Seed Quality of Velvet Bean Seeds (*Mucuna pruriens* L. Dc) In Western Kenya

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Abstract

Farmers in Bungoma County are actively involved in velvet bean (Mucuna pruriens L. DC) production due to its multiple uses. It can be used to improve soil fertility, nutrition, drought tolerance, pest and disease tolerance, food security and medicinal. Variation in seed characteristics has been reported to be useful for crop improvement programs. However, production of quality seed is a challenge for farmers in this county. The purpose of this research was to investigate the quality of velvet bean seed. Four types of velvet bean seeds were obtained from farmers during a survey. Morphological variability in seed characteristics like seed coat color, hilum color, seed length, width and thickness (IBPGR descriptor) were evaluated in four replications of 50 seeds each. Fifty seeds replicated 3 times were used to determine the electrical conductivity (EC) for each seed type. Germination test of these seeds was laid out in a CRD. To investigate the dynamics and rates of imbibition, thirty seeds replicated 3 times for each seed type were subjected to temperatures of 20, 25, 30, 30/20°C and in ambient condition during a germination test. Data were subjected to analysis of variance (ANOVA) using GENSTAT software release 14.1 and the mean separations was done using least significant difference (LSD) at 5%. Pearson correlation coefficient was used to analyze seed traits of the morphotypes. Germination percentage data was analyzed using Microsoft Office (Excel) V.2013. There were significant differences ($P \leq 0.05$) among seed types in all seed traits measured except seed coat thickness. White seed type recorded a lower electrical conductivity (more vigorous), higher final germination percentage, higher speed of germination index and higher imbibition rate at 30°C for both substratum (sand and filter paper) than other seed types. This study therefore recommends that the barriers to germination in dark colored seed types need to be addressed by seed scientists to improve velvet bean seed quality at farm level hence increase the production. Further study can be done on genetic and physiological properties of velvet bean black seed type in order to improve its germination capacity.

Keywords: Seed morphology, Electrical conductivity, Germination, Imbibition, Velvet bean

INTRODUCTION

Mucuna pruriens Linn (Family-Fabaceae), commonly known as velvet bean, is an annual climbing legume. This leguminous crop is endemic in India and other tropical regions, including South and Central America and native to tropical Asia and Africa (Katzenschlager et al., 2004). Just like other legumes for example soybean, common bean, and mung bean velvet bean grows under moist and warm conditions in both cultivated and wild forms (Pugalenthi et al., 2005). There is tremendous morphological variability in velvet bean (Sathyanarayana et al., 2016).

Velvet bean can be used as human and livestock feed and it has medicinal value. It also improves soil fertility through N fixation. It has a higher nitrogen fixing ability than other legume crops (FAO, 2011). It can contribute to sustainable agriculture and crop production improvement (Saria et al., 2018). Farmers in Western Kenya face a number of food production constraints (land degradation, low soil fertility, poor rainfall, climate variability), resulting in high food insecurity. Adoption of legumes with high protein content such as velvet bean can reduce food insecurity in Kenya (Wabwoba and Mutoro, 2019).

Farmers in Bungoma County were actively involved in velvet bean (Mucuna pruriens L. DC) production due to its multiple uses. It can be used to improve soil fertility, nutrition, drought tolerance, pest and disease tolerance, food security and medicinal. Despite its multiple uses, farmers in Bungoma County are facing problems due to its poor germination and crop establishment in the field (Melavanki and Kumar, 2020; Diop et al., 2021).

Variation in seed characteristics has been reported to be useful for crop improvement programs (N'Danikou et al. 2022). However, production of quality seed was a challenge for farmers in this county. The purpose of this research was to investigate the quality of velvet bean seed.

MATERIAL AND METHODS

Seed Source

Velvet bean seeds were sourced from farmers in Bungoma County during a survey which targeted velvet bean farmer groups and carried out on the 10th to 15th June 2019. The County covers an area of about 3,032 km2 and is located in Western Kenya on the southern slopes of Mount Elgon. It borders to the west and southwest Busia County, to the northeast Trans-Nzoia County, to the east and southeast Kakamega County and to the northwest the Republic of Uganda (Ralph et al., 2005). Bungoma County is divided into 9 sub counties namely Bungoma North, Bungoma Central, Bungoma West and Bungoma South, Webuye West and East, Kimilili, Mt. Elgon and Bumula.

