectare
ISTA:	International Seed Testing Association
Kg:	Kilogram
KPa:	Kilopascal
LSD:	Least Significance Difference
N:	Azote
P:	Potassium
PDA:	Potato Dextrose Agar
PPM:	Parts Per Million
SM:	Soil Moisture
Zn:	Zinc
%:	Percentage

ABSTRACT

Common bean (*Phaseolus vulgaris* L.) is mostly cultivated in different areas where seasonal rainfall is inconsistent and soil moisture stress limits grain yield production . This study aimed at identifying biofortified common bean varieties with high seed weight and high seed quality performance under different soil moisture levels. Two trials comprising of five biofortified common bean varieties (PVA, HM21-7, RWR21-54, RWR22- 45 and CDBIOB27) were subjected to 5 soil moisture levels (20; 40; 60; 80 and 100% of the pot capacity) in a split-plot arranged in a complete randomized design at Kabete Field Station, University of Nairobi. At harvest time, data on pod number and number of seeds per pod were taken. Pod weight, seed weight and the rate of seed weight decrease due to water stress were also detemined. Seed lots from greenhouse experiments were used in the laboratory conditions to evaluate seed quality parameters such as germination rate, thousand seed weight, seed vigor index, seed moisture content, seed health, Iron and Zinc content for different biofortified common bean under various soil moisture levels. Variety CDBIOB27 performed poorly for all yield components while variety RWR21-54 performed better than other varieties followed by variety RWR22-45 and HM21-7. No significant differences in seed weight were observed under 100%, 80% and 60% pot capacity while 20% and 40% pot capacity gave the poorest lowest seed weight. All other yield components had significantly low performance under 20% pot capacity followed by performance under 40% pot capacity. The germination rate, seed vigor index and thousand seed weight differed significantly depending on the different biofortified varieties and soil moisture levels. There was no correlation between soil moisture and the seed moisture content. The Iron and Zinc content differed significantly depending on the common bean varieties and soil moisture levels. There was an interaction between soil moisture levels and variety on seed Zinc and Iron content. Overall, variety RWR21-54 performed well and gave the highest seed weight per plot (161.64g), 1000 seed weight (626.53g) and highest Iron content (66.64mg/kg) at 60% pot capacity. The fungus incidence ranged from 14.5-52.5% (*Aspergillus spp*), 6-36% (*Rhizoctonia spp*), and 0-9.5% (*Penicillium spp*) depending on the varieties and soil moisture level. Incidence of *Aspergillus spp* decreased with decreased soil moisture content. The study demonstrates that the soil moisture level can be maintained at 60% without compromising the seed quality, Iron and Zinc content for the investigated biofortified common beans. The general performance indicates that variety RWR21-54 performed better than other varieties at 80% and maintained this performance at 60% soil moisture level. Field trials should be done in different climatic conditions to evaluate the adaptation of variety RWR21-54 and RWR22-45 to different regions.

Keywords: Biofortified Common Bean, Soil Moisture Levels, Seed Quality, Seed Weight

CHAPTER ONE: INTRODUCTION

Background

Common bean, an important food legume crop, is mainly cultivated in areas with inconsistent seasonal rainfall conditions limiting grain yield production (Wortmann *et al.,* 1998; Assefa *et al.,* 2013; Beebe *et al.,* 2013). It is recognized that over 60% of beans in the developing world are cultivated under drought stress with short and unpredictable rain season (Beebe, 2012; Assefa *et al.,* 2013; Rao, 2014). This includes large spaces in Central America, Mexico, and Africa where beans are planted after maize or is intercropped with cereals (Graham et al., 2003). Drought stress and diseases are the principal constraints to bean production causing a reduction of more than 50% of grain yield in eastern and southern Africa (Wortmann *et al.,* 1998). Moreover, drought stress is also contributing to low quality of the seeds (Beebe, 2012).

Seed quality remains an important part of any crop system because it is the source of crop life. Therefore, enhancing seed quality is the basis for agricultural productivity improvement (Louwaars and De Boef, 2012; Etwire *et al*., 2013). Availability and accessibility to improved varieties and quality seed remain a big challenge for seed entrepreneurs (CTA, 2014).

The common bean is a significant food worldwide. Unfortunately, due to biotic and abiotic constraints, its yield is relatively low in many regions where the crop is grown (Fageria *et al*., 2000). In particular it is a big challenge for farmers to buy quality seed of open pollinated varieties including the common bean and other pulses. To contribute to food security and improved nutrition, HarvestPlus, a project of the International Center for Tropical agriculture (CIAT), developed biofortified beans with high concentrations of iron and zinc. Common bean has been improved for key productivity characters and have become more attractive to farmers in terms of nutritional value and adaptability to poor soil fertility and water stress. However, such tolerances can be very different from one genotype to another (Beebe *et al*, 2000).

Beans and other legume crops are affected by many abiotic constraints including drought stress which lead to significant abortion of flowers and pods particularly when it happens during the flowering period (Graham and Ranallii, 1997).

Problem statement

Phosphorus deficiency and limited content of moisture in the soil are two problems limiting the productivity of crops including common bean in Sub-Saharan Africa. This condition is aggravated by climate change warranting the need to select varieties tolerant to water stress (Beebe *et al*., 2013; Margaret *et al*., 2014).

Several authors (Beebe, 2012; Assefa *et al.,* 2013; Rao, 2014) reported that almost 60% of beans in the developing world are cultivated under drought stress. In some cases, drought reduced seed yield from 22 to 80% (Acosta-Gallegos and Kohasashi-Shibata, 1989; Ramirez-Vallejo and Kelly, 1998; Zadraznik *et al.,* 2012). The reuduction was greater when drought was imposed during the flowering stage (Miller and Burke 1983). The situation is more critical where drought stress is severe.

In addition to grain yield loss, drought stress causes poor seed quality when it occurrs during flowering and filling stages (Halterlein, 1983; Farooq et al., 2016; Assefa *et al.,* 2017). The intensity, type and duration of drought stress determine the level of reduction in grain yield (Thung and Rao, 1999; Rao *et al.,* 2016). The effect of water stress is highly variable in response due to interacting factors such as variety and temperature. The expression of most traits of common bean was reduced under water stress conditions including loss of leaf area (Acosta-Gallegos, 1988; Ramirez-Vallejo and Kelly (1998). , There is no doubt as to the importance of water on plant growth and development and that limitation in water availability affect crop productivity (Nahar *et al*., 2011). Therefore, it is crucial to select genotypes adapted to limited soil moisture.

Justification

Adaptation to drought stress include morphological, physiological, and biochemical mechanisms such as deeper root system development, stomatal closure, and improved photosynthate remobilization (Beebe *et al.,* 2011). Increasing yield under drought conditions has been achieved for numerous crops by developing drought-resistant cultivars (Cattivelli *et al.,* 2008; Beebe *et al.,* 2010; Polania *et al.,* 2016).

Development of drought-tolerant common bean cultivars in addition to soil moisture management is a strategic approach to reducing yield loss (Beebe *et al.,* 2013). Therefore, this study focused on the influence of different soil moisture levels on seed quality and yield of different varieties of common bean used in Kenya and the Democratic Republic of Congo.

Objectives

1.1.1. Main objective

This project aims at contributing to increasing yield of biofortified common bean by studying the influence of soil moisture on seed quality and productivity of the crop.

1.1.2. Specific objectives

1. To identify biofortified common bean varieties with high yield and adapted to different soil moisture levels.

2. To evaluate the influence of soil moisture content on seed germination, seed vigor, seed health, and levels of Iron and Zinc in biofortified common bean seed.

Hypothesis

1. There are no varietal differences in yield of common bean varieties under different soil moisture levels.
2. Soil moisture content has no influence on seed germination, seed vigor, seed health, iron and zinc content in biofortified common bean.

CHAPTER TWO: LITERATURE REVIEW

2.1 Production and utilization of biofortified common beans

Micronutrient deficiency, known as "hidden hunger", is a major public health worldwide problem and affects over two billion people. Iron and Zinc deficiency can lead to anaemia, cause alteration of the immune response and decrease output in performance. Iron deficiency affects over 60% of the world's population because of low consumption of this nutrient especially by women at reproductive age and developing adolescents (Welch and Grahan, 2004).

To minimize these issues of deficiencies, biofortification has been developed to ameliorate and preserve the nutritional status of the population (Brigide *et al*., 2014). This strategy was developed to address widespread deficiencies in Fe and Zn that remain prevalent largely in developing countries (Sadeghzadeh and Rengel, 2013). According to Marquez-Quiroz *et al. (*2015), biofortification is a process of increasing concentration of essential elements in the edible portions of staple food plants through soil application and foliar application by adding the elements to irrigation water. Usually, biofortification is focused on micronutrients and vitamins, although potential protein and essential secondary metabolites could be targets for biofortification (Welch *et al*., 2000).

Biofortification refers to enhancing the quality of crops to produce nutritious and safe foods in sufficient and sustainable ways. The biofortification of beans can be achieved through transgenic, conventional, and agronomic approaches (Garg *et al*., 2018). The transgenic approach involves the use of biotechnology. It is favourable to crops that have limited or no genetic variation in nutrient content. The approach includes the transfer and expression of desirable genes from one crop species to another, independently of their evolutionary and taxonomic status (Gascuel *et al*., 2017). The aim is to utilize genes to engineer plant metabolism. These genetic modifications are targeted to disseminate micronutrients between tissues, which enhance concentration, efficiency, and reconstruction. The development of transgenically biofortified crops is cost-effective and efficient in achieving sustainable human well-being by reducing malnutrition (Altman and Hasegawa, 2012). For instance, high lysine maize, high unsaturated fatty acid soybean, high provitamin A and iron rich cassava, and high provitamin A Golden rice are successful examples of transgenic methods whose biofortification enhanced the food crop nutritional level (Hefferon, 2015).

The conventional approach involves crop breeding. It is favourable to crops that have sufficient genotype variation. As parent lines are crossed with recipient line, productivity of desired nutrient and agronomic traits are enhanced, improving the levels of minerals and vitamins in crops (Garg *et al*., 2018). With the increasing concern of health development and food safety, the international community has invested in breeding programs such as the Health Grain Project, Consultative Group for International Agricultural Research, and HarvestPlus that, through this program, focuses on enhancing the productivity of specific nutrients that are essential for the population target (HarvestPlus, 2014). It is the most expedient method because it produces staple food crops that have measurable impacts on eradicating malnutrition and food crisis (Anderson, Saltzman, and Pfeiffer, 2017).

The agronomic approach involves fertilization strategies. It refers to applying mineral fertilizers regularly to the soil in order to increase the solubilisation and mobilisation of the crop from the soil (Garg *et al*., 2018). Simple and inexpensive, agronomic biofortification enhance the contribution of macrominerals to the crop yields. Soluble inorganic fertilizers are applied to the roots of the targeted crop, making the soil available with nutrients (Olaofe and Sanni, 1988).

Beans are important sources of Iron which is absorbed by human for about 1-7% in dietary sources (Brigide *et al*., 2014). Common bean has been a good candidate of biofortification because of rich content of Fe and Zn and other microelements compared to cereals and other crops (Blair *et al.,* 2013). It has been anticipated that biofortified beans could be adapted to growing environments in many african countries, and that one to two million people in DR. Congo and Rwanda would consume it each year (HarvestPlus, 2010).

A bowl of beans is the centrepiece of an East African diet. *Phaselous vulgaris* is commonly used by the people as the major grain legume crop, though it is third in importance after soybean and peanut. It is a valuable source of protein, minerals, and vitamins (Graham and Vance, 2003). Because of its widespread consumption throughout the region, efforts to improve the beans' micronutrient content have led to the need to biofortify the common bean. This process would eradicate the common type of hunger called hidden hunger caused by the lack of essential micronutrients in the diet (Sharma *et al*., 2016).

2.2 Effect of soil moisture on common bean

Changes in the water equilibrium and the quantity of water available in the soil are crucial for crop yield (Kramer and Boyer, 1995). Physiological consequences of water stress are complex and extremely different in responses to related factors (Ramirez-Vallejo and Kelly, 1998). Two major parts of drought resistance have been detected in common bean namely avoidance and tolerance. Avoidance is due to development of lengthy roots and efficient stomatal closure (Barradas *et al.*, 1994). Tolerance mechanism occurs through cellular osmotic adjustment and consists of membrane system protection (Mullet and Whitsitt, 1996). Water stress affects crop growth because of stomatal closure and low intake of CO_2 (Adiku *et al*., 2001).

In most developing countries, common bean yield is less than 900 kg due to water stress (Singh, 2001). Brougthon *et al. (*2003), reported that bean yield can be less than 400 kg when rainfall is lower than 400mm within three months following the sowing date. Such yield is lower than the optimum yield obtained in eastern Africa which is estimated to be 1500 kg.

According to several authors, the effects of drought depend on its magnitude (severe or moderate), genotypes, and the stage of plant development when the stress occurs (Boutraa and Sanders, 2001; Terán and Singh, 2002). Several research findings have shown differences in development between cultivars when they are subjected to drought conditions. Ramirez-Vallejo and Kelly (1998) recorded a decrease of 22 to 71% seed yield when the crop was subjected to water stress.

Halterlein (1983) estimated that short duration of drought negatively affected bean seed quality and yield while Stoker (1974) suggested that loss of yield under drought was mainly due to abscission of flowers and young pods. Acosta- Gallegos (1989) found that morphological characters were negatively influenced by water stress, including loss of leaf surface, leaf reduction and inhibition of expanding foliage. Foster *et al*., (1995) observed reduction of water use efficiency and low harvest index under moderate moisture stress.

Common bean is more sensitive to drought during the reproductive stage (Nielsen and Nelson, 1998). Many researchers have shown that some genotypes of beans are resistant to soil moisture stress (Porch *et al*., 2009; Porch *et al*., 2013).

A number of indirect techniques are used to assess drought tolerance, but seed yield is the best indicator as it represents the harvested product (Ramirez-Vallejo and Kelly, 1998). Drought stress interacts with other constraints including low or high temperature, limited soil conditions and diseases to reduce seed yield (Porch *et al*., 2009).

