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Seed coat structural and imbibitional characteristics of dark and light coloured Bambara groundnut (*Vigna subterranea* L.) landraces

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Abstract

Bambara groundnut is cultivated using landraces of different seed coat colours. However, very few studies have associated the seed coat colour (morphological feature) with other physiological and biochemical processes as underlying the observed differences in seed quality among landraces. This research sought to investigate seed quality characteristics (viability and vigour) of landraces on the basis of seed coat colour with the hypothesis that; seed coat colour could be linked to other properties (physical, physiological, biochemical and ultra-structure) that may account for seed quality with respect to germination, vigour and storage potential. Four landraces were analysed for differences in seed coat colour and seed coat thickness using a scanning electron microscope (SEM). Seed imbibition, electrolyte conductivity, tetrazolium test, and standard germination tests were combined to evaluate the viability of seeds after deterioration through accelerated ageing (AA) at 42 °C and 100% relative humidity (RH) over 5 durations, namely 24, 48, 72, 96 and 120 hours. There were significant differences ($P < 0.001$) among landraces with respect to seed coat colour, seed coat thickness, electrical conductivity (EC), hydration rate,

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germination rate and length of the measured seedling axis. The light coloured landrace, Kazai, had the highest germination (66.9%) whereas the dark coloured landrace, G340A, had the lowest final germination (53.6%) after 120 hours of seed ageing. Likewise, G340A and Kazai had the highest (110.33 μS cm^{-1} g^{-1}) and lowest EC (92 μS cm^{-1} g^{-1}), respectively. Electron microscope revealed that dark and light seeds had the thickest (127 μm) and the thinnest (104.6 μm) seed coats, repsectively. This study highlighted that (1) seed coat thickness and colour alone do not account for hydration pattern of Bambara groundnut landraces and (2) Bambara groundnut seeds viability may not necessarily imply good seed vigour.

Keywords: Agriculture, Plant biology

1. Introduction

Under different eco-physiology, plants have several strategies to perpetuate themselves (Sax and Gaines, 2008). The production of heterogeneous seeds is one such strategy which ensures the survival of next generation (Imbert, 2002). An individual plant species might produce seeds that are heterogeneous with respect to the extent of dormancy, dispersion, and persistence. Not only can heterogeneity affect certain physiological and molecular properties related to seed germination, but also it could be expressed through characteristics such as the colour, the size and the shape, often used as seed heterogeneity parameters (Matilla et al., 2005). In heterogeneous seeds, the above features determined seed behaviour and altered their timing of germination (Smith et al., 2004).

In this work, an emphasis is placed on the existence of Bambara groundnut landraces having major variations in the characteristics of the seed coat colour. These landraces constituted a valuable tool for elucidating the mechanism of germination and perpetuation of seeds. The Bambara groundnut seed coat is the modulator of the interactions between the internal structures of the seed and the external environment (Sano et al., 2016). Seed coat modulates the gas exchange with the environment and water absorption during the germination process (Souza and Marcos-Filho, 2001). The seed coats exercised a special function with regard to the supply of nutrients to the embryo during seed development (Chen et al., 2015).

The physiological quality of Bambara groundnut seeds from certain regions had been compromised by elevated levels of deterioration due to humidity and the rupture of the coat associated with mechanical damage at harvest and during post-harvest (Rao et al., 2017). This situation is due to the fragility of the coat, observed in some landraces currently available for agricultural use. However, the prominence of this characteristic can vary following the landraces' seed coat colours. Kaptso

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et al. (2008) established that dark-coloured seed coats exhibited a semi-impermeability to water, which confers greater resistance to deterioration in the field after physiological maturity and slower soaking during the germination process, thus allowing a better reorganization of the membrane system and less mechanical damage in comparison with light-coloured permeable seed coats which are characteristic of the traditionally favoured landraces.

Despite the knowledge on the properties of dark-coloured seed coats (Amarowicz and Pegg, 2008; Boesewinkel and Bouman, 1984; Deaker et al., 2004), such as semi-permeability, greater epidermal thickness, higher concentrations of phenolic compounds and lignin, which confer to them superior characteristics in relation to the landraces with light seed coat, research aimed at elucidating other attributes of this type of seed has not been adequately explored. As an example, the limited information generated in respect to the physiological characteristics of these contrasting seeds as well as their hydration patterns can be mentioned. The present study aimed to evaluate the physiological quality and hydration pattern of the seeds of four Bambara groundnut landraces with seed coat colours ranging from light to dark.

2. Materials and methods

2.1. Plant material and seed multiplication

Bambara groundnut landraces (Mana, Kazai, Kazuma and G340A) were sourced from the Department of Research and Specialist Services (DR&SS), Harare, Zimbabwe in June 2017. Seed multiplication was done at the University of KwaZulu-Natal, Ukulinga research farm, Pietermaritzburg (29°40′1.65″S, 30°24′28.61″E), in the 2017/2018 rain season. Weed control was done using hand-hoe. Application of pyrethroid insecticide lambda cyhalothrin was done as a preventative measure for cutworms at a dose of 150 ml ha-1. Harvesting was done manually and the seeds collected in the same manner to preserve their integrity. The moisture content of seeds was monitored at 12.2 ± 0.3% using the Intelligent Grain Moisture meter KM-21G, Durban, South Africa.

