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RESEARCH ARTICLE

Seed storage protein changes and mobilization pattern in Bambaranut (*Vigna subterranea*) (L.) Verdc. during germination

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ABSTRACT

The germination of seeds involves series of events during which mobilization and utilization of seed storage proteins occur. This study is aimed at determining the changes in total and fractions of seed storage protein in six bambaranut landraces during 96 hrs germination period. The study assessed the changes in seed storage protein content, storage protein profile, endopeptidase activity, free amino acids and gibberellic acid levels. Significant ($p<0.05$) decrease in total storage protein after 24 hrs, albumin from 48 hrs and globulin and glutelin after 72 hrs germination period were observed in the studied landraces. Prolamin showed significant ($p<0.05$) decrease after 48 hrs in all the landraces. Five peptide bands were detected in the six landraces with molecular weights corresponding to 97.4 kDa, 45 kDa, 29 kDa, 20.1 kDa and 18 kDa. Peptide bands with molecular weight of 97 kDa and 29 kDa decreased in intensity after 48 hrs of germination in four landraces. Free amino acids content significantly ($p<0.05$) increased following 24 hrs germination period in all the landraces. The activity of endopeptidase increased significantly ($p<0.05$), reaching maximum after 96 hrs germination. Significant ($p<0.05$) increase in gibberellic acid level throughout germination period was also observed. Although slow degradation rate of storage proteins was observed, there was variation in the rate at which storage protein and its fractions decreased among the bambaranut landraces during germination. This variation could be utilized towards obtaining improved bambaranut genotypes with better germination characteristics.

Introduction

Bambaranut (*Vigna subteranea* (L) Verdc.) is a drought tolerant legume known to have the ability to grow in soils with low fertility (1). The crop is widely cultivated using landraces of different seed colours. Only few studies have associated the seed coat colour with physiological and biochemical processes and the notable differences in seed quality among landraces (2). A number of issues including photoperiod and poor and slow germination are known to affect the production of bambaranut; these problems need to be solved in the near future to ensure its potentials towards global food, nutritional and environmental security are harnessed (3, 4).

Plants accumulate abundant reserves as carbohydrates, oils and proteins in their seeds during maturation which are crucial for seed germination and seedling establishment (5). Germination begins with imbibition of seeds with water and is concluded by the emergence of the radicle from the seed coat (6). Germination is accompanied by mobilization of food reserve from the storage organs (cotyledons or endosperm), providing essential energy to fuel growth until the seedlings become autotrophic (7). Seed storage protein mobilization during germination is among the vital events in the growth and development of seedlings (8). During germination, storage proteins are degraded by the action of endo and exopeptidases, proteolytic enzymes which convert the storage proteins into soluble peptides that are further hydrolyzed into free amino acids which are then mobilized to the embryonic axis to support growth (9).

Storage protein is the main reserve been frequently mobilized and used during germination of legume species (10). The relationship between seed reserves mobilization and germination has been reported in many economically-important species such as *Oryza sativa* (11) and *Glycine max* (12). The rate of degradation of storage proteins during germination is important in proper seedling establishment. For instance, fast rate of degradation of high molecular weight globulin polypeptides were reported during the early stages of germination of soybeans and faba beans (13).

Germination is a complex process affected by a number of integrating external (moisture, temperature, light and pH) and internal (gibberellic acid (GA3), abscisic acid (ABA) and seed reserves) factors (14-17). The environmental factors affect germination through the regulation of biosynthesis

and catabolism of GA3 and ABA respectively (18, 19). This study is aimed at determining the changes in seed storage protein mobilization in six bambaranut landraces during 96 hrs germination period.

Materials and Methods

Sample collection

Seeds of six landraces of bambaranut were locally obtained and identified in the Department of Plant Biology, Bayero University, Kano with a voucher number '0509'. The seeds were named Niger cream, Yobe cream, Yobe black, Kano mottled, Kano maroon and Kano brown based on their place of collection and colour. Eighty (80) seeds were randomly selected from each landrace and divided into four sets of 20 seeds; each set was then subjected to different germination period of 24, 48, 72 or 96 hrs.

