

ORIGINAL ARTICLE OPEN ACCESS

Phytic Acid, Protein, and Oil Contents and Their Relationship With Seed Quality During Seed Maturation of Bambara Nut (*Vigna subterranea* (L.) Verdc.) Landraces

Gerard Oballim^{1,2}  | Wilson R. Opile¹ | Julius O. Ochuodho¹

¹Department of Seed, Crop and Horticultural Sciences, University of Eldoret, Eldoret, Kenya | ²National Agricultural Research Organization, Abi Zonal Agricultural Research and Development Institute, Arua, Uganda

Correspondence: Gerard Oballim (obalcnkene@gmail.com)

Received: 11 October 2023 | **Revised:** 22 February 2024 | **Accepted:** 23 February 2024

Funding: This study was funded by the Intra-Africa Academic Mobility Scheme of the European Union/African Union.

Keywords: Bambara nut | oil | phytic acid | protein | seed maturation | seed quality

ABSTRACT

Phytic acid, proteins, and oils are seed storage compounds that play a role in germination and seedling growth and may determine seed quality. The pattern of accumulation of these compounds and the relationship of their contents with the seed quality of the Bambara nut (BN) are poorly understood. Seeds of three BN landraces, AbiBam001 (black/cream), LocalBam (brown speckled), and TVSU544 (cream), were harvested from two field experiments at different maturation stages and tested for final germination percentage (FGP), germination velocity index (GVI), and seedling dry weight (SDW). Seed samples from the same experiments were analyzed for phytic acid and proximate composition. Kendall's ranked correlation was used to describe relationships between phytic acid, protein, and oil contents and the seed quality of the landraces. Results showed no differences in the phytate, protein, and oil contents of landraces during seed maturation ($p > 0.05$), except for the phytate content of AbiBam001 ($p < 0.05$), which increased in the first experiment. At mass maturity, AbiBam001 and LocalBam had higher phytate and less protein and oil contents than TVSU544, implying that seed coat color may influence the phytate, protein, and oil contents of BN landraces. Higher phytate content in landraces appeared to relate positively with FGP, GVI, and SDW and vice versa. Phytic acid may positively affect seed germinability in BN landraces with high phytate content but may affect it negatively in low-phytate landraces. The oil content of all landraces had negative correlations with most seed quality characteristics, suggesting that BN oils either are not priority reserves or play a minimal role in germination and seedling growth.

1 | Introduction

Grain legumes such as the Bambara nut (BN) (*Vigna subterranea* (L.) Verdc.) are rich in carbohydrates, proteins, fats, and minerals and supply valuable affordable nutrients to the human population worldwide, especially in under-developed countries (Affrifah, Uebersax, and Amin 2023; Singh et al. 2022; Semba et al. 2021; Mohan, Tresina, and Daffodil 2016; Vaz Patto et al. 2015). Proteins provide a source

of amino acids and nitrogen in plants, for growth and development (Batra et al. 2023; Dhull, Kinabo, and Uebersax 2023; Kermode 2011), and in the germinating seed, for seedling growth and early establishment before the seedling becomes self-supporting (Bewley and Nonogaki 2017; Kermode 2011; M. Zhao et al. 2018). On the other hand, carbohydrates and lipids serve as sources of energy for metabolism (Bewley and Nonogaki 2017; Kermode 2011; M. Zhao et al. 2018). Besides the major storage reserves, grain legumes also accumulate

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2024 The Authors. *Legume Science* published by Wiley Periodicals LLC.

a comparatively high content of phytic acid (myo-inositol-1,2,3,4,5,6-hexakisphosphate), normally stored in the seed as salts of the acid called phytates (Karmakar et al. 2020; Mohan, Tresina, and Daffodil 2016; Silva et al. 2021). Phytic acid and phytates are secondary plant metabolites that perhaps are better known for their antinutritional properties (Dhull, Punia, Kidwai, et al. 2020; Dhull et al. 2021; Mohan, Tresina, and Daffodil 2016; Punjabi et al. 2018; Raboy 2020). However, phytic acid and phytates do play a role in plant nutrition and plant protection (Dhull, Punia, Sandhu, et al. 2020; Dhull, Sandhu, et al. 2020). Phytates are the principal storage form of phosphorus (P) in most cereals and legume seeds (60%–90% of all P in seeds) (Karmakar et al. 2020; Mandizvo and Odindo 2020; Raboy 2009; Silva et al. 2019). They serve as a source of inorganic phosphates for the germinating seed (Daffodil, Fathima, and Mohan 2013) and are involved in plant protection against insects, pathogens, nonarthropod herbivores, and abiotic stresses (Pilu et al. 2005; Sangronis and Machado 2007; Sparvoli and Cominelli 2015; Wink 2010). In many cultivated and wild species, seed reserves differentially or similarly (spatial-temporarily) accumulate in the seeds during seed development and maturation (Bewley and Nonogaki 2017; Bewley et al. 2013). Studies have shown that lipid/oil accumulation in legumes tends to occur early in seed development whereas proteins accumulate mostly in the later stages (Huang et al. 2018; Warsame et al. 2022; Xu et al. 2016). Upon maturation, seeds show varying contents of stored reserves, between and within species (Bewley and Nonogaki 2017; Bewley et al., 2013). In BN, light-colored accessions have been shown to have higher protein and oil, but less minerals compared to dark-colored accessions (Donkor et al. 2022). Similarly, landraces of cream and white (light) colors had higher protein content than dark-colored (red, brown, and black) ones (Hlanga, Modi, and Mathew 2021). The patterns of oil and protein accumulation towards mass maturity have not been fully described before for any BN landrace. In addition, landraces from different regions have different maturation periods and different contents of storage reserves, but these have also not been described before for Ugandan landraces. Accumulation of phytates in legume seeds for the most part follows a pattern similar to the major seed components (Dhole and Reddy 2015; Mandizvo and Odindo 2020; Shunmugam et al. 2015; Sparvoli and Cominelli 2015; Urbano et al. 2000). A contrasting scenario to this was, however, observed in soybean whereby seed phytate accumulation followed a linear trend throughout most of seed development (Raboy and Dickinson 1987). Similarly, Pandey et al. (2016) showed that the phytate content of both yellow and black Indian soybean genotypes increased in a linear pattern from early seed development to maturation. A study of phytate accumulation in BN landraces revealed that the phytate content of landraces gradually increased in the early stages and then rapidly increased afterwards until maturity (Mandizvo and Odindo 2020). The study by Mandizvo and Odindo (2020) described the accumulation of phytates only up to an apparent physiological maturity and not beyond. However, it is likely that some of the landraces studied, and even the Ugandan landraces, may have longer maturity period, hence the need to determine phytate content beyond 65 days after flowering (DAF). It has been suggested that the storage contents of seeds may be related to the seed quality of certain species. The

