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Overcoming the Physical Seed Dormancy in Bambara Groundnut (*Vigna subterranea* L.) by Scarification: A Seed Quality Study

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Abstract: Bambara groundnut (*Vigna subterranea* L.) is a neglected African crop legume with potential to play a significant role as a staple and industrial crop in sub-Saharan Africa. The crop can compete with *Phaseolus vulgaris* and *Glycine max* under harsh condition associated with climate change. However, there are some challenges associated with successful production of bambara groundnut, such as poor crop establishment due to physical seed dormancy. This study was conducted to investigate the effect of scarification on overcoming seed dormancy in bambara groundnut. Bambara groundnut landrace seeds were characterized by seed coat colour (cream, light brown and brown) in order to determine the effects of mechanical (sand paper) and chemical (sulphuric acid) seed scarification on germination and emergence. A completely randomized design with three replications was used. Thousand grain mass (TGM), mean germination time (MGT), germination vigour index (GVI), seedling root to shoot ratio, seedling dry mass and field emergence were determined. Thousand grain mass increased with the decrease in seed coat colour pigmentation. Brown seeds had the highest final germination and field emergence, and cream seeds had the least. Imposing chemical or mechanical scarification improved germination as compared to no scarification. However, seedling establishment was vigorous in seeds that were not scarified. Seed quality in bambara groundnut is highly influenced by scarification and seed coat colour. The practical implications of the study are that producers can use scarification to improve bambara groundnut germination, however further research through seed enhancements is still needed for seedling establishment under field conditions.

Key words: Germination, scarification, seed coat, seedling, vigour.

1. Introduction

Bambara groundnut is the third most important indigenous African legume after peanut (*Arachis hypogaea*) and cowpea (*Vigna unguiculata*) [1]. Bambara groundnut possesses great potential for global food security as a drought tolerant crop that is adapted to low input agriculture. It is a staple crop that provides a cheap and rich source of protein [1] and can be used as a healthy substitute of animal protein [2]. The crop is also considered to be a complete food, since humans can survive on it alone due to its rich nutrient composition [3]. While this crop is popular among subsistence or smallholder farmers, it faces a challenge of poor germination and stand establishment which hinders success of its production. This may be attributed to poor seed quality which consequently poses deleterious effect on yield [4-6]. Bambara groundnut has poor emergence of about < 30% [7]. While high potential yield greater than 3,000 kg/ha of the crop can be achieved through breeding [8], yields of 649-1,582 kg/ha [9] and seed yields of 68.5-159.9 kg/ha [10] were reported with no breeding.

Poor seed quality of most legumes is attributed to physical seed dormancy due to their characteristic hard seed coats. Seed dormancy occurs when viable seeds fail to germinate under favourable conditions [11], which is accompanied by temporal suspension of growth and reduced metabolic activities. The metabolic activities that are usually suppressed

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include sugar accumulation and utilization [12], transcription, translation, photosynthesis, respiration and protein turnover, among others [13]. Albeit all types of dormancy involve metabolism suppression, physical dormancy commonly found in legumes is also characterized by palisade or radially elongated cells in seeds causing hardseededness and impermeability. This hampers the passage of water to the embryo [14] which is necessary for germination. Moreover, water impermeability due to physical seed dormancy (hardseededness) was previously reported to be influenced by phenolic compounds [12].

Phenolics are one of the essential plant secondary metabolites that are directly associated with pigmentation in plants, including seed coat colour. Seed coat colour is correlated to mechanical resistance to radicle protrusion due to dormancy in plants [11, 15], which may have direct impact on water imbibition during germination [16, 17]. Germination is hindered by over-accumulation of pigments in seed coats [17]. Kantar et al. [18] further alluded that particularly flavonoid compounds in legume seeds caused physical seed dormancy associated with water-impermeability which hinders germination. A study by Asiedu and Powell [16] reported that pigments in seed coats of most legumes might reduce water absorption, while this is rapid in unpigmented seeds. Therefore, dark seeds germinate slower than lighter coloured seeds due to the presence of strong dormancy [17]. However, unpigmented seeds are highly susceptible to imbibition damage and they hastily deteriorate compared to pigmented seeds [16, 17]. This shows that seed coat colour may play a pivotal role in seed quality and physical seed dormancy.

The challenge of physical seed dormancy may be overcome by seed scarification. Scarification is a technique that is used to promote seed coat permeability [19] through disruption of seed coat while keeping the seeds viable. Aliero [20] previously reported that physical dormancy may be successfully overcome by chemical and mechanical scarification. Mechanical scarification physically creates scars on the surface of the seed [21-23], while chemical (acid) scarification employs chemicals that soften and melt hard seed coat [24]. Mechanical scarification was presented as the most successful method in alleviating legume seed dormancy, achieving 96%-100% seed germination [25]. Uzun and Aydin [26] and Ibiang et al. [19] agreed with these findings using sand paper. In another study, it was reported that sulphuric acid was effective in disrupting the seed coat of *Parkia biglobosa*, which consequently breaks seed dormancy [20]. However, Uzun and Aydin [26] found no germination response of *Medicago* and *Trifolium* species to sulphuric acid.

