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Seed Performance of Selected Bottle Gourd (*Lagenaria siceraria* (Molina) Standl.)

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: Bottle gourd is a useful crop to include in climate change adaption strategies for agronomy. However, diversity in plant and seed forms makes it difficult to predict performance under field management. There is a dearth of knowledge on the relationship between seed morphology and seed performance, namely, germination and early establishment of seedlings. This led to a need to evaluate seed morphology of different bottle gourd landraces and its effect on seed quality as defined by germination and vigour.
Methodology: Six mature fruits of different bottle gourd landraces were collected from subsistence farmers and seeds from each fruit were morphologically characterized. Standard germination test, root: shoot ratio, seedling fresh mass, seedling dry mass, germination velocity index (GVI) and electrical conductivity were used to establish seed quality and vigour.
Results: Although all traits were significantly different, most of them were not good indicators of seed quality. Seed coat thickness isolated varieties by provenance and was inversely proportional to root: shoot ratio as a measure of seedling establishment.
Conclusion: It is concluded that *Lagenaria siceraria* seed morphology could be a useful trait for selection of planting material in the context of seed germination as a trait.

Keywords: Bottle gourd (Lagenaria siceraria); landrace selection; seed quality; seed morphology.

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1. INTRODUCTION

Food security has become a crucial issue in rural Africa due to the possible effects of climate change, reduction in arable land, as well as increase in human population and poverty. In the past, research was mostly undertaken to enhance productivity of selected crops suitable for high potential areas, and the so called traditional/orphaned crops that are especially appropriate for low potential areas were abandoned [1]. However, most rural farmers were unable to afford the high input costs associated with such innovations, and production was affected by numerous biotic and abiotic constraints [2]. These initiatives were, therefore, unsuitable, and increased the poverty gap for most subsistence-based rural farmers. Before the turn of the century, the need to find cheaper alternatives to green revolutionary farming for subsistence farmers increased [3]. Research has since refocused its efforts on alleviating poverty in rural communities by using strategies relevant and indigenous to these communities. One such strategy employed was the re-introduction of neglected underutilized crops such as bottle gourd [4].

Bottle gourd (*Lagenaria siceraria* (Molina) Standl.) is one of the most important crops in the cucurbitaceae family, although it is considered as a poor man's crop due to the socio-economic restrictions governing its production and use. It has a pan-tropical distribution with regional economic importance and is used as a vegetable, container, musical instrument or float while its seeds are used for oil and protein. A lot of information is known on the medicinal aspects of bottle gourd [5]; however its potential as a possible food security crop has been lowly documented. In nature, bottle gourd exhibits great morphological and genetic variability [6]. This alone could indicate its wide environmental adaptation [7]. The plant also demonstrates an indeterminate growth habit when there is enough supply of water. This allows farmers to have a constant supply of fresh green leaves for consumption and animal fodder. Young immature fruits are consumed in the same manner as pumpkin fruits, while the seeds are a rich source of essential amino and fatty acids [8, 7]. Bottle gourd does not require complex field management practices. It grows well with small amounts of nitrogen fertilizer and it is a natural weed smother [7]. It is often intercropped with cereal crops and can act as a live mulch [9]. Given such benefits, it is surprising that bottle gourd is the cucurbit with the least amount of scientific research directed at enhancing utilization, let alone productivity. One important aspect in crop production that is often overlooked by many resource limited farmers is seed quality. There is limited and diffused information on seed technology of bottle gourd [10,11].

Seed quality has been described as a multiple concept comprising several components [12 - 14]. Hampton [15] described seed quality as the standard of excellence in certain characteristics that will determine performance when the seed is either stored or sown. Seed germination capacity and vigour are, therefore, the key measures of seed quality [16,17]. When varieties occur in variegated seed forms, it is important to determine whether or not such variegation affects seed performance in terms of germination and vigor. Many scholarly articles have reported on the effect of seed size, colour, seed coat morphology and dimorphism (single plant produces two seed types with different morphology) on seed quality in several species [16,18-20]. It is difficult, however, to conclude that results obtained from these studies can be superimposed on bottle gourd, owing to the vast morphological variegation within a single species.

Practical considerations have shown that large seeds have better germination capacity and vigour, and will produce more competitive seedlings than smaller seeds, hence high seed quality [20]. The possible effect of seed size on seed quality (germination and vigour) is associated with the longer duration and the rapid provision of energy by the large endosperm to the developing seedling [18]. On the other hand, research has also demonstrated that there is an association between seed physical parameters such as seed coat thickness [22] and endosperm size [23] with seed quality. Of interest is the effect of seed coat thickness on seed electrical conductivity, which is another measure of seed quality.

Electrical conductivity test measures the amount of electrolyte leakage from seed during imbibition. This leakage of electrolytes is due to reorganization of membrane components and conformational changes occurring in cell membranes upon drying and ageing of seeds [24]. Increase in conductivity has been found to be correlated with a decrease in seed quality. The seed coat acts as a barrier restricting the diffusion of nutrients and electrolytes from the seed into the soil [25]. However, this will depend on the seed coat integrity.

Currently, bottle gourd is considered as a neglected underutilized species (NUS) with regional importance in Africa. It has immense benefits, but possible widespread adoption is restricted by the lack of evidence regarding its morphology, husbandry and nutritional benefits for improving human nutrition. The objective of this study was to review the bottle gourd and evaluate possible variation in seed morphology of different landraces in relation to seed quality on the basis of germination and vigour.

1.1 History of Bottle Gourd

Bottle gourd (*Lagenaria siceraria* (Molina) Standl.), also known as calabash gourd or white flowered gourd plant, is a member of the Cucurbitaceae family, Cucurbitoideae sub family, and Benincaseae tribe [26]. The family Cucurbitaceae is comprised of 118 genera and 825 species [27]. The genus *Lagenaria* consists of five other wild species, namely *L. brevifilora* (Benth) Roberty, *L. rufa* (Gilg) C Jeffrey, *L sphaerica* E Mey, *L. abyssinia* (Hook. F.) C Jeffrey and *L. guineensis* (G Den) C Jeffrey, of which *L. siceraria* is the most cultivated [28]. Within the species *siceraria*, two morphologically distinct sub-species of bottle gourd have been recognized *viz. L. siceraria* ssp. *siceraria* and *L. siceraria* ssp. *asiatica* [29]. Bottle gourd has a bi-hemisphere distribution with regional and sub-regional importance [11]. Archeological findings have shown that the independent use and possible cultivation of the crop started from around 9 000 to 10 000 BP (before present) in the Americas (New world), 6 000 – 10 000 BP in East Asia and 4 000 – 5 000 BP in Africa. Based on this archeological evidence, bottle gourd is said to be one of the first species domesticated by humans [27,28,30].

Bottle gourd has long attracted an interesting debate about its centre of origin [26,31]. In that debate, there is strong evidence, that bottle gourd originated from Asia or, despite the lack of early remains but commonly thought, Africa south of the equator to be more precise [32]. The centre of origin of a crop can be described as the area containing the highest number of the wild relatives of that crop and its subsequent domestication. Both continents contain wild species of bottle gourd; however, the discovery of an additional wild indigenous species (*L. breviflora*) in Zimbabwe in 2004 by Decker-Walter *et al* [33] reinforced the latter hypothesis of Africa as the centre of origin.

The origins and subsequent dispersal of bottle gourd still perplexes many scientists. The crop is said to have reached Asia and the Americas about 10 000 to 8 000 years ago, possibly as a wild species whose fruits and seed had floated across the seas and oceans with the aid of currents [33,28]. Whitaker and Carter [34] demonstrated this hypothesis to be possible through experiments that showed that bottle gourd fruit still contained viable seeds even after floating in sea water for more than 7 months. Upon reaching Asia and the Americas, the wild bottle gourd is said to have evolved in to two subspecies, *L. siceraria* ssp. *siceraria* and *L. siceraria* ssp, *asiatica*, respectively [27]. It has been hypothesized that multiple domestications of bottle gourd should have occurred. Through DNA analysis and comparison, it is now certain that two separate events of domestication occurred [30,11,27].The first in Asia around 10 000 BP, then in Africa at around 3 000 BP [26].