2.3 Influence of soil humidity to common bean diseases

Kivisi (2015) findings reported that common bean seeds in Kenya are infected by root rot and bacterial blight disease which is likely to result in severe disease infections and low yields. Soil moisture influences bean root rots caused by *Pythium spp*, *Fusarium spp*, and *Sclerotium spp*. *Pythium* usually causes seedling and post-emergence damping off. The pathogen can cause deterioration of hypocotyls and the main root system (Hagedorn and Inglis, 1986). Pods can also be infected and develop a mass of white fungal mycelia if they are in contact with soil.

Symptoms caused by *Fusarium* root rot appear 1-2 weeks after emergence as thin, longitudinal reddish-brown lesions or streaks on the hypocotyl and primary root. Later, the lesions convert to brown and spread to the soil surface and roots are normally killed and detached (Buruchara *et al.*, 2010).

2.4 Seed quality charactistics

The grain during storage is affected by a number of factors such as temperature, humidity, oxygen surplus, bacteria, fungal, insects and rodents. Seed quality is a major factor determining the production of horticultural and field crops (Ferguson *et al* 1991).

Seed quality considers factors such as varietal purity, percentage of germination, freedom from disease, moisture content and weight of seeds. Such seed attributes ensure good germination, rapid emergence and vigorous growth and play a major role in maximizing the productivity of crops (Singh *et al*., 2014).

According to ISTA (2015) and FAO (2012), physical quality, physiological aspects of seed, genetic factors and seed health are four parameters related to seed quality. Physical qualities of the seed are classified according to the minimum number of damaged seed, weed seed, diseased seed, and the uniform size of the seeds in a seed lot while physiological qualities relate to seed performance and include high germination and vigor. Physical parameters can be assessed by visual examination and physiological parameters are determined through laboratory analysis of seed samples.

The germination percentage indicates the capacity of seed to emerge from the soil and to develop a plant in the field under normal conditions for germination (Komotho and Dulloo, 2014). Seed vigor is the ability of seed to emerge from the soil and survive under stressful conditions and to grow quickly under favorable conditions (FAO, 2010). Loss of seed germination capacity is the last stage of deterioration. Seed with high germination rate might have low vigor because of other physiological changes that could have occurred before loss of germination (FAO, 2012).

It is important to assess seed health because the diseases that are originally found in seeds can lead to the progressive development of the disease and reduce economic value of the seed while on the other hand imported seed lots can introduce diseases or pests into new areas (Islam *et al*., 2000).

2.5 Constraints to Common Bean Production

The common bean is the most affordable source of proteins for all East Africans. With nearly four fifth of the population in East Africa who belong to the low income class level, this crop has become an important source of nutrition because it contains iron, potassium, magnesium, zinc, and folic acid (Ssekandi *et al.,* 2016). However, the nutritional and economic importance is hindered by the poor yield efficiency of the crop. In order words, the cultivation of the common bean is affected by the many kinds of biotic and abiotic stresses, whose potential is aggravated by the effects of global warming causing droughts and floods (Pandey *et al.,* 2017).

Nevertheless, market constraints also hinder the production of common bean. For instance, the countries of East Africa suffer from high cost of inputs, low farming revenues, unequal distribution of profits, high interests on credit, price instability, and discrimination in access to inputs. These issues are extended to the poor adoption to new technology, limited technical assistance to farmers, and poor agronomic practices (De Luque and Creamer, 2014). Though the market demand is high due to the popularisation of bean-based products and its quality protein nutrients, the supply is low. The productivity remains inefficient due to constraints at environmental and social levels (Henchion *et al.,* 2017).

In Burundi for instance, a study was conducted with 380 farmers to determine the factors that delay common bean production. The findings unveiled the constraints that delayed production and therefore lowered supply, which included the lack of productive assets, lack of improved varieties, and inadequate use of fertilisers. Common bean is the major staple food in Burundi, but the surged demand of this crop in the country must significantly correlate with the social and agricultural inputs. The results of this study showed that a unit increase in the value of productive assets can generate 10% increase of bean production, the use of one kilogram increase in fertiliser can raise bean production by 10%, and changes in the bean varieties can increase production by 22%. Consequently, the increase of production would contribute to 30% increase in market supply and availability (Birachi *et al*., 2011). The case of Burundi is familiar to many regions in East Africa. In definitive, collective action in land use is required in order to produce more, thus adding marking surplus (Thornton, 2010).

CHAPTER THREE: IDENTIFICATION OF BIOFORTIFIED COMMON BEAN VARIETIES WITH HIGH YIELD COMPONENT AND ADAPTED TO DIFFERENT SOIL MOISTURE LEVELS

Abstract

Sub-saharan Africa is characterized by seasonal rainfall and soil moisture stress which limits the yield of common bean. This research aimed at identifying biofortified common bean varieties with high yield under different soil moisture levels. Two trials comprising five biofortified common bean varieties (PVA, HM21-7, RWR21-54, RWR22- 45 and CDBIOB27) were subjected to 5 soil moisture levels (20, 40, 60, 80 and 100% pot capacity) namely SM1, SM2, SM3, SM4 and SM5, respectively. These were tested using a split-plot arranged in a complete randomized design in a greenhouse. At harvest time, data on pod number, number of seeds per pod, pod weight and seed weight were determined. The percentage reduction of seed weight due to water stress was calculated. Variety RWR21-54 performed better than other varieties for all yield components while CDBIOB27 variety was poor performer for all yield components. Soil moisture levels has significant effect on the performance of the various varieties of common beans. Seed weight was high and significantly different under SM4 and was lower under SM5. All yield components had significantly low performance under SM5 followed by performance under SM4. Varietal performance of RWR21-54 under SM2 or 80% pot capacity was the highest and would be recommended for optimal common bean yield production.

3.1. Introduction

Phaseolus vulgaris L. is an important food crop for human diet (Silva *et al*., 2012; Garden-Robinson and McNeal, 2013) because of high protein, vitamins and mineral content (Broughton *et al*., 2003). It plays a crucial role in soil fertility improvement (Asfaw, 2011), and has socio-cultural value (Dinstel, 2012). In Africa, the Great Lakes Region and East African Community are the major producers (Beebe, 2012; Mushagalusa *et al*., 2016). In these areas, common bean is grown by smallholder farmers under seasonal rainfall disturbance associated with diseases and low soil fertility. Therefore, common bean yields remain low (Wortmann *et al*., 1998; Beebe *et al*., 2013).

Performance of common bean is affected by drought stress which is a major problem in Africa (Acosta-Gallegos and Adams, 1991; Wortmann, 1998; Asfaw *et al*., 2013). Yield reduction ranging from 10-100% were reported (Thung and Rao, 1999; Rao, 2001; Polania *et al*., 2016). Drought stress affects photosynthesis activity and can cause flower abortion, pod drop, reduces seed filling (Masaya and White, 1991) and deteriorate seed quality (Beebe, 2012). Usually, days to maturity are reduced and root length is increased (Asfaw *et al*., 2012). Reductions of yield components were recorded by several researchers when drought occurrs during flowering and post-flowering period (Khaghani *et al*., 2008; Rezene *et al*., 2013). Moreover, Santos *et al. (*2004) reported a reduction of P and N uptake under drought conditions. Reduction of grain yield depends on drought intensity, type and duration of the stress (Thung and Rao, 1999; Rao *et al*., 2016).

Several regions in Africa will suffer from climate change over the next few decades (Jones and Thornton, 2003), which will intensify losses in bean yield (Beebe *et al*., 2013). Hence, drought stress remains a big problem for many crops. Drought problem can be addressed by using irrigation, unfortunately many African farmers lack access to irrigation water because of limited financial resources (Ambachew *et al*., 2016). Thus, this study aimed at evaluating different biofortified common bean varieties for performance under different soil moisture levels.

3.2 Materials and methods

3.2.1 Description of the study site

The trial was carried out at the field station of the University of Nairobi, College of Agriculture and Veterinary Sciences. The site is located at latitude $1^0$14'20'' to $1^0$15'15'' south and longitude $36^0$44' to 36^0 45' east, at an altitude of 1940 meters. The agro-ecological zone of the area is upper midland zone three (UM). Two rain seasons are experienced with an annual rainfall of 1000mm. The long rains season occurs from early March to late May, whereas the short rain period goes from October until the end of December (Jaetzold and Schmidt, 2009). Temperature and rainfall conditions from September 2016 to June 2017 during experimentation are shown in appendix 1.

3.2.2 Greenhouse experiment

Five biofortified common bean varieties from HarvestPlus Project in the Democratic Republic of Congo were grown in plastic pots in a greenhouse and two trials were conducted; the first one from September 2016 to January 2017 and the second one from February 2017 to June 2017. Each experimental pot (16.5cm*30cm in size) contained 5kg of a mixture of topsoil, sand and manure at 3:1:1 ratio. Soil samples used for experiment were collected from Kabete field station in a fallow land. Each experimental plot comprising three seedlings was fertilized with 2g of Di-Ammonium Phosphate (18-46-0) at planting time. Each experimental unit comprised 4 plastic pots in triplicate. A total of 300 plastic pots were used.

Plants were exposed to 5 soil moisture levels (20, 40, 60, 80 and 100% of the pot capacity) 10 days after emergence to maturity. Amount of water was applied until macropores were filled. The amount of water drained from the soil by gravity action was collected 24 hours after into a basin and measured. The water pot capacity (100%) was determined as the difference between the applied water and the collected water (Sibomana *et al*., 2015; Lagat, 2016).

To minimize evapotranspiration pots were covered with a plastic sheet, in order to maintain the soil moisture level at the pot capacity level (100% PC). Four replicated pots were used to calculate the average volume of water to be applied. Other soil moisture levels (80, 60, 40 and 20%PC) were estimated using the amount of water calculated for 100%PC as the reference (Sibomana *et al*., 2015).

Emam *et al.* (2010)'s method was used to determine the amount of water to compensate the loss by evapotranspiration and to restore the appropriate moisture levels. Hence, additions of water to each pot were estimated through the difference in weight at two-day intervals to calculate the total amount of water consumed (litre per plant) by the cultivars under various soil moisture levels. A tensiometer was used to monitor soil water potential and to measure the actual availability of water in the soil. The plants were watered to pot capacity using a watering can (Lagat, 2016). The pot soil moisture was maintained at 100, 80, 60, 40 and 20% of PC for each treatment, respectively.

3.2.3 Data collection and analysis

At harvest time, three plants from each experimental pot were harvested, pod number and seeds per pod were taken. In addition, the pod weight and seed weight per experimental plot were determined. The % decrease of seed weight due to water stress was determined using the following formula (Darkwa *et al*, 2016; Ntukamazina *et al*., 2017) :

$Y_d=(1-Y_t/Y_c)*100$.

Where Y_d is the percentage reduction of seed weight, Y_c is the average seed weight obtained under the control growth which was the soil moisture at pot capacity (100% soil moisture), and Y_t is the average seed weight obtained under each treatment. The data was then processed using Microsoft Excel 2010 and the Analysis of Variance was caried out on yield components using statistical software GenStat (VSN International, 2011). Significance of variation among mean values was analysed using LSD method at p=0.05. The mean value was used as the final value as the experiment was replicated during two different periods.

3.3 Results

3.3.1 Identification of biofortified common bean varieties with high yield components under different soil moisture levels

Pod weight, seed weight, pod number and number of seed per pod showed highly significant differences($p<0.001$) under different soil moisture levels. There was no interaction between varieties and soil moisture levels. This indicated that varieties' behaviors are same at all soil moisture levels (Table 3.1). Varietal yield components are presented in Table 3.2. Means of varietal yield components under different soil moisture levels are presented in Table 3.3.

Table 3.1 Analysis of variance (F-statistics) for yield components

Source of variation	Df	Yield component			
		Number of seed per pod	Pod Weight per experimental plot (g)	Seed Weight per experimental plot (g)	Pod number per experimental plot
Variety (A)	4	8.63***	20.74***	18.31***	10.37***
Soil moisture(B)	4	4.14**	14.96***	20.04***	9.83***
A*B	16	0.90^{ns}	0.51^{ns}	0.79^{ns}	0.87^{ns}
CV (%)		11.8	26.9	26.6	26.3

Cv: coefficient of variation; ***: p<0.001; **: p<0.01; *: p<0.05; ns: non-significant

Table 3. 2 Yield components for different biofortified common bean varieties independent to soil moisture

Varieties	Means			
	Pod number per experimental plot	Pod Weight per experimental plot (g)	Number of seed per pod	Seed Weight per experimental plot (g)
PVA	63.63^{b}	150.62^{b}	4.07^{a}	115.98^{b}
RWR 21-54	79.57^{a}	220.08^{a}	3.95^{ab}	161.64^{a}
HM21-7	64.63^{b}	158.95^{b}	4.06^{a}	116.33^{b}
RWR 22-45	80.77^{a}	168.60^{b}	3.77^{b}	130.06^{b}
CDBIOB27	56.43^{b}	119.27^{c}	3.49^{c}	91.47^{c}
LSD(0.05)	9.93	23.18	0.23	17.16

Means followed by the same letter within the column are not significantly different at 0.05 level of probability, according to LSD: least significance difference

RWR21-54 and RWR22-45 varieties had significantly high pod weight than other varieties. Pod weight was significantly different between and among the varieties. Variety CDBIOB27 had significantly low weight pod while variety RWR21-54 had significantly high pod weight. Regarding seed weight, Variety RWR21-54 had higher seed weight while the low performing variety was CDBIOB27.

Table 3. 3 Yield components of common bean varieties under different soil moisture levels independent to variety

Soil moisture	Means			
	Pod number per experimental plot	Pod Weight per experimental plot (g)	Number of seed per pod	Seed Weight per experimental plot (g)
SM1	70.77^{ab}	176.35^{a}	3.89^{a}	135.27^{a}
SM2	77.57^{a}	188.71^{a}	4.04^{a}	146.41^{a}
SM3	78.80^{a}	187.59^{a}	4.00^{a}	141.18^{a}
SM4	64.33^{b}	149.21^{b}	3.82^{ab}	110.89^{b}
SM5	53.57^{c}	115.66^{c}	3.61^{b}	81.73^{c}
LSD (0.05)	9.93	23.18	0.23	17.16

Means followed by the same letter within the column are not significantly different at the 0.05 level of probability, LSD: least significance difference; SM1 (control): 100% of pot capacity; SM2: 80% of pot capacity; SM3: 60% of pot capacity; SM4: 40% of pot capacity and SM5: 20% of pot capacity.