Laboratory Experiment

Seeds obtained from the survey were taken to the Seed Physiology Laboratory, Department of Seed, Crop and Horticultural Sciences, University of Eldoret and experiments set up to determine the quality of farmers' seeds in Bungoma County. These experiments were carried out on the 17th to 30th June 2019.

Morphological Characterization of Velvet Bean Seed Types from Bungoma County

Seeds were categorized into different types. Morphological variability in seed characteristics like seed coat color, and hilum color, was evaluated in four replications of 50 seeds each type using the IBPGR descriptor. To determine seed variability (length, width and thickness) a seed caliper was used. Seed length was measured over the seed coat along the longest axis of the seed. The seed width measurement was taken on one of the widest faces at the middle of the seed. Then seed coat thickness was measured on one of the smallest faces at the middle of the seed without removal of seed coat.

Electrical Conductivity of Velvet Bean Seed Types from Bungoma County

For each seed type fifty seeds were weighed and this was replicated 3 times. The clean seeds were then immersed in 100ml of distilled water at 25°C temperature for 24 hours. The conductivity meter is warmed for about 30 minutes before testing. Conductance of distilled water in a beaker was measured. The electrode was then cleaned with some tissue paper. It was inserted into leachate and conductance of the leachate was read. The electrode was thoroughly washed using a wash bottle and wiped with a clean tissue paper before reusing.

To get the electrical conductivity (EC) of leachate the reading of distilled water was subtracted from the sample reading then divided by the weight of the seed in grams:

$$\text{Electrical conductivity} = \frac{\text{EC Reading} - \text{Control}}{\text{Weight}}$$

Results represent the mean of 3 measurements ± standard error (SE) and are expressed as mS g^{-1}.

Germination Test of Velvet Bean Seed Types From Bungoma County

Three replications of 50 seeds each from each type of velvet bean were used for preconditioning stage and germination tests. In order to reduce the chances of contamination, petri dishes and plastic containers were sterilized with 1% sodium hypochlorite. The seeds were chipped at the hilium, then placed into petri dishes and put into the incubator. A container filled with distilled water was placed below the petri dishes. This setup was incubated at 25°C for 2 days.

Sand was obtained from University of Eldoret farm, washed and dried at the greenhouse and then sterilized in an oven set at 150°C. Three replications of fifty seeds from the 4 types of velvet bean was counted and placed into their respective plastic containers containing the sand. The plastic containers (covered with lids) were placed into the incubator at altering temperature 30/20°C in the light/dark (8h/16h a day) (ISTA, 2015). Seeds were incubated for 14 days while monitoring germination count daily, according to ISTA (2011). Seeds were considered germinated if their radicle had protruded at least 2 mm. Final germination percentage (FGP) and Speed of germination index (SGI) were calculated according to ISTA (2011) as follows:

$$FGP = \frac{NT\ x\ 100}{N}$$

Where:
FGP = Final Germination Percentage
NT = Total Number of Seeds Germinated
N = Number of Seeds Sown.

$$SGI = \sum \frac{n}{d}$$

Where:
n = number of seedlings emerging o day ‘d’
d = number of days

Imbibition Rate of Velvet Bean Seed Types from Bungoma County

In order to know the optimum media for water uptake of velvet bean seeds, two media were used: sand and filter paper. Three replications of thirty seeds were subjected to temperatures of 20, 25, 30, 30/20°C in the light/dark (8h/16h a day) and in ambient condition at the following times 2, 4, 6, 8, 24 and 48 h. Water imbibing capacity of the seeds (ws) was calculated the formula by Hidayati et al. (2001).

$$\%Ws = \frac{(Wi - Wd)}{Wd} X100$$

Where Ws: water imbibing capacity of the seeds, Wi: seed wet weight after each imbibition time and Wd: initial weight of dry seeds (g).

Data Analysis

Seed morphological data, germination percentage, speed of germination index was subjected to one-way analysis of variance (ANOVA) using GENSTAT software release 14.1. Mean separation was done using least significant difference (LSD) at 5% probability. Pearson correlation coefficient was used to relate the seed traits with germination percentage using Microsoft Office (Excel) V.2013. For the electrical conductivity (EC) and imbibition rates, data was subjected to descriptive analysis and presented as line graph using Microsoft Office (Excel) V.2013.