1.2 Botany

Bottle gourd is an annual herbaceous plant with a prostrate or branching type growth habit. The leaves are alternate and variable, and tendrils (Fig. 1) are almost always present. Flowers of *L. siceraria* are monoecious in nature, where solitary male and female flowers are found on different plant axis of the same plant, thus cross pollination is highly favorable.

Fig. 1. Different bottle gourd plants

Seed shape of fruits displayed: A and D – Calabash; B – Cucumber; C – Bean shaped; E – Pumpkin; F and G – Cylindrical; H – Club.

Dioecious and andromonoecious sex forms bearing hermaphrodite flowers also exist in wild, non–cultivated types. Like most cucurbits, the sex ratio (male: female) for bottle gourd is high [35]. The proportion of male to female flowers has been shown to affect yield significantly. According to Desai [36], environmental conditions (precipitation, temperature and light intensity) and various growth regulators (Auxins, ethylene, gibberellic acid etc) can be used to alter this ratio.

Bottle gourd fruit vary widely in shape and size, and this is within or among cultivars [37] (Fig. 1). According to Morimoto et al. [38], among the six known species, *L. siceraria* exhibits the widest variations in fruit shape; these are either long, cylindrical, necked, oblong flat or round, conical pyriform to club shaped, while skin texture varies from warted to smooth (Fig. 1). Fruit size varies from 5 to 40 cm wide, and 20 to 90 cm long [35]. Seed forms also differ according to shape, size, presence or absence of frills and seed lines, and seed coat surface texture. The large genetic variability in bottle gourd is a much desirable trait as it also reflects on its wide adaptation it possesses.

1.3 Uses and Opportunities as a Food Security Crop

Bottle gourd is mainly grown as a vegetable for human consumption. However, hard dry shell is often used in utensil and instrument making, hence calabash gourd [39]. Furthermore, in India different plant parts, especially the fruit juice, can be used as medicine to cure stomach elements [40]. In many parts of the world the young green fruit is a popular cooked vegetable [41]. In Southern Africa, the leaves are commonly consumed as a vegetable relish and at times mixed with other vegetable plants. They can also be added fresh to maize porridge [42]. The leaves can also be dried and stored for later use in the off season. In Asia, bottle gourd is used as rootstock for watermelon (*Citrullus lanatus*) against soil-borne diseases and low soil temperature [11]. In West Africa, mature seed of bottle gourd are roasted and ground to a paste, which is used to thicken sauces [39]. In Botswana, Zimbabwe and South Africa, oil is extracted from the seed and used as an alternative to vegetable oil [42] while the defatted cake can be used as a protein supplement. According to Loukou et al. [39], the untapped potential of bottle gourd lies in the use of its seed kernel in the food and livestock feed industry; it is a rich source of oil (45%) and protein (35%).

In Southern Africa, similar to many developing countries, there is a high incidence of protein-energy malnutrition (PEM) in rural and urban populations, with the consumption of a predominantly maize based diet. It has been observed that there are approximately 38.6 percent stunted, 28.4 percent underweight, and 8 percent wasted children under 5 years old, all symptoms of kwashiorkor and marasmus. Although modern science has been able to boost the proportion of essential amino acids (lysine and tryptophan) in maize, the penetration into rural communities is still slow. Due to the inadequacy of maize based diets in supplying much needed essential amino acids the use of bottle gourd seed or defatted seed cake could boost availability as it contains most if not all of the essential amino acids.

According to Axtell and Fairman [43], oil extracted from bottle gourd seed is rich in fatty acids (high in essential fatty acids, chiefly linoleic acid) and sterolic compounds (eg spinasterol) and is comparable to semi-siccative oils such as sunflower or grapeseed oil. The human body is not able to produce essential fatty acids on its own, so it is necessary that one consumes a diet rich in these crucial building blocks in order to maintain a healthy body. The use of oil extracted from bottle gourd seed could possibly provide resource limited farmers, and more importantly pregnant and lactating women, and children under 5 years old, with much needed essential fatty acids. Therefore, bottle gourd has potential to contribute to food security and plug dietary gaps.

Other than the provision of essential fatty and amino acids, the young edible fruits of bottle gourd are rich in dietary fiber with very low fat and cholesterol levels and have about 80% water content in its flesh. It contains some amount of iron content and is rich in vitamin B and vitamin C and also contains sodium, potassium and essential minerals as well as trace elements. High sodium and potassium content makes bottle gourd an excellent vegetable for hypertension patients.

Livestock also play a significant role in most small-scale farming systems throughout the world. Despite the importance of livestock, poor livestock nutrition is a common problem in developing countries, and a major factor affecting the viability of livestock industries in these countries [44]. Observations in rural communities of Zimbabwe have shown that leaves, fruit and seed of bottle gourd are being used to supplement livestock grazing and feed resources [44]. Cattle have been observed consuming young tender leaves of bottle gourd, while goats and pigs prefer the fruit [44]. In the wake of increased rural malnutrition, rural farmers still have to cope with high cost of livestock feed, depleting pasture lands and water resources. Increasing production and use of bottle gourd to compliment and augment feed could assist in increasing availability of nutritious feed to livestock, thus increasing food security within these regions.

On the other hand, bottle gourd, like all cucurbits, produces trace quantities of complex substances known as cucurbitacins, which produce a distinctive aroma and help protect the plant from insects and animal predators [45-47]. Cucurbitacins are bitter compounds and have a tetracyclic triterpenoid structure. Bitter bottle gourds have abnormally high levels of these cucurbitacins than the less bitter types. The amount of bitter juice that is consumed decides the level of toxicity. The ingestion of 50 ml of bitter bottle gourd juice can cause complications, while over 200 ml has proved to be fatal [45]. Cucurbitacins present in the juice results in gastrointestinal toxicity which causes abdominal pain, vomiting, diarrhea, hypotension and upper gastrointestinal bleed. Though toxic to animals, the bitter taste is said to deter humans from consuming large amounts, thus, prevents poisoning. Higher levels of these cucurbitacins are triggered by environmental stress, like wide temperature swings, low pH, high temperature, too little water, low soil fertility and improperly stored or over matured fruits [45-47]. Therefore, it is important to have an appreciation of the ecology where bottle gourd is found before introducing it to new locations so as to avoid increasing the toxicity.

1.4 Ecology and Productivity

As stated earlier, adaptation and distribution of bottle gourd is bi-hemisphere and therefore grows well within the tropical and temperate regions of Africa, Indo-Malayasia, the Americas and neo-tropics. Sillotoe [37] and Grubben and Dento [42] observed good adaptation in high elevated sub-tropical, tropical and temperate climates, as well as low-lying semi-arid to arid climates. Bottle gourd grows well in areas with rainfall of between 400 – 1 500 mm per annum; however, moderate, rather than excessive soil water is desired for good harvest [40]. Therefore, bottle gourd is intolerant of water logging. According to Grubben and Dento [42], bottle gourd grows well under warm temperatures (25 – 35ºC). Under frost–free, low temperature conditions it will also grow well provided the plants have attained sufficient vegetative growth before the onset of cool weather. Optimum germination temperature is between 20 and 25ºC. Temperatures below 15ºC and above 35ºC reduce the germination rate [35]. This cucurbit has been observed to do well in a range of soils, which are fertile and well–aerated. Flowering is highly sensitive to photoperiod. Short days, coupled with low night temperatures and high relative humidity, promote the development of male flowers, while the reverse promotes female flowers [40]. Agronomic practices that promote the production of more female flowers than male flowers could increase yields; however, Haque et al. [40] observed less seed set due to the reduction of pollen. It is, therefore, important to determine the optimum ration of male and female flowers to optimize fruit and seed set.

Not much information is available on the production of bottle gourd, especially in the southern African context where women are the main custodians of its husbandry. The FAO provides combined production data for pumpkin, squashes and gourds; as such there is difficulty in ascertaining the exact amount of global bottle gourd production. In Bangladesh, Haque et al. [40] observed yields of 35 t/ha in sub-tropical to tropical conditions and less than 20 t/ha in semi–arid conditions. Hybrid varieties in Asia have recorded yields of more than 40 t/ha under optimum conditions, while local landrace varieties produced less than 25 t/ha. In view of changing climatic conditions, serious poverty and malnutrition, there is a need to unlock the potential of neglected underutilized species, through the generation of information on general crop husbandry.