Germination procedure

Germination was carried out according to standard procedure (20). Seeds were surface sterilized with 3.5% sodium hypochlorite solution for 5 min and then thoroughly rinsed with distilled water. The seeds were then soaked in 200 ml distilled water for 6 hrs. The water imbibed seeds were germinated in petri dishes lined with water-soaked filter paper at room temperature. Germination was carried out in duplicate for each landrace and only 10 ml distilled water was sprayed at a period of 5 hrs interval daily. Cotyledons from germinating seeds were collected at 24, 48, 72 and 96 hrs and frozen to stop the germination process. The seeds were then dried and crushed using a mortar and pestle, sieved and stored in air tight plastic containers. Water absorption capacity was determined from the weight of 100 seeds before and after imbibition. Radicle length was measured using a vernier caliper.

Extraction and quantification of storage protein

Seeds flour (10 gm) was defatted with n-hexane which was subsequently removed by decantation and leaving the flour to dry at room temperature. The extraction of total proteins was done as per standard method (21). Seed flour (1 gm) was dissolved in 10 ml extraction buffer (50 mM phosphate buffer, pH 7.8), the homogenate was then vortexed and centrifuged at 10000 x gm for 10 min at 4 °C. The supernatant was used for total protein estimation and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

Storage protein fractions were sequential extracted based on their solubility (22). Seeds flour (1 gm) was dissolved in 10 ml distilled water, the homogenate incubated for 30 min at 30 °C and subsequently centrifuged at 3000 x g for 20 min. The supernatant was recovered and pellet resuspended with 5 ml distilled water, incubated for 30 min at 30 °C and centrifuged at 3000 x g for 20 min. The supernatants were combined as albumin fraction. Globulin, glutelin and prolamin fractions were respectively extracted in a similar manner using 1% NaCl, 0.1 M NaOH and 70% ethanol as the solvents. Total protein was also quantified (23).

Storage protein mobilization pattern

SDS-PAGE analysis of total storage proteins of the 6 bambaranut landraces was carried out to determine peptides degradation pattern (24).

Free amino acids determination

Free amino acids were quantified (25). Seed flour (1 gm) was boiled with 10 ml 80% ethanol for 10 min. The homogenate was centrifuged at 3000 x gm for 10 min, supernatant collected and pellet re-extracted twice; the supernatants were pooled together and filtered. The filtrate was used for the estimation of free amino acids by ninhydrin method using L-tyrosine as standard (26).

Endopeptidase assay

Crude endopeptidase extract was prepared (20). Seed flour (2 gm) was mixed with chilled 0.05 M Tris-HCl buffer (pH 7.2), containing 2 mM β-mercaptoethanol (1:4 w/v). The extract was filtered through four layers of cheesecloth and the filtrate centrifuged at 10000 x gm for 15 min at 4 °C. The supernatant was used for the enzyme assay. Endopeptidase activity was measured (27) using casein as substrate (25).

Endogenous gibberellic acid determination

Endogenous gibberellic acid (GA3) was extracted using 80% cold methanol at 4 °C (28). The concentration of endogenous GA3 was determined spectrophotometrically at 254 nm from a standard curve (29).

Statistical analysis

All data are presented as mean ± standard deviation of three replicate analyses. For comparison, data were analyzed by ANOVA with Turkey's post-hoc test. All statistical analysis was based on a significance level of 0.05. Analyses were done with SPSS software version 16.0.