metabolism of stored reserves during seed germination and in seedling growth has been documented in several species (for example, Bewley and Nonogaki 2017; Soriano et al. 2011; M. Zhao et al. 2018). M. Zhao et al. (2018) studied the mobilization of seed reserves in germinating seeds of wild grass species and found that the germination rate had a positive relationship with both soluble sugar and soluble protein contents at different stages of germination. On the contrary, protein and fat reserves in dry seeds did not have any relationship with the rate and extent of germination (M. Zhao et al. 2018). Further reports indicated that the germination rate had a positive association with nitrogen and lipid concentrations of forest tree seeds (Soriano et al. 2011), while the fat content and germination percentage of flax seeds did not show any relationship (Kanmaz and Ova 2015). Similarly, the phytate content of legume seeds and their seed quality have largely been shown to be positively related (for example, Pramitha et al. 2020; Soares et al. 2014; Zhou et al. 2018). Interestingly, low phytic acid (*lpa*) mutants have been suggested to result in negative gene pleiotropy that may undermine seed viability and germination (Colombo et al. 2020; Meis, Fehr, and Schnebly 2003; Pilu et al. 2005). The relationship between phytate, protein, and oil contents and the seed quality of BN landraces has not been detailed before. The purpose of this study was therefore to determine the pattern of phytic acid, protein, and oil accumulation and the relationship of their contents with seed quality during the maturation of BN landraces from Uganda. This would elucidate their roles in determining seed quality during seed maturation and germination, which will in turn help refine aspects of seed quality management for better yields.

2 | Materials and Methods

The procedures and materials of the field experiments and seed germination tests presented below have been described in full detail in Oballim et al. (2023).

2.1 | Field Experiment Sites

Two field experiments were set up at on-farm sites in *Ocetoke*, Kitgum District (2020/2021), and *Koro*, Omoro District (2021/2022), both in the northern region of Uganda. Both *Ocetoke* (03°22'33.071" N/032°51'55.69" E, 1020 masl) and *Koro* (02°39'22.92" N/032°18'44.27" E, 1085 masl) lie in the warm subhumid zone of the northern moist farmlands' agro-ecological zone (AEZ) (Wortmann and Eledu 1999). The rainfall in this AEZ is unimodal (>1200 mm annual average), stretching from April to October, and the average annual temperature is >20°C. The zone has a long dry spell usually from November to March (Nsubuga et al. 2014; Wortmann and Eledu 1999). The soils are mostly sandy and sandy clay, having low organic matter and low nutrient availability (Wortmann and Eledu 1999).

2.2 | Plant Material

BN seeds of three landraces, namely, *AbiBam001*, *LocalBam*, and *TVSU544*, were obtained from a market in Arua, in the north-western region of Uganda, and planted in randomized complete

blocks with three replicates. AbiBam001 is small, round, and cream with black stripes (intermediate color), and LocalBam has large oblong mottled seeds with brown or purplish specs and is of dark color. TVSU544 has medium-sized cream seeds with black eyes and is of light color (Oballim et al. 2023).

2.3 | Field Operations and Seed Sampling

Standard crop management practices were applied at all stages of the crop growth. The crop was irrigated twice a week during periods of extended dry spells, especially in December and January. Pods were harvested from randomly selected rows in each plot at predetermined days after sowing (DAS) of 103, 113, and 123 (*Ocettoke*) and 123, 130, and 138 (*Koro*). Pods were sun dried and temporarily kept at room temperature and then later transferred to a -20°C freezer until use. Pods were hand threshed and used for the seed quality and biochemical analyses.

2.4 | Seed Size Measurements

Seed dry weight was measured with a weight balance. Twenty seeds were drawn twice from each replicate; each set of 20 seeds was weighed, and the average of the two measurements was recorded as the 20-seed weight. The result was expressed as the 100-seed weight by multiplying by 5.

2.5 | Germination Tests

A germination test was performed in triplicate on seeds sterilized with 1% sodium hypochlorite solution on sterilized moistened sand media in plastic trays. Seeds were incubated in a growth chamber at alternating conditions of 30°C 8-h light and 20°C 16-h dark, and the number of seeds germinated was recorded on a daily basis for 16 days. A seed was considered germinated when the plumule has emerged from the sand surface. At 16 days, the final germination percentage (FGP) was calculated using the following formula:

$$FGP = (N_g / N_T) 100$$

where N_g is the number of germinated seeds and N_T is the total number of seeds sown (Damalas, Koutroubas, and Fotiadis 2019) after modification.

The germination velocity index (GVI) was calculated as proposed by Maguire (1962) as

$$GVI = G_1/N_1 + G_2/N_2 + \dots + G_n/N_n$$

where $G_1, G_2, \dots, G_n$ are the number of seeds germinated on the first, second, and last counts. $N_1, N_2, \dots, N_n$ are the number of days at the first, second, and last counts from the day of sowing.