As a way of understanding the amount of diversity for bottle gourd, different landrace selections need to be collected and stored in regional seed gene banks. This will allow genetic preservation and ease access for researchers. In the context of Africa, research is lacking on agronomic management of crop; fertilizer requirements, plant densities, planting dates and water requirements over different agro-ecological zones. Other areas of research that could be looked into include the efficiency of bottle gourd as live mulch for weed suppression; its water utilization and effect of drought stress on growth, development and yield. It is also clear that there is a dearth in information on seed technology of bottle gourd.

The desire of any farmer is to see the germination and growth of all seeds planted in a field. This way the farmer is assured to obtain reasonable yield if all growing conditions are optimal. This is not always the case with resource limited farmers practicing agriculture in sub-optimum conditions while growing poor quality seed. According to numerous researches, the main source of plant material grown by resource limited farmers is seeds that have been saved from the previous season or exchanged. These sources of seed are often of inferior quality in terms of genetic purity and germination, leading to poor crop stands, lower yield and food insecurity. Although bottle gourd is an ancient crop, there is little information on its seed quality. Since seed is important for crop establishment, it is important to study it as a major element of an underutilized species.

1.5 Seed Quality

Seed quality has been described as a multiple concept comprising several components [12, 13]. Hampton [15] described seed quality as the standard of excellence in certain characteristics that will determine seed performance when the seed is either stored or sown. According to De Geus et al. [12] it is the physiological (seed germination ability and seed vigor) and genetic quality. Thomson [48] included aspects of genetic purity, analytical purity (the absence of contaminants from foreign species and matter), pure seed, healthy seed (the absence of seed borne pathogens), correct moisture content and uniformity in mixing and blending of seed size. On the other hand, Burgrass and Powell [49] stated that seeds with poor quality will show symptoms of typically aged seed such as low viability, reduced germination, poor emergence and seedling growth, and poor tolerance to suboptimum conditions. From all these components of seed quality, Odindo [50] stated that germination capacity and physiological vigour are the two most important indicators of seed quality, because they are intrinsic properties of the seed.

The effect of different seed morphologies on seed quality has been studied; however, the main focus was seed poly-morphism (single plant produces two or more seed types with different morphology) and seed quality. There is a dearth of information on seed quality for different varieties belonging to the same sub-species possessing different seed forms such as bottle gourd. Seed morphology is determined both by seed genotype and parental environment [51]. Most quality characteristics of seeds have been described as polygenically inherited, and will, therefore, be influenced by the environment, to a large extent. For example, Ye et al. [52] observed a genotype by environment interaction on seed quality of cotton, Krishnan and Suryarao [53] in rice varieties, while Cowling and Tarr [54] observed these differences in lupin. Adebisi and Ajala [55] observed significant seed quality differences between different cultivars of sesame harvested from plants grown in diverse populations.

Seed quality of landraces/populations or open pollinated varieties (OPVs) has been shown to be of a lower standard than hybrid seed due to differences in genotype composition. Mabhaudhi and Modi [56] observed better seed quality for hybrid than landrace varieties under optimum conditions, while the reverse was observed under sub-optimum conditions. Wongvarodon and Naulkong [57] observed better germination of bambara landraces than hybrid seed when accelerated aging had been induced. Idikuta et al. [58] observed better germination and vigor for popcorn landrace varieties when compared with hybrid seed under salt and high temperature conditions. Therefore, for a cross pollinated crop like *L. siceraria*, differences in seed quality can be expected between plants, fruits within the same plant and seeds at different positions within the fruit. While genetic makeup determines the base line potential of seed quality, other heritable factors, such as seed size have been found to have an equal role to play towards enhanced seed quality.

Theoretical considerations predict that large seeds will yield better and more competitive seedlings than smaller seed, hence high seed quality [59–62,21]. According to Soltani et al. [63], the possible effect of seed size on seed quality (germination and vigour) is associated with the duration and the rapid provision of energy to the developing seedling. That is, there is a higher seed reserve utilization rate in bigger seeds than small ones. Chastin et al. [23] suggested that larger seeds produce seedlings with better early growth and increased competitive ability against weeds and pests. Amico et al. [64] concluded that higher vigour that occurred in larger seeds was due to the larger food reserves in these seeds. They also noted a positive linear relationship between seed weight and emergence in the field. Baalbaki and Copeland [65] reported that in wheat, seed size not only influenced emergence and establishment but also affected yield components and ultimately grain yield. A similar observation was made by Arunachalam et al. [66] while working with the tree species, and this was attributed to the larger food reserves in the larger seeds. Also, these results indicated that seed size had greater effect on percent than index of germination and emergence. With increased seed size, higher germination and emergence were determined in triticale, but besides higher germination percentage declined median germination time were determined in some forage plants [67]. In another study, Willenborg et al. [68] stated that germination was increased with increasing seed size in oats (Avena sativa L.). In pea (Pisum sativum L.) cultivars with low 100 seed weight had higher germination percentage than larger seed ones [69].

Seed size is considered as one of the least plastic traits of seed morphology [59], and according to Hossain et al. [70] its heritability varies for different species. For example, the heritability in Medicago sativa was 0.14, while Hevea brasiliensis had 0.90. Mendez [71] observed variation in seed size and quality between and within plant species, within plants for inflorescences produced at different growth stages, and for seeds developing at different positions within the fruit. According to Vaughton and Ramsey [72], such evidence is contrary to the theoretical concept that the mother plant will partition all resources equally to developing seed. Causes of variation could be due to the relative position of the fruit [71], differences in nutrient supply by mother plant to developing seeds, often related to genetic quality of the seed [69], parental/sibling conflict and sibling rivalry [73] and parent fitness [74]. Creating trade-offs between seed size and seed number [59,75,18].

Seed is a key input in crop production. All cultural practices are designed to exploit the full genetic and physical potential of seeds sown. No agricultural practices (for example tillage, cultivation, weeding, fertiliser, pest and disease control) can increase crop yields beyond the limit set by the seed quality. Seed is therefore the baseline for success or failure of the crop planted. To increase available information on bottle gourd and to translate effective breeding programs and agronomic practices, it is necessary to obtain information on seed morphology as it may affect seed quality [38,76]. A study was, therefore, done to evaluate the magnitude of variation in seed morphology of different bottle gourd landrace selections and its effect on seed quality on the basis of germination, vigour and EC.

2. MATERIALS AND METHODS

2.1 Plant Material

Six mature fruit of bottle gourd landraces were randomly collected from farmers' fields in Richards Bay (28°19'S; 32°06E; 30 m above sea level (masl)), in northern KwaZulu-Natal, South Africa and Chimbwanda East (18º19'S; 31º12'E; 1484 masl), in Mashonaland East province, Zimbabwe. Before the experiment, seeds were extracted from each fruit and surface sterilised by immersing them in a 5% solution of sporekill for 5 minutes. Seeds were then dried at room temperature (21 to 28ºC) for 24 hours. Table 1 gives a brief description of the landrace selections and agro-ecological characteristics of where they were collected while Fig. 2 is a picture of seeds for the different landrace selections.

Table 1. Description of the bottle gourd landraces and agro-ecological characteristics of where they were collected

Landrace	Fruit shape	Shell texture	Fruit length (cm)	Location	Climate
Cal	Calabash	Smooth	45.6	Richards Bay	Sub-tropical
ZIM 1	Oval	Smooth	29.85	Chimbwanda east	Semi-arid
ZIM 2	Spherical	Warted	26.96	Chimbwanda east	Semi-arid
S	Pumpkin shaped	Smooth	9.49	Richards Bay	Sub-tropical
C	Club shaped	Warted	24.97	Richards Bay	Sub-tropical
R	Spherical	Smooth	11.55	Richards Bay	Sub-tropical

2.2 Experimental Design and Data Collection

All experiments where laid out in a randomised complete block design with three replicates at the University of KwaZulu-Natal's (UKZN) seed technology laboratory. The number of seeds used per replicate varied for each experiment. Details of each experiment are given below.