PVA, RWR21-54 and HM21-7 varieties had significantly higher number of seed per pod compared to RWR22-45 and CDBIO27 which had the lowest. CDBIOB27 variety had significantly low seed weight than other varieties. RWR21-54 behaved better than other varieties for all yield components while CDBIOB27 variety was inefficient performer for all yield components.

Pods number were significantly high for varieties tested in SM2 and SM3 without being significantly different from SM1, SM4 and SM5 where performance was comparatively low. Performance of variety pod weight under SM1, SM2 and SM3 were not significantly different but were different at SM4 and SM5. Seed per pod under SM1, SM2, and SM3 were significantly different from SM5 but not significantly different from SM4. There were no significant differences for seed weight under SM1, SM2 and SM3. However, seed weight was high and significantly different under SM4 and was lower under SM5. All yield components had significantly low performance under SM5 followed by performance under SM4. Varietal performance under SM2 or 80% pot capacity was the highest. Soil moisture levels has significant effect on the performance of the various varieties of common beans.

3.3.1.1 Number of pods for the different biofortified common bean

Based on the pod number per experimental plot, the different biofortified common bean varieties significantly differed ($p<0.05$) from each another. Both RWR22-45 and RWR21-54 were recorded to be the best performing with 79.57 and 80.77 pods per experimental plot, respectively (Figure 3.1). Whereas varieties CDBIOB27, PVA and HM21-7 were found to be significantly different with 56 and 63 and 64 pods per plot, respectively (Figure 3.1).

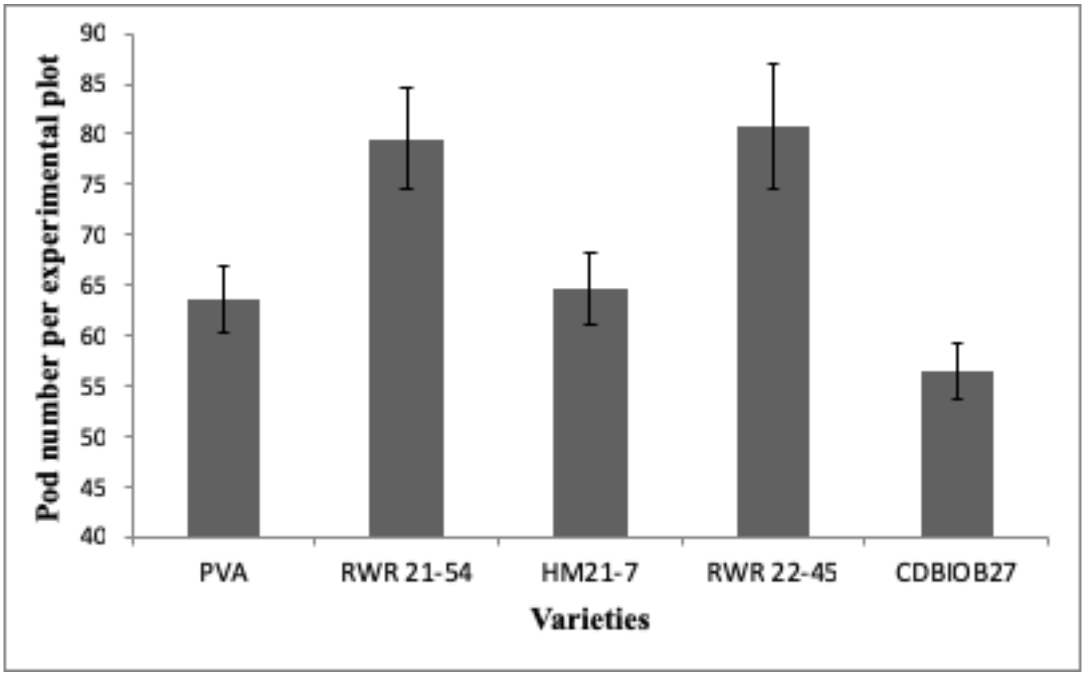


Figure 3.1 Pod numbers for different common bean varieties per experimental plot independent to soil moisture level. Plotted data are mean ± standard error of three replicates.

3.3.1.2. Pod weight per experimental plot for the different biofortified common bean varieties

In this study, pod weight for the different bio-fortified common bean varieties significantly ($p<0.05$) differed and variety RWR21-54 had the highest pod weight (220.08g). Pod weight values for RWR22-45, HM21-7 and PVA varieties were not significantly different and ranged from 150.62g to 168.6g whereas CDBIO27 variety had the lowest pod weight (119.27g). The pod weight in this study varied from 119.27 to 220.08 g depending on the variety (Figure 3.2).

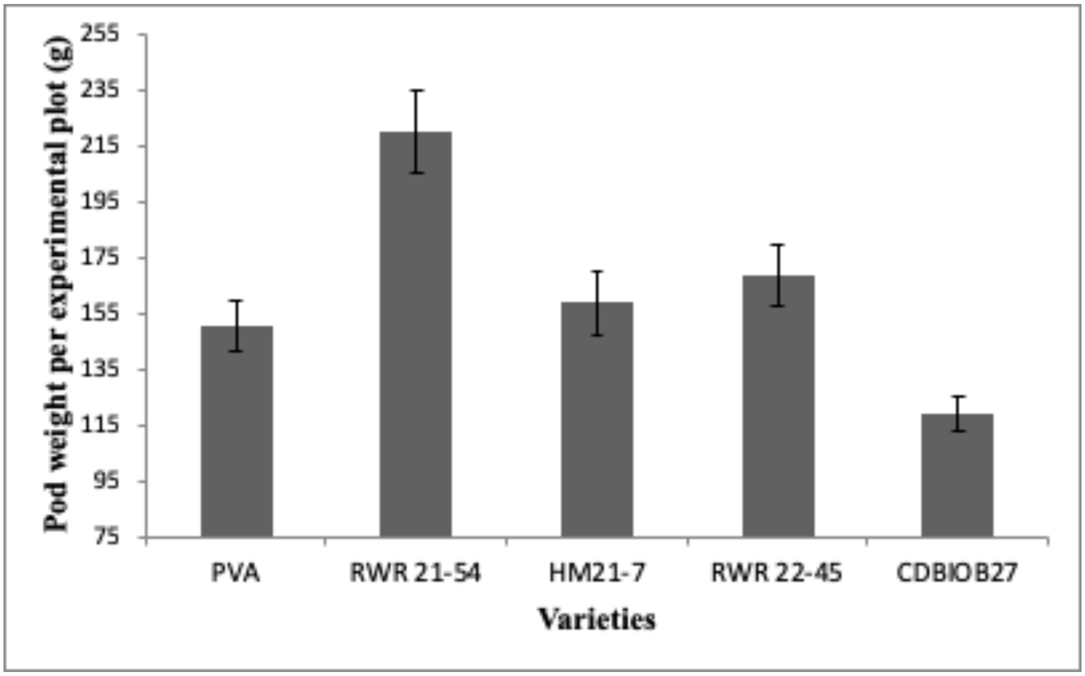


Figure 3.2 Pod weight for different common bean varieties independent to soil moisture level. Plotted data are mean ± standard error of three replicates.

3.3.1.3. Seed number per pod for the different varieties

The results on the number of seed per pod significantly differed ($p<0.05$) from one variety to another. Number of seed per pod ranged from 3.49 to 4.07 and PVA variety had the highest (4.07), followed by HM21-7 (4.06), RWR21-54 (3.95), RWR22-45 (3.77) and CDBIOB27 had the lowest number (3.49) as illustrated in Figure 3.3.

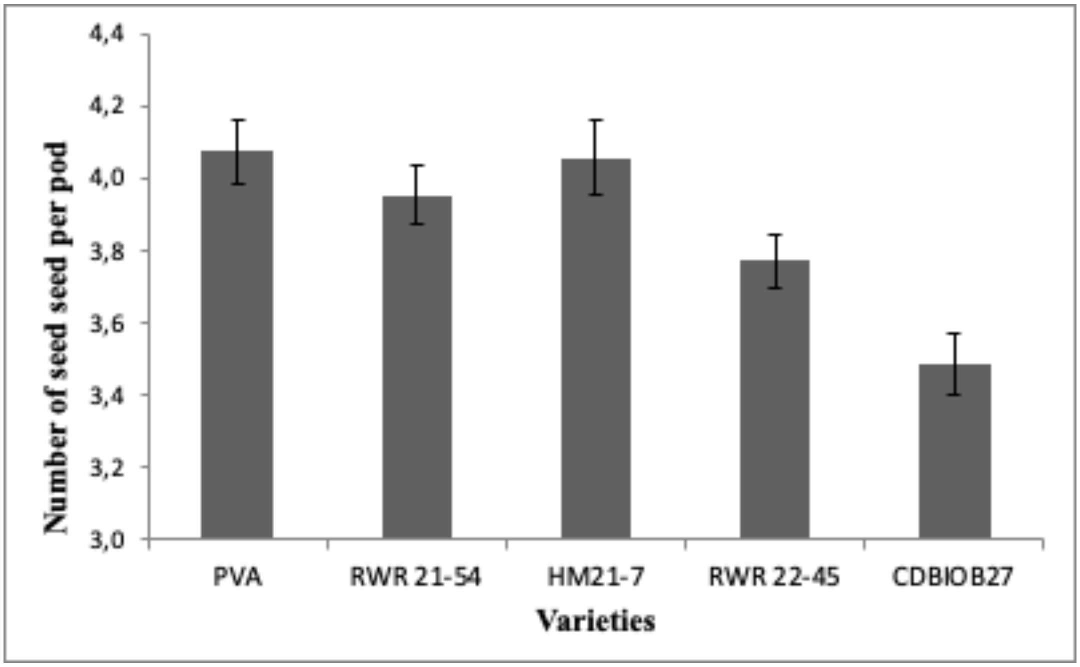


Figure 3.3 Number of seed per pod for different common bean varieties independent to soil moisture. Plotted data are mean ± standard error of three replicates.

3.3.1.4. Seed weight for the different biofortified common bean

Mean values of seed weight for the different biofortified common bean significantly differed among varieties. Variety RWR21-54 had the highest value (161.64g) while variety CDBIOB27 had the lowest seed weight per experimental plot (91.47g). Seed weights for PVA, HM21-7 and RWR22-45 were equal and were significantly different from others (Figure 3.4).

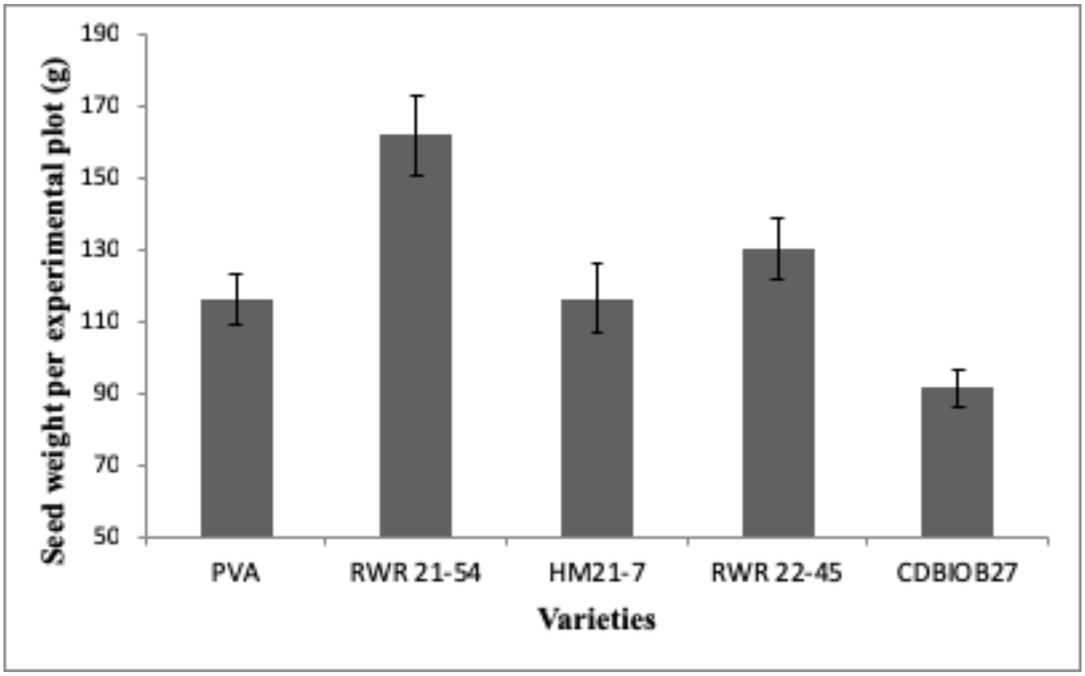


Figure 3.4 Seed weight for common bean varieties independent to soil moisture level. Plotted data are mean ± standard error of three replicates.

3.3.2 Effect of different soil moisture levels on seed yield components

The analysis of variance for varietal performance under various soil moisture levels are presented in Table 3.1. There were significant differences for number of seed and high significant differences for other yield components. Therefore, yield components were influenced by soil moisture levels regardless of varieties. Mean performance of varietal components such as pod number, pods weight, seeds number per pod, and seeds weight under various soil moisture levels are illustrated in Figures 3.5, 3.6, 3.7 and 3.8.

3.3.2.1 Effect of soil moisture level on pod number

Soil moisture level significantly affected the number of pods. The value ranged from 53.57 (20% pot capacity) to 78.80 (60% pot capacity). Soil moisture level of 60% (78.80 pods) and 80% (77.57 pods) pot capacities were highest and significantly different from the others. However, there was insignificant reduction in number of pods at 100% pot capacity. When compared with 80% and 60% moisture levels, 20% and 40% significantly reduced the pod number as illustrated in Figure 3.5.