Although scarification has proven to be effective in overcoming dormancy of many legumes, there is still a need to better understand its effect on seed quality and establish whether dormancy is related to seed coat colour of bambara groundnut. The aim of this study was to investigate the effect of scarification on dormancy breaking in bambara groundnut using chemical and mechanical scarification on seeds with cream, light brown and brown testa, and further establish whether there is an interactive effect of seed coat colour and scarification on seed quality of bambara groundnut.

2. Materials and Methods

2.1 Experimental Design

Bambara groundnut landrace seeds were donated by the local farmers of Pongola in KwaZulu-Natal. The seeds were characterized according to seed coat colours which were cream, light brown and brown (Fig. 1) from a landrace with a mixture of these different seed coat colours. The seeds were scarified before germination and prior to seedling establishment tests in the field for emergence determination. The three scarification treatments were: T_1—no scarification (control), T_2—mechanical scarification and T_3—chemical scarification. The experimental design

Fig. 1 Bambara groundnut landrace seeds characterized by seed coat colour.

was completely randomized design with three replications for both germination and seedling establishment tests. The treatment structure was a combination of three seed coat colours and three scarification treatments, with three replications.

2.2 Thousand Grain Mass

Before scarification treatments were imposed on seeds, three replicates of thousand grains from each seed coat colour were randomly selected. These seeds were measured for thousand grain mass using a digital sensitive balance (Masskot, FX320, Switzerland).

2.3 Seed Scarification

Mechanical scarification was carried out using 150-fine sandpaper. The seeds were scraped 10 times on four regions on the circumference region with sandpaper. Chemical scarification was carried out using 95%-99% undiluted sulphuric acid as per description by Bonner et al. [27]. Seeds were completely immersed in the acid for 10 min at room temperature. Thereafter, seeds were immediately washed thoroughly for 5-10 min in running distilled water.

2.4 Germination Test

Standard germination test was conducted in the laboratory using the paper towel method [28]. Twenty five randomly selected seeds for each treatment were germinated between brown paper towels. The seeds were placed equidistantly on two moist to saturation germination paper towels. Another set of two moist paper towels were used to cover the seeds after placing the seeds on the paper towels. The paper towels were rolled, fastened with elastic bands on opposite ends and then 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 h) for 14 d. Germination was rated daily until 14 d, and seeds were considered germinated if their radicle had protruded at least 2 mm.

Germination vigour indices included germination vigour index (GVI) [29] and mean germination time (MGT) [30], as calculated by Eqs. (1) and (2), respectively:

$$GVI = G_1/N_1 + G_2/N_2 + \ldots + G_n/N_n \quad (1)$$

where, G_1, $G_2 \ldots G_n$ = number of germinated seeds in the 1st, 2nd… last count, and N_1, $N_2 \ldots N_n$ = number of sowing days at the 1st, 2nd… last count.

$$MGT = \frac{\Sigma D_n}{\Sigma n} \quad (2)$$

where, D is the number of days from the beginning of germination, and n is the number of seeds that have germinated on day D.

After 14 d of germination, seedling root and shoot length were measured with a ruler, which was used to calculate root to shoot ratio. Thereafter, seedlings were dried in an oven (70 °C for 72 h) in order to determine seedling dry mass using a digital sensitive balance (Masskot, FX320, Switzerland).

2.5 Field Emergence Test

Another experiment was conducted in the field for determination of field emergence under rainfed conditions at University of KwaZulu-Natal, Pietermaritzburg. The crop was hand planted under planting density of 150,000 plants/ha. Weed control was manual, and there was no infestation of pest, therefore no pesticides were used. Emergence percentage was calculated by counting the number of emerged seeds divided by the number of planted seeds, all multiplied by 100.

2.6 Statistical Analysis

All the data collected were subjected to analyses of variance (ANOVA) using GenStat® Version 17 (VSN International, United Kingdom) at the 5% probability level. Duncan's test in GenStat® at a probability level of 5% was used to compare means. Standard errors were also used to indicate statistical differences which were accepted at $P \leq 0.05$.

3. Results and Discussion

3.1 Thousand Grain Mass

There were no significant differences ($P > 0.05$) in thousand grain mass between seed coat colour treatments (cream, light brown and brown). However, cream seeds were heavier (62.3 g) than light brown (58.9 g) and brown seeds (56.7 g) (Fig. 2). These results were not in line with those of van Molken et al. [31] who reported that darker seeds were heavier than lighter seeds. These results are in disagreement maybe due to the different species used in these studies. In addition to possible different genetic potential to accumulate biomass in these species, the variation in environment and availability of resources might have played part in the inconsistency of these results.

3.2 Standard Germination

3.2.1 Effect of Seed Coat Colour on Germination

Seed coat colour showed significant differences ($P < 0.05$) with respect to germination rate. It was previously reported that seed coat colour significantly influence germination [18]. Dark coloured seeds (light brown followed by brown) were not significantly different, but were superior compared to cream seeds. The final germination was 2.3% greater in pigmented seeds (brown and light brown) than cream seed (Fig. 3). These results agree with those of Sinefu [32]; Mabhaudhi and Modi [33]; van Molken et al. [31].