<u>2.2.1 Seed morphology</u>

Ten seeds of each landrace selection were randomly selected and the following quantitative morphological parameters were determined: seed length (SL), width (SW), size (SZ = SL x SW), mass (SM), seed coat mass (SCM), seed coat thickness (SCT), embryo length (EL), embryo width (EW), embryo size (EZ = EL x EW), and embryo dry mass (EDM). Seed and embryo lengths and widths were measured using a digital vernier calliper (VT Zero (limited), while mass was measured using a digital scale. Seed coat thickness was determined using a Zeiss EVO scanning electron microscope (SEM) in a vapour pressure mode (Chakrabarti *et al*. 2003).

Lignin was determined using the modified acetyl bromide procedure of Liyama and Wallis (1988) except that three replicates of 20 mg samples each were weighed into 4-ml brown vials and 2.0 ml of 25% acetyl bromide containing perchloric acid (70%, 0.08 ml) was added. After digestion, the samples were dissolved in acetic acid and then transferred to 50 ml volumetric flasks containing 2M sodium hydroxide (5 ml) and acetic acid (12 ml). The flasks were made to the mark with acetic acid.

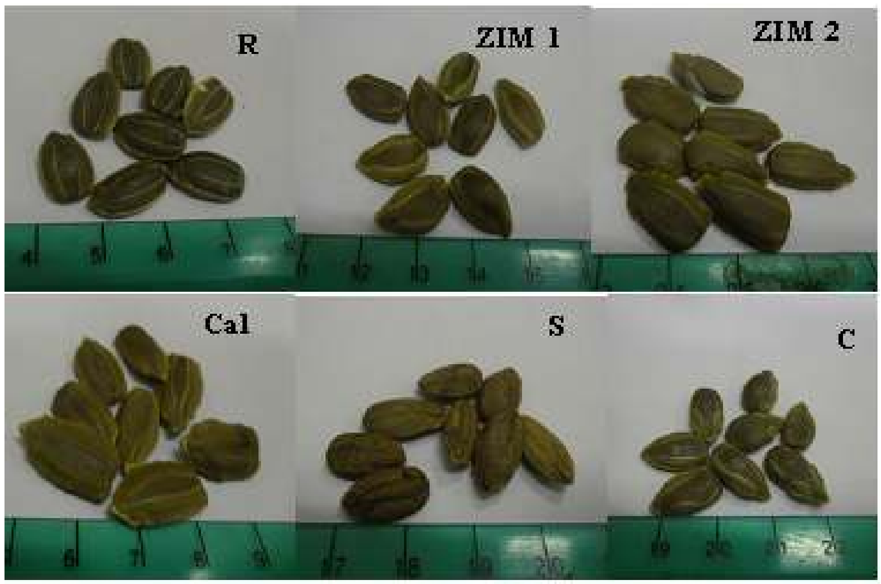

Fig. 2. Pictures of seeds for the different landrace selections (ZIM1, ZIM2, R, S, C and Cal)

2.2.2 Seed quality test

For the standard germination test, three replications of 20 seeds per replicate were germinated between double layered moistened paper towels [77] in an illuminated germination chamber set at 20ºC/30ºC (16 hours day/ 8 hours night) for 14 days [78]. Germination counts were taken daily, with radicle protrusion being the criterion used to indicate germination. Final germination count was based on visual observation of normal seedlings according AOSA [78] guidelines. On day 14, root and shoot lengths, root: shoot ratio and seedling fresh mass were measured. Fungi infection was visually scored with 1 representing no infection and 5 representing heavily infested. Germination velocity index (GVI), was calculated based on a formula by Maguire [79]:

$$GVI = G_1N_1 + G_2N_2 + \dots\dots + G_nN_n$$

Where: G_n is the number of germinated seeds in count n
N_n is the number of sowing days at n count.

Seed electrical conductivity was determined using three replicates of initially weighed 20 seeds per treatment following imbibition in 100 ml of de-ionized water at 25ºC for 24 hours. Following this, electrical conductivity (EC) was measured using an EC meter (Hanna H1 991300).

Imbibition was done on a seed testing water bath (Grant Instruments, England). Ten seeds per landrace selection were placed in a completely randomised design experiment with three replicates per selection. Seeds were imbibed for 0, 15, 30, 60, 120, 240, 720, 840, 960, 1440, 2160, 2880, 4320, 5760 and 7200 minutes and the percentage change in seed mass during imbibition was measured at each time interval.

2.3 Data Analysis

All data were subjected to analysis of variance (ANOVA) using GenStat® (Version 14, VSN International, UK). Means of significantly different variables were separated using least significant differences (LSD) at a probability level of 0.05. Data on morphological traits were then subjected to principal component analysis to establish traits that contributed to seed variation. Raw data were standardized to give a mean of zero and a standard deviation of +/- 1. This was followed by computing and construction distance matrix using variance-covariance coefficients. Eigen values and eigen vectors of the variance-covariance matrix were then computed to generate PC1 and PC2 scores. The landrace selections were then plotted on a bi-plot using the first two principal component scores (PC1 and PC2). Correlation analysis was done on selected variables to establish relationships.

3. RESULTS

3.1 Seed Morphology

3.1.1 Seed and embryo length

Significant differences (P<0.001) were observed across the landrace selections for both seed and embryo length (Fig. 3). Landrace selection Cal had the longest seed length and this was followed by ZIM 2. The shortest landrace selections were R, C and S, respectively. A similar trend of length was observed in embryo length (Fig. 3).

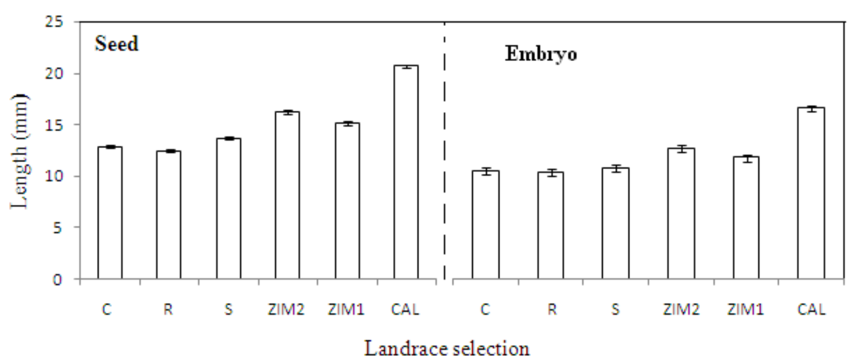

Fig. 3. Seed and embryo length of selected bottle gourd landrace selections (ZIM1, ZIM2, R, S, C and Cal)

Landrace selections significantly different from each other for seed and embryo width at P < 0.001; Mean values ± Standard error of means of six landrace selections.

3.1.2 Seed and embryo width

Highly significant (*P* < *.001*) and significant (*P* = *.04*) differences were observed among the landrace selections for seed width and embryo width, respectively (Fig.4). Landrace selection Cal had the widest seeds and this was followed by R, ZIM 1 and ZIM 2; and these were not significantly different from each other (Fig. 4). The narrowest seeds belonged to landrace selection R. Similar to seed width landrace selection Cal had the widest embryo. Landrace selection ZIM 1 had the narrowest embryo and its width was not significantly different from those of ZIM 2, R, C and S.