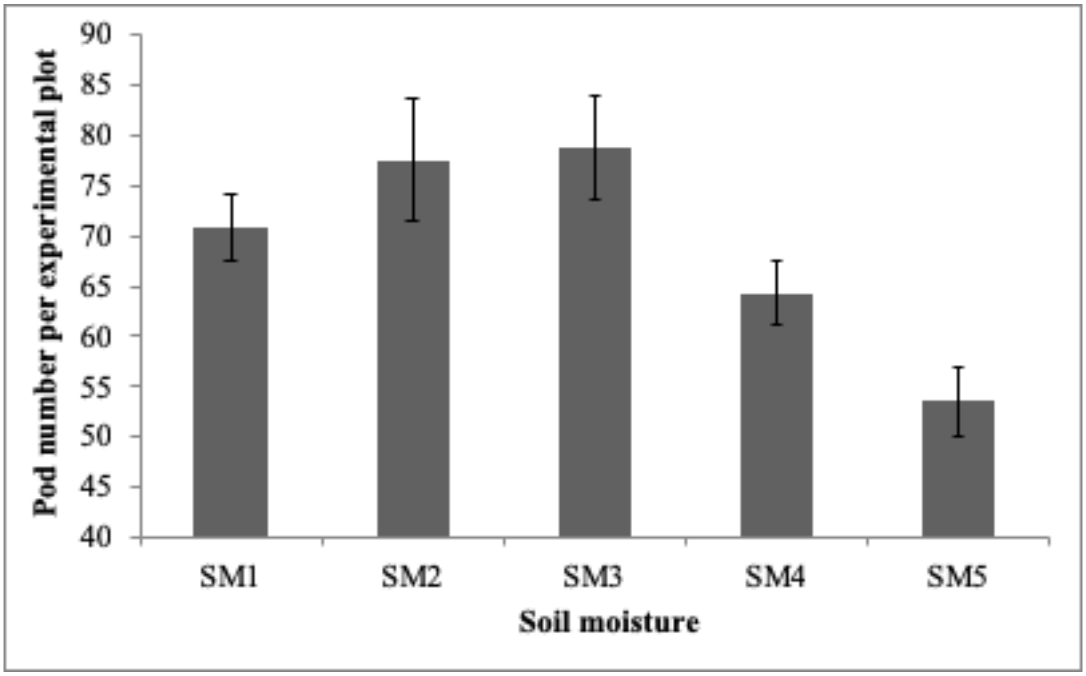


Figure 3.5 Effect of soil moisture level on pod number of common bean independent to variety. Plotted data are mean ± standard error of three replicates. SM1 (control): 100% pot capacity; SM2: 80% pot capacity; SM3: 60% pot capacity; SM4: 40% pot capacity; SM5: 20% pot capacity

3.3.2.2 Effect of soil moisture level on pod weight

The pod weight was significantly affected by soil moisture levels. The 80% (SM2) and 60% pot capacity (SM3) were significantly different from 100% pot capacity (SM1) with 188.71 and 187.59gm, respectively. On the other hand, the 20% pot capacity had the lowest pod weight (115.66g) followed by 40% pot capacity (149.21g) (Figure 3.6).

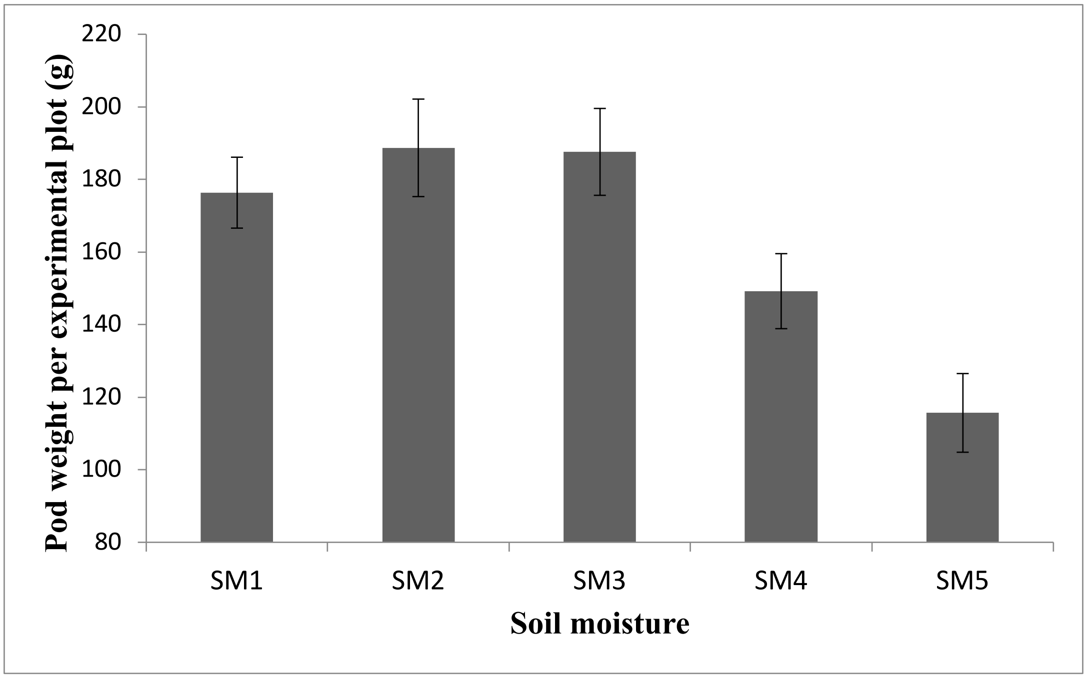


Figure 3.6 Effect of soil moisture level on the pod weight of common bean per experimental plot independent to variety. Plotted data are mean ± standard error of three replicates. SM1 (control): 100% pot capacity; SM2: 80% pot capacity; SM3: 60% pot capacity

3.3.2.3 Effect of soil moisture level on the number of seed per pod

The soil moisture level affected significantly the number of seeds per pod for the different bio-fortified common bean varieties. Mean values for seeds number per pod are illustrated in Figure 3.7. At 80% (SM2) and 60% (SM3) pot capacities, seed number per pod were higher and not significantly different to 100% pots capacity (SM1). A severe water stress (40% and 20% pot capacities) significantly decreased the number of seeds per pod. The lowest number of seed per pod was observed under 20% pot capacity of soil moisture.

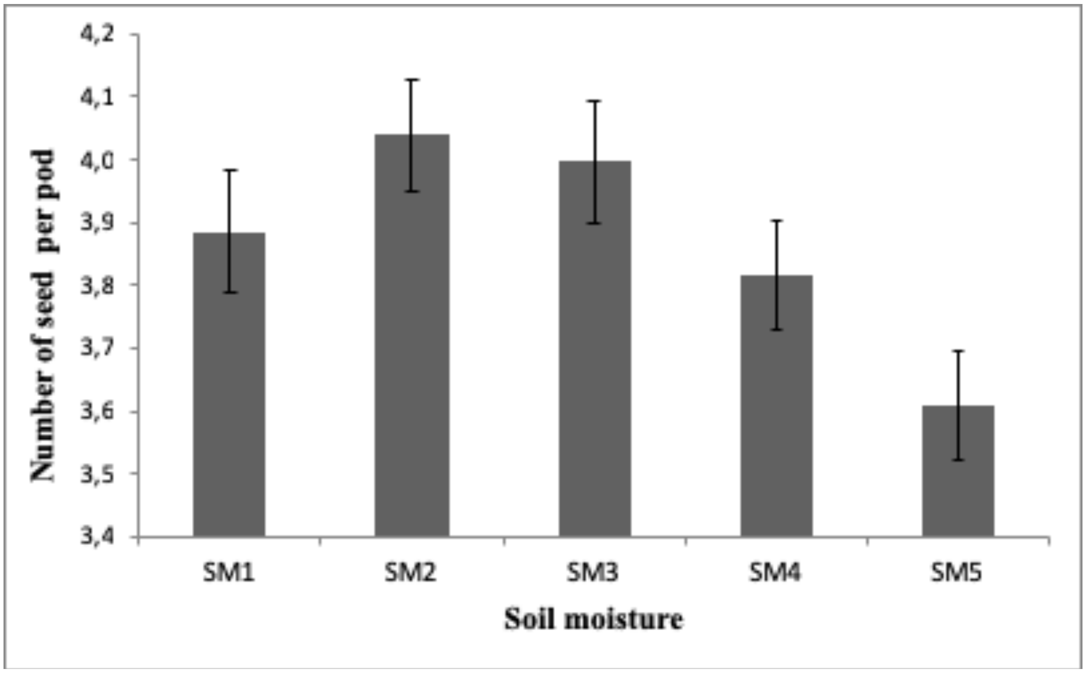


Figure 3.7 Effect of soil moisture level on number of seed per pod independent to common bean varieties. Plotted data are mean ± standard error of three replicates. SM1 (control): 100% pot capacity; SM2: 80% pot capacity; SM3: 60% pot capacity; SM4: 40% pot capacity; SM5: 20% pot capacity

3.3.2.4 Effect of soil moisture level on seed weight

The seed weight for different common bean varieties significantly different and ranged from 81.73g for SM5 (20% pot capacity) to 146.41 for SM2 (80% pot capacity). The 80% (SM2) and 60% (SM3) pot capacities were not significantly different at 100% pot capacity. Moderate water stress (60% pot capacity) didn't affect seed weight. Therefore, increasing the severity of water stress up to 20 or 40% pots capacities decreased seed weight (Figure 3.8).

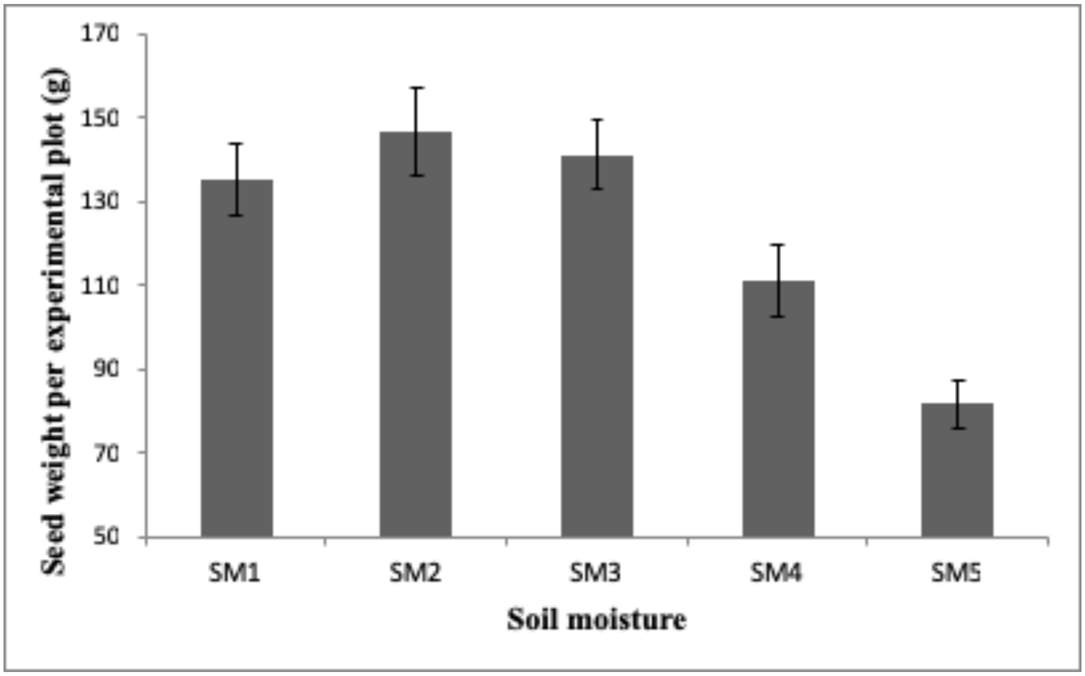


Figure 3.8 Effect of soil moisture level on seed weight of common bean per experimental plot independent to variety. Plotted data are mean ± standard error of three replicates. SM1 (control): 100% pot capacity; SM2: 80% pot capacity; SM3: 60% pot capacity; SM4: 40% pot capacity and SM5: 20% pot capacity

3.3.2.5 Effect of water stress on seed weight

Percentage reduction due to water stress is presented in table 3.4. Average means for varietal seed weight under various soil moisture levels were used for calculation.

Seed yield loss increased with decreased soil moisture levels and ranged from 5-47.4% depending on the varieties and the soil moisture level. All varieties were affected by drought stress (40 and 20%). On other hand, no reduction of seed weight was observed under 80 and 60% pot capacity of soil moisture. However, decreasing soil moisture from 100% pot capacity to 80 and 60% pots capacities increased seed weight per experimental plot.

Table 3. 4 Percentage reduction of yield due to water stress on seed weight.

Variety	SM2	SM3	SM4	SM5	Mean
PVA	-1.2	1.9	15.0	35.7	10.28
RWR21-54	-24.6	-5.3	5.0	37.5	2.5
HM21-7	3.0	-14.5	29.7	47.4	13.12
RWR22-45	-17.3	-7.3	18.5	36.4	6.06
CDBIO27	6.1	5.9	26.5	41.8	16.06
Mean	-6.2	-3.86	18.94	39.76	

SM2: 80% pot capacity; SM3: 60% pot capacity; SM4: 40% pot capacity and SM5: 20% pot capacity. Negative value indicates increasing of seed weight while positive values indicates a decrease.

At 60% pot capacity (SM3), the highest reduction of seed weight was observed for CDBIOB27 followed by PVA. The lowest reduction was observed for RWR21-54 (2.5%) followed by RWR22-45 (6.06%) which appeared to be tolerant varieties compared to others.