RESULTS

Morphological Variability

Among seed types, there were significant differences ($p \leq 0.05$) in all seed traits measured except seed coat thickness (Table 1). White and Brown white seed types had significantly longer and wider seed than other seed types.

Table 1: Morphological variation in seed length, width and thickness among four seed type of velvet bean in a laboratory experiment conducted between 17th to 30th June 2019 at the University of Eldoret

Seed type	Seed length (mm)	Seed width (mm)	Thickness (mm)
Black	13.10 a	9.27 a	9.101 a
Dark brown black	13.18 a	9.59 a	9.455 b
Brown white	15.30 b	11.73 b	9.484 b
White	15.55 b	11.82 b	9.491 b
LSD	1.8579	1.8916	0.3470
CV (%)	8.4	11.6	2.4
F pr.	0.020	0.019	0.086

LSD: Least significant differences, CV: coefficient of variation, F pr.: F probability. Mean followed by a different letter within a column are significant different at p≤0,05.

Correlation of seed traits showed that seed length correlated with seed width ($r= 0.996$, $p \leq 0.05$) and seed thickness ($r=0.660$, $p \leq 0.05$) (Table 2). Seed width and seed thickness also showed a significant positive correlation ($r=0.713$, $p \leq 0.05$). Seed length and width positively correlated with germination percentage ($p \leq 0.05$).

Table 2: Mean correlation coefficient (r) between seed traits and germination percentage of velvet bean seed type in a laboratory in the experiment

Seed type	Seed length (mm)	Seed width (mm)	Thickness (mm)
Seed length (mm)			
Seed width (mm)	0.996		
Thickness (mm)	0.660	0.713	
FGP (%)	0.929	0.935	0.829

FGP = Final germination percentage

Using the Royal Horticultural Society (RHS) colour chart (RHS, 2015) colour chart 4 seed, types were identified (Plate 1 and Table 3). Velvet bean seed coats ranged from black to dark greyish, yellowish brown to yellowish grey, dark greyish yellowish brown (dark brown black) and yellowish grey (brown white seed type) spot were observed respectively on the seed coat. Pinkish grey pattern colour was observed on the seed coat of the white seed type. All seed type had a yellowish white hilium colour but the group differed from yellow white to white (Table 4).

Plate 1: Different seed types collected from farmers during the survey in Bungoma County

Table 3: Seed Colour (RHS Chart) in a laboratory experiment conducted between 17th to 30th June 2019 at the University of Eldoret

Type	Seed colour	*Code*	*Group*	Spot colour	*Code*	*Goup*	Pattern colour	*Code*	*Group*
Black	Black	203 B	Black group						
Dark brown black	Black	203 B	Black group	Dark Greyish Yellowish Brown	N200 A	Brown group			
Brown white	Dark Greyish Yellowish Brown	N199 B	Grey Brown group	Yellowish Grey	156 A	Greyed-white groupe			
White	Yellowish Grey	156 A	Greyed-white group				Pinkish Grey	201 D	Grey group

Table 4: Hilium Colour (RHS Chart) in a laboratory experiment conducted between 17th to 30th June 2019 at the University of Eldoret

Type	HiliumColour	*Code*	*Group*
Black	Yellowish white	158D	Yellow white group
Darkbrown black	Yellowish white	158D	Yellow white group
Brown white	Yellowish white	155B	White group
White	Yellowish white	155B	White group

Electrical conductivity (EC)

Among the seed types, no significant difference in the average EC was noted. White seed type with average electrical 0.0012mSg^{-1} was more vigorous than dark brown black, followed by black and brown white seed type (Table 5). Lower the EC value greater is the vigor of the seed.

Table 5: Electrical conductivity of velvet bean seed in a laboratory experiment conducted between 17th to 30th June 2019 at the University of Eldoret

Seed Type	Average EC (mSg-1)
Black	0.0016a
Dark brown black	0.0019a
Brown white	0.0014a
White	0.0012 a
LSD	0.00084
CV (%)	36.0
F pr.	0.427

LSD: Least significant differences, CV: coefficient of variation, F pr.: F probability. Mean followed by the same letter within a column are not significantly different at p ≤0.05

Germination Test

Final germination percentage (FGP) and speed of germination index (SGI) significantly (p≤0.05) differed with seed type. White seed type had significantly (p≤0.05) higher FGP (76.67%) and SGI (5.31) than the others seed types, followed by brown white (57.33% ; 4.02). FGP and SGI were low for dark brown black and black seed type (Figure 1 and Figure 2).