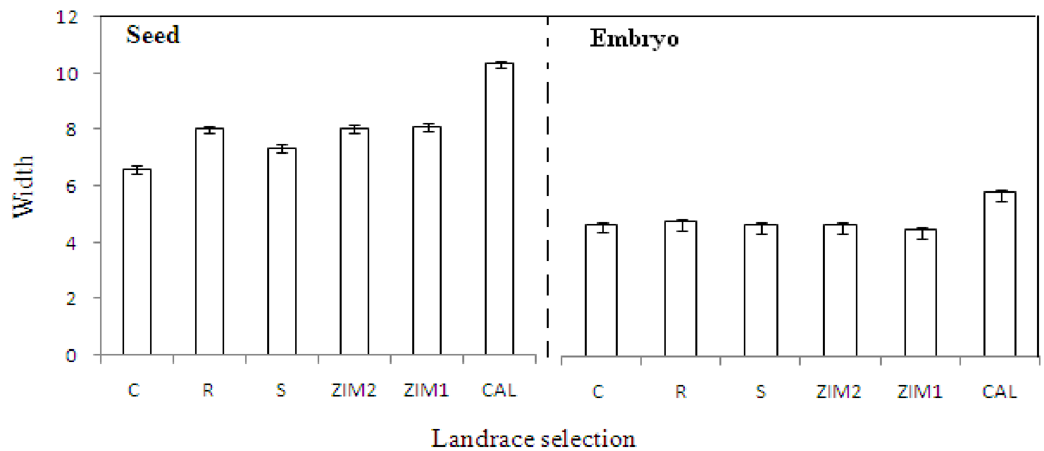

Fig. 4. Seed and embryo width of selected bottle gourd landrace selections (ZIM1, ZIM2, R, S, C and Cal)

Landrace selections significantly different from each other for seed and embryo width at P < 0.001 and P < 0.05, respectively; Mean values ± Standard error of means of six landrace selections

3.1.3 Seed and embryo size

There were highly significant (*P* < *.001*) and significant (*P* = *.02*) differences observed across the landrace selections for seed size and embryo size, respectively (Fig. 5). High standard deviation was also observed for the landrace selection for seed and embryo size (46.23 and 22.36, respectively). As expected, landrace selection Cal had the largest seed size and it was followed by ZIM 2. The landrace selection with the smallest seed size was C (Fig. 5). Landrace selection Cal also had the largest embryo size and this was also followed by ZIM 2. There were no significant differences of embryo size between landrace selections C, R, S and ZIM 1 (Fig. 5).

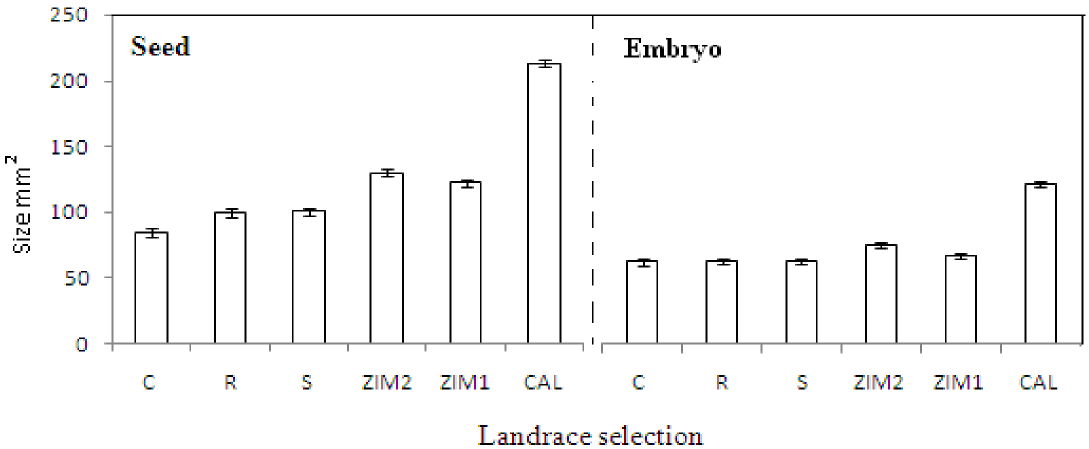

Fig. 5. Seed and embryo size of selected bottle gourd landrace selections (ZIM1, ZIM2, R, S, C and Cal)

Landrace selections significantly different from each other for seed size at P < 0.05; Mean values ± Standard error of means of six landrace selections.

3.1.4 Seed, embryo and seed coat weight

Significant differences (*P* = *.03*) were observed among the landrace selections for seed, embryo and seed coat weight (Fig. 6). Results of the analysis showed that landrace selection Cal had the heaviest seeds while there were no significant differences in seed mass for the other landrace selections. Similarly, Cal had the heaviest seed coat but it showed no significant difference with selections ZIM 1 and C. What was interesting to note was that the embryo mass of landrace selection R was similar to that of Cal, although it had the lightest seed coat and had the smallest embryo size.

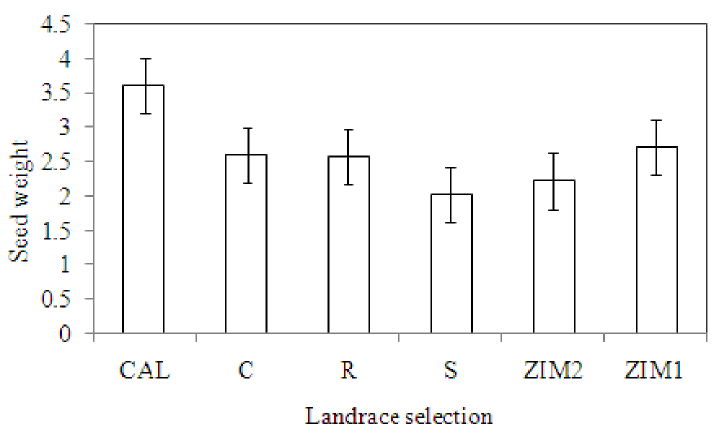

Fig. 6. Seed and embryo lengths of selected bottle gourd landrace selections (ZIM1, ZIM2, R, S, C and Cal)

Landrace selections significantly different from each other for seed weight at P < 0.05; Mean values ± Standard error of means of six landrace selections.

The proportion of seed coat weight contributing towards total seed weight differed for some landrace selections while it was somewhat constant in others (Fig. 7). Seed coat for landrace selection R contributed 53% towards total seed weight, while 47% was contributed by the embryo. Seed coats of landrace selections C, ZIM 2, Cal and S contributed lightly towards the total seed weight with 30%, 33%, 35% and 37%, respectively, while embryos contributed 70%, 67%, 65% and 63%, respectively (Fig. 7).

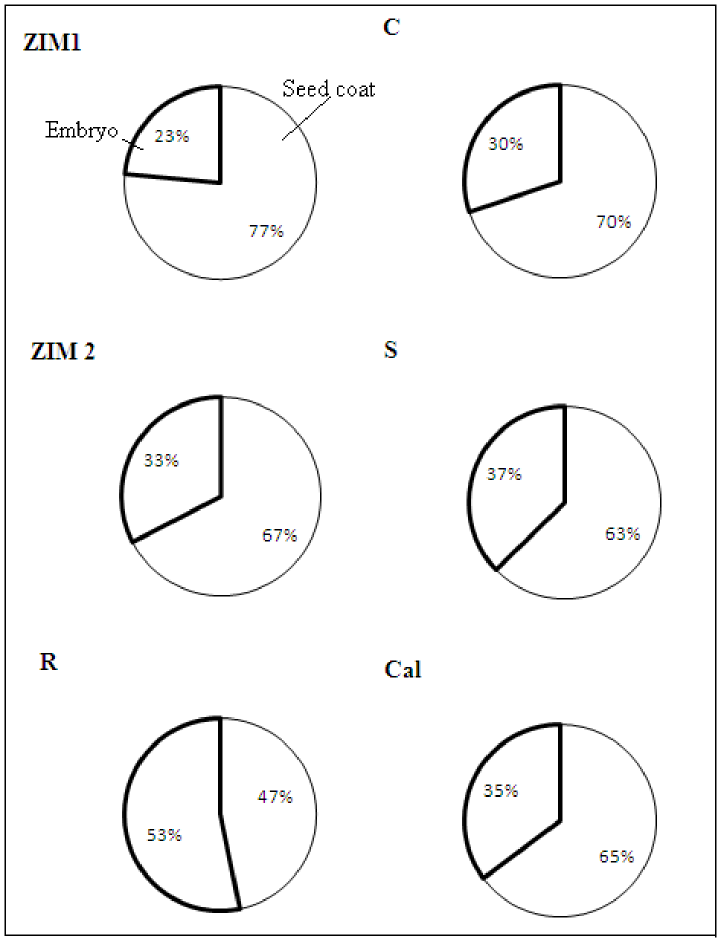

Fig. 7. Percentage contributions of seed coat and embryo weight to total seed weight for six bottle gourd landraces (ZIM1, ZIM2, R, S, C and Cal)

3.1.5 Seed coat thickness

Highly significant differences ($P < .001$) were observed among the selections for seed coat thickness (Fig. 8). High standard deviation of 39.88 was also observed for seed coat thickness across the landraces. Landraces with the thickest seed coats were Zimbabwean selections ZIM 1 and ZIM 2 (Fig. 8). And these selections were not significantly different from each other. Landrace selections with the thinnest seed coats were S and C and these were not significantly different from the other South African landrace selections.