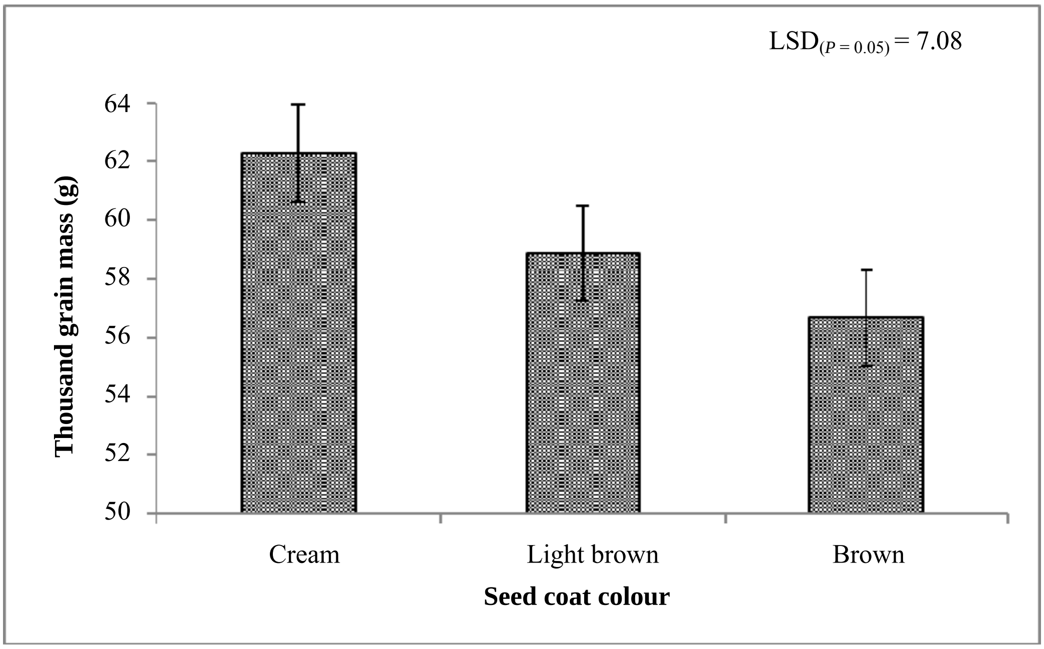

Fig. 2 Thousand grain mass of bambara groundnut seeds based on seed coat colour.

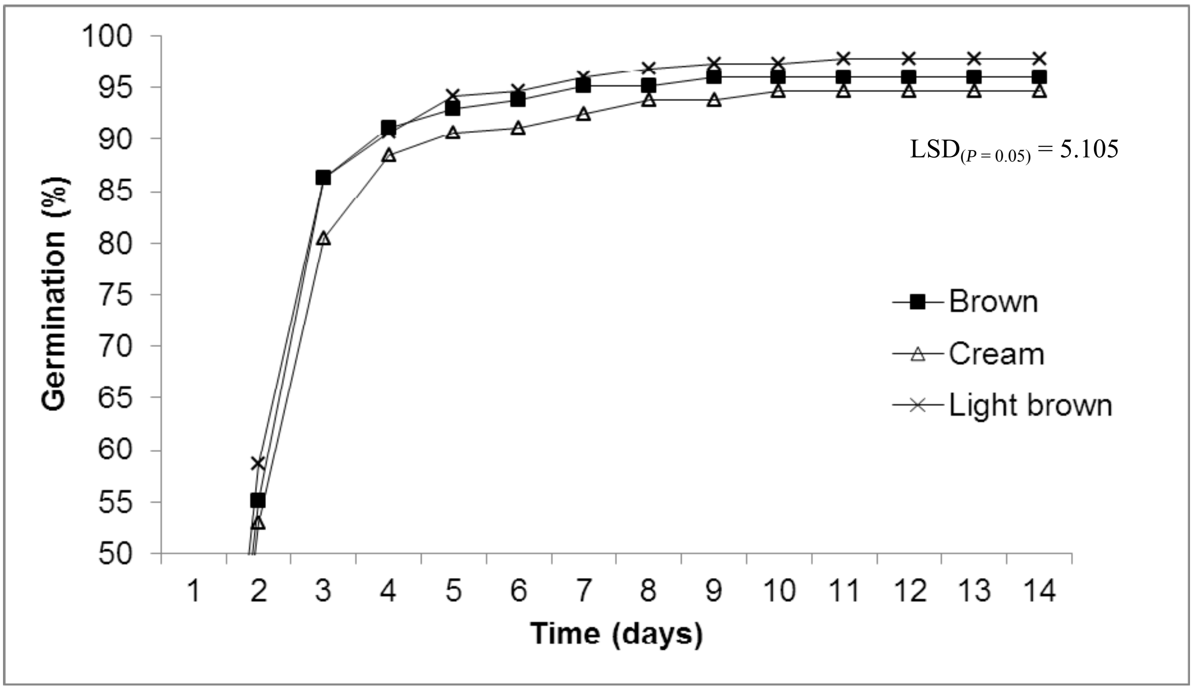

Fig. 3 Germination of bambara groundnut seeds with different seed coat colours.

Cream seeds in this study are larger and heavier than light brown and brown seeds. Even though large seeds are expected to have higher germination [31] due to abundant nutritional reserves available for germination [34], this was not the case in this study. It was also not the case in the study of Upadhaya et al. [35] who further alluded that large seeds also have thick seed coat which hinders radicle protrusion and hence germination.