RWR21-54, HM21-7 and RWR22-45 varieties managed to maintain their seed yield without loss, whereas PVA and CDBIOB27 varieties recorded up to 6% seed weight decrease at moderate water stress (SM3). The low performing variety was CDBIOB27 at all soil moisture levels. All tested varieties recorded seed yield loss when the soil moisture level decreased up to 40% pot capacity (SM4) and the seed yield loss was much higher (35-47.4% seed weight loss) for 20% pot capacity (SM5) for all the varieties. Forty percent pot capacity is considered as the beginning of severe water stress, the best variety at this level was RWR21-54 as the yield loss was only 5%.

3.4 Discussion

There were highly significant differences among soil moisture levels and varieties for yield components. There were no interactions observed between the two factors for most yield components including number of pods per plant, seed number per pod and seed weight. At moderate water stress (60% pot capacity), yield reductions due to water stress were higher in CDBIOB 27 followed by PVA varieties which seems to be susceptible varieties. RWR21-54, HM21-7 and RWR22-45 varieties appeared to represent valuable genetic sources for drought resistance. Ambachew *et al. (*2015) reported that yield components performance in common bean are correlated to the genotype under drought conditions. Several studies revealed a positive and significant genotypic (Rezene *et al*., 2011) and phenotypic (Singh, 2001; Beebe *et al*., 2008) correlations between pod harvest index and seed yield under non-stress and stressful environments (Assady *et al*., 2005; Rezene *et al*., 2011; Sadeghi *et al*., 2011).

Ambachew *et al*. (2015) reported that pod numbers are correlated positively with seed yield. In this study, variety RWR22-45 as well as RWR21-54 that were producing higher pod numbers also maintained high flower set and yields (Rezene *et al*., 2011). Variety is categorized as high, medium, or low yielding if it has 16-20, 10-15 or 10 pods per plant, respectively. Differences observed are attributed to varietal characteristics when other environmental factors are uniform (Katungi *et al*., 2015). Pod weight in this study varied with the varieties and was associated with vegetative growth in each variety. However, genotypes with less vegetative growth might be more efficient in yield per plant (Emam et *al.*, 2012). Abiot (2018) findings reported a significant effect of soil moisture levels on vegetative growth of common bean. In his study, 75% field capacity was found to be the best level to minimize water wastage. In this study, vegetative parameters were not included but as confirmed by Sadeghipour (2012), effects of water stress on seeds weight depends on the stage at which the stress is occurred.

From the study by Acosta-Gallegos *et al. (*1989), number of pods was most adversely affected by water stress and was correlated with seed yield. Darkwa *et al. (*2016) research reported a reduction of pod number and seeds number per pod leading to yield reduction of 29.8% in the drought stress relative to no-stress treatment. In the present study, data showed a reduction of yield weight ranging from 1.9% to 47% depending on the level of water stress. Severe water stress treatments (40% and 20% pot capacity) caused a reduction in yield components for all varieties compared with 100% pot capacity. Yield components were higher at 80% pot capacity soil moisture than 100% which was used as the control.

The significant reduction of seed per pod is likely to be due to excess moisture at 100% pot capacity (SM1) and the lack of water for 40 and 20% pot capacities. Pod development and seed filling stages are recognized to be more responsive to drought (Darkwa *et al*., 2016). Emam *et al*. (2010) as well as Miorini and Saad (2012), reported that yield reduction under water stress conditions might be associated to lower number of pods due to flowers abscission and embryo abortion related to low photosynthesis activity (Rezene *et al*., 2011). Positive correlation of seed weight with various other yield components have been reported by Sofi *et al.* (2014). This is true for performance of all varieties evaluated under 20% soil moisture levels in this study. Effects of drought depend on timing when the stress occurred, genotypes, and magnitude of the stress (Frahm *et al*., 2004).

At 60% pot capacity which was the moderate water stress, all yield components were stable and comparable to the yield components under 100% and 80% soil moisture levels and differed among varieties. The significant effect of the soil moisture levels and varieties for the various yield components with no interaction between the two factors indicated that the expressions of the varieties across the various soil moisture levels was static and responsive. This result is consistent with Asfaw and Blair (2014) who reported differential response of common bean varieties to drought stress which caused early maturity of flowers and poor pod-partitioning. Pod setting and flowering are reported to be sensitive to drought and water logging respectively (Ntukamazina *et al*., 2017).

Reduction of seed weight was recorded for all varieties evaluated under 20% of soil moisture. The reduction in seed yield is related to low number of pods, low number of seeds per pod and it is consistent with Mathobo *et al. (*2017) findings. Positive and significantly correlation of seed weight with leaflet length, pod width, and seed width have been mentioned. Negative correlation with day to flowering, seeds per pod and pods per plant was observed by Rana *et al*. (2015). The findings were consistent with those of Asfaw and Blair (2014), who reported significant reduction in all yield components of common bean under water-stress conditions.

Data under 20% and 40% soil moisture levels were consistent with Schneider *et al.* (1997) and Rosales-Serna *et al. (*2004) findings on common bean under water stress. Effects of drought depend on timing when the stress occurred, genotypes, and magnitude of the stress (Frahm *et al*., 2004). Reduction in seed weight under 20% and 40% soil moisture levels suggested that water stress accelerated maturity and resulted in the development of small seeds. Variety HM21-7 has been classified by Lubobo *et al. (*2016) as drought resistant in South-Kivu province in the Democratic Republic of Congo. In the present study, variety RWR21-54 had a good performance under favorable moisture conditions (80%) and also displayed high yield potential under moderate water stress (60%) than other varieties. The results from this study suggest that varieties RWR21-54, and RWR22-45, be subjected to adaption tests and yield tests with objective of releasing them for cultivation. Variety HM21-7 be used for developing drought tolerant lines.

CHAPTER FOUR: EFFECT OF DIFFERENT SOIL MOISTURE LEVELS ON SEED QUALITY PARAMETERS OF BIOFORTIFIED COMMON BEANS

Abstract

Erratic rainfall and diseases remain a big challenge for common bean production. This research aimed at evaluating the effect of different soil moisture levels on common bean seed quality. Seed lots from previous experiments comprising five common bean varieties were subjected to 5 soil moisture levels (100%, 80%, 60%, 40% and 20% of Pot Capacity) and were used for seed quality evaluation under laboratory conditions. Germination test and seed vigor index were evaluated using ISTA rules (2015). Seed moisture content was evaluated using the low constant temperature oven method and agar plate method was used for detecting seed borne pathogens. Iron and Zinc content were analyzed by atomic absorption spectrophotometer. Germination rate, seed vigor index and thousand seed weight (TSW) had very high significant differences among common bean varieties while soil moisture level affected only the TSW. Fe and Zn content differed significantly depending on varieties and soil moisture levels. RWR21-54 variety performed well and gave the highest TSW (626.53g) and highest Iron content (66.64mg/kg) at 60% pot capacity. Germination rate of this variety was low probably because of seed borne diseases. The incidence of fungal diseases differed significantly among varieties and soil moisture levels. The study demonstrated that the soil moisture level at 60% did not compromise the seed quality. In contrast, soil moisture of 100%, 40% and 20% pot capacity compromised common bean seed quality.

4.1 Introduction

Common bean is well known for high-protein content and plays a major role in human diet (Beebe, 2012; Broughton *et al*., 2003; Garden-Robinson and McNeal, 2013). Grain legumes are affected by environmental stresses (Vyas, 2014). Among them, water stress plays a major role in limiting crop productivity (Fang *et al*., 2010). Drought reduces yield by decreasing leaf development (Emam *et al*., 2005).

Common bean is cultivated under rainfall season and it is subjected to water stress in many areas (Souza *et al*., 2003; Zlatev and Stoyanov, 2005; Machado and Durães, 2006). Around sixty percent of common bean production occurs under significant drought stress in developing world. Consequently, common bean yields remain low in many countries (<900Kg/ha) (Graham and Ranalli, 1997; Singh, 2001). Crop growth can be affected by drought stress at any stage of development which can result in grain yield loss. Water stress during reproduction as well as grain filling is more devastating. Aggressivity of water stress depends on prolongation and intensity of the stress, genetic trait and crop stage (Farooq *et al*., 2016).

In bean production, water stress during flowering stage results to a lower percentage of pod formation due to embryos abortion (Emam, 1985). Generally, grain yield decreases as the number of days under drought increases (Emam and Seghatoleslami, 2005; Zlatev and Stoyanov, 2005). Furthermore, common bean cultivars appear to respond differently to soil moisture stress (Szilagyi, 2003; Zlatev and Stoyanov, 2005).

Water stress accelerates seed maturity and reduces nutrient content. A considerable reduction in seed protein content was observed in beans subjected to drought (Ghanbari *et al*., 2013). Therefore, developing strategies to reduce water stress in grain legumes is important to reduce yield loss. Genotypes with high water-use efficiency have been developed to improve yield in dry environments (Ulemale *et al*., 2013). Shorter duration is also among technologies developed for adaptation to water stress (Beebe *et al*., 2013; Farooq *et al*., 2016).

Fungi, especially *Penicillium* and *Aspergillus* are associated with post-harvest losses (Marcenaro and Valkonen, 2016). Special attention is given to *Aspergillus*, *Penicillium* and *Fusarium* as they are the major mycotoxin producers in seeds (Bragulat *et al*., 2008; Bhat *et al*., 2010). *Aspergillus flavus* is given more focus due to its production of aflatoxin which is known to be carcinogenic (Frisvad, 1995; Samson *et al*., 1995). This research aimed at evaluating the effect of different soil moisture contents on the quality of common bean seed.

4.2 Material and Methods

4.2.1. Sample collection and preparation

Two previous experiments comprising 5 common bean cultivars subjected to 5 soil moisture levels (100%, 80%, 60%, 40% and 20% of Pot Capacity) were done in the greenhouse. A split-plot arranged in a complete randomized design was used and seed lots produced were used for seed quality analysis in laboratory conditions.

4.2.2. Determination of germination rate

Germination test was done in the laboratory using paper towel method with 200 seeds in four replicates of 50 seeds from each seed sample . Samples were randomly selected from all treatments and sterilized in 2% sodium hypochlorite for three minutes, rinsed in three changes of sterile distilled water and blot dried on sterile paper towel. Inside the sandwich box, three layers of absorbent paper towel were placed, moistened with sterile distilled water and the seeds were sown on the paper towel. Two layers of paper towels were placed on top of the seeds and then moistened with sterile distilled water. The sandwich boxes were closed and the seeds incubated under natural source of light. Germination count was recorded every two days for 10 days after sowing (Wareham *et al*., 1996; ISTA, 2015). Germination percentage was expressed based on normal seedling as:

$$\text{Germination rate (\%)} = \frac{\text{Number of germinated seeds (normal seedlings)}}{\text{Total number of seeds}} * 100$$

4.2.3. Determination of seedling vigor index (SVI)

From the seed reserved for the germination test, randomly selected samples of 20 normal seedlings from each treatment in all replication were used for seedling vigor. Seedling length was measured from the tip of primary leaf to the root tip and the mean seedling length was expressed in cm. SVI was determined by considering the germination % and seedling length of the same seed lot. Normal seedlings were counted and seedlings' lengths of 5 randomly selected seeds were measured. The following equation was used according to Adebisi *et al., (*2013).

$$\text{SVI} = \frac{\text{Germination (\%)*mean of seedling length}}{\text{Number in seed lot}}$$

4.2.4. Determination of seed moisture content

Moisture content of seed is the quantity of water in a sample expressed as a percentage of the weight of the original sample (Alberta Government, 2016). Seed moisture content was evaluated using the low constant temperature oven method: 103°C for 17 hours, according to ISTA (2015). The wet weight basis was applied. To dry the sample, the following procedure was used according to Reeb et Milota (1999) and Desai (2004). The plate was weighed and recorded as tare, then 8-10gram of seed was weighed and the sample was dried at ±103° C for 17 hours in the oven. Finally, sample was allowed to cool in an incubator for 15 minutes before weighing and the moisture content was calculated as:

$$\text{Seed moisture content} = \frac{(\text{Weight of the fresh seeds} - \text{Weight of dry seeds})}{\text{Weight of fresh seed}} * 100$$

4.2.5. Thousand seed weight determination

Samples of 400g were divided into four replicates of 100g each. Each fraction from each replicate was weighed separately and the thousand seed weight was determined using (ISTA, 2015) as follows:

TSW=(weight of seeds in pure sample/Number of seeds in pure sample)*1000

4.2.6. Determination of Iron and Zinc content

The beans were harvested and dried at 105°C for 2hours. This was milled using a mill model PX-MFC 90D, manufacturer: POLYMIX. Beans flour was ready for analysis using dry ashing procedure. Minerals were analyzed using the AOAC (1999) method. A sample of 0.5-1.0 grams were weighed in a porcelain crucible and put in the muffle furnace at a temperature of 450^0C for 2hours. It was allowed to cool, and the ash dissolved in 5mL 6M hydrochloric acid and mixed together. The acid was evaporated on a hot plate at 100^0C. The residue was dissolved in 0.1M HNO_3 and volume was topped up after 15-20minutes using distilled water, mixed and then filtered using a filter paper.

Iron and Zinc content were analyzed by Atomic Absorption Spectrophotometer (AAS) model AA 6300230V, Serie no: A30524602445AE, manufacturer: Shimadzu Corp. The calibration curve for the determination of Iron levels in the bean samples by AAS was established using the procedure by Okalebo *et al.* (1993). A series of working standard solutions were prepared from the stock solution (50 mg/kg Fe) as follows. In a clean set of 100ml volumetric flask, 0, 2,4,8,12,16 and 20ml were pipetted and topped up by adding distilled water. This working standard series contained 0, 1, 2,4,6,8 and 10 mg/kg Fe respectively. The working standard series were aspirated into the Atomic Absorption Spectrophotometer calibrated for Iron measurement at wavelength 248.3nm and the calibration curve was evaluated from the absorbencies of the standard series and the sample were read.

Zinc determination procedure was similar to that of Iron. A series of working standard solutions were prepared from the stock solution (50 mg/kg Zn). In a clean set of 100ml volumetric flask, 0, 1,2,4,8 and 10ml were pipetted and topped up by adding distilled water. This working standard series contained 0, 0.5,1,2,4 and 5 mg/kg Zn and aspirated using wavelength 213.9nm and the calibration curve was evaluated from the absorbencies of the standard series.