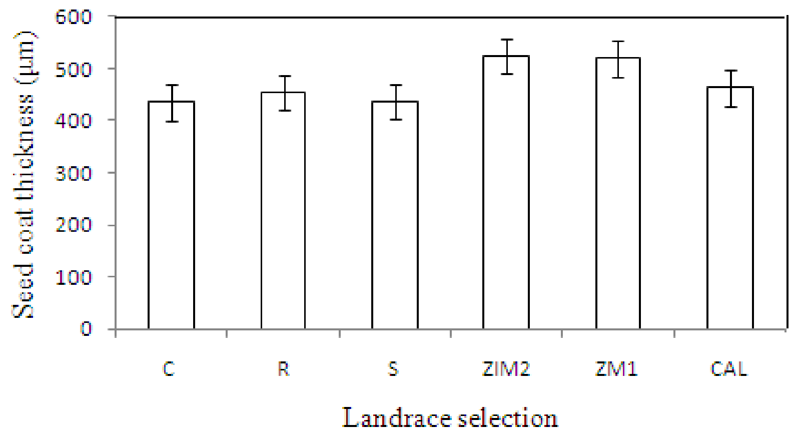

Fig. 8. Seed coat thickness of selected bottle gourd landrace selections (ZIM1, ZIM2, R, S, C and Cal)

Landrace selections significantly different from each other for seed coat thickness at P < 0.001; Mean values ± Standard error of means of six landrace selections.

3.1.6 Total fibre content in seed coat

Significant differences (*P* = *.03*) for total fibre content were observed among landrace selections (Fig. 9). Results show that landrace selection C had the least amount of fibre in their seed coats and selection S had the most (Fig. 9).

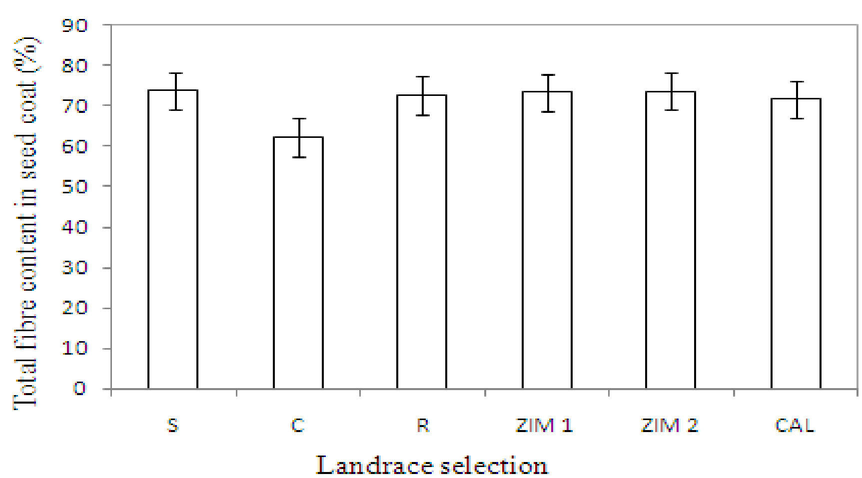

Fig. 9. Total fibre content in seed coats of selected bottle gourd landrace selections (ZIM1, ZIM2, R, S, C and Cal)

Landrace selections significantly different from each other for total fibre content in seed coat at P < 0.05; Mean values ± Standard error of means of six landrace selections.

3.1.7 Principal component analysis

Results of the principal component analysis for the 10 morphological traits indicated that the first two PCs explained 98.64% (PC 1: 65.68% and PC 2: 34.16) of the variation among the landrace selections. Seed size had the largest contribution to PC 1 and this was followed by EZ and SCTH. On the other hand, SCTH had the largest contribution to PC 2 and this was followed by SZ and EZ (Table 2).

Table 2. The first two principal component scores of 10 measured traits on six landrace selections of bottle gourd (ZIM1, ZIM2, R, S, C and Cal)

TRAIT	PC1	PC1
EL[1] (mm)	0.046	0.013
EW (mm)	0.008	0.009
EZ	0.390	0.223
EDWT (g)	0.003	0.004
SL (mm)	0.056	0.011
SW (mm)	0.022	0.006
SZ	0.855	0.263
SWT (g)	0.008	0.006
SCTH (µm)	0.333	-0.939
SCWT (g)	0.007	0.001

[1] *Embryo length (EL), embryo width (EW), embryo size (EZ = EL x EW), embryo dry mass (EDM), seed length (SL), width (SW), size (SZ = SL x SW), mass (SM), seed coat mass (SCM) and seed coat thickness (SCT).*

Fig. 10 shows principal component (PC) analysis plot of first two principal components depicting relationships among bottle gourd landrace selections. The selections were separated into 3 distinct groups. In a clockwise direction, the first cluster was a single landrace selection group which consisted of Cal. The following cluster comprised of the two Zimbabwean selections (ZIM 1 and ZIM 2) while the final cluster had the three local landraces (C, R and S) (Fig. 10).

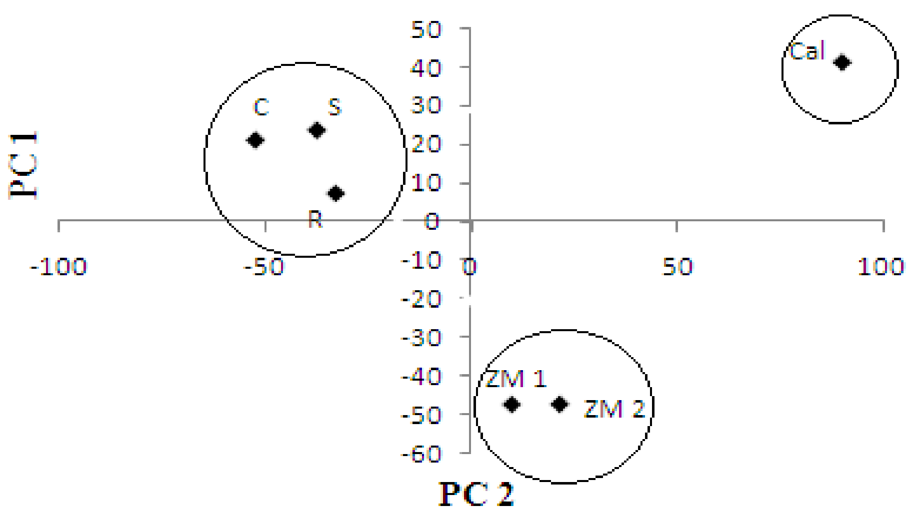

Fig. 10. Principal component (PC) analysis plot of first two principal components depicting relationship among bottle gourd landrace selections (ZIM1, ZIM2, R, S, C and Cal)

Looking at the trait that contributed to seed variation, according to PC 1 and PC 2 at a cluster level, it is evident that the first cluster had thinner seed coats and had smaller seed and embryo (Fig. 10). The second cluster comprised of landrace selections with the thickest seed coats, but had moderately larger seed and embryos than those in the first cluster (Table 3). The landrace selection in the third cluster also had a thinner seed coat, though thicker than the first cluster, but had the largest seed and embryo sizes (Table 3).

Table 3. Characteristics of landrace selection cluster groups based on morphological traits that contributed much variation to seed

Trait	Clusters		
	1	2	3
SCTH[2] (µm)	442.22	521.84	463.53
SZ (g)	95.11	126.43	213.36
EZ (g)	60.20	68.04	116.86

[2]SCTH – seed coat thickness; SZ – seed size; EZ – embryo size.

3.2 Seed Quality

3.2.1 Seed germination

Germination of landrace selections started on the fifth day of incubation with large number of seeds germinating for landrace selection C and ZIM 2 (Fig. 11). Seeds continued to germinate steadily until they reached their peak of 84% on day 12. For R, Cal and ZIM 1, fewer seeds germinated on day 5 and the maximum numbers of germinated seeds were recorded on day 10 for landrace selection R, day 11 for S and day 12 for ZIM 1 and Cal (Fig. 11).