Results and Discussion

The dry weight of 100 seeds per landrace, water absorption capacity (WAC) and radicle length following 96 hrs germination period for the 6 bambaranut landraces are presented in Table 1. Kano brown had the highest weigh of 75 gm per 100 seeds and the least weight was found in Niger cream and Kano mottled landraces with 69 gm per 100 seeds. Yobe cream landrace had the highest WAC (9.72%) following imbibition while Niger cream landrace had the least (2.17%). Radicle emergence through seed coat was observed after 48 hrs in 4 of the landraces, after 72 hrs in Kano maroon landrace and until 96 hrs in Niger cream landrace (Fig. 1). Significant ($p<0.05$) increase in radicle length was observed across the various germination periods in all the landraces and the radicle lengths varied between the genotypes (0.48 - 3.82 cm) (Table 1). Generally, landraces with higher WAC were the first to germinate (Fig. 1) and also possessed longer radicles.

Results indicated that the plain cream (Yobe cream) landrace has better germination indices compared to the other landraces. Previous studies

Table 1. Seed weight, water absorption capacity and radical length during germination of bambaranut seeds.

Parameters	bambaranut landrace					
	Niger cream	**Yobe cream**	**Yobe black**	**Kano mottled**	**Kano maroon**	**Kano brown**
100 Seeds Weight (g)	69.00	72.00	70.00	69.00	70.00	75.00
WAC (%)	2.17	9.72	5.71	7.25	3.86	8.93
Radical length (cm)						
24 hours	0.00	0.00	0.00	0.00	0.00	0.00
48 hours	0.00	0.57^{a}±0.95	0.34^{a}±0.13	0.42^{a}±0.10	0.00	0.85^{a}±0.20
72 hours	0.00	2.54^{b}±0.32	1.50^{b}±0.48	1.58^{b}±0.44	0.61^{a}±0.11	1.97^{b}±0.34
96 hours	0.48b±0.10	3.82^{c}±0.39	2.90^{c}±0.48	2.96^{c}±0.46	1.81^{b}±0.40	3.16^{c}±0.30

Data expressed as mean ± standard deviation, n=10. Means with different superscript across columns are significantly different ($p<0.05$). WAC= water absorption capacity.

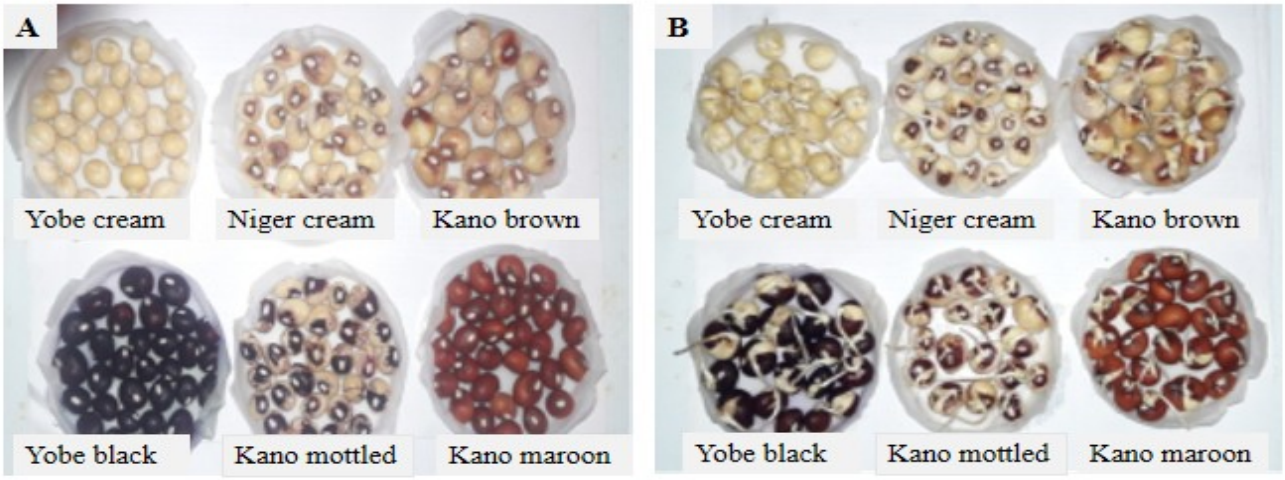

Fig. 1. Bambaranut seeds showing radicle emergence during germination period.(A. seeds at 24 hrs, B. seeds at 96 hrs germination periods).