4.2.7. Determination of seed health status

Agar plate method was used for detecting seed borne pathogens. Sample were randomly selected from all treatments and sterilized in 2.5% sodium hypochlorite for three minutes, rinsed three times in sterile distilled water and blot dried on sterile paper towel. The Potato dextrose agar (PDA) medium amended with streptomycin antibiotic was dispensed into 9cm plastic Petri dishes under sterile conditions in laminar flow cabinet. Using sterile forceps, five seeds were plated on the surface of non-solidified agar medium.

Samples were incubated at 20°C in the darkness for 5-7 days. Each fungal type presented was then sub cultured on PDA to obtain pure cultures. Two techniques; visual observation in Petri dishes and microscopic observation were used for identification of fungi. Growth and colony appearance were examined every day. Identification of fungi was done based on microscopic features at magnification x400 power and morphological characteristics such as hyphal septation, conidial shape and size were examined (Domsch et *al*., 1980; Mathur and Kongsdal, 2001; Bhale *et al*., 2003).

Number of seeds infected in each Petri dish was counted and used to determine the incidence of each pathogen as follows :

$$\text{Percentage infected seed} = \left(\frac{\text{Number of infected seed per plate}}{\text{Number of total sample}}\right) * 100$$

4.2.8 Statistical analysis

The Analysis of Variance (ANOVA) was caried out on seed quality parameters using statistical software GenStat (VSN International, 2011). The significance of variation among mean values was analysed using LSD method at p=0.05. The mean value was used as final value since the experiment was repeated for two seasons. Spearman's correlation was applied to analyse the relationship between soil moisture and micronutrient content (Iron and Zinc) of common bean seeds.

The statistical analyses of disease score was carried out using Stata 15.0 software (StataCorp, 2017). Generalized Linear Models (GLM: Poisson and negative binomial families) were used to investigate the effect of varieties and soil moisture as explanatory variables on the number of infected seeds. Predictions of incidence were performed after each GLM procedure in Stata software. Standards of linear predictor were used to compute confidence intervals (IC) of the predicted incidence and plotted for multiple comparison.

4.3. Results

4.3.1. Effect of soil moisture on common bean seed quality

There were very high significant differences among varieties independentely to soil moisture level for all parameters except for seed moisture content (MC). There were no significant differences for seed quality parameters in all soil moisture levels except for TSW. There were no interactions of varieties with soil moisture levels (Table 4.1). Means of germination rate, seed vigor index and thousand seed weight in various soil moisture levels are presented in Table 4.2 .

Table4.1 Analysis of variance (F-statistics) for seed quality parameters

Source of variation	Df	Seed quality parameters			
		GR	SVI	MC	TSW
Variety (A)	4	8.09***	32.25***	0.36^{ns}	24.57***
Soil moisture (B)	4	2.02^{ns}	1.12^{ns}	0.76^{ns}	2.68*
A*B	16	0.96^{ns}	1.45^{ns}	0.76^{ns}	1.40^{ns}
CV (%)		17.4	33.8	29.7	9.12

GR: Germination rate; SVI: Seed Vigour Index; MC: Seed Moisture Content; TSW: Thousand Seed Weight. Cv: coefficient of variation; ***: p<0.001; **: p<0.01; *: p<0.05; ns: non-significant.

Table 4.2. Mean performance of seed quality parameters for various common bean varieties independent to soil moisture level.
Table

Varieties	GR (%)	SVI	MC (%)	TSW (g)
PVA	92.5^{a}	593.75^{bc}	8.99	528.37^{cd}
RWR 21-54	75.5^{b}	512.17^{c}	9.22	626.53^{a}
HM21-7	89.9^{a}	620.75^{b}	9.05	544.77^{c}
RWR 22-45	86.0^{a}	679.32^{b}	8.54	512.70^{d}
CDBIOB27	90.6^{a}	1051.13^{a}	8.90	585.20^{b}
LSD (0.05)	6.6	103.16		26.07

Means followed by the same letter within the same column are not statistically significant at p=0.05. GR: Germination rate; SVI: Seed Vigor Index; TSW: Thousand Seed Weight.

Variety RWR21-54 had the lowest germination rate (75.5%) compared to other varieties. Considering seed vigor index, variety CDBIOB27 had the highest while RWR21-54 did not perform well but recorded a higher thousand seed weight (626.53).

4.3.1.1. Germination rate for different common bean varieties

The germination rate for the different common bean varieties differed significantly

($p<0.05$) from one variety to another and ranged from 75.5 to 92.5%. Varieties PVA, CDBIOB27, HM21-7, and RWR22-45 had 92.5%, 90.6%, 89.9%, 86% germination rate respectively while variety RWR21-54 had the lowest (7.55) (Fig. 4.1).

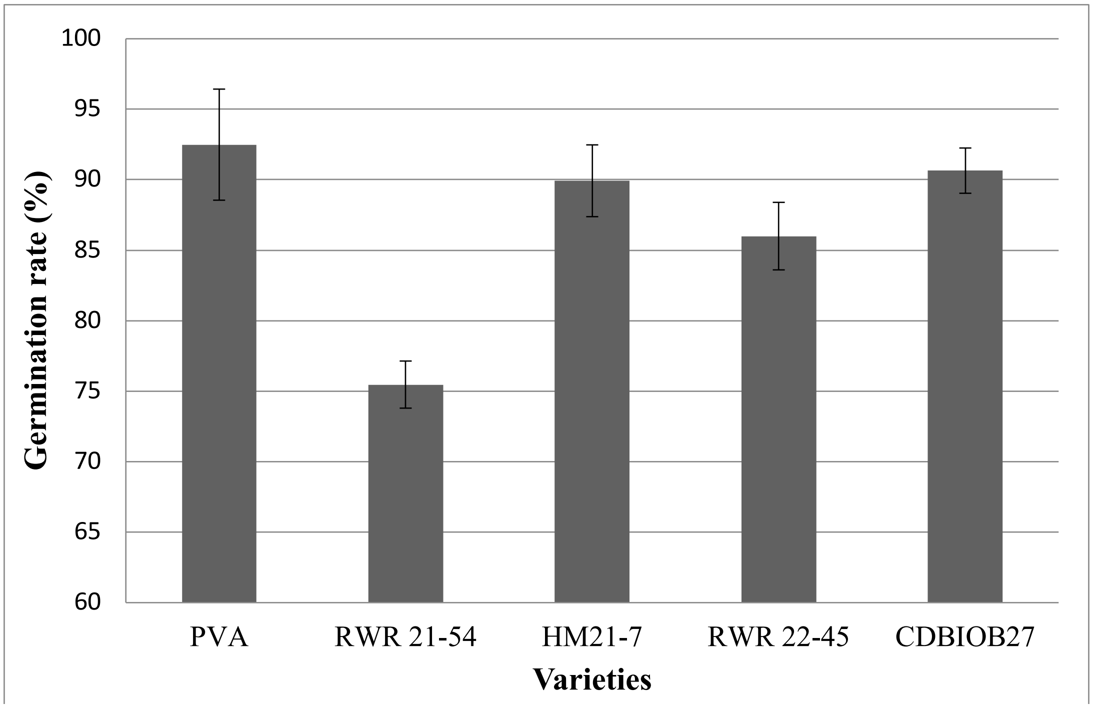


Figure 4.1 Germination rate for different bean varieties independent to soil moisture level.

4.3.1.2. Seed vigor index for the different biofortified common bean varieties

Variety CDBIOB27 had the highest SVI (1051.13) and was significantly different from the other. RWR21-54 had the lowest SVI than CDBIOB27 but not significantly different from PVA. Overall, variety CDBIOB was the highest in SVI than all other varieties and variety RWR21-54 had the lowest (Fig 4.2).

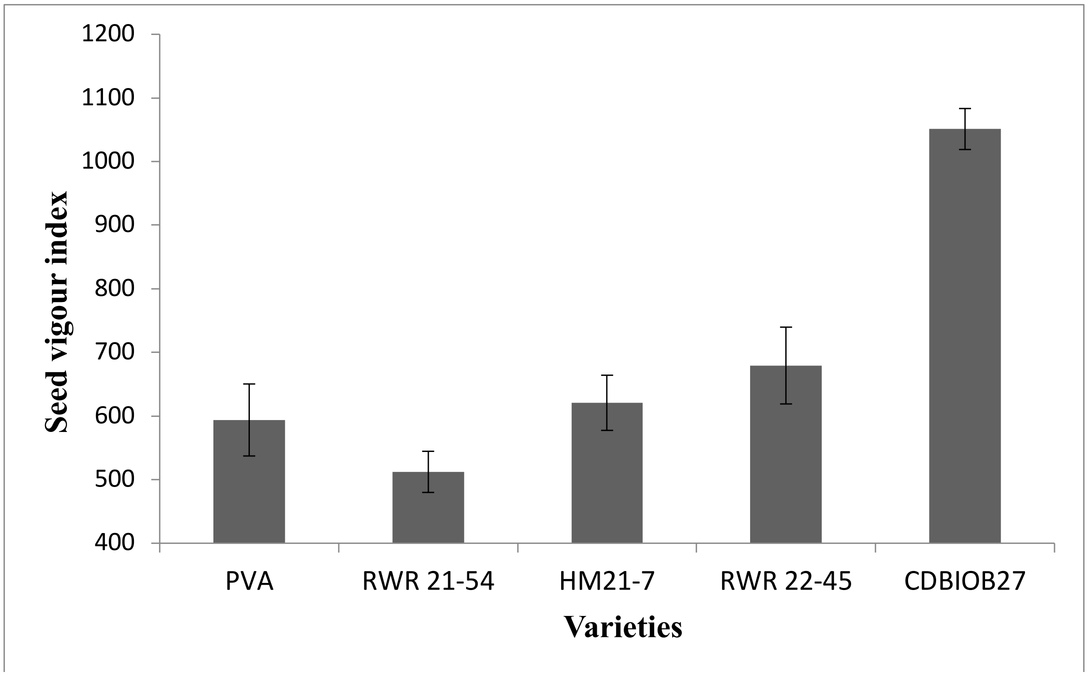


Figure 4.2 Seed vigor index for different common bean varieties independent to soil moisture level.

4.3.1.3. Thousand seed weight for common bean varieties under different soil moisture level

Results on the seed weight indicated significant differences ($p<0.05$) among common bean varieties. Thousand seed weight of variety RWR21-54 (626.53g) was significantly higher than all the others. The mean TSW of variety RWR22-45 (512.70g) was significantly lower than RWR21-54, HM21-7, and CDBIO27 but not significantly different to PVA. Thousand seed weight for varieties RWR22-45 and HM21-7 were significantly different but not significantly different from variety PVA (Fig 4.3).

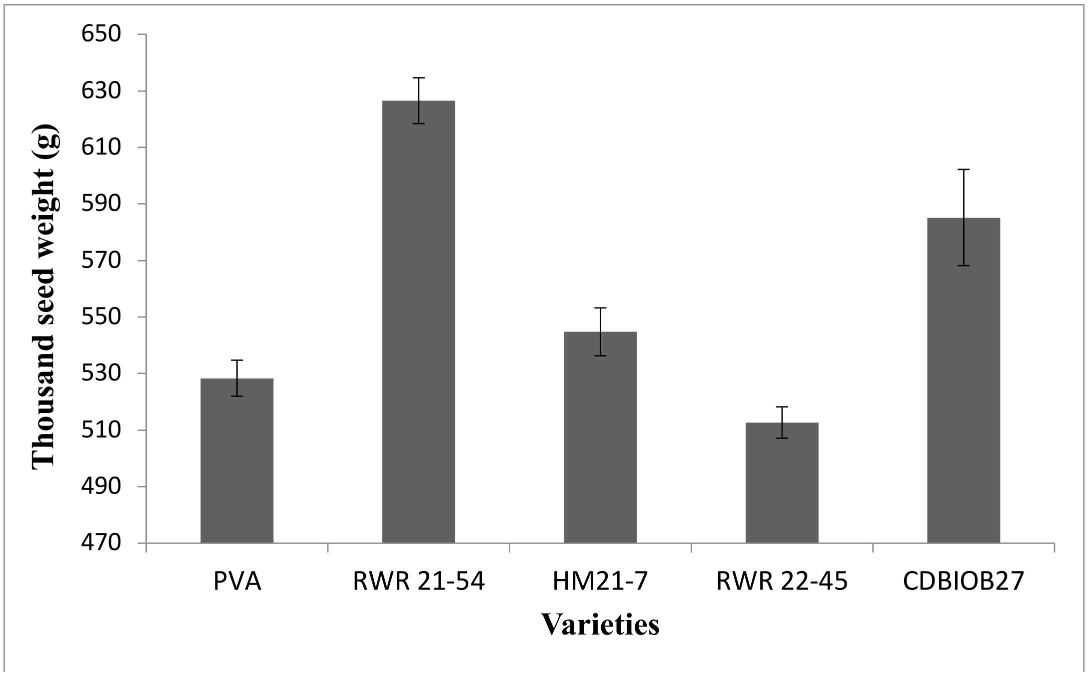


Figure 4.3 Thousand seed weight for different common bean varieties independent to soil moisture level.

The performance of TSW in SM1, SM2, SM3 and SM4 were not significantly different. However, severe water stress under SM5 (20% pot capacity) significantly reduced TSW than other soil moisture levels. The severe water stress had significant effect on TSW and in reference to seed yield (Fig 4.4).