2.2. Seed coat colour

Seed coat colour measurements were done on samples of harvested seeds using a stereomicroscope integrated with a computer software (Leica Application Suite 4.0, South Africa). Measurements of the colour codes red, green and blue (RGB) were recorded and converted to hue, saturation and lightness (HSL), Bautista et al. (2014).

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2.3. Seed coat thickness

Seed coat thickness was measured using a Zeiss EVO scanning electron microscope (SEM) (Zeiss, Oberkochen, Germany). Seeds were cryofractured in liquid N2 and split into halves. The seeds were mounted on stubs and secured with carbon insulating tape. The seeds were gold-coated using a gold sputter coat instrument (Quorum Q150R ES) and viewed under the Zeiss EVO SEM in high vacuum mode. Images were captured on the scanning microscope at 5 kV and seed coat thickness and adherence of seed coat with the cotyledon were measured using analysis software (Soft Imaging System, Münster, Germany).

2.4. Accelerated seed ageing

Harvested seeds were subjected to the Accelerated Aging test. The experiment was laid out as a 4 × 5 factorial treatment using a completely randomized design and replicated three times. Accelerated seed ageing was done for 24, 48, 72, 96 and 120 hrs according to Demir et al. (2004), using an incubator consisting of 11 × 11 × 3.5 cm inverted plastic sandwich boxes containing a 10 × 10 × 3 cm copper wire mesh tray. One hundred millilitres of saturated NaCl solution were poured in each plastic box and a dry screen tray was inserted. Twenty seeds of each landrace were weighed using an analytical scale (Presica 125A, Presica Ltd, UK) and placed on the surface of the screen trays. After levelling the seed sample on the surface of the screen tray the lid was secured on each plastic AA box. The AA boxes were placed in a germination chamber (Labcon L.T.I.E, South Africa) set at 42 ± 0.3 °C.

2.5. Electrolyte conductivity and pH

Ten grams aged seeds and unaged seeds (control) of each landrace selection were weighed and placed into beakers filled with 100 ml distilled water. Initial electrolyte conductivity and pH of distilled water were recorded using H198129 pH/EC waterproof tester (HANNA Instruments, Romania). Readings on electrolyte conductivity and pH were taken at 4 hrs intervals over a 24 hr duration. The pH/EC waterproof tester probe was rinsed in distilled water after each successive measurement to eliminate cross contamination of measurements.

2.6. Imbibition pattern

Three samples (replicates), each comprising of ten grams unaged seeds per landrace selection were weighed and each sample immersed in a glass flask filled with 50 ml distilled water and kept for 12 hrs at room temperature (25 °C). The samples were removed from the glass flask after 2 hours, blotted and weighed to 2 decimal places. Percentage change in seed mass during imbibition was

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determined according to Chimonyo and Modi (2013). For comparison of water uptake among landraces, seeds were soaked using beakers for 24 hours, with images taken at three representative time points (8-hourly intervals) during early imbibition. After each interval soaked, seeds were removed from the beaker and the seed blotted on dry tissue. The seeds were subsequently excised with a scalpel blade and placed on a 60 mm glass slide, which was then placed into the bore of the Magnetic Resonance Imaging (MRI) machine (SGI Silicon Graphics Octane2 GE MRI, Germany). Images (autoradiograms) were captured in the transverse (horizontal) orientation.

2.7. Tetrazolium test

Seed samples were preconditioned by soaking in distilled water for 18 hrs, at 25 °C in an incubator, and then excised with a scalpel blade through the embryo. The excised seeds were immersed in a 1% tetrazolium salt solution for 12 hours at 25 °C in an incubator. Seed viability evaluation was done by placing the seeds in categories as viable and non-viable according to the coloration of the embryonic axis, computing only the percentage of viable seeds (TeKrony, 2001).

2.8. Germination test and seedling vigour

Ten seeds for each treatment (ageing duration) were germinated using the paper towel method (Hosseini et al., 2002). Four paper towels were moistened using running tap water and allowed to drain until there was no water dripping. The seeds were placed equidistantly on two moistened germination paper towels and covered using the remaining two. The paper towels were rolled, fastened with elastic bands on opposite ends and sealed in zip-lock bags. The zip-locks were incubated under illumination in a germination chamber (Labcon, L.T.I.E, South Africa) set at 20/30 °C (16/8 hrs) for 14 days. Germination was assessed by counting seeds which exhibited at least 2 mm of radicle protrusion. Seed germination rates were calculated according to Hegarty (1978) and used as an indicator of seed vigour. Seedling vigour evaluation was done by measuring root and shoot length on the 14^{th} day using string and ruler.

2.9. Statistical analysis

Data were analysed by analysis of variance (ANOVA) using Genstat statistical analysis software 18^{th} edition to determine seed coat colour differences and effects for investigated traits. Bar graphs were used to present analysed data. Means were separated using Fisher's Protected least significant difference when treatments showed significant effects on measured parameters at $P < 0.05$.

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3. Results

3.1. Seed coat colour

Hue, saturation and lightness values were significantly different ($P < 0.05$) among landrace selections. Hue, saturation and lightness values ranged from (8−38°), (61.83−83.53%) and (14.33%−33.9%) respectively. Kazai topped the lightness ranking 33.9% and G340A was the least with14.33% (Table 1).

3.2. Seed coat thickness and nonadherence

There were significant differences in seed coat thickness and non-adherence of seed coat to the cotyledon ($P < 0.05$) among landraces (Fig. 1). The thickest coat (127 μm) was observed on G340A followed by Mana (114.2 μm) and the thinnest seed coat (104.6 μm) was observed on Kazai (see Fig. 2).