In another study, light coloured seeds germinated earlier due to their quick imbibitions, yet suffered more imbibition damage which disturbs seed vigour and viability compared to darker seeds [18]. However, secondary metabolites, such as polyphenols (flavonoids) mostly found in darker seeds are important in seed quality, as they protect seeds against imbibition damage in addition to benefits of defence from oxidative stress and solute leakage [15]. Khan et al. [36] reported that imbibition damage was commonly accompanied by poor emergence and low vigour of crop seeds. Therefore, cream seeds in this study might have sustained greater imbibition damage and consequently scantly germinated, compared to brown and light brown seeds.

3.2.2 Effect of Scarification on Germination

Bambara groundnut germination significantly responded to scarification. Scarification of seeds yielded 6.6% higher germination than the seeds not scarified. Mechanical scarification was more effective in overcoming dormancy than chemical scarification, while seeds without scarification had the lowest germination rate (Fig. 4). These findings were congruent with these researches where mechanical method of breaking seed dormancy was also found most successful [19, 25, 26]. Mechanically scarified seeds reached 98.22% germination, which was within the range of 96%-100% seed germination reported by Baskin et al. [25]. However, Aliero [20] found chemical scarification using sulphuric acid to be the most effective method of breaking seed dormancy. On the contrary, when *Medicago* and *Trifolium* species were scarified with sulphuric acid, there was no germination response [26]. These inconsistent results may have been attributed to different species used in these studies. Another reason may be the differences in dormancy breaking enzymes (e.g., alpha-amylase) and phytohormones (e.g., gibberrelic acid) that are found within the seeds, since these are vital in seed

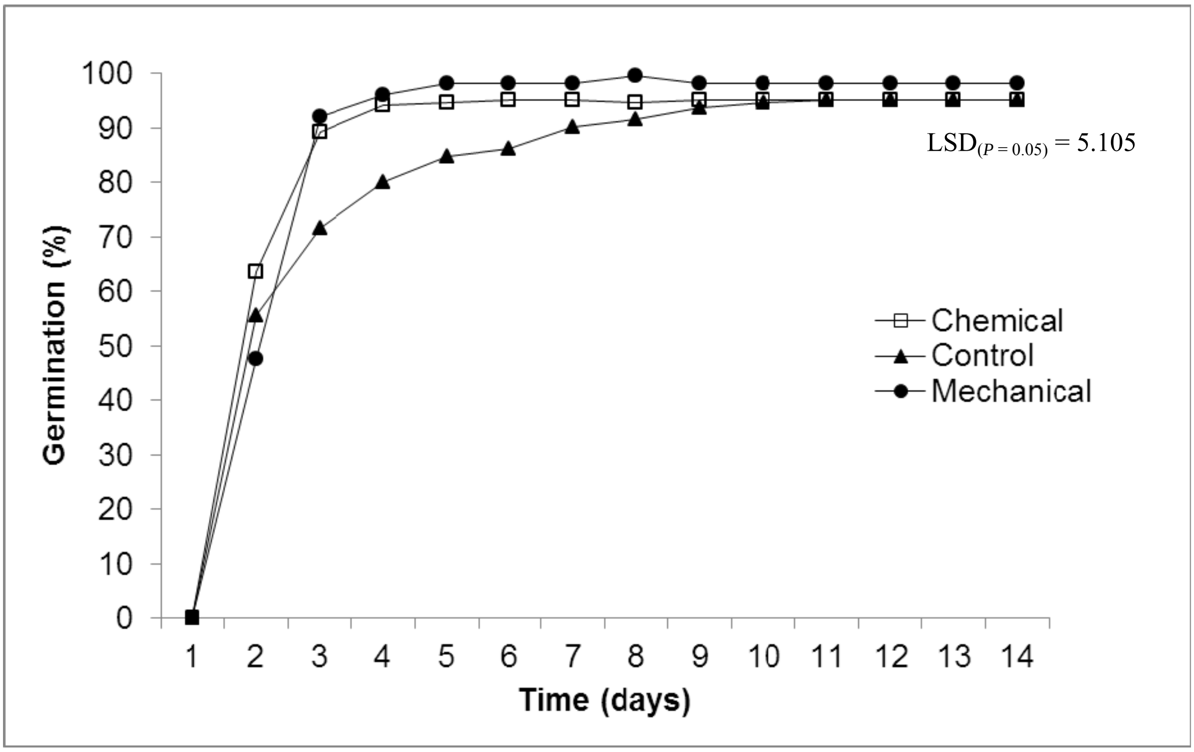

Fig. 4 Bambara groundnut germination response to mechanical, chemical and no scarification.

viability and germination [37].

3.3 Germination Indices

In terms of germination vigour indices, albeit the interaction between seed coat colour and scarification had no significant effect on mean germination time and germination vigour index, it is interesting to note that light brown, chemically scarified seeds were among the hastily germinated seeds (6.2 d), which was also associated with the highest germination vigour index of 43.31. However, the slowest germinated seeds were light brown seeds with no scarification (6.4 d) which was accompanied by poorest germination vigour index of 35.8. The same trend was observed in final germination (results not shown). Mean germination time of treatment interactions was in line with their associated germination vigour indices (Fig. 5). Furthermore, these results were supported by biomass accumulation presented by seedling dry mass in Fig. 6. Light brown, chemically scarified seeds were superiour with respect to seedling dry mass. However, the poorly performing treatments were both brown seeds with chemical scarification and brown seeds without scarification (Fig. 6).