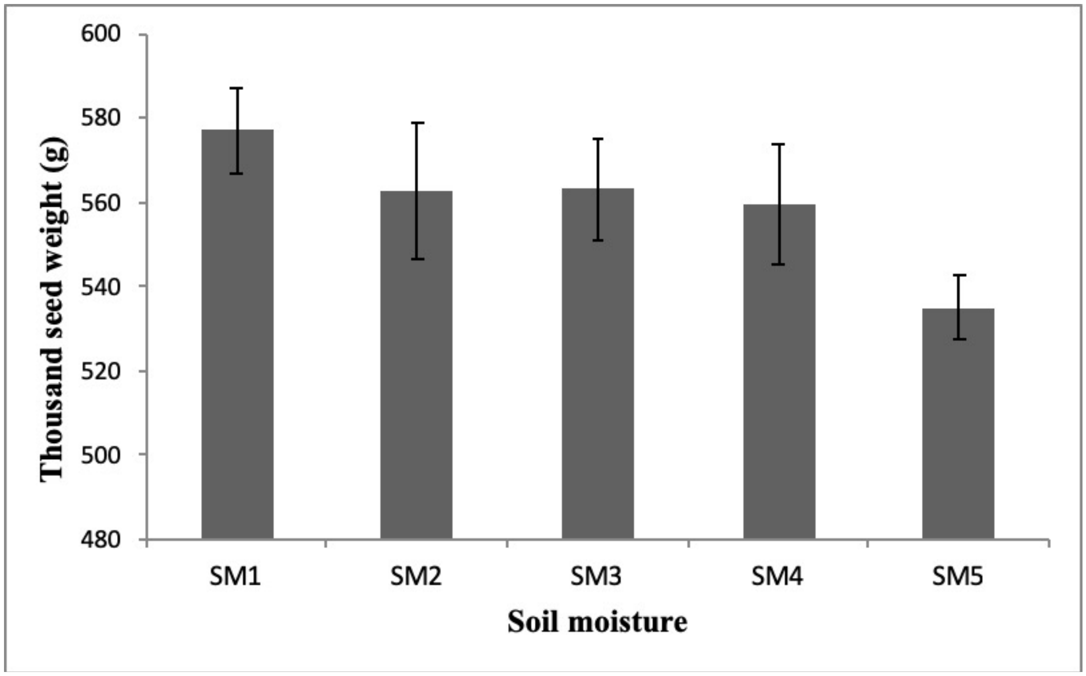
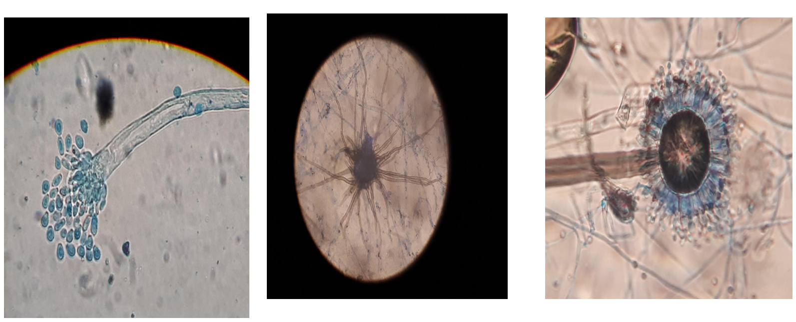


Figure 4.4 Effect of soil moisture levels on thousand seed weight of common bean independent to variety. SM1: 100% soil moisture level; SM2: 80% soil moisture level; SM3: 60% soil moisture level; SM4: 40% soil moisture level; SM5: 20% soil moisture

4.3.2. Fungus incidence for different biofortified common bean seeds

Three fungi namely *Aspergillus spp, Penicillium spp and Rhizoctonia spp* were identified (Plate 1).



Penicillium spp ***Rhizoctonia spp*** ***Aspergillus spp***

Plate 1: Fungal pathogens identified in common bean seed lots

The incidence of pathogens differed significantly ($p<0.001$) among varieties under various soil moisture levels (Appendices 2, 3, 4). Significantly low incidences of *Aspergillus spp* (27.5%) were recorded at 20% pot capacity (SM5), for *Penicillium spp* at 60% (SM3) and 40% (SM4) pot capacities, and for *Rhizoctonia spp* at 80% pot capacity. PVA variety had the highest incidence of *Aspergillus* at 100% soil moisture levels. The incidence increased with increasing soil moisture level and the highest incidence was recorded for *Aspergillus* (51.5%) at 100% soil moisture level while *Penicillium* had the lowest incidence (2%) at 100% and 20% pots capacities respectively.

4.3.2.1. Incidence of *Aspergillus spp* in different biofortified common bean

Incidence of *Aspergillus spp* differed significantly (Appendix 2) among the different biofortified common beans and various soil moisture levels (Table 4.3). Variety CDBIOB27 was found to have lowest incidence of *Aspergillus spp* (14.5%), followed by HM21-7 (38.5%), RWR22-45 (39%), RWR21-54 (49%), and PVA had the highest incidence of *Aspergillus spp* (52.5%).

Variety RWR 22-45 had the lowest incidence of *Aspergillus spp* while HM21-7 had the highest incidence followed by RWR21-54, CDBIOB27, and PVA at 100% pot capacity (Table 4.4). Variety CDBIOB27 had the lowest *Aspergillus spp* incidence followed by RWR 22-45 and variety HM21-7 had the highest incidence at 80% pot capacity.

Table 4.3 Incidence (%) of *Aspergillus spp* in common bean varieties under different soil moisture levels

Variety	Soil moisture					
	SM1	SM2	SM3	SM4	SM5	Mean
PVA	55	60	60	45	42.5	**52.5**
RWR 21-54	60	62.5	45	40	37.5	**49**
HM21-7	75	75	0	25	17.5	**38.5**
RWR 22-45	10	45	67.5	32.5	40	**39**
CDBIOB27	57.5	0	0	15	0	**14.5**
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	**<0.001**
Mean	**51.5**	**48.5**	**34.5**	**31.5**	**27.5**	**38.7**

SM1: 100% soil moisture level; SM2: 80% soil moisture level; SM3: 60% soil moisture level; SM4: 40% soil moisture level; SM5: 20% soil moisture level.

4.3.2.2. Incidence of *Penicillium spp* in the different biofortified common bean varieties

The results on incidence of *Penicillium* spp in different biofortified common bean are presented in Table 4.4. There was no incidence of *Penicillium* spp in both RWR21-54 and RWR22-45 varieties (Appendix 3). An incidence of 2% was recorded in both PVA and HM21-7. Variety CDBIOB27 had the highest incidence of *Penicillium* spp of 9.5%.

Soil moisture level had significant difference in incidence of *Penicillium* spp (Table 4.4). However, 60% (SM3) and 40% (SM4) pot capacities suppressed *Penicillium* spp incidence in different varieties. An incidence of 2% was observed at 20% and 100% pot capacities. The highest incidence of *Penicillium spp* 9.5% was observed at 80% pot capacity (SM2).

Table 4.4. Incidence (%) of *Penicillium spp* in common bean seed under different soil moisture levels

Variety	Soil moisture levels					
	SM1	**SM2**	**SM3**	**SM4**	**SM5**	**Mean**
PVA	10	0	0	0	0	**2**
RWR 21-54	0	0	0	0	0	**0**
HM21-7	0	0	0	0	10	**2**
RWR 22-45	0	0	0	0	0	**0**
CDBIOB27	0	47.5	0	0	0	**9.5**
p-value	<0.01	<0.01			<0.01	**<0.01**
Mean	**2**	**9.5**	**0**	**0**	**2**	**2.7**

SM1: 100% pot capacity; SM2: 80% pot capacity; SM3: 60% pot capacity; SM4: 40% pot capacity and SM5: 20% pot capacity

4.3.2.3. Incidence of *Rhizoctonia spp* in the different biofortified common bean

Highly significant differences (appendix 4) were found among varieties for incidence of *Rhizoctonia spp* at various soil moisture levels (Table 4.5) and there was a very highly significant interaction between varieties and soil moisture levels. The lowest incidence was recorded in PVA, followed by RWR21-54 and RWR22-45. Besides, the highest incidence was observed in CDBIOB27 and HM21-7. Considering soil moisture, the highest incidence was recorded for HM21-7 and CDBIOB when soil moisture was below 80% pots capacity.

Table 4.5. Incidence (%) of *Rhizoctonia spp* among biofortified common bean under different soil moisture levels

Variety	Soil moisture					
	SM1	**SM2**	**SM3**	**SM4**	**SM5**	**Mean**
PVA	2.5	0	0	10	17.5	**6**
RWR 21-54	17.5	2.5	35	12.5	22.5	**18**
HM21-7	5	0	72.5	47.5	27.5	**30.5**
RWR 22-45	42.5	37.5	0	20	15	**23**
CDBIOB27	0	22.5	57.5	50	50	**36**
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	**<0.001**
Mean	**13.5**	**12.5**	**33**	**28**	**26.5**	**22.7**

SM1(control): 100% pot capacity; SM2: 80% pot capacity; SM3: 60% pot capacity; SM4: 40% pot capacity and SM5: 20% pot capacity

4.3.2.4. Influence of soil moisture level on Fe and Zn content in biofortified common bean varieties

There were significant differences among varieties under different soil moisture levels for Fe and Zn content (Appendix 5). Means comparison of Iron and Zinc content in different common bean seed under different soil moisture levels are presented in Table 4.6 and Table 4.7, respectively.

Table 4.6. Iron content (mg/kg) in common bean varieties under different soil moisture levels

Varieties	SM1	SM2	SM3	SM4	SM5
PVA	64.9 a	64.9 ac	62.5 a	70.4a	48.6 b
RWR 21-54	66.0 a	69.9 a	66.6 a	65.5ac	48.4 b
HM 21-7	60.4 a	48.7 b	59.5 a	64.6ad	69.8 a
RWR 22-45	68.4 a	52.8abc	61.0a	51.3 bd	56.4 a
CDBIOB 22	60.4 a	57.6b	53.1 a	48.0 b	45.6 b

SM1(control): 100% pot capacity; SM2: 80% pot capacity; SM3: 60% pot capacity; SM4: 40% pot capacity and SM5: 20% pot capacity. Means followed by the same letter are not statistically significant at p=0.05;LSD=13.72.

There were no significant differences among varieties on Fe at 100, 80, and 60% pots capacities of soil moisture (Table 4.6). However, significant differences were observed among varieties at 40%, 20% pot capacity. Variety HM21-7 had the highest Fe content. In general, Fe content of the variety HM21-7 was not affected by the changes in soil moisture level. The Iron content of variety RWR21-54 was not significantly different from HM21-7 iron content at 40%PC but its Iron content was low at 20% pot capacity (Table 4.6).

Variety CDBIO27 had lowest Iron content when soil moisture level was low (20%, 40% pot capacity). On other hand, the Iron content of variety RWR22-45 was not affected by soil moisture levels. This variety had a response not significantly different from HM21-7 Iron content under different soil moisture levels, decreasing soil moisture level decreased Iron content in common bean seed (Table 4.6).

There were no significant differences among varieties in Zn levels at 100, 80, and 60% pots capacities of soil moisture (Table 4.7).There were significant differences among varieties in Zn content for common bean seed at all soil moisture levels. Variety RWR21-54 had significant differences in Zn content under various soil moisture levels. The lowest Zn content was observed under 100, 80% soil moisture content than fewer than 40 and 20% pot capacity(Table 4.7).

Table 4.7. Zinc content (mg/kg) in common bean varieties under different soil moisture levels

Varieties	SM1	SM2	SM3	SM4	SM5
PVA	35.4 a	38.9a	35.1 a	39.3 a	31.8 c
RWR 21-54	17.9 c	20.7 b	54.5 d	25.5 b	24.5 b
HM 21-7	24.3 b	23.7 b	21.0 b	18.8 c	20.9 b
RWR 22-45	22.3 b	23.3 b	25.2 b	40.7 a	36.3 a
CDBIOB 22	36.9 a	37.926 a	38.0 a	35.3 d	35.5 a

SM1(control): 100% pot capacity; SM2: 80% pot capacity; SM3: 60% pot capacity; SM4: 40% pot capacity and SM5: 20% pot capacity. Means followed by the same letter are not statistically significant at p=0.05; LSD=4.44

There were significantly differences for Zn content in RWR22-45 variety at different soil moisture levels. The highest Zn content was observed at 40, 20% pot capacity of soil moisture levels. However, the lowest Zn content was found at 100, 80% pot capacity. Therefore, variety RWR21-54 response was not significantly different to RWR22-45. However, variety RWR22-45 had the highest Zn content than RWR21-54 under water stress conditions. The content of Zinc in CDBIO27 variety was not affected by the changes in soil moisture level. On the other hand, variety PVA had significantly high Zn content at 40% pot capacity than 20% pot capacity which had the lowest. Overall, Zn content of varieties PVA and CDBIOB were not affected by the variation of soil moisture levels (Table 4.8). Whereas, RWR21-54 and HM21-7 had similar responses, decreasing soil moisture level increased Zn. On the other hand, increasing soil moisture level decreased Zn content of variety RWR22-45 (Table 4.8).

Table 4.8. Spearman's correlation between soil moisture and zinc and between soil moisture and iron

Variety	Zinc-soil moisture		Iron-soil moisture	
	Spearman's rank R	**P**	**Spearman's rank R**	**P**
PVA	0.20	0.21	0.20	0.21
RWR 21-54	0.58	<0.001	0.34	0.03
HM21-7	0.57	<0.001	-0.53	<0.001
RWR 22-45	-0.67	<0.001	0.20	0.21
CDBIOB27	0.25	0.11	0.33	0.03

4.4. Discussion

This study revealed a useful variation in seed quality parameters, fungus incidence as well as the Iron and Zinc content among biofortified common bean varieties. Germination rate, seed vigor index and thousand-seed weight differed depending on bean varieties.

The germination rate ranged from 75.5 - 92.5% depending on the variety and this is in agreement with Gharib and Hegazi (2010) who reported a germination rate of 93-97.5% in six different common bean varieties. The seed vigor index ranged from 512-1051 among different varieties confirming genetic and environment influence on seed quality (Grusak, 2002; Coelho and Benedito, 2008; Ghanbari *et al*., 2015). Variety CDBIOB27 produced the higher TSW and had highest SVI due probably to large size of seeds. Seed vigor maybe influenced by the size seeds that depends on the quantity of stored food (Ghassemi-Golezani and Mazloomi-Oskooyi, 2008). However, Pereira et *al*. (2013) observed that under sub-optimal conditions of water availability, smaller seeds were the best performers, while larger seeds provided higher levels of germination and resulted in more vigorous seedlings under ideal conditions of no water stress. This is in accordance with the present results, variety RWR21-54 had a higher TSW but developed a low SVI. In contrast, CDBIOB27 had a high TSW and developed a higher SVI. Haig and Westoby (1991) reported that a larger amount of reserves may increase the probability of successful seedling establishment.