Five normal seedlings from each tray (each replication) were air oven dried at 95°C for 24 h and weighed to determine the seedling dry weight (SDW). A combined ranking of FGP, GVI, and SDW for sampling days was used to estimate stages of highest seed quality. Mass maturity stages were estimated as the point of diminished increase in seed dry weight (Oballim et al. 2023).

2.6 | Determination of Total Phosphorus and Phytic Acid Contents Using the Megazyme Kit

Samples were analyzed using the K-PHYT standard assay procedure (Neogen Megazyme, Wicklow, Ireland). Colorimetric determination of phosphorus was performed in a Synergy HTX microplate reader (Agilent Technologies, California, USA).

2.6.1 | Sample Extraction

Dried seeds were ground into a fine powder using the Cyclotec 1093 mill (FOSS Analytics, Hillerød, Denmark). One gram of sample material was weighed in a 75-mL glass beaker, and 20 mL of 0.66-M hydrochloric acid was added. The beaker was then covered with aluminum foil and stirred vigorously on a reciprocal shaker for at least 3 h at room temperature. One milliliter of the extract was transferred to a 1.5-mL microfuge tube and centrifuged at 13,000 rpm for 10 min. Immediately, 0.5 mL of the resulting extract supernatant was transferred to a fresh 1.5-mL microfuge tube and neutralized by the addition of 0.5 mL of 0.75-M sodium hydroxide solution. The neutralized sample extract was then used in the enzymatic dephosphorylation step.

2.6.2 | Enzymatic Dephosphorylation Reaction

For the “total phosphorus” isolation, 0.6 mL of distilled water, 0.2 mL of K-PHYT solution 1 (buffer), 0.05 mL of sample extract, and 0.02 mL of phytase suspension were pipetted into 1.5-mL microfuge tubes. They were mixed by vortex and incubated in a water bath at 40°C for 10 min. After 10 min, 0.2 mL of K-PHYT solution 3 (buffer) and 0.02 mL of alkaline phosphatase suspension were added to the reaction tube, mixed by vortex, and incubated in a water bath at 40°C for 15 min. The reaction was terminated by the addition of 0.3 mL of 50% (w/v) trichloroacetic acid. The solution was then centrifuged at 13,000 rpm for 10 min and left to stand. The supernatant was carefully recovered for the colorimetric determination of phosphorus. The procedure for the “free phosphorous” was the same, except in the first step where 0.62 mL of distilled water and no phytase were added, and in the second step, 0.02 mL of distilled water and no alkaline phosphatase were added.

2.6.3 | Preparation of Color Reagent

A color reagent was freshly prepared on the day of use by mixing two solutions, “A” and “B.” To make Solution A, 10 g of ascorbic acid was added to 90 mL of distilled water, followed by 5.35 mL of concentrated sulfuric acid (95%–98%), and the ascorbic acid was dissolved. The volume of the solution was adjusted to 100 mL with distilled water. Solution B was made by dissolving 1.25 g of ammonium molybdate in 20 mL of distilled water, and the solution was adjusted to a volume of 25 mL with distilled water. The color reagent was formed by adding one part of Solution B to five parts of Solution A.

2.6.4 | Colorimetric Determination of Phosphorus

A standard solution of 0.2-mL sample and 0.1-mL color reagent was transferred into a 0.65-mL microfuge tube and mixed by vortex. The solution was incubated in a water bath at 40°C for 1 h. Afterwards, it was again mixed by vortex, and then 0.2 mL of the

mixture was transferred to a known position in a 96-well microtiter plate. A blank (distilled deionized water, 0.2 mL) was also added to a known position in the microtiter plate. Absorbance for each of sample, standard and blank, was read at 655 nm within 3 h in a Synergy HTX microplate reader (Agilent Technologies, California, USA). The absorbance values for both “total phosphorus” and “free phosphorus” were entered into Megazyme’s Mega-Calc Excel worksheet, which generated the corresponding phosphorus and phytic acid values for the samples.

2.6.5 | Preparation of the Phosphorus Calibration Curve

Standard phosphorus solutions were prepared in 13-mL polypropylene tubes using 0.00, 0.05, 0.25, 0.50, and 0.75 mL of a 50- μ g/mL phosphorus standard solution (Megazyme K-PHYT solution 5) and adjusted to a final volume of 5 mL with distilled water. The standards’ absorbances generated a phosphorus standard curve that was used in the determination of the phosphorus contents of samples. The regression coefficient (R^2) of the phosphorus standard curve was 0.9999, denoting the reliability of the curve in the determination of phosphorus and phytic acid contents of seeds.

2.7 | Proximate Analysis

2.7.1 | Crude Fat

Total fat was determined by the acid hydrolysis method using the Soxhlet apparatus (Horwitz and Latimer 2012; Puwastien et al. 2011). Samples were digested with hydrochloric acid, filtered with a Whatman filter paper, and the filter paper was washed with warm distilled deionized water and dried overnight in an oven at 50°C–60°C. Samples were then extracted by immersing them in warm petroleum ether for 3 h and then drying.

2.7.2 | Crude Protein

Protein was determined using the Kjeldahl method (Horwitz and Latimer 2012; Puwastien et al. 2011). Samples were digested with concentrated sulfuric acid and hydrogen peroxide to release nitrogen from proteins. Samples were then analyzed with the UDK 169 Automatic Kjeldahl Nitrogen Protein Analyzer (Velp Scientific, New York, USA). Protein content was estimated by multiplying the amount of detected nitrogen by 6.25.

2.7.3 | Ash Content

Ash content was determined by the dry ashing method (Horwitz and Latimer 2012; Puwastien et al. 2011). Samples were incinerated in a muffle furnace (Bioeurope FNC-BX1400, Shandong, China) at 500°C–550°C until the residue was uniformly white or nearly white. Ash content (dry weight basis) was determined as the percentage loss in weight on ashing.