3.3. Accelerated seed ageing

3.3.1. Seed moisture content

Percentage seed moisture content increased with ageing (Table 2). The greatest percent moisture increase was observed in Kazuma (dark coloured) which showed a water content of 11.9% at 0 h and increased overall by 83.2 % to 21.8% after 120 hrs. Contrastingly, the landrace Kazai landrace with a water content of 12.3% at 0 h, increased overall by 60.2%−19.7% for 120 hours. The landraces Mana (82.6%) and G340 (82.8%) did not markedly differ from Kazuma (83.2 %).

3.3.2. Electrical conductivity of seed leachate and pH

The electrical conductivity (EC) of seed leachate increased with ageing duration (Fig. 3). The EC values for the control treatments varied from 76 μS cm-1 g-1 in

Table 1. Hue, saturation and lightness for Bambara groundnut landraces.

Landrace	Hue (°)	Saturation (%)	Lightness (%)
Mana	8.00^{c}	81.47^{b}	14.77^{c}
Kazai	38.00^{a}	61.83^{d}	33.90^{a}
Kazuma	11.00^{b}	68.43^{c}	18.60^{b}
G340A	8.00^{c}	83.53^{a}	14.33^{c}
P-value	<.001	<.001	<.001
s.e.d	0.707	1.193	1.207
l.s.d	1.631	2.752	2.783
cv%	5.3	2.0	7.2

Means in the same column followed by the same letter are not significantly different according to Fisher's test (P<0.05). Figures with the **same footnote** are not **significantly different**, while figures with *different footnote* are *significantly different.*

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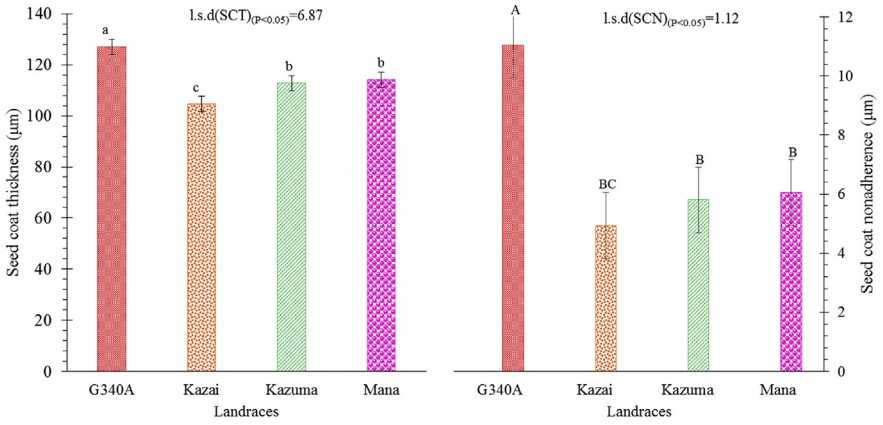

Fig. 1. Seed coat thickness and nonadherence of Bambara groundnut landraces.

Kazai to 90 µS cm-1 g-1 in G340A after 24 hrs. Electrical conductivity increased considerably with accelerating ageing to reach 110.33 µS cm-1 g-1 in G340A and 103 µS cm-1 g-1 in Mana after 120 hrs ageing. Minimum EC was observed in Kazai (92 µS cm-1 g-1) after 120 hrs seed ageing. The potents of hydrogen of seeds leachate decreased with time of seed ageing. The pH of the control treatment dropped most in G340A from 6.98 (initial water pH) to 6.84 after 24 hrs of soaking in water. A sharp drop in pH was observed on 72 hrs ageing treatment and 120 hrs ageing treatment, pH dropped from 6.98 to 6.31 and 5.7 respectively (Fig. 4).

3.3.3. Seed imbibition pattern

Significant differences ($P < 0.05$) were observed between landraces with respect to increase in seed mass after 2 hrs of soaking. The values ranged from 0.28 g (G340A) to 0.15 g (Kazai) (Table 3). The trend showed a general increase on average until after 24 hrs. The landrace G340A showed the highest seed mass (5.25 g) which translates to 52.5% gain of their initial mass. Kazai recorded the lowest increase in seed mass (4.94 g) corresponding to a 49.4 % increase of the initial seed mass.

The results showed that dark coloured seeds imbibed water more rapidly than light coloured seeds (Fig. 5). These findings are contrary to those by Chibarabada et al. (2014) who reported that light coloured Bambara seeds absorbed water rapidly than dark coloured seeds, this was related to permeability of the seed coat governed by their thickness (Coen and Magnani, 2018; Powell et al., 1986; Qutob et al., 2008).

The results from the Magnetic Resonance Imaging autoradiograms revealed that G340A (dark coloured) imbibed water more rapidly, within a period of 24 hrs. G340A and Mana were completely imbibed while Kazai (light coloured) and Kazuma were still imbibing (Fig. 6).