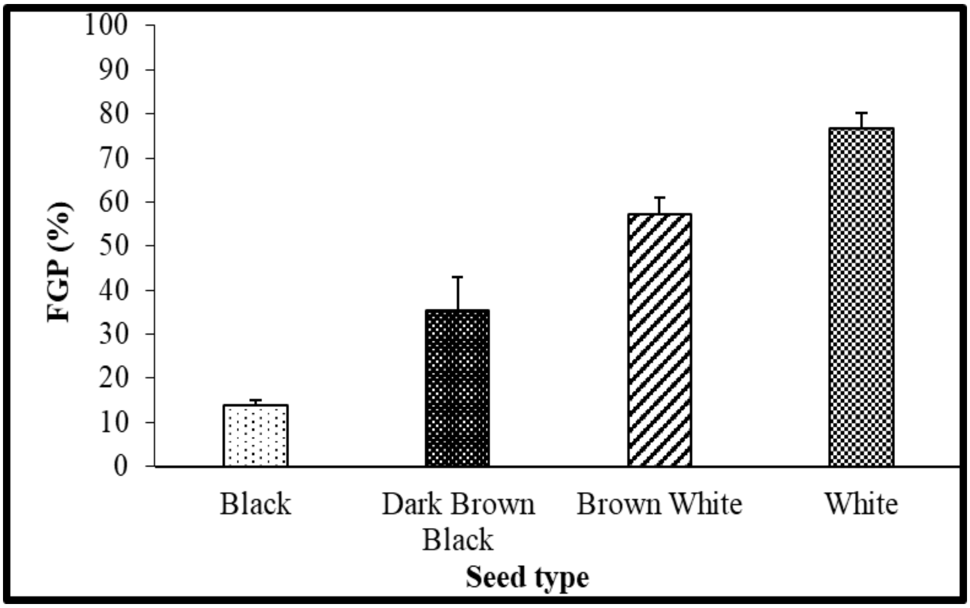

Figure 1: Germination percentage of four velvet bean seed types in a laboratory experiment conducted between 17th to 30th June 2019 at the University of Eldoret

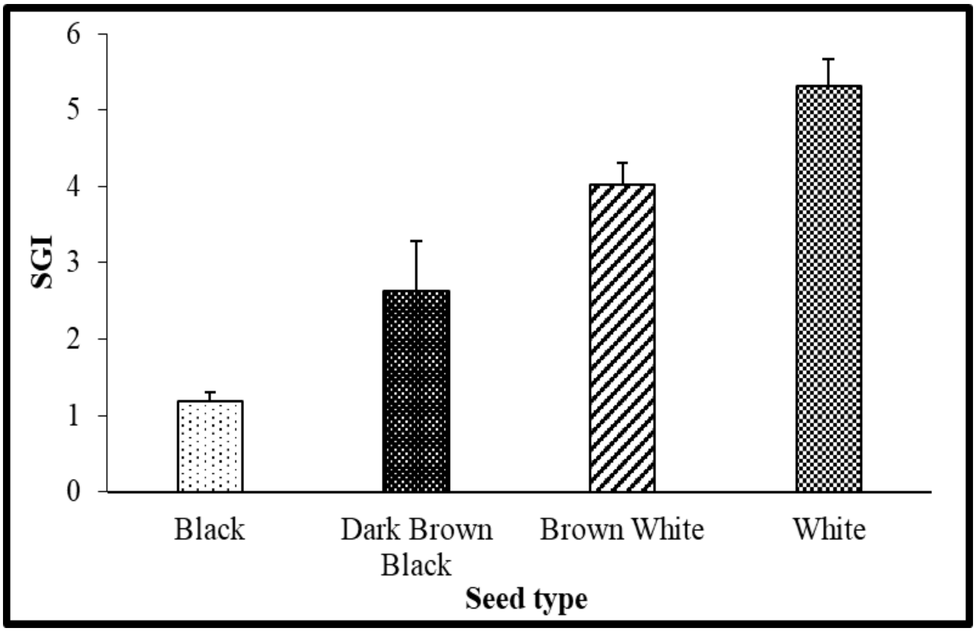

Figure 2: Speed of germination index of four velvet bean seed type in a laboratory experiment conducted between 17th and 30th June 2019 at the University of Eldoret