2.8 | Data Processing and Analysis

Data were entered in Excel, and analysis of variance (ANOVA) was performed on the proximate composition of seeds at measured stages of seed development in GenStat 14th edition (VSN International Ltd, Hemel Hempstead, UK). ANOVA was

performed to compare the following: the developmental stages for the proximate composition of landraces during maturation, the proximate composition of landraces at the respective stages of highest seed quality and mass maturity, and the phytate content of landraces at mass maturity. Means were separated using Tukey’s procedure at the 5% level. *t*-tests were performed (also in GenStat 14th edition) to compare landraces for phytic acid contents at 113 and 123 DAS in the *Ocettoke* experiment and at points of highest seed quality to compare only AbiBam001 and LocalBam (at 113 DAS for both landraces). This was because phytic acid was determined only from 113 DAS onwards for all landraces (the stage of highest seed quality for TVSU544 was at 103 DAS). Kendall’s rank correlation coefficient (τ) (Kendall’s tau-b) was calculated in IBM SPSS Statistics (Version 20) statistical software (IBM Corporation, Armonk, New York, USA) to explore relationships between the protein, oil, and phytic acid contents of seeds and different germination variables (Oballim et al. 2023).

3 | Results

3.1 | Phytic Acid Content of Seeds During Seed Maturation

There were no differences in the phytate content of seeds of all landraces in both experiments ($p > 0.05$), except for AbiBam001 at *Ocettoke*, which showed significant differences in phytate content at 113 and 123 DAS ($p < 0.01$) (Table 1). Nevertheless, for both AbiBam001 and TVSU544, there were 33% and 7% increases in phytate content, respectively, from 113 to 123 DAS, while for LocalBam, it decreased by 22% from 113 to 123 DAS at *Ocettoke* (Table 1). At *Koro*, AbiBam001 showed a steady decrease in phytate content at all seed maturation stages, but both LocalBam and TVSU544 showed an increase and then a decline in phytate content (Table 1). At the stage of highest seed quality (113 DAS for both AbiBam001 and LocalBam), the two landraces showed significant differences in their phytate content with LocalBam having a much higher phytate content than AbiBam001 (Table 2). Similarly, at mass maturity, there were significant differences in the phytate content of the landraces with LocalBam having the highest content followed by AbiBam001 and TVSU544 having the lowest phytate content (Table 2).

3.2 | Proximate Composition of Seeds During Seed Maturation

There were no differences in the ash, protein, and oil composition of seeds of all landraces in both the *Ocettoke* and *Koro* experiments ($p > 0.05$) (Table 3). However, both protein and oil contents exhibited increasing trends in *Ocettoke*, except for LocalBam, which showed a decreasing trend (Table 3). Ash content also increased with seed maturation for both AbiBam001 and LocalBam but decreased for TVSU544 in the *Ocettoke* experiment (Table 3). At *Koro*, the ash, protein, and oil contents of AbiBam001 overall increased, whereas those of both LocalBam and TVSU544 either decreased or leveled off. At the stage of their respective highest seed quality, landraces showed no differences in their oil, protein, and ash contents, but at mass maturity, they differed significantly in protein and oil contents, but not in ash content (Table 4). The landrace

TABLE 1 | Phytic acid composition of Bambara nut seeds during seed maturation.

Location	Seed developmental stage (DAS)	Phytic acid content of landraces (g/100 g)		
		AbiBam001	LocalBam	TVSU544
<i>Ocettoke</i>	113	439.5 a	956.3 a	514.5 a
	123	653.8 b	786.1 a	552.3 a
	<i>p</i> value	0.003	0.137	0.185
	<i>s.e.d.</i>	33.19	70.96	23.56
<i>Koro</i>	123	710.59 a	647.59 a	541.06 a
	130	669.60 a	658.15 a	777.71 a
	138	581.29 a	627.03 a	689.25 a
	<i>p</i> value	0.817	0.356	0.150
	<i>s.e.d.</i>	197.284	16.622	71.075
	<i>l.s.d.</i>	848.845	71.520	305.809

Note: Means followed by different letters within a column are significantly different from each other (Tukey's test, $p < 0.05$).

TABLE 2 | Phytic acid composition of seeds at the stage of highest seed quality and at mass maturity.

Landrace	Phytic acid content (g/100 g)	
	At stage of highest seed quality	At mass maturity
AbiBam001	439.53 a	668.32 ab
LocalBam	956.26 b	956.26 b
TVSU544	—	514.54 a
<i>p</i> value	0.002	0.037
<i>s.e.d.</i>	72.76	109.28
<i>l.s.d.</i>	—	303.40

Note: Means followed by different letters within a column are significantly different from each other (Tukey's test, $p < 0.05$). Stages of highest seed quality: 113 DAS (AbiBam001 and LocalBam), 103 DAS (TVSU544); mass maturity stages: 113 DAS (LocalBam and TVSU544), 103 DAS (AbiBam001) (Oballim et al. 2023).

with the highest ash, protein, and oil contents at mass maturity was, respectively, AbiBam001, LocalBam, and TVSU544. The lowest ash content at the same stage was recorded in TVSU544, and the lowest protein and oil contents were both recorded in AbiBam001 (Table 4).

3.3 | Relationship Between Phytate Content and Seed Quality of Landraces

Phytate had a positive relationship with FGP for AbiBam001 and LocalBam in both experiments (at *Ocettoke* and *Koro*) but a negative relationship with FGP for TVSU544 in both experiments (Tables 5 and 6). For SDW, there were negative relationships with phytate content for all landraces in both experiments, except for TVSU544 at *Koro* (Tables 5 and 6). Phytate content related positively (for LocalBam) and negatively (for TVSU544) with GVI at both sites, whereas for AbiBam001, the relationship was negative at *Ocettoke* and positive at *Koro* (Tables 5 and 6). Phytate content had positive (for TVSU544) and negative (for LocalBam) relationships with seed weight at both sites. For AbiBam001, the relationship was negative at *Ocettoke* and positive at *Koro* (Tables 5 and 6).