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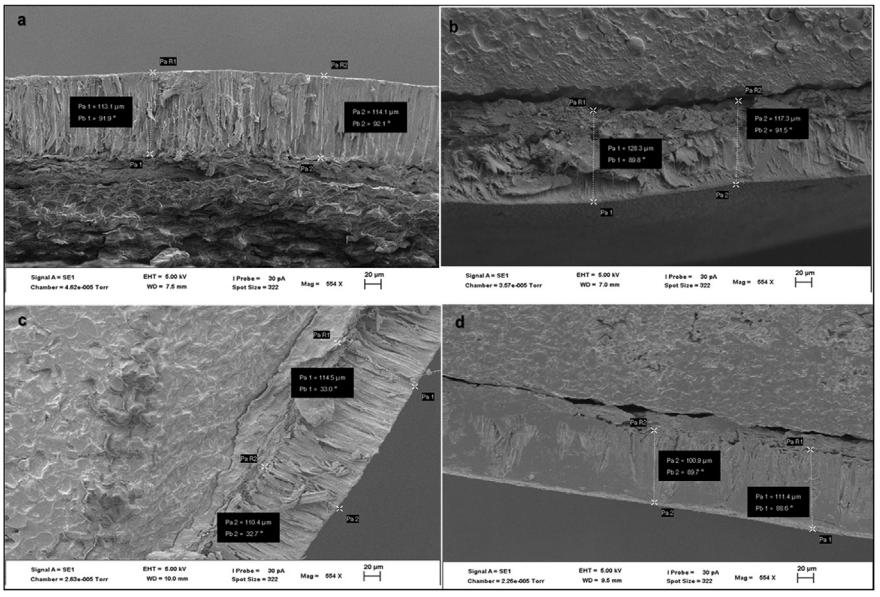

Fig. 2. Seed coat thickness of Bambara groundnut landraces (a) Kazuma (b) G340A (c) Mana (d) Kazai.

Table 2. Effect of accelerated ageing treatment time on seed moisture content (%) for 4 Bambara landraces. Values for each ageing treatment represent percent water content by weight. Percent increase was calculated as: (Difference/0hr) $\times 100$.

Bambara landrace	Ageing treatments (hrs)						Differences	
	0	24	48	72	96	120	(120−0)	% Increase
G340A	12.2	13.3	15.6	18.2	20.4	22.3	10.1	82.8
Kazai	12.3	12.8	13.6	15.1	18.3	19.7	7.4	60.2
Kazuma	11.9	13.1	14.9	17.4	20.2	21.8	9.9	83.2
Mana	12.1	13.5	15.3	17.8	20.6	22.1	10	82.6
l.s.d $_{(0.05)}$	*	0.3766	0.38	0.3261	0.3261	0.3394		
P $_{(0.05)}$	*	0.014	<.001	<.001	<.001	<.001		
s.e.d	*	0.1633	0.1633	0.1414	0.1414	0.1472		
cv%	*	1.5	1.3	1	0.9	0.8		

The footnote (*) represents that there were no values recorded at 0 hrs and the weight did not change hence no values for **l.s.d $_{(0.05)}$**, **P $_{(0.05)}$**, **s.e.d** and **cv%** at 0 hrs.

3.3.4. Seed viability

Significant differences ($P < 0.05$) were observed between the landraces with respect to seed viability (tetrazolium staining) after 120 hrs of accelerated seed ageing (Table 4).

Kazuma and G340A recorded the highest (68.3%) and least (60.3%) viability after 120 hrs of AA. Other AA treatments did not show significant differences, however

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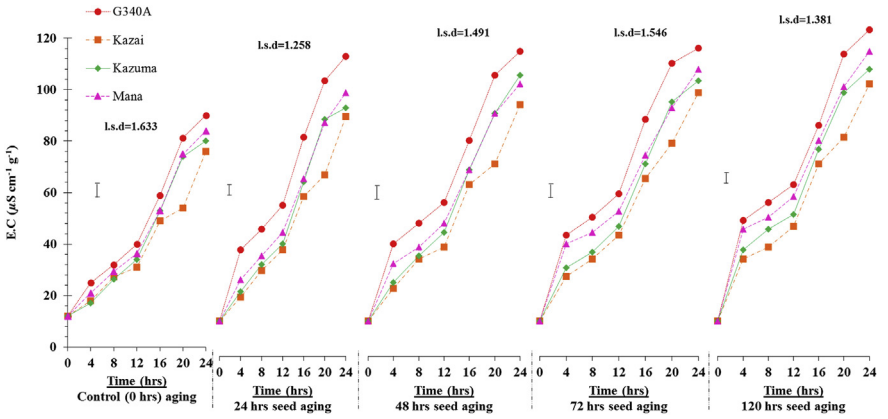

Fig. 3. Effect of accelerated ageing on electrical conductivity of seed leachate (EC) of 4 Bambara groundnut landraces.

they followed a general trend, reduction in percentage viability was proportional to AA duration (Table 4).

The TZ-test furnish quick estimates of seed germinability. The mechanism of this test is based on the fact that all living tissues which respire are capable of reducing a colourless chemical 2,3,5 triphenyl tetrazolium chloride (TTC) into a red coloured compound formazan (Fig. 6) by hydrogen transfer reactions catalysed by dehydrogenases (Ellis, 2013).

Staining of embryos (Fig. 7A) indicates the presence of dehydrogenase enzyme in their active and stable state, hence the seeds will be viable if planted. Dehydrogenases belong to the class of enzymes called oxidoreductases. During germination, stored food in cotyledons is liberated by anaerobic respiration. Anaerobic respiration is made possible by dehydrogenases which catalyse catabolic chemical processes in anaerobic conditions (Chiu et al., 1995). Non-viable seeds will not stain their embryos (Fig. 7B), this is due to denaturation of oxidoreductases, and therefore the ability to reduce colourless (TTC) to red formazan is lost.