Mean germination time and germination vigour index were significantly influenced by scarification. Chemically scarified seeds germinated quicker (6.179 d) than mechanical scarification (6.279 d) and control (6.342 d). Germination vigour index also showed similar trend of chemical > mechanical > control. On the other hand, seed coat colour had no significant influence on mean germination time and germination vigour index. However, it is noteworthy that light brown seeds took the shortest time to germinate, followed by brown and cream seeds, respectively. Germination of light brown seeds was vigorous than brown and cream seeds, respectively.

Contrary to reports from literature, cream seeds which are larger had poor germination vigour. Larger seed mostly germinates faster owing to higher amounts of food substances found in seeds, which promote quicker metabolic activities and consequently faster germination [38]. Moreover, less time is taken for heavier seeds to germinate compared to lighter

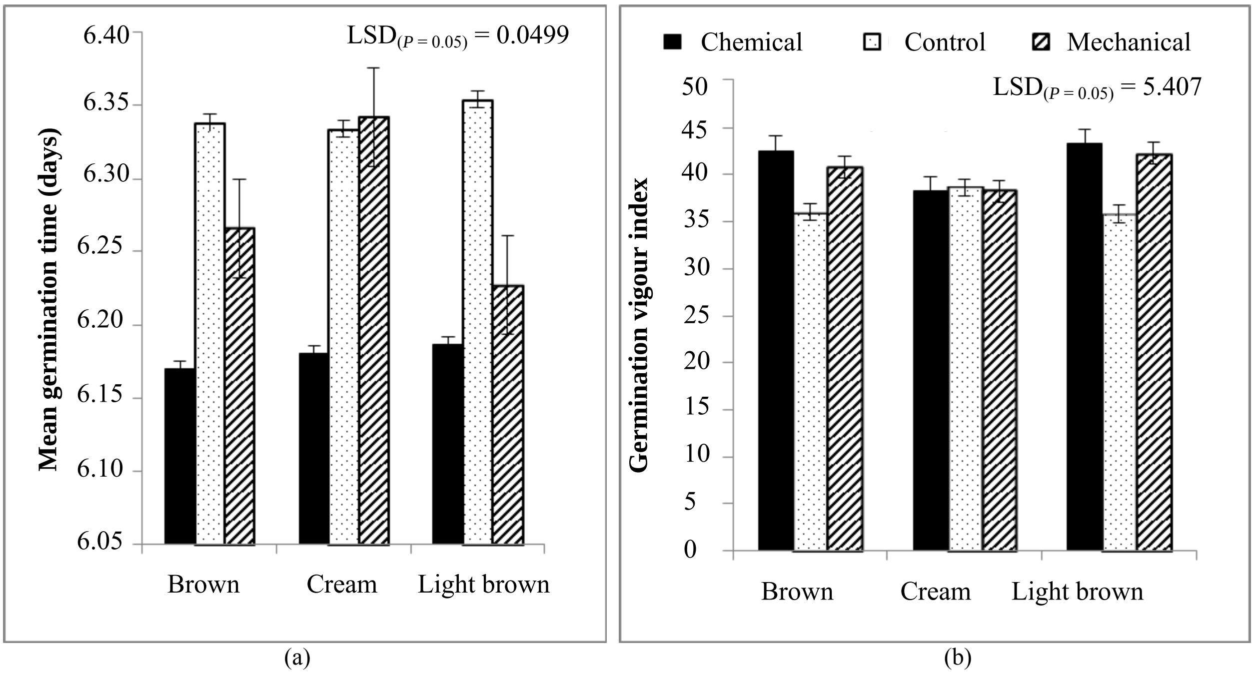

Fig. 5 The interactive effect of seed coat colour and scarification on mean germination time (a) and germination vigour index (b).