3.4 | Relationship Between Oil and Protein Contents and Seed Quality of Landraces

Oil content had mostly negative correlations with the seed quality characteristics of FGP, GVI, SDW, and seed weight in both experiments for LocalBam and TVSU544. For AbiBam001, oil content had positive relationships with all seed quality characteristics in the *Ocettoke* experiment, except SDW (Tables 5 and 6). In the *Koro* experiment, the oil content of AbiBam001 had negative relationships with FGP and GVI and positive relationships with SDW and seed weight (Tables 5 and 6). For protein content, there were positive or no relationships with FGP and GVI in both experiments for LocalBam, whereas for AbiBam001, the relationships of protein content with FGP and GVI were positive at *Ocettoke* and negative at *Koro* (Tables 5 and 6). The protein content of TVSU544 was negatively related with FGP, GVI, and SDW at both sites. SDW had negative relationships with the protein content of all landraces at *Ocettoke* and positive relationships with the protein contents of AbiBam001 and LocalBam at *Koro*. For seed weight, there were positive relationships with the protein content of all landraces at both sites, except for LocalBam at *Ocettoke* (Tables 5 and 6).

4 | Discussion

4.1 | Phytic Acid Content of Seeds During Seed Maturation

Both AbiBam001 and TVSU544 showed an initial increase and then an overall decline in phytate content from the first experiment (*Ocettoke*) to the second experiment (*Koro*). For LocalBam, however, there was an overall declining trend of phytate content from the first experiment to the second experiment. Accumulation of seed storage compounds, that is, carbohydrates, proteins, and oils, in most species is known to gradually increase up to a point, and then there is a rapid increase towards physiological maturity and eventual leveling off at and decline after physiological maturity (Bewley and Nonogaki 2017; Bewley et al., 2013). It has been observed that phytates accumulate in legume seeds in much the same way as the major seed components (Dhole and Reddy 2015; Mandizvo and Odindo 2020; Shunmugam et al. 2015; Sparvoli

TABLE 3 | Proximate composition of seeds of landraces at different developmental stages from the *Ocettoke* and *Koro* sites.

Location	Seed developmental stage (DAS)	Landrace								
		AbiBam001			LocalBam			TVSU544		
		Crude protein ^a (%)	Crude fat (%)	Ash (%)	Crude protein (%)	Crude fat (%)	Ash (%)	Crude protein (%)	Crude fat (%)	Ash (%)
<i>Ocettoke</i>	103	17.69 a	7.389 a	3.360 a	20.53 a	7.792 a	2.860 a	18.70 a	6.819 a	3.820 a
	113	18.44 a	7.667 a	3.440 a	21.42 a	6.722 a	3.640 a	19.97 a	7.861 a	3.540 a
	123	19.08 a	8.181 a	3.440 a	20.40 a	7.389 a	2.960 a	18.99 a	9.111 a	3.320 a
	<i>p</i> value	0.093	0.544	0.200	0.253	0.194	0.641	0.597	0.167	0.094
	<i>s.e.d.</i>	0.32	0.62	0.03	0.46	0.37	0.80	1.14	0.73	0.11
	<i>l.s.d.</i>	1.36	2.67	0.14	1.96	1.62	3.45	4.91	3.13	0.49
<i>Koro</i>	123	14.25 a	4.382 a	3.667 a	13.54 a	5.327 a	3.622 a	13.54 a	5.127 a	4.411 a
	130	14.80 a	4.991 a	4.297 a	13.86 a	5.118 a	3.987 a	13.90 a	4.775 a	3.731 a
	138	14.78 a	5.084 a	4.095 a	13.85 a	5.265 a	3.213 a	13.57 a	5.376 a	3.569 a
	<i>p</i> value	0.539	0.111	0.420	0.598	0.827	0.088	0.503	0.459	0.246
	<i>s.e.d.</i>	0.472	0.191	0.387	0.317	0.333	0.17	0.284	0.393	0.361
	<i>l.s.d.</i>	3.023	0.822	1.665	1.363	1.431	0.73	1.224	1.692	1.552

Note: Means followed by different letters within a column are significantly different from each other (Tukey's test, $p < 0.05$).

^aCrude protein, crude fat, and ash measured as percentage of dry matter on a dry weight basis.

TABLE 4 | Protein, oil, and ash contents of seeds of BN landraces at respective stages of highest seed quality and mass maturity.

Landrace	Seed component					
	At stage of highest seed quality			At mass maturity		
	Proteins (%)	Oil (%)	Ash (%)	Proteins (%)	Oil (%)	Ash (%)
AbiBam001	18.44 a	7.67 a	3.44 a	14.80 a	4.99 a	4.30 a
LocalBam	21.42 a	6.72 a	3.64 a	21.42 b	6.72 b	3.64 a
TVSU544	18.70 a	6.82 a	3.82 a	19.97 b	7.86 c	3.54 a
<i>p</i> value	0.250	0.196	0.251	0.008	0.003	0.242
<i>s.e.d.</i>	1.34	0.36	0.16	0.45	0.10	0.33
<i>l.s.d.</i>	5.78	1.56	0.67	1.92	0.44	1.41

Note: Means followed by different letters within a column are significantly different from each other (Tukey's test, $p < 0.05$). Stages of highest seed quality: 113 DAS (AbiBam001 and LocalBam), 103 DAS (TVSU544); mass maturity stages: 113 DAS (LocalBam and TVSU544), 103 DAS (AbiBam001) (Oballim et al. 2023).