Imbibition

Water imbibing capacity of the seeds significantly ($p \leq 0.05$) differed among seed types. As temperature increased, the water imbibing capacity also increased but the alternative temperature 30/20°C resulted in completely interruption of the water uptake for both media. In all two cases, the seeds revealed the lowest water imbibing capacity at ambient condition. The maximum imbibition was reached at 25°C (black, dark brown black seed types) and 30°C (white, brown white seed types) using filter paper as media (Figure 3). In contrast, dark brown black seed type reached maximum imbibition capacity at 30°C using sand as media (Figure 4).

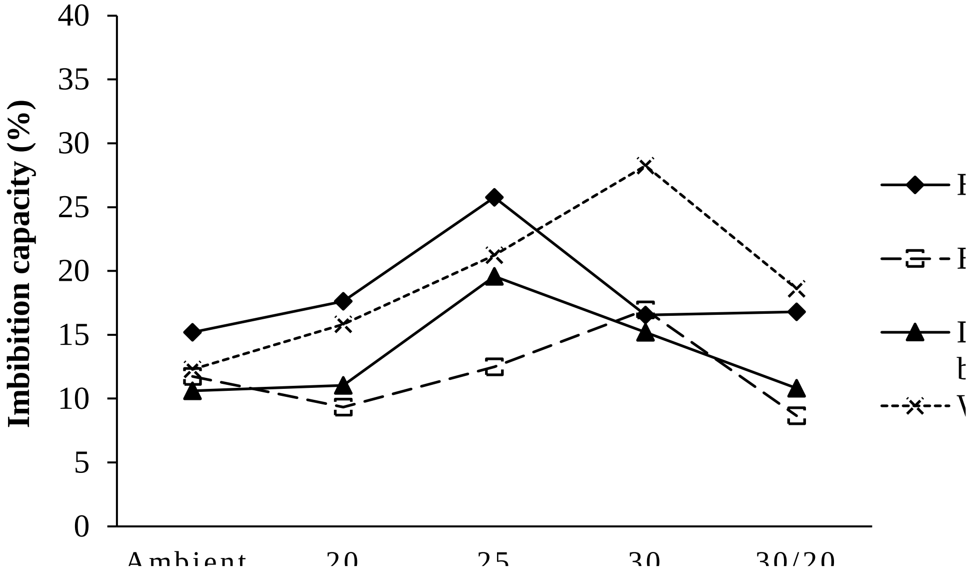

Figure 3: Imbibition capacity of velvet bean seed type using filter paper as media in a laboratory experiment conducted between 17th to 30th June 2019 at the University of Eldoret

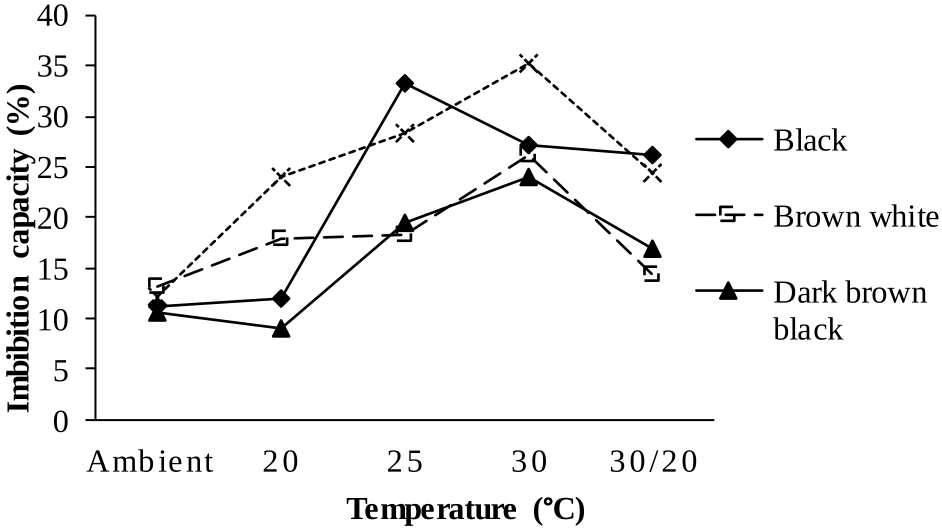

Figure 4: Imbibition capacity of velvet bean seed type using sand as media in a laboratory experiment conducted between 17th and 30th June 2019 at the University of Eldoret