(30, 31) on plain bambaranut indicated that dark-coloured seeds performed better compared to light-coloured seeds. This was related to tannins, particularly polyphenols, present in dark-coloured seeds (31, 32). Polyphenols have antioxidant properties as such can confer a degree of stress tolerance during germination and seedling emergence. Since in the present study plain cream landrace performed better in term of water absorption, radicle emergence and length, it is suggested that water absorption capacity could be a useful selection criterion for seed quality in bambaranut towards solving the problem of poor and slow germination.

Sequential decrease in the concentration of total seed storage protein and protein fractions was generally observed in the bambaranut landraces during germination. With the exception of Niger cream landrace, significant ($p<0.05$) decrease in total seed storage protein was observed in all the landraces by 48 hrs germination period with further decreases by 96 hrs (Table 2). Similar decreases were observed in the levels of albumins (Table 3) and globulins (Table 4). As presented in Table 5, the concentration of glutelins was only observed to decrease significantly ($p<0.05$) after 72 to 96 hrs germination period while the level of prolamins was significantly ($p<0.05$) decreased by 48 hrs and subsequently continued to decrease but insignificantly (Table 6). In all the landraces of bambaranut, differences were observed in the pattern of decrease in seed storage protein and its fraction.

The storage proteins are important because they determine both the quantity and quality of seed proteins for various uses in metabolism (33). The concentration of total seed storage proteins and its fractions of the 6 bambaranut landraces decreased significantly ($p<0.05$) upon germination for 96 hrs. The decrease in storage protein concentration could be attributed to the degradation of storage proteins by proteases (25). Water imbibition and germination lead to several physiological and metabolic changes in plant seeds as all the nutrients and energy necessary for the growth and development of the embryonic axis are derived from storage compounds within the seeds (34). Slow depletion of seed storage proteins at the initial stages (24 to 48 hrs) of germination followed by gradual increase in depletion of protein content was observed in many legume species: Indian bean (35), broad bean (36) and horse gram seeds (37). Thus, the degradation of major storage protein can be considered to be species dependent (38).

Fig. 2 presents the SDS-PAGE electrophoregram of storage protein of bambaranut seeds during 96 hrs germination period. There was no observed difference in the peptide bands pattern of Niger cream (L1-L4) and Yobe cream (L5-L8) landraces throughout the germination period. From 48 to 96 hrs germination period there was an observable decrease in intensity of 97.4 kDa molecular weight peptide bands in Kano brown landrace (L21-L24). Throughout the studied germination period, in all the landraces, the intensity of a 45 kDa peptide band remained relatively unchanged. Decrease in intensity

Table 2. Total seed storage protein concentration during germination of bambaranut.

Germination time (hours)	Total seed storage protein (mg/ml)					
	Niger cream	Yobe cream	Yobe black	Kano mottled	Kano maroon	Kano brown
24	0.087^{a}±0.002	0.100^{a}±0.002	0.099^{a}±0.001	0.082^{a}±0.001	0.082^{a}±0.001	0.118^{a}±0.003
48	0.082^{a}±0.003	0.084^{b}±0.002	0.088^{b}±0.003	0.074^{b}±0.000	0.078^{b}±0.001	0.111^{b}±0.000
72	0.076^{b}±0.003	0.065^{c}±0.001	0.073^{c}±0.000	0.072^{b}±0.002	0.073^{b}±0.002	0.096^{c}±0.001
96	0.065^{c}±0.002	0.058^{d}±0.003	0.060^{d}±0.002	0.062^{c}±0.003	0.068^{c}±0.000	0.083^{d}±0.003

Data are expressed as mean ± standard deviation of triplicate measurements. Means with different superscript across a column are significantly different ($p<0.05$).