and Cominelli 2015; Urbano et al. 2000). In contrast, however, Raboy and Dickinson (1987) showed that phytates accumulate in a linear trend in soybean throughout most of seed development. A similar report by Pandey et al. (2016) indicated that the phytate content of both yellow and black Indian soybean genotypes increased linearly from early seed development to maturation. Nonetheless, Mandizvo and Odindo (2020) demonstrated that the phytate content of BN landraces had a gradual increase between 14 and 42 DAF (approximately 49–77 DAS) and then had a rapid increase afterwards until physiological maturity at 65 DAF (approximately 100 DAS). The patterns of phytate accumulation shown by the landraces in the present study concur with the findings of Mandizvo and Odindo (2020). The following scenarios can therefore be inferred. For AbiBam001, the initial increase suggests the phase of rapid increase before mass maturity; for LocalBam, the gradual decrease suggests the phase of decline after mass maturity; and for TVSU544, the gradual decrease suggests the phase of leveling off prior to mass maturity. Subsequently,

however, beyond mass maturity (for all landraces), there is an apparent decline in phytate content as seen in the *Koro* experiment. Landraces also showed differences in phytate contents at stages of highest seed quality and at mass maturity. A study by Mandizvo and Odindo (2020) revealed that BN landraces of cream seed coat had lower phytate content compared to the dark-colored landraces. Another report by Dhole and Reddy (2015) showed that mung bean genotypes of yellow and green seed coats contained much less phytic acid content compared to those with black seed coat. A contrasting report by Pandey et al. (2016), however, revealed that black soybean genotypes had lower phytate content than yellow genotypes. In the present study, LocalBam, a dark brown speckled type, had the highest phytate content followed by AbiBam001 (of intermediate color), with TVSU544 (of light/cream color) having the lowest phytate content, thus corroborating the results of previous studies. These observations are suggestive of the inherent genetics of the landraces with regard to phytate content, possible influence of seed coat color on phytate content

TABLE 5 | Kendall's rank correlation coefficients (τ) among oil, protein, phytic acid, and seed quality parameters of the landraces at *Ocettoke*.

Landrace	Parameter	Oil	Protein	Phytic acid	FGP	GVI	SDW	Seed weight
AbiBam001	Oil	1.0						
	Protein	1.0**	1.0					
	Phytic	—	—	1.0				
	FGP	1.0**	1.0**	1.0**	1.0			
	GVI	0.3	0.3	−1.0**	0.3	1.0		
	SDW	−1.0**	−1.0**	−1.0**	−1.0**	−0.3	1.0	
	Seed weight	1.0**	1.0**	1.0**	1.0**	0.3	−1.0**	1.0
LocalBam	Oil	1.0						
	Protein	−0.3	1.0					
	Phytic	—	—	1.0				
	FGP	−0.3	1.0**	1.0**	1.0			
	GVI	−0.3	1.0**	1.0**	1.0**	1.0		
	SDW	−0.3	−0.3	−1.0**	−0.3	−0.3	1.0	
	Seed weight	−0.3	−0.3	−1.0**	−0.3	−0.3	1.0**	1.0
TVSU544	Oil	1.0						
	Protein	0.3	1.0					
	Phytic	—	—	1.0				
	FGP	−1.0**	−0.3	−1.0**	1.0			
	GVI	−1.0**	−0.3	−1.0**	1.0**	1.0		
	SDW	−0.3	−1.0**	1.0**	0.3	0.3	1.0	
	Seed weight	1.0**	0.3	1.0**	−1.0**	−1.0**	−0.3	1.0

**Correlation is significant at the 0.01 level.

of landraces, and potential influence of phytate on the seed quality of each landrace.

4.2 | Proximate Composition of Seeds During Seed Maturation

The protein and oil contents of AbiBam001 continued to increase with seed maturation, whereas those of LocalBam and TVSU544 leveled off or decreased as the seeds matured. Dry matter accumulation in orthodox seeds of many cultivated species including legumes such as BN follows a distinct pattern after embryogenesis, with variations to a lesser or greater extent between species, cultivars, and maternal environment (Bewley and Nonogaki 2017; Bewley et al., 2013). An initial gradual phase is typically followed by a rapid phase, in which there is a heightened rate of cell expansion and reserve deposition, and a final phase characterized by diminished reserve deposition and maturation drying (Bewley and Nonogaki 2017; Bewley et al., 2013). A study of seed development and maturation in soybean revealed that substantial lipid accumulation occurred early in seed development whereas protein accumulated mostly in the later stages (Xu et al. 2016). Warsame et al. (2022) demonstrated that storage protein accumulation in faba bean began later in seed development (at about 45 days after pollination), compared to other functional proteins that were abundant in the earlier stages. In the model legume *Medicago truncatula*, it has been shown that the onset of oil accumulation, evidenced by oil body biogenesis, is overlapped by embryo maturation (Kurdyukov et al. 2014).

A similar report showed a prominent increase in seed oil content of *Pongamia (Milletia pinnata)* from the embryogenesis phase to the early seed-filling phase, which steadied towards a maximum at maturation (Huang et al. 2018). Taken together, these reports strongly suggest that accumulation of lipids/oils in legumes occurs early in seed development whereas proteins accumulate mostly in the later stages. In the present study, the decreasing or leveling off of the mineral, protein, and oil contents for LocalBam and TVSU544 implies that the seeds of the two landraces are at a stage beyond mass maturity (113 DAS for both) (Oballim et al. 2023) when dry matter and seed mineral reserves' accumulation are in decline. For AbiBam001, however, the increasing trend can be explained by the fact that its mass maturity stage is approximately 130 DAS (Oballim et al. 2023); hence, it is plausible that minerals and dry matter accumulation still show an increasing trend. At mass maturity, landraces had significantly different levels of both proteins and oils. BN landraces have been shown to have varying contents of starch, proteins, oils, and minerals (Amarteifio, Karikari, and Modise 2002; Donkor et al. 2022; Hlanga, Modi, and Mathew 2021; Tan et al. 2020; Yao et al. 2015). According to Donkor et al. (2022), light-colored BN accessions had higher protein and oil, but less minerals compared to dark-colored accessions. Similarly, BN landraces of cream and white (light) colors were found to have higher protein content than the dark-colored (red, brown, and black) ones (Hlanga, Modi, and Mathew 2021). The report by Hlanga, Modi, and Mathew (2021) did not, however, find any clear relationship between BN seed coat color and oil content. The light-colored landrace

TABLE 6 | Kendall's rank correlation coefficients (τ) among oil, protein, phytic acid, and seed quality parameters of the landraces at Koro.