DISCUSSION

Morphological variability of farmers' velvet bean seed

The present study indicated that the variation in velvet bean seed morphology is manifested mainly in seed size, thickness, colour, and coat ornamentation. White and Brown white seed type had significantly longer and wider seed than other seed types. The difference (seed length and width) was no significant between black and dark brown black seed type also between white and brown white. This observation can be explained by the interplay of both genetic and varying environmental conditions (Leidinger et al., 2021). Correlation of seed traits showed that seed length correlated with seed width ($r=0,996$, $p \leq 0.05$) and seed thickness ($r=0,660$, $p \leq 0.05$). Seed length and width positively correlated with germination percentage. This is in line with studies that have indicated that big seed size goes hand in hand with seed viability and vigour (Massimi, 2018). The role of genetics and environment in seed morphology is also supported by the correlation between seed traits. Seed traits are important for identification of species. This is in line with some studies which indicated that seed characters are very helpful for identification of a large number of species or genera (Juan et al., 2000; Moro et al., 2001; Seggara and Mateu, 2001).

The colour of velvet bean seed is very variable. The seeds color varies from black to dark greyish yellowish brown to yellowish grey. Four colours of seeds were recognized: black seed type (black colour), dark brown black seed type (black colour) brown white seed type (dark greyish yellowish brown) and white seed type (yellowish grey). These four seed types corresponded respectively to four varieties of velvet bean (*Mucuna pruriens* utilis noir, rajada, yardghana, and *Mucuna pruriens* IRZ) described in Madagascar, regarding the color of integument (Kantiono, 2012). Seven different accessions of velvet bean (*Mucuna pruriens* var utilis (L.) DC) were indicated by Gurumoorthi et al. (2003), namely thachenmalai (white– coloured seed coat), thachenmalai (black– coloured seed coat), mundanthurai (white – coloured seed coat), kailasanadu (white – coloured seed coat), valanad (black – coloured seed coat), mundanthurai (black –coloured seed coat), mylaru (white – coloured seed coat), have also been gathered from different agroecological regions of Western Ghats, South India which were evaluated in their agrobotanical traits.

Dark Greyish Yellowish Brown and Yellowish Grey seed coat spot colour were recognized respectively in Dark Brown Black and Brown White seed type. Pinkish Grey seed coat pattern colour were recognized in White seed type. All seed types had a yellowish white hillium colour but the group differed from Yellow White to White. Pattern and hilium colour are also used for species identification. One study indicated that seed coat pattern or the micro-ornamentation on the surface of the outer cell wall can be considered of taxonomic value in the identification of the species (Zoric et al., 2010; Aniszewski et al., 2001). Some studies have also indicated that the structure and morphology of the seed coat can be used as a useful taxonomic feature (Algan and Büyükkartal, 2000; Seggara and Mateu, 2001; Bobrov et al., 2004; Hassan et al., 2005).

Electrical conductivity (EC) of farmers' velvet bean seed

There was no significant difference in the average EC among seed type. However, white seed type was more vigorous than Dark brown black, followed by black and brown white seed type. The lower the value of EC, greater is the seed vigor. It revealed that the lower the membrane integrity, the greater the electrolyte leakage in the steep water, thus the greater the conductivity measurement (ISTA 2011). The result is in agreement with Mavi (2010) who found that in watermelon, the electrical conductivity decreased from light colored to dark colored seeds. There are factors which influence the EC values such as initial water content, temperature and time of seed soaking, the number of seeds per sample, seed size and genotype (Gilvaneide et al., 2016). Conductivity test can be used to predict the field emergence and standard germination. This is consistent with a study which indicated that the EC test has been proved as indicator of seed vigor in wide range of crop species and has been successfully related to field emergence and stand establishment (Khodratien et al., 2017).