3.3.5. Seed germination

For all Bambara groundnut landraces, unaged (control) seeds had the highest percent germination, ranging from 97 % (Mana) to 94 % (Kazuma) (Table 5). Percentage germination steadily decreased for all landraces from 24 to 120 hours after being subjected to the Accelerated Aging test. The G340A landrace had 95 % germination at 0 hrs (control) as well as the lowest germination rate after 120 hrs of ageing (54 %) which constitutes a greatest decrease in germination of over 41.5% compared to other landraces. In contrast, Mana and Kazai (light coloured) landraces had among the highest percent germination for both the 0 hours control (97.3% and 96.1%,

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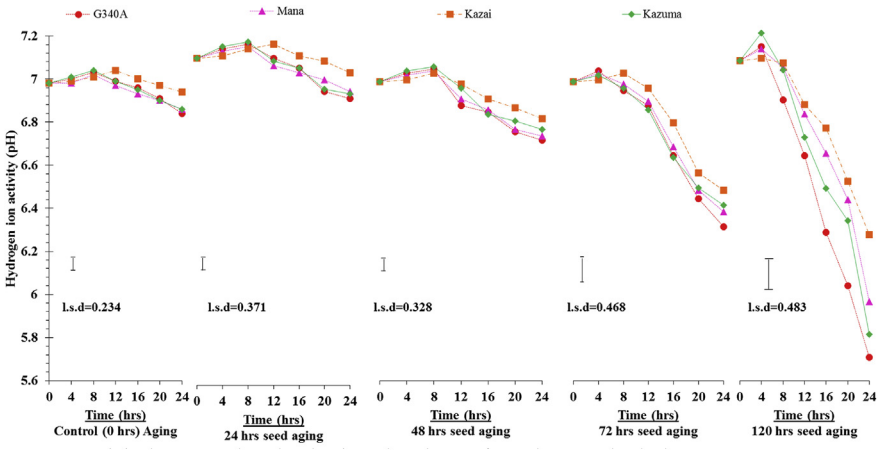

Fig. 4. Association between accelerated seed ageing and pH changes of 4 Bambara groundnut landraces.

Table 3. Change in average fresh mass of Bambara groundnut landrace seeds imbibed in distilled water for 24 hours.

Time (hours) after starting of imbibition	Bambara groundnut landraces				
	G340A	**Kazai**	**Kazuma**	**Mana**	**l.s.d $_{(0.05)}$**
0	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}	*
2	10.28^{a}	10.15^{d}	10.17^{c}	10.22^{b}	0.03564
4	10.43^{a}	10.19^{d}	10.25^{c}	10.29^{b}	0.03746
6	10.68^{a}	10.26^{c}	10.46^{b}	10.41^{b}	0.01633
8	10.84^{a}	10.37^{c}	10.63^{b}	10.65^{b}	0.03768
10	11.26^{a}	10.74^{d}	10.93^{c}	11.08^{b}	0.03261
12	12.84^{a}	11.55^{d}	11.85^{c}	12.5^{b}	0.1911
14	13.76^{a}	12.31^{d}	12.86^{c}	13.28^{b}	0.03756
16	14.24^{a}	13.04^{d}	13.57^{c}	13.9^{b}	0.1911
18	14.66^{a}	13.78^{d}	14.06^{c}	14.2^{b}	0.03261
20	14.93^{a}	14.26^{d}	14.57^{c}	14.64^{b}	0.03562
22	15.16^{a}	14.67^{d}	14.82^{c}	14.95^{b}	0.03716
24	15.25^{a}	14.94^{d}	15.16^{c}	15.05^{b}	0.03261

Means in the same row followed by the same letter are not significantly different according to Fisher's test ($P<0.05$). Figures with the **same footnote** are not **significantly different**, while figures with *different footnote* are *significantly different.*

respectively) and the 120 hours (56.1% and 66.9%, respectively) seed ageing treatments.

3.3.6. Seeding vigour

There were significant differences ($P < 0.001$) on performance of seedling axis (shoot length, root length and seedling length). Interestingly, G340A which had

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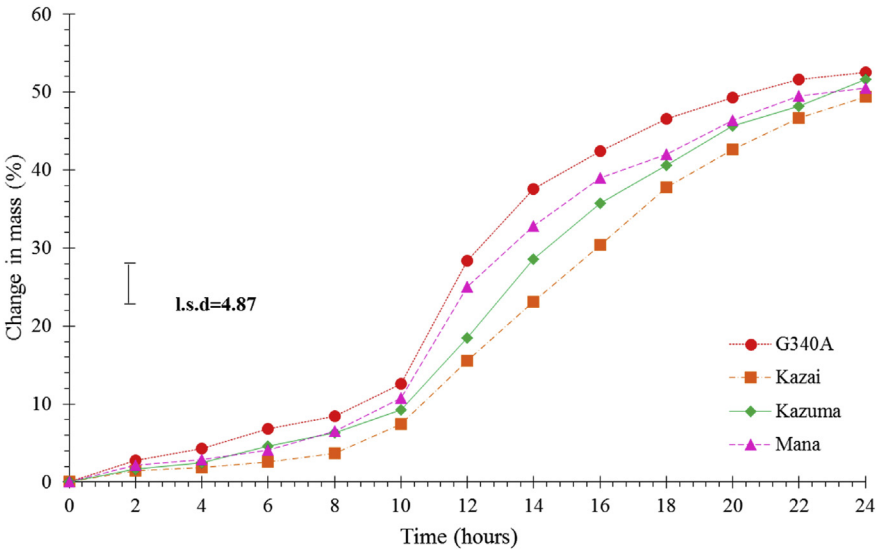

Fig. 5. Percentage accumulated mass of Bambara groundnut landraces.

lowest viability percentage according to TZ-test had the highest performance with respect to seedling axis; shoot length (49.83 mm), root length (111.3 mm) and seedling length (161.2 mm) (Table 6).