Landrace	Parameter	Oil	Protein	Phytic acid	FGP	GVI	SDW	Seed weight
AbiBam001	Oil	1.0						
	Protein	0.3	1.0					
	Phytic acid	−1.0**	−0.3	1.0				
	FGP	−1.0**	−0.3	1.0**	1.0			
	GVI	−1.0**	−0.3	1.0**	1.0**	1.0		
	SDW	1.0**	0.3	−1.0**	−1.0**	−1.0**	1.0	
	Seed weight	1.0**	0.3	−1.0**	−1.0**	−1.0**	1.0**	1.0
LocalBam	Oil	1.0						
	Protein	−1.0**	1.0					
	Phytic acid	−0.3	0.3	1.0				
	FGP	0.0	0.0	0.8	1.0			
	GVI	−0.3	0.3	1.0**	0.8	1.0		
	SDW	−0.3	0.3	−0.3	−0.8	−0.3	1.0	
	Seed weight	−0.3	0.3	−0.3	−0.8	−0.3	1.0**	1.0
TVSU544	Oil	1.0						
	Protein	−0.3	1.0					
	Phytic acid	−0.3	1.0**	1.0				
	FGP	−0.3	−0.3	−0.3	1.0			
	GVI	−0.3	−0.3	−0.3	1.0**	1.0		
	SDW	−0.3	−0.3	−0.3	1.0**	1.0**	1.0	
	Seed weight	−0.3	1.0**	1.0**	−0.3	−0.3	−0.3	1.0

**Correlation is significant at the 0.01 level.

TVSU544 in the present study had higher oil and lower mineral content than the darker colored landraces (LocalBam and AbiBam001), as elucidated in previous studies. However, its protein content was comparable to that of LocalBam, though higher than that of AbiBam001. Differences in storage reserve and phytochemical composition of legumes including BN are known to be genetically controlled (Kanmaz and Ova 2015; Karmakar et al. 2020; Silva et al. 2021) and are related to their adaptation to biotic and abiotic stresses in their environments (Arockianathan, Rajalakshmi, and Nagappan 2019; Sparvoli and Cominelli 2015). Seed coat color in BN and other legumes is largely determined by the polyphenolic content of the seed coat, mostly the tannins and flavonoids (Mabhaudhi and Modi 2013; Ren, Liu, and Wang 2012; Taahir, Jideani, and Hill 2018; P. Zhao et al. 2022). The darker seeded BN landraces have been shown to have higher tannins and flavonoids than the lighter seeded types (Mabhaudhi and Modi 2013; Taahir, Jideani, and Hill 2018).

4.3 | Relationship Between Phytate Content and Seed Quality of Landraces

Seed quality characteristics of landraces showed both positive and negative relationships with phytate content with varying patterns for any landrace. The relationship between phytate content of legume seeds and their respective seed quality parameters such as germinability, rate of germination, and seedling vigor has generally been shown to be positive (for example, Pramitha et al. 2020; Soares et al. 2014; Zhou et al. 2018).

This is because the hydrolysis of phytates releases inorganic phosphate that is used in the growth of the embryonic axis and later in seedling growth upon completion of germination (Dong and Saneoka 2020; Lazali et al. 2014). Phytate is further reported to be involved in the synthesis of abscisic acid and gibberellins, the hormones that regulate seed germination and dormancy (Silva et al. 2021). Moreover, *lpa* mutants have been suggested to result in negative gene pleiotropy, which may undermine seed viability and germination (Colombo et al. 2020; Meis, Fehr, and Schnebly 2003; Pilu et al. 2005). This may in part explain the negative relationships observed between the phytate contents of the landraces and some seed quality parameters. Nevertheless, a more comprehensive study covering a wider range of developmental and maturation stages would shed more light on the relation of the phytate content and seed quality of BN landraces.

4.4 | Relationship Between the Oil and Protein Contents and Seed Quality of Landraces

The oil content of all landraces had mostly negative relationships with seed quality characteristics in both experiments. There were, however, both positive and negative relationships between protein content and seed quality characteristics in both experiments, with no clear pattern among landraces or seed quality characteristics. Nevertheless, SDW mostly had negative relationships with both protein and oil contents of all landraces in both experiments. It is suggested that in certain species, the most abundant reserve is the first or the

main reserve to be mobilized (M. Zhao et al. 2018) and that different reserves play different roles during seed germination and seedling growth (Bewley and Nonogaki 2017; Soriano et al. 2011; M. Zhao et al. 2018). In general, proteins provide a source of amino acids and nitrogen necessary for the growth and development of the germinating seed (Kermode 2011). On the other hand, carbohydrates (starch, soluble sugars, and low molecular weight oligosaccharides) and lipids provide a source of energy for the various metabolic processes (Bewley and Nonogaki 2017; Kermode 2011; M. Zhao et al. 2018). According to M. Zhao et al. (2018), the germination rate of wild grass species had a positive relationship with both soluble sugar and soluble protein contents at different stages of germination. In dry seeds, however, there was no relationship of protein and fat reserves with the rate and extent of germination (M. Zhao et al. 2018). Another study on seeds of tree species in a tropical deciduous forest showed that the germination rate had a positive association with nitrogen and lipid concentrations of seeds (Soriano et al. 2011). Furthermore, no relationship was found between the fat content and germination percentage of flax seeds (Kanmaz and Ova 2015). It is apparent, therefore, that the influence of seed reserves on germination depends on the type and amount of reserve and the plant species in question (Kanmaz and Ova 2015; Soriano et al. 2011; M. Zhao et al. 2018). Unsurprisingly, the relationships of protein and oil contents with seed quality characteristics of landraces seem to be in agreement with most reports. However, there is a need for further in-depth investigation to better understand the relationship of stored reserves with seed quality and their metabolism during germination of BN landraces.