Germination capacity and speed of germination index of farmers' velvet bean seed

White seed type had significantly ($p \leq 0.05$) higher final germination percentage (76.67%) and speed of germination index (5.31) than the others seed types, followed by brown white. Seed colour plays a role in seed dormancy and germination. The result observed in black and dark brown black affirms with Chachalis and Smith (2000) who indicated_that light coloured seeds absorb water rapidly than dark soybean seeds and consequently have greater rate of imbibition and germination. This present study is in agreement with Ochuodho and Modi (2010) who indicated that in coloured seed of some legumes, the lower germination was attributed to the tight adherence of the seed coat to the embryo and the presence of phenolic compounds. Seed coat structure can be affected by different seed colours resulting from various level of pigment accumulation. This can be explained by the fact that different seed coat colours exert differential germination restrictive actions by providing different levels of impermeability to water and/or oxygen or the mechanical resistance to radicle protrusion (Debeaujon et al., 2000). In our study, the lower germination of black seed type could be attributed to the greater number of pigmentations that solidify the seed coat and increase its impermeability to water and/or oxygen (Marcos, 2005 and Sousa et al., 2017). Germination, longevity and dormancy are controlled by seed coats that are resistant to environment factors (Debeaujon 2000). Similarly, it was reported that various factors, such as water uptake by the seed (De Souza and Marcos-Filho 2001), seed quality due to color pigments in seed coat, germination (Nerson 2002) and seed dormancy (Baskin et al., 2000) are affected by seed coat. Therefore, it is important to understand dormancy because it is correlated with water content, chemical properties of endosperm or embryo, germination traits, seed shape, seedling size and seed production (Westoby et al., 2002; Forbis et al., 2002; Hamilton et al., 2013).

Imbibition capacity of farmer velvet bean seed

Water imbibing capacity of the seeds significantly ($p \leq 0.05$) differed from seed type. As temperature increased, the water imbibing capacity also increased. The same observation was indicated for three sorghum species which are *Sorghum bicolor, Sorghum sudanense, Sorghum vulgare* (Golubinova and Vasilevska-Ivanova, 2008). However, the alternative temperature 30/20°C resulted in complete interruption of the water uptake for both media. In all two cases, the seeds revealed the lowest water imbibing capacity at ambient condition. The maximum imbibition was reached at 20°C (black, dark brown black seed types) and 30°C (white, brown white seed types) using filter paper as media. In contrast, dark brown black seed type reached maximum imbibition capacity at 30°C using sand as media. These results mean that the water imbibing capacity depends on conditioning temperature, media and seed type that is specific characteristics of the seeds. The seed coat colour is associated with water absorption. This is in line with Ertekin and Kirdar (2010) who indicated that in several legume species, the reduction of imbibition rates is correlated with seed coat pigmentation. This was demonstrated by honey locust seeds, whose swelling percentage rose increased more rapidly in yellow coated seeds (100% in 48 h) than in light and dark brown seeds. Similar result was found using soybean seeds for seeds with black seed coats (Chachalis and Smith, 2000). Also, the hilium can prevent water uptake by acting as a hygroscopic valve (Hanna, 2006). It is known that imbibition capacity is important for germination. This is consistent with the Ashraf and Foolad (2005) who indicated that during the imbibition process seeds are hydrated to a moisture level sufficient to initiate early events of germination but not sufficient to permit radicle protrusion. Germination starts with imbibition, a process of water uptake by the seed, caused by different water concentration between seed and environment (Osmosis).

CONCLUSION AND RECOMMEDATION

There was a wide variety of seed types grown by the farmers. These seed types differed morphologically from each other in parameters like seed colour, seed size, germination percentage and vigour. Dormancy could have been a factor that limited the germination of dark-coloured seed types. To increase production of velvet bean seed, selection criteria needs to be adopted by farmers and the barriers to germination in dark colored seed types needs to be addressed. Training on production, management of seed and marketing aspects should be organised each time before planting of velvet bean to improve seed quality of farmer saved seeds. There is a need for stakeholders such as Kenya Agricultural and Livestock Research Organization (KALRO) to standardize agronomic and seed management practices of velvet bean in order to improve seed quality and yield at farm level. Genetic diversity exists among the evaluated seed of velvet bean. Genetic characterization of velvet bean found in western Kenya should be done in order to help differentiate the various morphotypes for breeding purposes. Results showed that black seed type have low germination percentage compared to other seed types. Further study can be done on genetic and physiological properties of velvet bean black seed type in order to improve its germination capacity.

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