Table 3. Albumin fraction concentration during germination of bambaranut.

Germination time (hours)	Albumin fraction (mg/ml)					
	Niger cream	Yobe cream	Yobe black	Kano mottled	Kano maroon	Kano brown
24	0.019^{a}±0.001	0.026^{a}±0.002	0.034^{a}±0.001	0.022^{a}±0.004	0.026^{a}±0.001	0.023^{a}±0.002
48	0.018^{a}±0.000	0.018^{b}±0.002	0.029^{b}±0.001	0.022^{a}±0.000	0.022^{a}±0.001	0.022^{a}±0.000
72	0.009^{b}±0.001	0.012^{b}±0.002	0.023^{c}±0.000	0.021^{a}±0.003	0.018^{b}±0.001	0.018^{b}±0.002
96	0.005^{b}±0.002	0.005^{c}±0.003	0.021^{c}±0.002	0.014^{b}±0.002	0.018^{b}±0.000	0.017^{b}±0.000

Data are expressed as mean ± standard deviation of triplicate measurements. Means with different superscript across column are significantly different ($p<0.05$).

Table 4. Globulin fraction concentration during germination of bambaranut.

Germination time (hours)	Globulin fraction (mg/ml)					
	Niger cream	Yobe cream	Yobe black	Kano mottled	Kano maroon	Kano brown
24	0.057^{a}±0.002	0.063^{a}±0.001	0.056^{a}±0.000	0.046^{a}±0.001	0.043^{a}±0.002	0.085^{a}±0.002
48	0.054^{a}±0.003	0.058^{b}±0.001	0.051^{a}±0.000	0.041^{b}±0.002	0.039^{b}±0.000	0.081^{b}±0.000
72	0.050^{b}±0.001	0.056^{b}±0.000	0.046^{b}±0.001	0.038^{c}±0.001	0.032^{c}±0.001	0.073^{c}±0.002
96	0.046^{b}±0.001	0.049^{c}±0.000	0.040^{b}±0.001	0.034^{c}±0.001	0.023^{d}±0.003	0.070^{c}±0.001

Table 5. Glutelin fraction concentration during germination of bambaranut.

Germination time (hours)	Glutelin fraction (mg/ml)					
	Niger cream	Yobe cream	Yobe black	Kano mottled	Kano maroon	Kano brown
24	0.006^{a}±0.003	0.007^{a}±0.001	0.013^{a}±0.001	0.009^{a}±0.002	0.007^{a}±0.001	0.007^{a}±0.001
48	0.005^{a}±0.001	0.006^{a}±0.000	0.012^{a}±0.001	0.007^{a}±0.002	0.006^{a}±0.000	0.004^{b}±0.000
72	0.003^{b}±0.001	0.004^{b}±0.000	0.012^{a}±0.000	0.004^{b}±0.000	0.005^{a}±0.001	0.003^{b}±0.001
96	0.002^{b}±0.000	0.003^{b}±0.001	0.009^{a}±0.002	0.004^{b}±0.000	0.004^{a}±0.000	0.001^{c}±0.000

Table 6. Prolamin fraction concentration during germination of bambaranut.

Germination time (hours)	Prolamin fraction (mg/ml)					
	Niger cream	Yobe cream	Yobe black	Kano mottled	Kano maroon	Kano brown
24	0.002^{a}±0.000	0.003^{a}±0.001	0.002^{a}±0.000	0.003^{a}±0.000	0.002^{a}±0.001	0.0023^{a}±0.001
48	0.001^{b}±0.000	0.003^{a}±0.001	0.002^{a}±0.000	0.002^{b}±0.001	0.002^{a}±0.001	0.002^{b}±0.000
72	0.001^{b}±0.000	0.002^{c}±0.000	0.001^{b}±0.000	0.002^{b}±0.000	0.001^{b}±0.001	0.002^{b}±0.0001
96	0.001^{b}±0.000	0.002^{c}±0.001	0.001^{b}±0.002	0.001^{c}±0.001	0.001^{b}±0.000	0.002^{b}±0.0001

Data are expressed as mean ± standard deviation of triplicate measurements. Means with different superscript across column are significantly different ($p<0.05$).

of 29 kDa and 20.1 kDa bands was observed by 48 hrs with a prominent decrease by 96 hrs germination period in four of the bambaranut landraces.