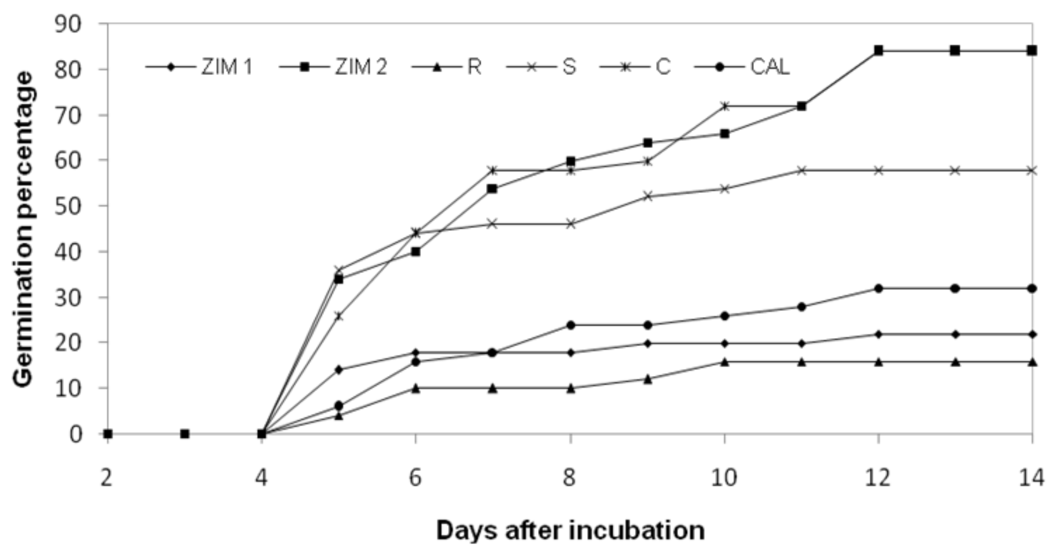

Fig. 11. Progress in daily germination percentages of landrace selections (ZIM1, ZIM2, R, S, C and Cal) during the first 14 days

3.2.2 Electrical conductivity

There were significant differences (*P = .03*) observed for EC across the landrace selections. High EC values were observed for landrace selection R and this was followed by ZIM 2. Cal had the least EC and this was not significantly different from those of ZIM 1, S and C (Fig. 12).

3.2.3 Fungi scores

There were significant differences (P = .05) observed for fungi score across the landrace selections. High fungi scores were observed for Cal, ZIM 1 and R while ZIM 2, S and C had low fungi scores (Fig. 12).

3.2.4 Germination velocity index

There were significant differences (*P = .03*) observed for GVI across the landrace selections. Results showed that Cal and ZIM 1 had the lowest GVI. The rest of the landrace selections had higher GVI and these were not significantly different from each other (Fig. 12).

3.2.5 Seedling dry mass

There were significant differences (*P = .05*) observed for seedling dry mass across the landrace selections. Results showed that landrace selection Cal had the heaviest seedling dry mass. Landrace selection C had the lowest seedling dry weight and it was not significantly different from R and S.

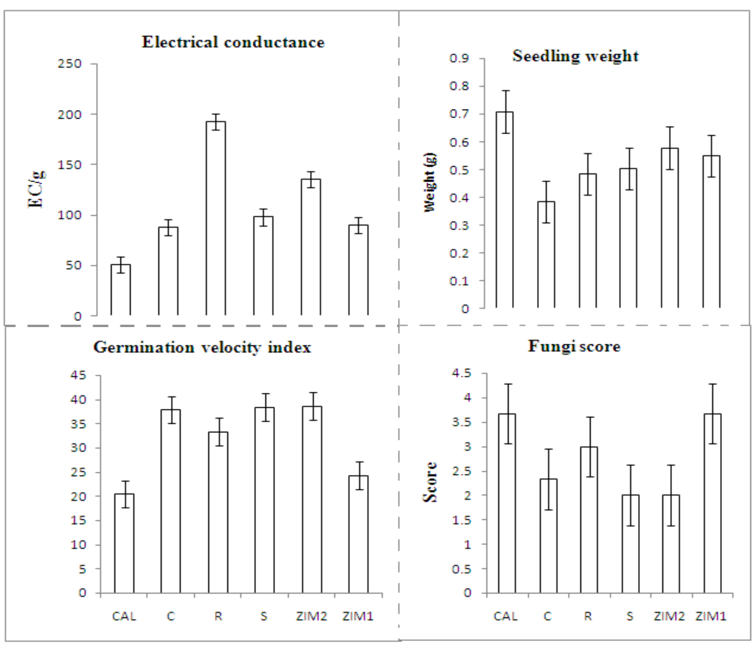

Fig. 12. Electrical conductivity, seedling weight, germination velocity index and fungi scores of different landrace selections (ZIM1, ZIM2, R, S, C and Cal)

Mean values ± Standard error of means of means of six landrace selections.

3.2.6 Seed imbibitions

Results of the imbibition experiment showed that the rate of imbibition differed across landrace selections and at different times measured (Fig. 13). The rate of imbibition for landrace selections R and S was higher than the other varieties as explained by the largest seed weight increment at the shortest time. Landrace selections S, ZIM 1, ZIM 2, and Cal had somewhat the same imbibition rates from the beginning of the experiment till 7200 minutes (Fig. 13). What was interesting to note was that landrace selection C actually lost weight at two time intervals (15 and 60 minutes of imbibition). Similarly, Cal lost seed weight 960 minutes into the experiment of which it there afterwards (Fig. 13).

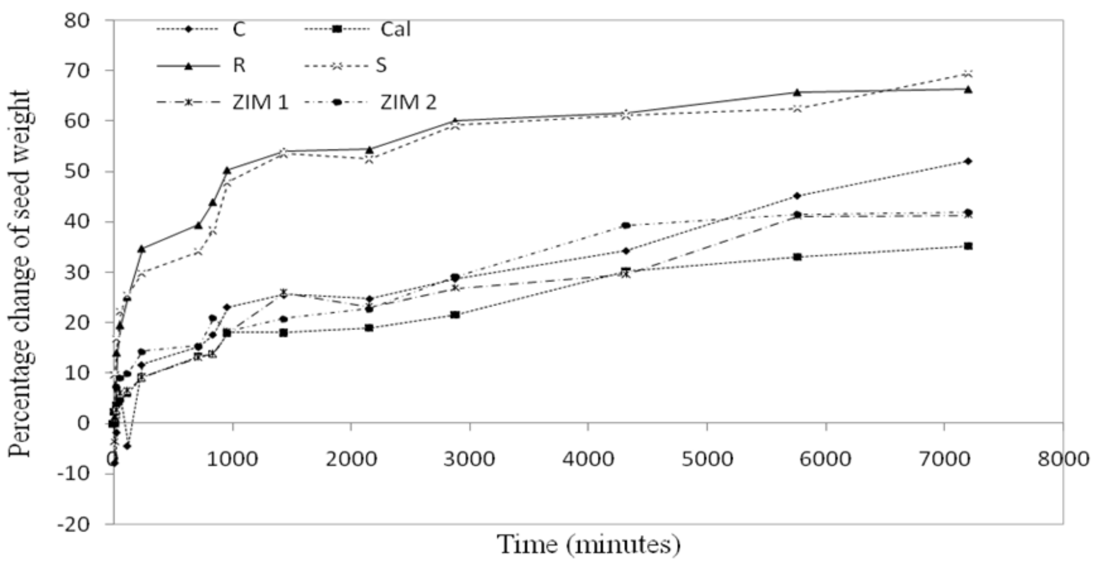

Fig. 13. Percentage change in seed weight of imbibed landrace selections (ZIM1, ZIM2, R, S, C and Cal)

Percentage change of seed weight was recorded at 15, 30, 60, 120, 240, 720, 840, 960, 1440, 2160, 2880, 4320, 5760 and 7200 minutes after incubation on a water bath table.

3.3 Correlations among Morphological Traits and Seed Quality Indices

Positive and significant correlations were observed for most seed morphological traits. Embryo length (EL) was positive and significant correlations with embryo width (EW) (r = 0.872, $P = .02$), embryo size (EZ) (r = 0.980, $P = .001$), seedling mass (r = 0.920, $P = .012$), seed length (SL) (r = 0.993, $P < .001$), seed width (SW) (r = 0.914, $P = .013$), seed size (SZ) (r = 0.990, $P < .001$). Embryo width had positive and significant correlations with EZ (r = 0.951, $P = .011$), SL (r = 0.818, $P = .012$), SW (r = 0.844, $P = .011$), seed weight (SWT) (r = 0.834, $P = .012$) and SZ (r = 0.880, $P = .012$). Embryo size had significant and positive correlations with SL (r = 0.955, $P < .001$), SW (r = 0.915, $P < .001$) and SZ (r = 0.978, $P < .001$).