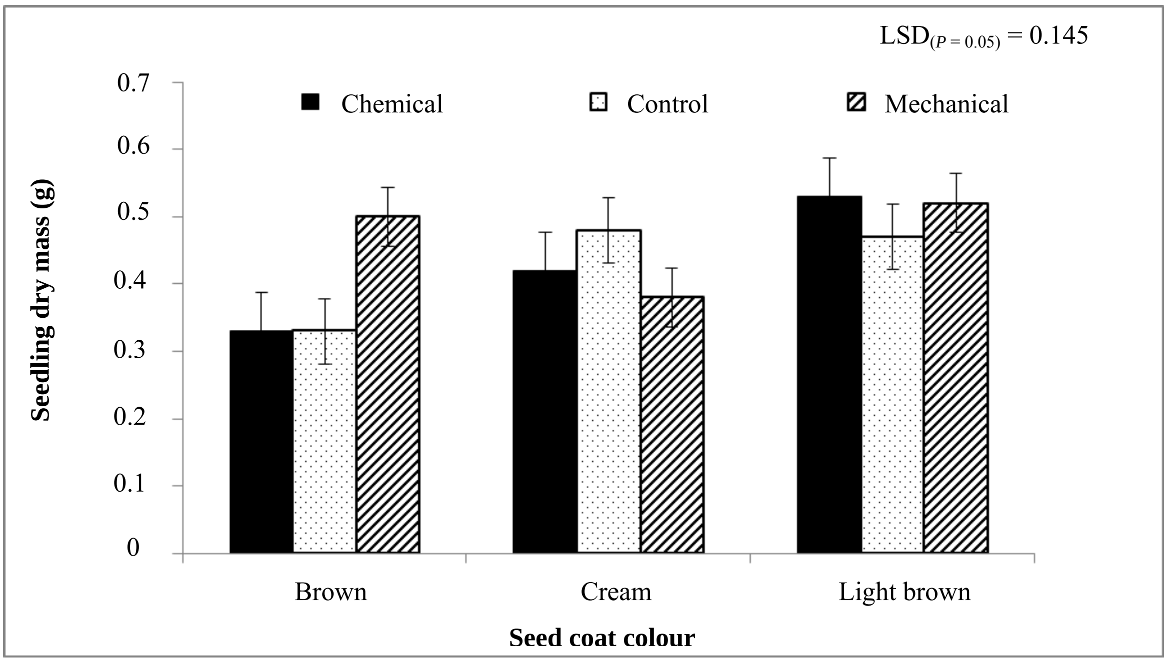

Fig. 6 The interactive effect of seed coat colour and scarification on seedling dry mass.

seeds [39, 40]. These conflicting results may have been caused by using different species in these studies.

3.4 Seedling Dry Mass

Scarification had no significant influence on seedling dry mass and there was no significant interaction between seed coat colour and scarification. Mechanical scarification had 8.5% greater dry matter accumulation based on seedling dry mass, compared to chemical scarification and control. However, seedling dry mass was significantly different between different seed coat colours. Light brown seeds produced heavier seedlings (0.51 g) than brown seeds (0.49 g) and cream (0.42 g). Yet, though brown and light brown seeds were significantly different, cream seeds were not significantly different to both of them. Albeit Chibarabada et al. [41] reported that seed coat colour has an effect on seed quality, contrary to this study, their results pointed out that plain cream landrace showed high vigour particularly with respect to seedling dry mass after germination.

According to literature, heavier and larger seeds produce heavier seedlings [38, 35], which is contrary to this study. Larger and heavier seeds are characteristic of earlier and quicker germination with consequences of larger seedlings that develop into larger plants [42] with high vigour [43]. These reports may be contradictory to the current study due to different plant species used.

3.5 Root to Shoot Ratio

Scarification, seed coat colour and their interaction thereof were significantly different with respect to root to shoot ratio. Light brown seeds without scarification had the highest root to shoot ration and brown seeds without scarification had the lowest ratio (Fig. 7). Chemical scarification and control equally increased root to shoot ratio by 28% to mechanical scarification. Therefore, chemically scarified seeds are better adapted to stressful environments due to their healthier roots. Light brown seeds were 5.7% greater than cream seeds and 31% greater than brown seeds with respect to root to shoot ratio. These results indicate that poor root system for initial seedling establishment can be caused by mechanical scarification while brown seeds also had poor root system.

Scarification method of overcoming physical dormancy depends on seed coat colour in bambara groundnut. In terms of seed coat colour, dark coloured seeds of bambara groundnut landraces tends to be more vigorous than light coloured seeds [32, 33]. The current study agrees with this trend. This is owing to that polyphenols found in dark coloured seeds [33, 44] was associated with antioxidant properties in plants with consequences of stress defence and tolerance during germination and emergence [41].

3.6 Field Emergence

With respect to overcoming dormancy, scarified seeds showed great vigour than seeds that were not scarified. However, this does not confirm stand performance

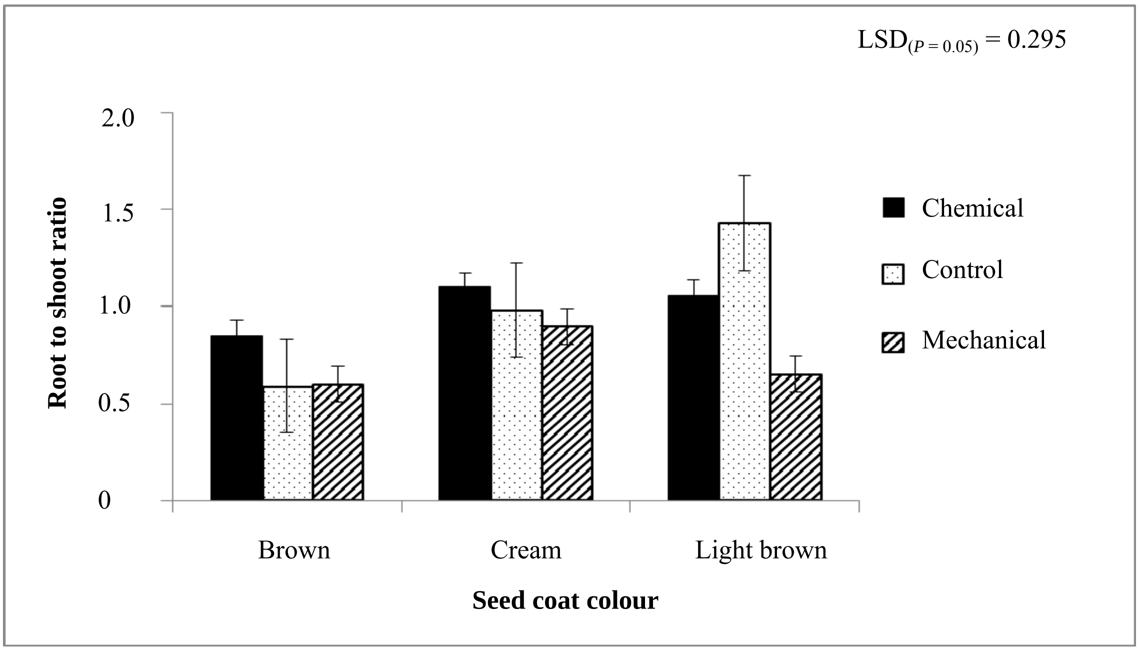

Fig. 7 Root to shoot ratio in response to interaction between scarification and seed coat colour.