Gradual disappearance of 29 kDa band in L11 and L12 (Yobe black), L15 and L16 (Kano mottle), L19 and L20 (Kano maroon), and L23 and 24 (Kano brown) was observed. In the other two landraces (Niger cream and Yobe cream), no notable decrease in intensity nor gradual disappearance of peptide bands was observed. The differences seen in the pattern of degradation of the peptides could be an indication of genetic variability among the studied landraces. SDS-PAGE has been widely used for monitoring protein mobilization at the early stages of seed germination (39). It was reported that major storage protein (vicilin-type globulins) follow distinct degradation patterns during germination in different plant species (38). The morphological and phenotypic differences in the seeds of bambaranut landraces are pointers of genetic difference. Even though there were similarities among the studied landraces, differences in number of peptides, degradation pattern and evident preferential degradation of some peptides over others during the germination were observed.

The free amino acids content of the 6 bambaranut landraces during 96 hrs of germination are presented in Table 7. Significant ($p<0.05$) increase in free amino acids concentration was seen throughout the germination period; observable differences were also evident between the landraces. Following seed imbibition and germination, storage proteins enlarge as a consequence of increase in osmotic potential due to proteolysis which releases free amino acids (25, 40). Studies have reported increase in free amino acids content during germination in legumes species such as horse gram, Indian bean and broad bean (25, 35, 36). The activities of proteolytic enzymes lead to the

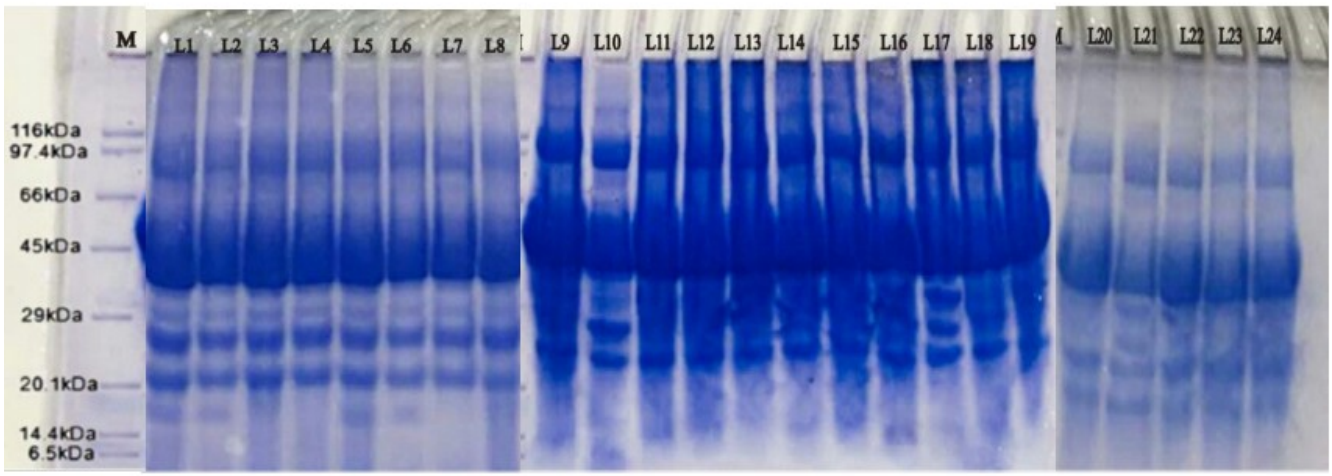

Fig. 2. SDS-PAGE electrophoregram of seed storage proteins of six bambaranut landraces from 24 to 96 hours germination period. Lanes: M = molecular weight marker; L1-L4 = Niger cream; L5-L8 = Yobe cream; L9-L12 = Yobe black; L13-L16 = Kano mottled; L17-L20 = Kano maroon; L21-L24 = Kano brown following 24, 48, 72 and 96 hrs germination periods respectively.