In any seed lot, losses of seed vigour are related to a reduction in the ability of seeds to carry out all the physiological functions that allow them to perform. Seeds lose vigour before they lose the ability to germinate. That is why seed lots that have similar high germination values can differ in their physiological age (the extent of deterioration) and so differ in seed vigour and therefore the ability to perform.

4. Discussion

The present study evaluated the physiological quality and hydration pattern of Bambara groundnut landraces seeds varying in coat colour, Hue is the measure of true colour by wavelength and this can be influenced by phenolics in *Arabidopsis* (Nakayama and Komatsu, 2015) or anthocyanins in soybean (*Glycine max*) (Atanassova et al., 2004). We perceive colour differently and there is a complexity on the colour trait for Bambara landraces with diverse colour schemes. Hue (colour) was used in this study to mitigate physical characters such as seed coat topography and possible translucent reflection from the cotyledon. A thin seed coat permits a passage of incident light to illuminate the internal cotyledon pigments such as carotenoids (Hossain et al., 2011). Similarly, epidermal cells in flower petals influences apparent flower colour through light reflection and refraction (Pfündel et al., 2008). Furthermore, in chickpea (*Cicer arietinum*), pleiotropic action between factors responsible for cotyledon colour and seed coat colour has previously been reported (Hossain et al., 2011). This highlights likely subjective errors when using

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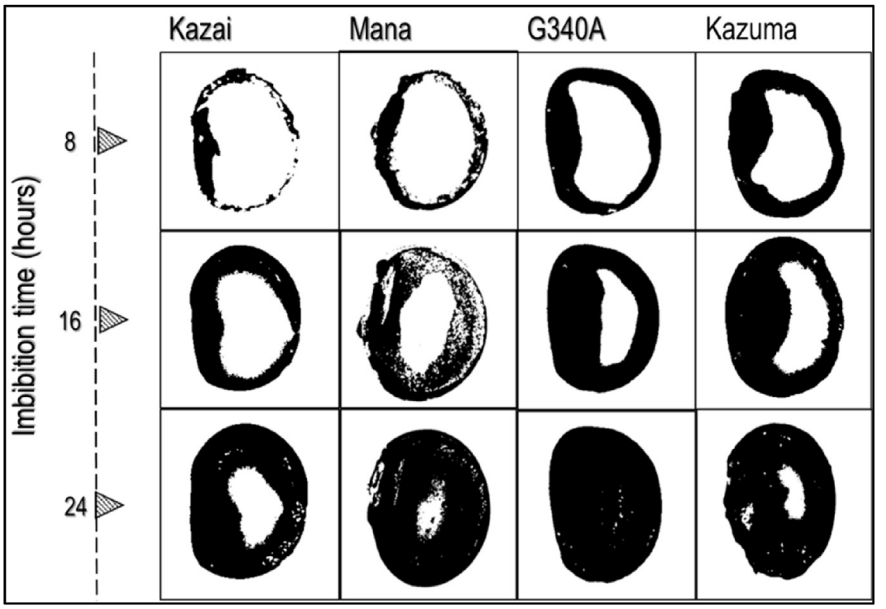

Fig. 6. Magnetic Resonance Imaging autoradiograms showing water movement into Bambara groundnut landraces after 8, 16 and 24 hours of imbibition.

Table 4. Percentage viability of four Bambara groundnut seed lots by tetrazolium test after different seed ageing periods.

Bambara landrace	Ageing treatments (hours)					
	0	**24**	**48**	**72**	**96**	**120**
G340A	97.7[a]	95[a]	83.3[a]	82.3[a]	70.3[a]	60.3[c]
Kazai	97.7[a]	97.7[a]	83.3[a]	80[a]	74.7[a]	64.3[b]
Kazuma	97.3[a]	96.7[a]	85[a]	82[a]	72[a]	68.3[a]
Mana	96[a]	97.3[a]	84[a]	82[a]	72.3[a]	66[a]
l.s.d $_{(0.05)}$	2.977	7.04	7.91	4.861	4.416	4.210
P $_{(0.05)}$	0.550	0.824	0.955	0.862	0.237	0.014
s.e.d	1.291	3.06	3.43	2.108	1.915	1.826

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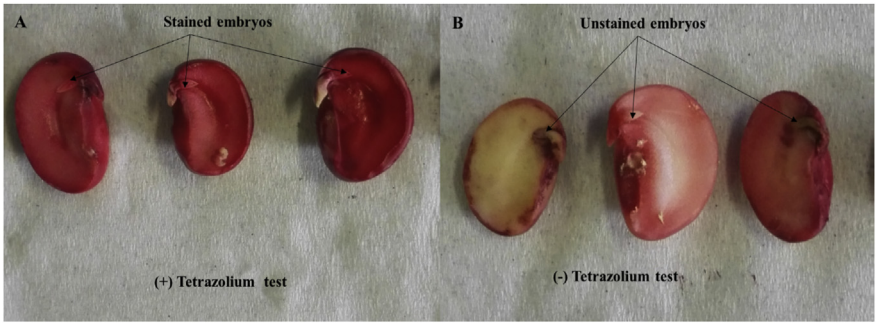

Fig. 7. Tetrazolium tests among Bambara groundnut landraces (A) positive test (B) negative test.