Soil moisture level had no significant difference on seed quality parameters except for the thousand-seed weight. Muasya *et al.* (2008) reported a general trend indicating that conditions that are conducive to high yield components generally induce high quality of seeds. Previous studies reported no effect of drought on seed vigor index for various crops (Vieira *et al*. 1992). High quality seeds of common bean can be produced under both well and limited irrigation conditions when plants are harvested at physical maturity (Ghassemi-Golezani and Mazloomi-Oskooyi, 2008)

Thousand-seed weight decreased with decreased soil moisture level, suggesting an acceleration of seed maturity and resulted in development of small seeds and can be related to low photosynthesis activity. Gohari (2013) reported decreasing of 100 seed weight in beans and dry bean respectively under drought. The results also indicated that the soil moisture level at 40% pot capacity (SM4) reduced significantly the thousand seed weight and are consistent with Stoyanov (2005) findings who reported a reduction of seed yield when drought occurred at the grain-filling periods. Ozbahce *et al.* (2014)

reported a value of 285-416g for thousand seed weight in common beans depending on the season.

A total of three fungi namely *Aspergillus* spp*; Penicillium* spp. and *Rhizoctonia* spp*.* were isolated from the bean seeds samples. The incidence of *Aspergillus spp, Rhizoctonia spp* and *Penicillium* spp ranged from 14.5-51.5, 6-36 and 0-9.5 %, respectively, depending on the varieties and soil moisture level as well. *Aspergillus* spp was the most fungal isolated, confirming the results of Oshone et al. (2014) in Ethiopia. The incidence of *Aspergillus* spp decreased with decreased soil moisture level. Tylkowska *et al.* (2010) reported that, *Penicillium* spp were eradicated from seeds exposed to microwaves for 45 and 60 seconds.

Iron and Zinc content revealed a significant difference among the different varieties due to soil moisture level. A moderate correlation was observed (R:-0.53) between soil moisture level and Iron content of bean seeds for only variety HM21-7, increasing soil moisture level decreased Iron content. For all the other varieties, Iron content was not related to soil moisture level. For variety HM21-7, Iron content ranged from 48.7 mg/kg (80%pot capacity) to 69.8 mg/kg (20%pot capacity). Casinga *et al* (2017) reported similar results (44-76mg/kg iron content) for a soil moisture level of 48%. In addition, for a soil moisture level of 29 and 37%, they reported a range of 23-29mg/kg, 31-59mg/kg Iron content, respectively. The variation of Iron content depending on the soil moisture ranged within 29-60mg/kg and 24-49mg/kg Iron content in HM21-7 and RWR22-45, respectively (Casinga *et al*., 2017). Shimelis and Rakshit (2005) reported iron content ranging from 61.8-84mg/kg in 8 cultivars of common bean in Ethiopia and were consistent with Blair *et al., (*2009) findings (40-84.6 mg/kg). These values were higher than those reported by Iqbal *et al.* (2006) for different legumes from Pakistan, 30mg/kg (chickpea), 26 mg/kg (cowpea), 31 mg/kg (lentil) and 23 mg/kg for green pea. Martinez Meyer *et al.,* (2013) also reported similar results (61.8-80.6mg/kg) of Fe content in Finland common beans. A research reported by Lubobo *et al.,* (2016) on 4 cultivars of common beans revealed 50-72mg/kg Iron content and 62mg/kg Iron content in HM21-7 common bean.

A positive and moderate correlation (R:0.57-0.58) was observed between soil moisture level and zinc content for varieties HM21-7 and RWR21-54. However a negative correlation (R:-0.67) was observed between soil moisture level and Iron content for variety RWR22-45, increasing soil moisture level decreased Iron content in seed bean.

Previous studies reported a zinc content of 19-26mg/kg, 25-29mg/g and 28-34mg/kg depending on the season under a soil moisture level of 29%, 37% and 48%, respectively. Based on the common bean varieties, 20-33mg/g and 22-32mg/g zinc content was reported in HM21-7 and RWR2245 depending on the soil moisture level as well as the season (Casinga *et al*., 2017). These values are comparable to those reported in this study. Furthermore, zinc content ranged within 30-35mg/kg in 4 cultivars of biofortified common beans and 33mg/kg zinc content was reported in HM21-7 particularly under South-Kivu conditions (Lubobo *et al*., 2016). The reported Zn content in this study, 17.8-39.2 mg/kg is comparable to Onwuliri and Obu (2002) in northern Nigeria, and Shimelis and Rakshit (2005) findings in Ethiopia. Meyer *et al.,* (2013) reported zinc content in Finland common bean seeds at the level of 21-25 mg/kg. The Zn content of different varieties of common beans varied within a wide range from 17.7 to 42.4 mg/kg (Blair *et* *al*., 2009). The zinc content reported in this study was much lower than the zinc content of bean seeds (32.42-65.32 mg/kg) reported by Głowacka. *et al. (*2015).

Seed quality was most influenced by genetic differences amon varieties, the best performer were varieties CDBIOB27 and RWR21-54. The study demonstrates that the soil moisture level can be maintained at 60% without compromising the seed quality, Iron and Zinc content for the investigated biofortified common beans.

CHAPTER FIVE: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 General discussion

Among yield components, highly significant differences between soil moisture levels and varieties were observed with no interaction between the two factors for number of pods per plant, seed number per pod, seed weight and thousand seed weight. Based on moderate water stress (60% pot capacity), overall, yield reductions due to water stress treatments were higher in CDBIOB27 followed by PVA varieties which proved to be susceptible to water stress. RWR21-54, HM21-7 and RWR22-45 varieties represents valuable genetic resources for drought resistance. From the study by Acosta-Gallegos *et al. (*1989), number of pods was most adversely affected by water stress and was correlated with seed yield. Darkwa *et al. (*2016) research reported a reduction of pod number and seed number per pod leading to yield reduction of 29.8% in the drought stress relative to no-stress treatment. In the present study, data showed a reduction of yield depending on the level of water stress. Severe water stress treatments (40% and 20% pot capacity) caused a reduction in yield components for all varieties compared with 100% pot capacity. Yield components were higher at 80% pot capacity soil moisture than at 100% which was used as the control and this is consistent with Abiot (2018) findings who recommended an average of 75% soil moisture for better vegetative growth on common bean.

Based on 60% pot capacity which was the moderate water stress, all yield components were stable and comparable to yield components under 100% and 80% soil moisture levels and differed from one variety to another. The significant effect of the soil moisture levels and varieties for the various yield components traits with no interaction between the two factors indicated that the expressions of the varieties across the various soil moisture levels was static and responsive. This result is consistent with Asfaw and Blair (2014) who reported differential response of common bean varieties to drought stress. Drought caused early maturity of flowers and poor pod-partitioning. Pod setting and flowering are reported to be sensitive to drought and water logging respectively (Ntukamazina *et al*. 2017). The results of Emam *et al. (*2010) showed that plant height, number of leaves, leaf area, and number of pods, pod dry matter and total plant dry matter weights were significantly reduced under water stress. At 50 and 25% field capacity, plant pods of both cultivars tested completely aborted.

Data on yield components under 20% and 40% soil moisture levels were comparable with previous results reported for beans by Schneider *et al.* (1997) and Rosales-Serna *et al. (*2004) under water stress. Variety HM21-7 has been classified by Lubobo *et al. (*2016) as drought resistant in South-Kivu province in the Democratic Republic of Congo. In the present study, variety RWR21-54 had a good performance under favorable moisture conditions and also displayed high yield potential under moderate water stress (60%) than other varieties.

Based on seed quality parameters, significant differences were observed between varieties under various soil moisture levels. The reduction in seed yield and TSW due to drought maybe associated to low photosynthesis activity and poor partitioning of carbohydrates to grain development (Muñoz-Perea *et al*. 2007). Under drought, differential effects have been observed among dry bean cultivars for biomass accumulation, its translocation to yield components and differences exist between bean cultivars in terms of seed quality (Coelho and Benedito, 2008). Present results are consistent with Ghassemi-Golezani & Mazloomi-Oskooyi (2008) who reported that water supply had no significant effect on SVI of common bean. On the other hand, Muasya *et al., (*2008) reported that low seed quality under low rainfall conditions. In this study, variety CDBIOB27 performed well in terms of seed quality but had the lowest performance in yield components under various soil moisture level and was more sensitive to water stress. Therefore, variety CDBIOB27 can be used to improve seed quality performance such as germination rate and seed vigor index. However, small scale farmers usually reject variety with low yield performance (Blair *et al*., 2009)

From this study, Iron and Zinc content were significantly influenced by varieties and soil moisture levels and an interaction was observed between variety and soil moisture levels and this is consistent with Casinga *et al. (*2017) findings, who reported highly correlated Iron and zinc content with soil moisture regimes. Ghanbari *et al. (*2015) reported a reduction of Fe content in common bean seed when the crop was subjected to drought. Since increasing Zn and Fe contents can result in increases in grain yields, genotypes with high values of these elements had higher grain yields under stress conditions. In the present study, variety RWR21-54 had the highest Iron content at 60% pot capacity of soil moisture. In the study of Casinga *et al. (*2017), variety HM21-7 demonstrated better adaptability to the Sud-Kivu province climatic conditions compared to others tested varieties. All well performing varieties such as RWR21-54 and RWR22-45 were not involved by Casinga *et al. (*2017) research.

Several fungal species were detected of which *Aspergillus spp, Rhizoctonia spp,* and *Penicillium spp* were the most common. Incidence of pathogens influences varieties performance under different soil moisture levels. The incidence of *Aspergillus spp, Rhizoctonia spp,* and *Penicillium spp* ranged from 14.5-51.5, 6-36 and 0-9.5% respectively depending on the variety and soil moisture level. In general, the incidence of fungi decreased with decreased soil moisture level. Apart from *Rhizoctonia spp* these fungi are commonly associated with seeds, and they do not generally cause diseases in most cultivated species under field conditions. However, they may compromise seed quality, reducing germinative power and causing embryo death, especially when storage is inappropriate (Embaby *et al*., 2013).

Fungal incidence correlated with seed moisture levels. In the present study, seeds moisture was constant and ranged from 8-10%. In the research of Francisco and Usberti (2008) highest incidences of *Rhizoctonia spp* were recorded at 16-18% of seed moisture content.

5.2 Conclusion

Varieties PVA, RWR21-54 and RWR22-45 performed well under moderate water stress. This was shown by favourable performance of their yield components under 80% and 60% soil moisture levels. Yield components included seeds per pod, pods weight, seed weight as well as number of pods per plant. Based on the pod weight, seed weight and pod numbers, RWR21-54 and RWR22-45 varieties were the best performers under water stress. PVA variety had the highest number of seed per pod. Bean performance under different soil moisture levels were best under 80% pots capacity (SM2) and 60% pots capacity (SM3). These were found to be the optimum soil moisture levels for most yield components. Maximum soil moisture level (100% pots capacity) as well as lowest soil moisture levels from 40% pots capacity up to 20% pots capacity resulted in reduced performance of yield components. This was due to their capacities to maintain their seed yield. This study showed that 80% soil moisture level was the best for high yield performance for biofortified common bean.

Seed quality parameters including seed germination rate, seed vigor index and thousand seed weight differed significantly depending on the different biofortified varieties and soil moisture levels. There was no effect of soil moisture level on the seed moisture content meaning that drying of the seed lots was uniform. Germination rate as well as the moisture content had high values recorded for 60% pot capacity (SM3). Besides, the 100% pot capacity (SM1) showed high seed vigor index and the thousand-seed weight followed by the 60% pot capacity (SM3).

Fungus incidence, ranged from 0- 9.5% (*Penicillium spp*), 6-3.6% (*Rhizoctonia spp*), and 14.5-52.5 (*Aspergillus spp*) in different varieties and soil moisture levels. In addition, incidence of *Aspergillus spp* decreased with decreased soil moisture content. Iron and Zinc content differed significantly among common bean varieties under various soil moisture levels and a significant interaction was observed between varieties and soil moisture. This meant that in some varieties zinc content changed due to changes in soil moisture level. The best performing variety was RWR21-54 with a high Iron content at 60% pot capacity. This variety also produced the highest thousand seed weight.

5.3 Recommendations

- Field experiments comprising varieties evaluated in this study should be done under various environmental conditions to confirm the present findings to ascertain the performances of RWR21-54 and RWR22-45 as well as HM21-7 before release to the farmers.
- Varieties included in this study are not appropriate in case of severe water stress but should be used when the soil moisture is at least 60% field capacity.
- When planting biofortified common bean, 60-80% of field capacity should be recommended to farmers.
- Variety CDBIOB-27 should not be recommended to farmers because of its low yield but should be used by breeders to improve seed vigor index and germination rate of biofortified common bean.

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APPENDICES

Appendix 1. Characteristics of Kabete Agro-Meteorological station, University of Nairobi

Parameters	S	O	N	D	J	F	M	A	M	JN
Temp(^{0}C)	18.7	21.6	20.0	20.5	22.3	21.9	23.4	20.8	19.7	19.4
Average Rainfall(mm)	13.0	22.5	137.7	19.5	27.8	16.5	50.9	167.8	158.8	0.6

Appendix 2. Analysis of variance for Iron and Zinc content in common bean seed

Source	**Iron**					**Zinc**				
	DF	SS	MS	F	P	DF	SS	MS	F	P
Variety(A)	4	2700.6	675.142	3.49	0.009	4	7933.0	1983.24	98.11	<0.001
Soil Moisture(B)	4	2201.6	550.402	2.85	0.025	4	445.5	111.38	5.51	<0.001
A*B	16	6920.9	432.559	2.24	0.005	16	2706.6	169.16	8.37	<0.001
Error	175	33822.1	193.269			175	3537.4	20.21		
Total	199	45645.2				199	14622.5			
Grand Mean	59.372					29.404				
CV	23.42					15.29				