Table 7. Free amino acids concentration during germination of bambaranut seeds.

Germination time (hours)	Free amino acid (µg/ml)					
	Niger cream	Yobe cream	Yobe black	Kano mottled	Kano maroon	Kano brown
24	$102.72^{a}\pm0.35$	$118.93^{a}\pm0.35$	$113.41^{a}\pm0.00$	$106.61^{a}\pm1.05$	$105.37^{a}\pm2.24$	$171.69^{a}\pm2.26$
48	$180.43^{b}\pm0.20$	$207.21^{b}\pm0.59$	$189.04^{b}\pm0.41$	$192.61^{b}\pm0.53$	$176.06^{b}\pm1.39$	$247.21^{b}\pm2.16$
72	$197.63^{c}\pm2.04$	$251.80^{c}\pm0.40$	$246.52^{c}\pm0.90$	$242.04^{c}\pm0.91$	$192.72^{c}\pm1.80$	$293.87^{c}\pm3.09$
96	$257.90^{d}\pm0.69$	$330.31^{d}\pm0.59$	$286.17^{d}\pm2.00$	$267.32^{d}\pm1.38$	$246.97^{d}\pm2.41$	$363.07^{d}\pm1.58$

Data are expressed as mean ± standard deviation of triplicate measurements. Means with different superscript across columns differ significantly ($p<0.05$).

significant increase in free amino acids content during germination (41, 42).

In the present study, endopeptidase activity increased significantly ($p<0.05$) during the 96 hrs germination period (Table 8). It reached maximum activity at 72 hrs of germination and declined by 96 hrs in four of the 6 bambaranut landraces; however, the activity continued to rise in the other two landraces up to 96 hrs germination period. Furthermore, the endopeptidase activity was observed to be positively related to the free amino acid level. It was stated that free amino acid contents increase during germination due to resultant activities of proteolytic enzymes (42).

The positive correlation between endoprotease activity and protein depletion suggests the involvement of endoprotease in the degradation of proteins or peptides to free amino acids in the cotyledons of germinating seeds (25). Changes in endopeptidase activity, free amino acid and protein concentration have been reported by previous studies in legumes such as horse gram, Indian bean, soybean and broad bean (25, 35, 41, 43).

Significant ($p<0.05$) increase in the concentration of GA3 was observed by 48 hrs germination period and it continued to rise throughout the germination period (Table 9). Longitudinal growth and water uptake in the root and shoot within the growing axis are enhanced by endogenous gibberellin (44). GA3 stimulates storage protein mobilization by inducing synthesis of hydrolytic enzymes (19). Application of exogenous GA3 was reported to increase seed weight and delayed seed dehydration, suggesting the role of GA3 during seed germination and in later stages of seedlings development (45).

In the present study, WAC during seeds imbibition can be considered as a more important parameter in breaking the physical dormancy caused by the hard seed coat of bambaranut. Therefore, WAC can be considered to have more effect than storage protein degradation and GA3 at the early stages of germination (radicle emergence) before the onset active cell division and enlargement as well as proper root and shoot development. It was reported that endogenous GA3 is closely related to cell division and cell enlargement (46).

Endogenous GA3 content was reported to have effect on endopeptidase activity indicating its influence on protein degradation (47). Earlier studies have reported the influence of gibberellins on seed germination and protein mobilization by affecting endopeptidase activity (47, 48). Therefore endogenous GA3 might have its effect more pronounced at the late germination stages of bambaranut when proper roots and shoots were developed or during the onset of its seedlings establishment.