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Table 5. Effect of accelerated ageing treatment time on germination (%) of 4 Bambara landraces. Values for each ageing treatment represent percentage germination. Percent decrease was calculated as: (Difference/0hr) ×100.

Bambara landrace	Ageing treatments (hours)						Differences	
	0	24	48	72	96	120	(120−0)	% Decrease
G340A	95.1	91.2	82.6	74.7	65.3	53.6	−41.5	43.64
Kazai	96.1	94.2	89.6	82.4	77.3	66.9	−29.2	30.39
Kazuma	94.3	90.3	86.3	78.3	71.6	61.1	−33.2	35.21
Mana	97.3	93.3	83.3	76.2	68.6	56.1	−41.2	42.34
l.s.d $_{(0.05)}$	0.3766	0.2977	0.3843	0.3216	0.3646	0.3766		
P $_{(0.05)}$	<.001	<.001	<.001	<.001	<.001	<.001		
s.e.d	0.1633	0.1291	0.1667	0.1394	0.1581	0.1633		
cv%	0.2	0.34	0.2	0.51	0.28	0.62		

visual based methods to artificially place seed into colour groupings for scientific reporting.

Seed coats of legumes are composed of (1) a surface deposit of waxy material interfused with a lipoidal substance, β-sitosterol, (2) a subjacent encrustation of hemicellulose-cellulose complex and (3) a layer of palisade cells in which the secondary walls are impregnated with arabinan and the lumen contains tannin and phenolic compounds (Rangaswamy and Nandakumar, 1985). Our findings revealed that the dark-coloured seeds had the thickest seed coats, this concurred with previous findings by Maldonado et al. (1996) indicating the resistance of dark-coloured common bean cultivars to infestation by Mexican bean weevil (*Zabrotes subfasciatus*). The authors explained this to be due to an extensive deposit of tannin cells. The findings also agree with Chibarabada et al. (2014) who reported thick seed coats in dark-coloured seeds. It is apparent that seed coat thickness influenced both by hue and saturation. These results suggests that seeds of landraces with low degrees of hue can be classified as having thick seed coats. Generally, landraces that had the thickest seed coats also had the highest nonadherence. Data on seed coat thickness of landraces substantiate the association of seed coat thickness and seed coat nonadherence.

An increase in seed water content after ageing is attributed to absorption of water vapour by the seed coat. High temperature and moisture content increase the respiration of seeds, causing seeds to deteriorate rapidly (Goel et al., 2003). Kapoor et al. (2011) reported that prolongation of seed ageing led to deterioration of cell membranes and increased seed moisture. The darkest landrace G340A had the highest increase in seed water content (82.8%) and on contrary the lightest landrace Kazai had lowest percentage increase (60.2%). Differences in percentage increase of water content by landraces can be attributed to the structure of seed coats (Shao et al., 2007).

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Table 6. Performance of Bambara groundnut landraces seedling axis under the standard germination test.

Bambara landraces	Shoot length (mm)	Root length (mm)	Seedling length (mm)
G340A	49.8[a]	111.3[a]	161.2[a]
Kazai	20.3[c]	39.0[d]	59.3[d]
Kazuma	27.7[b]	86.0[b]	113.7[b]
Mana	25.2[b]	48.3[c]	73.5[c]
l.s.d	5.055	7.27	10.5
P-value	<.001	<.001	<.001
cv%	8.7	11.4	13.7

Figures with the **same footnote** are not **significantly different**, while figures with *different footnote* are *significantly different*.

The outer layer of dark coloured seed coats may consist of a gelatin like substance which take up free humid water rapidly. On other hand, the light coloured seed coats may have deposits of waxy material which prevents water absorption. Previous anatomical study established that the features consistently correlating with seed coat permeability to water were small cuticular cracks, visible with SEM, on the surface of the seed coat (Ma et al., 2004; Yaklich et al., 1986).

Autoradiography examination of seeds during imbibition provided both a non-destructive and detailed visual aid of water uptake and distribution in Bambara groundnut landraces seeds, as well as a clearer indication of water uptake at different time intervals (Fig. 6). According to our findings, dark coloured seeds (G340A and Mana) imbibed water rapidly compared to light coloured seeds (Kazai and Kazuma). This could probably attributed to a high matric potential of dark coloured seeds, hence very low water potential that could lead to a rapid water intake (Malik et al., 2004). This also explains the low germination percentage in dark coloured seeds as a result of imbibition damage (Kovach and Bradford, 1992; Obroucheva et al., 2017). The seed water potential is a sum of three distinct components which are, the osmotic potential Ψ_s due to dissolved solutes, the matric potential (Ψ_m) due to the tendency of proteins (phenolics) to attract water and the turgor pressure (Ψ_p) (Woodstock, 1988). Of the three components of seed water potential, it is the matric potential which is primarily responsible for imbibition. Dark coloured seeds are rich in phenolics and antioxidants than light coloured seeds (Van Ha et al., 2007; Yu et al., 2005).