A negative but significant correlation was observed between embryo weight and root length (r = -0.850, $P = .04$). Embryo size had a positive and significant correlation with seedling mean weight (SDLWT). Although fungal infection was not an experimental treatment, observations showed that it had a negative and significant effect on germination (GERM %)

(r = -0.819, *P = .04*), germination velocity index (GVI) (r = -0.953, *P = .012*), SWT (r = -0.811, *P = .013*) and shoot length (r = -0.877, *P = .02*). Seed weight was positive and significantly correlated with GERM% (r = 0.939, *P < .013*) but was negatively correlated with GVI (r = -0.863, *P = .03*). On the other hand, GVI had a negative and significant correlation with seed coat mean weight (SCWT) (r = -0.875, *P = .014*) and a positive and significant correlation with shoot length (r = 0.881, *P < .001*). Shoot length was positive and significantly correlated to root length (r = 0.0.842, *P = .012*); but it was negatively correlated to seed weight (r = -0.990, *P < .001*). Seed size was positive and significantly correlated with SDLWT (r = 0.948, *P = .013*). Imbibition was significant and negatively correlated to seed coat thickness (r = -0.650, *P = .04*) and seed germination (r = -0.500, *P = .05*). On the other hand, imbibition had a significant positive correlation to total fibre content of seed coats (r = 0.63, *P < .04*) and EC (r = 0.643, *P = .04*).

4. DISCUSSION

Morphological characterization has been used by many scientists as a method of distinguishing differences among plant species populations. Knowledge of existing variation and associations between various morphological traits is vital for many evaluation experiments as it allows the identification of superior performing genotypes [38,76]. Based on results obtained in this study, seed for landrace bottle gourd selections portrayed a wide range of diversity in terms of the quantitative traits measured. These results are similar to those obtained by Decker-Walters [33], Yetişir et al. [11] and Morimito et al. [38] who observed large variation in seed morphology across different bottle gourd landrace selections.

Based on traits contributing most to seed variation, the observed results for standard deviation corresponded well with those of principal component analysis, which lead to the clustering of the landrace selections by provenance and seed size. The observed results on seed coat thickness are similar to those observed by Nooden et al. [80] in soybean, who associated seed coat thickening with adaptation strategies to stress conditions such as moisture and heat. Seeds obtained from Zimbabwe were produced in semi-arid conditions whereas those obtained in Richards Bay were from a more sub-tropical climate. On the other hand, it was observed that thicker seed coat had negative effect on root: shoot ratio. According to Bewley [16] thick seed coats can act as a barrier against radicle protrusion. Radicles will therefore, take longer to emerge from the seed coat thence appearing shorter and reducing root: shoot ratio. During periods of low soil moisture, reduced root: shoot ratio can have a negative effect on seedling establishment since few roots occupy a smaller volume of soil, thus extracting less water. Such landrace selections can succumb more to the negative effect of drought and produce poor quality seedlings. This would suggest that thinner seed coats are more desirable for farmers practicing agriculture in water limited environments wanting faster and more uniform germination since seeds can quickly develop radicles that will absorb water faster before it is lost through negative water fluxes (drainage and evaporation).

Still on seed coat, the observed relationship between this trait and EC was similar to that observed by Borji et al. [81] who showed the positive interaction between the two variables. Borji et al. [81] demonstrated that an increase in seed coat thickness resulted in an increase in the availability of physiochemicals (water soluble compounds) within the seed coat that play an important role in the seed and its integrity when soaked in water. Therefore, seeds with thicker seed coats exude more physiochemicals thus giving off a high EC value. However, contrary to norm, EC was not a good indicator of seed quality. This could suggest that, for seed with thick seed coats, EC should always be accompanied by other seed quality tests to determine overall quality.

The observed differences in imbibition rates is similar to results obtained by Asiedu et al. [82] who observed different rates in different soybean selections. According to Borji et al. [81] the rate of water uptake is proportional to the diffusivity of water, which is determined by factors such as chemical composition, microstructure, moisture and temperature of the seed. Seed coat fibre, namely lignin has been found to affect permeability of seed; therefore, acting as a physical barrier affecting imbibition rate. The observed interactions between imbibition rate and seed coat fibre were also observed by Asiedu et al. [82] and de Sousa and Fihlo [22]. This could imply that seed treatments such as scarification with acid or abrasion could assist in increasing water absorption. On the other hand, other factors that should also be considered as factors that could have had an effect on imbibition are temperature of seed and water, pore size, density and distribution, seed coat colour, solute concentration and initial moisture content of the seed, which have all been found to be positively correlated with imbibition.

The observed changes in seed weight during imbibition are similar to those observed by Alencar et al. [83] who observed its decline in Cereus jamacaru D.C. ssp. jamacaru (Cactaceae). According to Doman et al. [84], cotyledons of most epigeic seedlings differentiate into photosynthetic organs after the hydrolysis and mobilization of stored reserves. During this time of hydrolysis and mobilisation the seed still gets its energy. This has been shown to result in a reduction of total embryo weight but not necessarily size. If seed reserves in the cotyledons are utilised at a faster rate than the rate of differentiation into photosynthetic organs, seeds could die before germination. With regards to bottle gourd, its embryo is rich in lipids and nitrogenous compounds. According to Alencar *et al*. (2012), lipids were the main reserve mobilized during germination because their levels were strongly reduced after seed germination, while proteins were the second most utilized reserve in this process. It would be interesting to establish the changes of biochemical composition of bottle gourd embryo in relation to the developing embryo in the seed axis.

Similar to observations by Nik et al. [85], obtained results in this study showed that large seeds had large embryos. According to Gracia et al. [86] seed size is primarily controlled by embryo size. On the other hand, the large contribution of seed coat weight towards the final seed weight of landrace selection R was similar to results obtained by Islam et al. [87] who observed differences in embryo weight for different varieties of coconut seeds with the same size. Seeds of landrace selections like R could be very misleading for resource poor farmers that extract oil and use the defatted seed cake as a protein supplement. This is because embryo weight and seed oil content have been found to be significantly positively correlated [88]. Therefore landrace selections like R could produce less oil and protein supplement per kilogram harvested.

Similar to results obtained by Akita et al. [89] heavy seeds did not always mean high plant growth rates (root length). Nevertheless, the observed relationship between seed size and seedling dry weight could be due to the longer duration and the rapid provision of energy to the developing seedling provided by larger seeds [64]. Maree [90] stated that the potential of seedling growth is a function of genetics since seed size is genetically controlled.

The obtained results between seed weight and GVI are contrary to those obtained by [91] who observed a positive correlation between the two traits. The lower GVI could be attributed to the high fungal infection observed on seed at day 14 of the experiment. This could be substantiated further with the observed interaction between germination percentage and fungal infection. According to Nik et al. [85], fungi can inhibit seed germination and cause death of emerged seedlings by suffocating seed nutrients required for seed germination and growth.

5. CONCLUSIONS

Seed morphology of the different landrace selections was indeed different. Seed and embryo size and seed coat thickness contributed most to seed diversity. The local landrace selections had either large or small seeds, and all had thin seed coats. On the other hand, seeds of landrace selections from Zimbabwe were medium in size and had thicker seed coats when compared to the local selections. The germination percentage and EC of bottle gourd varieties were different and there were no correlations with most seed morphological traits. Seed size was correlated with seed quality as determined by seed germination rate and seedling quality. Therefore, in this study most measured traits were not good indicators of seed quality. Although, fungus was not a treatment, it had a negative effect on seed germination and vigour. Seed coat thickness may be used as a parameter for recommending varieties into different agro-ecological areas which warrants further investigation. Furthermore, aspects of seed dormancy and seed physiology and their effect on seed quality need to be established. It should be noted that, weaknesses to the study were the number of landrace selections and seeds used per landrace selection. As a future direction, more landrace selections and seeds should be used to increase the accuracy of the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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