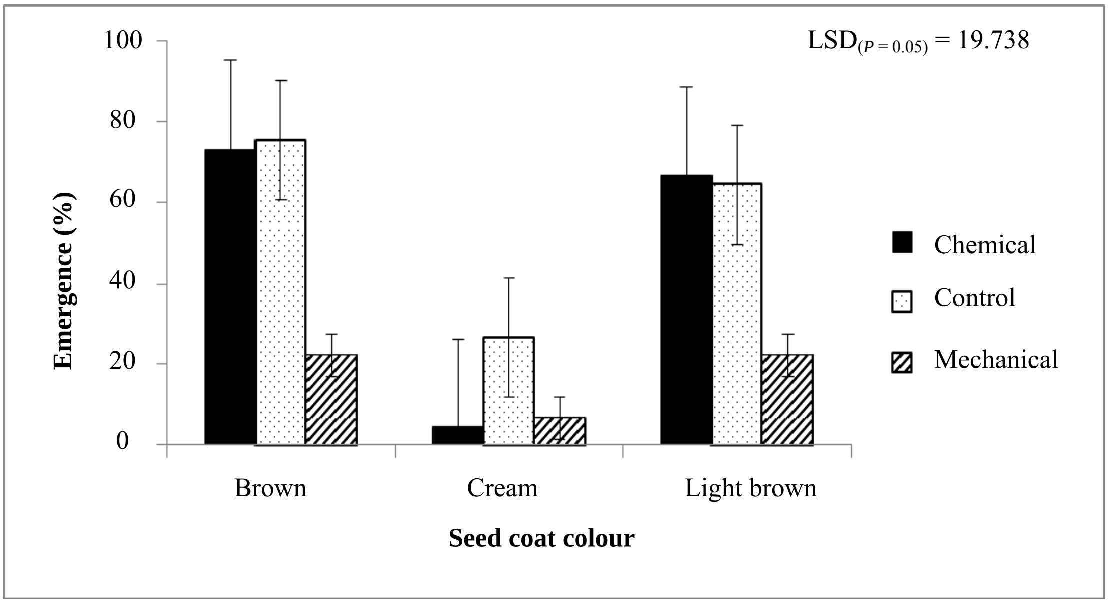

Fig. 8 Field emergence in response to scarification and seed coat colour interaction.

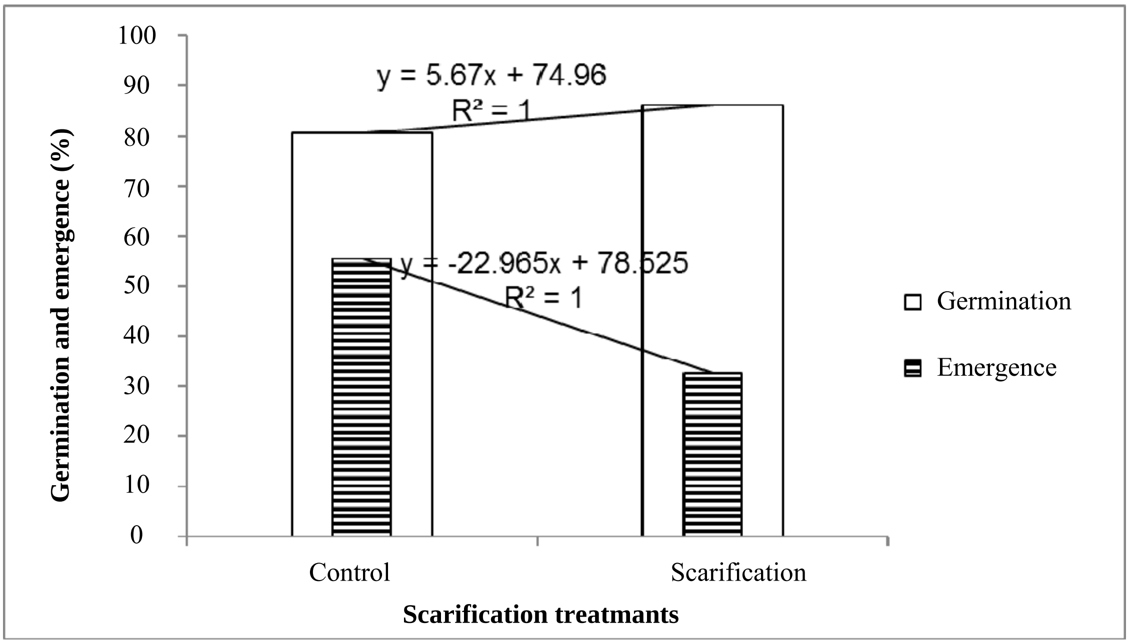

Fig. 9 Germination and emergence response to scarification.

under field conditions. Contrary to the laboratory results on bambara groundnut seed quality, seedling establishment tests showed that scarification was significantly detrimental to performance of the seeds (Figs. 8 and 9). This might have been attributed to seeds being exposed to some stressful biotic and abiotic factors in the field.