Seed deterioration can be evaluated by measuring seed solute leakage. It has been found that biological membranes regulate the influx and efflux of materials, so they play a key role in maintaining seed viability and vigour. Following rehydration, solute leakage from seeds accompanies seed imbibition during the membrane reorganization period. Therefore, seed leakage rates correlate with the degree of cell membrane damage and repair in response to ageing (Khan et al., 2005). Free

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radicals, are produced in cell membranes when exposed to accelerated ageing, and they are deleterious to membrane resulting in ion leakage (as determined by electrical conductivity). The general trend on pH of soak water was a slight increase in pH between 0−12 hours followed by a gradual decrease in pH. This phenomenon can be explained in terms of basic and acidic cations leaked into the soak water. Materials leached from seeds during imbibition include a variety of substances like inorganic ions, sugars, amino acids, enzymes, nucleosides and nucleotides, and fatty acids. Kinetics of potassium, phosphate, amino acid, sugar, protein, and total electrolyte efflux from aged and control Bambara groundnuts axes indicated that cell rupture as well as altered membrane permeability influenced the drop in pH.

The results showed that dark coloured seeds imbibe water more rapidly than light coloured seeds, these findings do not agree with those by Chibarabada et al. (2014). They reported that light coloured Bambara seeds absorb water more rapidly than dark coloured seeds, this was related to permeability of the seed coat governed by their thickness. The route that water imbibition follows is controversial, three structures (1) the hilum (2) the micropyle and the raphe have been proposed as possible sites of water entry (Ellis, 2007). Hence, seed coat thickness alone does not account for impermeability since even in a thick seed coat, water may access the embryo via specialized regions such as the hilum, lens or raphe. In contrast, the results from this study agree with those from Bertling et al. (2018), who reported that dark coloured seeds of chicory (*Cichorium intybus*) imbibed faster than light seeds during the remaining seven hours of imbibition. Research with peas (*Pisum sativum*) (Wojtyla et al., 2006) and cowpeas (*Vigna unguiculata*) (Mwangwela et al., 2006) has shown that water entry in legumes occurs primarily through the seed coat. With soybeans, most evidence suggests that water entry occurs through the seed coat. Qutob et al. (2008) observed that seed coat was the site of water entry in normal soybeans and the barrier to water entry in hard soybeans. On the hand, among the common bean (*Phaseolus vulgaris*), the seed coat is impermeable because of waxy cuticle layer. Avanza et al. (2012) studied water entry into beans and found that the micropyle was the location of greatest water entry, and that water movement was positively associated with micropyle size.

According to our findings, as seed deterioration increases, seed percentage viability progressively decreased. Progressive decline in viability can be explained by the biochemical manifestation of seed deterioration. Seed deterioration is associated with cellular, metabolic and chemical alterations including chromosome aberrations and DNA damage, impairment of RNA and enzyme denaturation (Kapoor et al., 2010, 2011).

The slow reduction in germination rate after 24 hours of seed ageing may be due to the impairment of metabolic processes caused by membrane aberrations and the need for repair mechanisms to take place in order to compensate for the accumulated

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damage. However, after 48 hours of ageing seeds, cells cannot tolerate the severe biochemical changes that's why seed germination decrease (Bewley et al., 2013). The decrease in percent germination may be due to degradation of the mitochondrial membrane, leading to a decrease in energy supply for germinating seeds. Decrease in germination ability was well correlated with increase in membrane deterioration, as assayed by electrical conductivity and electrolyte leakage in soaked seed.

By the results, it was verified that there was concordance between the classification obtained by the tetrazolium test using the methodology proposed in this study, and the germination test. Loss of seed viability with accelerated ageing in the four Bambara landraces tested was associated with increased seed water content (seed conductivity) and electrolyte leakage. High temperature, ambient relative humidity and seed moisture trigger biochemical and physiological changes of seeds leading to viability loss. The slow reduction in germination rate after 24 hrs of seed ageing is due to the impairment of metabolic processes caused by membrane aberrations and the need for repair mechanisms to take place in order to compensate for the accumulated damage. However, after 48 hrs of ageing seeds, cells cannot tolerate the severe biochemical changes that's why seed germination sharply decrease.

5. Conclusion

This study has revealed that seed coat thickness and colour alone do not account for hydration patterns in seeds. Based on the results of this study, the light coloured landrace (Kazai) imbibed water slowly than the dark coloured landrace (G340A) (Fig. 6). These findings reset the debate on the seed imbibition pattern in relation to their coat colour. This inconsistent behaviour amongst different landrace selections justifies the need to understand the microstructure of Bambara groundnuts. To perceive the imbibition pattern possessed by landraces of different seed coat colour, further examination of structural differences between dark and light Bambara seeds might be needed with a special focus on a SEM study of the three major water entry sites of the seed which are; the hilum, the micropyle and the raphe in dry seeds.

On the other hand, our study revealed that the seed viability may not necessarily imply good seed vigour. Indeed, the light coloured landrace of Bambara groundnuts used in this work was more viable compared to the dark-coloured landraces. Low viability in dark-coloured seeds can be explained by rapid water uptake which is the main cause of imbibition damage, seen in the death of cells of the cotyledons and high solute leakage, which has been shown to reduced viability of seeds. On contrary, although dark-coloured seeds exhibited low viability, their vigour was high in terms of seedling axis length. The possibility for the presence of greater stored energy reserves for emergence within dark-coloured seeds can explain this scenario. Literature and studies to scrutinize association between seed coat colour

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and cotyledon mineralogy are lacking and findings from this work suggested that future research should also focus on these characteristics. This may help to explain why dark-coloured seeds had low viability but high vigour.

Declarations

Author contribution statement

T Mandizvo: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

A.O. Odindo: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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