In the present study it was observed that landraces with dark coloured seed coat (black, mottle, maroon and brown) showed sign of faster protein degradation rate. Similarly, landraces with dark coloured seed coats had higher levels of

Table 8. Endopeptidase activity expressed as microgrammes of free amino acids released/hour/ml in germinating bambaranut seeds.

Germination time (hours)	Endopeptidase activity µg/hour/ml					
	Niger cream	Yobe cream	Yobe black	Kano mottled	Kano maroon	Kano brown
24	24.22^{a}±0.52	31.46^{a}±0.40	52.03^{a}±0.60	29.50^{a}±1.40	43.76^{a}±2.15	27.44^{a}±2.08
48	42.03^{b}±1.19	74.22^{b}±1.40	82.84^{b}±0.40	51.00^{b}±1.38	78.93^{b}±0.00	78.93^{b}±0.00
72	64.33^{c}±1.05	113.89^{c}±0.56	114.79^{c}±0.35	73.07^{c}±1.92	116.07^{c}±1.41	115.70^{c}±2.96
96	62.38^{c}±0.59	109.51^{c}±0.40	129.39^{d}±0.40	68.41^{d}±1.82	122.15^{d}±1.55	105.83^{d}±2.82

Data are expressed as mean ± standard deviation of triplicate measurements. Means with different superscript across columns differ significantly ($p<0.05$).

Table 9. Gibberellic acid concentration during germination of bambaranut.

Germination time (Hours)	Endogenous gibberellic acid (mg/ml)					
	Niger cream	Yobe cream	Yobe black	Kano mottled	Kano maroon	Kano brown
24	0.05^{a}±0.00	0.07^{a}±0.01	0.11^{a}±0.00	0.06^{a}±0.02	0.09^{a}±0.00	0.10^{a}±0.01
48	0.07^{b}±0.00	0.10^{b}±0.00	0.15^{b}±0.01	0.08^{a}±0.00	0.14^{b}±0.01	0.12^{b}±0.00
72	0.08^{b}±0.01	0.12^{c}±0.01	0.17^{b}±0.00	0.13^{b}±0.00	0.16^{b}±0.01	0.16^{c}±0.00
96	0.14^{c}±0.01	0.17^{d}±0.01	0.26^{c}±0.01	0.20^{c}±0.01	0.21^{c}±0.00	0.22^{d}±0.01

Data are expressed as mean ± standard deviation of triplicate measurements. Means with different superscript across columns are significantly different ($p<0.05$).

endopeptidase activity and GA3. Previous studies have reported that dark coloured bambaranut seeds performed better compared to light coloured seeds during germination and seedlings establishment which might be due to faster rate of protein degradation in addition to high content of polyphenols (30, 31).

The germination potential of a plant species is governed by a number of factors including environmental and genetic makeup. The observed variability among the bambaranut genotypes studied is a pointer of their potential towards breeding varieties with better germination rates and success.

Conclusion

The effect of germination on the assessed biochemical parameters indicates the existence of varied mechanism approach in storage protein degradation and mobilization during germination among the studied bambaranut landraces. Variation was observed in the pattern of storage protein, endopeptidase activities, free amino acid and endogenous GA3 contents. Among the studied bambaranut genotypes, two landraces Yobe cream and Kano brown indicated better germination ability, although neither of the two landraces has the highest GA3 level. The variability in storage protein mobilization and germination of bambaranut landraces could be exploited towards improvement of local genotypes to obtain accessions with better agronomic properties, including germination rate and success.

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Authors' contributions

NMS carried out the laboratory and statistical analyses and prepared the draft manuscript. YYM conceived the study and participated in its design and coordination. All authors read and approved the final manuscript.

Conflict of interests

Authors do not have any conflict of interests to declare.

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