Moreover, seed coat colour and interaction between seed coat colour and scarification had a significant influence on field emergence. In another study, seed coat colour did not influence seedling establishment [31]. Seed coat colour in this study had the same effect on germination and emergence. The vigour of germination and emergence increased with an increase in seed coat colour pigmentation (Fig. 10). This might have been attributed to the fact that dark seeds have heavier seed coats and consequently sustains less imbibition damage which supports its prolonged

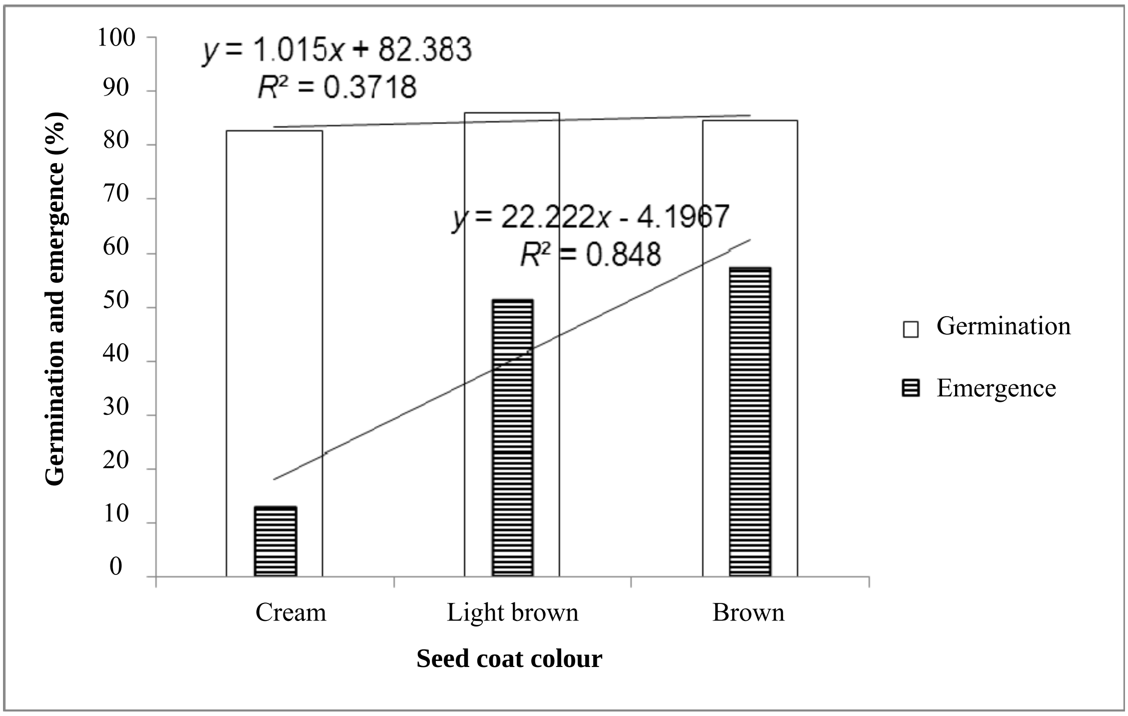

Fig. 10 Germination and emergence response to seed coat colour.

persistence especially in the soil [18]. Antioxidant properties exhibited by darker seeds may have supported their success in the field.

Contrary to the expected results, laboratory germination test is a close estimation of seed performance in the field. This study has established that there is a negative relationship between germination and seedling establishment due to seed scarification (Fig. 9). This might be owing to the fact that conditions are optimum in standard germination tests; hence even if scarification disturbs the seed coat, there is no negative effect on the final germination. Moreover, under these conditions, seeds do not suffer any external stresses, such as biotic and abiotic stress. In terms of stand establishment test, field conditions may not be optimum, and the fact that seed coat is disturbed by scarification might exacerbate the negative effect of the environment on final emergence.

This study has established that there is a positive relationship between germination and seedling establishment due to seed coat colour (Fig. 10). Brown and light brown seeds had greater germination and emergence. These seeds are smaller than cream seeds. The observed relationship may have been attributed to higher seed coat water permeability in smaller seeds [45].

4. Conclusions

This study showed that seed quality in bambara groundnut is highly influenced by scarification and seed coat colour, as well as their interaction. Scarification improved germination, but not field emergence of bambara groundnut. Dark coloured seeds (light brown and brown) had great vigour both in terms of germination and seedling establishment. Seeds with scarification treatments produced high quality seedlings. Therefore, producers can use scarification to improve bambara groundnut germination. However with the limited improvement of stand establishment, dark coloured seeds are recommended especially under limiting conditions. The limitation faced by seeds under field conditions proves that there are still improvements required in order to enhance stand establishment, which may be through seed enhancements and other methods. Further research is still needed to determine seed coat constituents that influence seed dormancy and seed quality especially in bambara groundnut.

Acknowledgments

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