5 | Conclusions

Accumulation of phytate, oil, and proteins in the seeds of landraces appears to follow the normal trend of dry matter accumulation in legume seeds, evidenced by diminished trends towards maturation and decreasing or leveled off trends at and after maturation. The apparent color-related seed contents of phytic acid, proteins, and oils at maturity suggest that seed coat color may influence the phytate, protein, and oil contents of BN landraces. The reciprocal relationship of phytate content with seed quality aspects implies that phytic acid content may positively affect seed germinability in BN landraces with high phytate content but negatively affect it in low-phytate landraces. The negative correlation of oil contents with seed quality aspects of landraces may mean that BN oils are either not priority reserves or play a minimal role in germination and seedling growth. A more extensive investigation is, however, required to better understand the relationship of phytate content and stored reserves with seed quality and their metabolism during germination of BN landraces.

Author Contributions

Gerard Oballim: Conceptualization; Data curation; Investigation; Methodology; Software; Validation; Writing – original draft. **Wilson R. Opile:** Resources; Supervision; Validation; Writing – review and editing. **Julius O. Ochuocho:** Funding acquisition; Project administration; Resources; Supervision; Validation; Writing – review and editing.

Acknowledgments

This study was funded by the Intra-Africa Academic Mobility Scheme of the European Union/African Union through the SCIFSA project, as part of a PhD training program.

Ethics Statement

We declare that the work presented in this paper is the original research work conducted at the University of Eldoret. It has not been manipulated in any way or submitted to another journal before. The authors of this paper are not engaged in any personal or financial undertakings that may impact this publication.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon request.

References

- Affrifah, N. S., M. A. Uebersax, and S. Amin. 2023. “Nutritional Significance, Value-Added Applications, and Consumer Perceptions of Food Legumes: A Review.” *Legume Science* 5, no. 4: e192. <https://doi.org/10.1002/leg3.192>.
- Amarteifio, J. O., S. K. Karikari, and O. J. Modise. 2002. “The Proximate Composition and Mineral Composition of Six Landraces of Botswana Bambara Groundnut.” *Tropical Science* 42: 188–191.
- Arockianathan, P. M., K. Rajalakshmi, and P. Nagappan. 2019. “Proximate Composition, Phytochemicals, Minerals and Antioxidant Activities of *Vigna mungo* L. Seed Coat.” *Bioinformation* 15, no. 8: 579–585. <https://doi.org/10.6026/97320630015579>.
- Batra, D., S. B. Dhull, J. Rani, M. Meenakshi, Y. Kumar, and J. Kinabo. 2023. “Effect of Heat Stress on Seed Protein Quality in Mung Bean [*Vigna radiata* (L.) Wilczek].” *Legume Science* 5, no. 4: e205. <https://doi.org/10.1002/leg3.205>.
- Bewley, J. D., and H. Nonogaki. 2017. “Seed Maturation and Germination.” In *Reference Module in Life Sciences*, edited by B. D. Roitberg, 1–9. Amsterdam, Netherlands: Elsevier. <https://doi.org/10.1016/B978-0-12-809633-8.05092-5>.
- Bewley, J. D., K. J. Bradford, H. W. M. Hillhorst, and H. Nonogaki. 2013. *Seeds: Physiology of Development, Germination and Dormancy*. 3rd ed. 27–83; 85–131. New York: Springer-Verlag.
- Colombo, F., P. Dario, E. Cominelli, F. Sparvoli, E. Nielsen, and R. Pilu. 2020. “MRP Transporters and Low Phytic Acid Mutants in Major Crops: Main Pleiotropic Effects and Future Perspectives.” *Frontiers in Plant Science* 11: 1301. <https://doi.org/10.3389/fpls.2020.01301>.
- Daffodil, D.-A. E., K. R. Fathima, and V. R. Mohan. 2013. “Effect of Different Water or Hydrothermal Treatments on Anti-Nutritional and In Vitro Protein Digestibility of Three Accession of Itching Bean (*Mucuna pruriens* (L.) DC var. *pruriens*): An Underutilized Tribal Pulse.” *International Journal of Biochemistry* 108: 152–172.
- Damalas, C. A., S. D. Koutroubas, and S. Fotiadis. 2019. “Hydro-Priming Effects on Seed Germination and Field Performance of Faba Bean Spring Sowing.” *Agriculture* 9, no. 201: 1–11. <https://doi.org/10.3390/agriculture9090201>.
- Dhole, V. J., and K. S. Reddy. 2015. “Genetic Variation for Phytic Acid Content in Mung Bean (*Vigna radiata* L. Wilczek).” *The Crop Journal* 3, no. 2: 157–162. <https://doi.org/10.1016/j.cj.2014.12.002>.
- Dhull, S. B., J. Kinabo, and M. A. Uebersax. 2023. “Nutrient Profile and Effect of Processing Methods on the Composition and Functional

- Properties of Lentils (*Lens culinaris* Medik): A Review." *Legume Science* 5, no. 1: e156. <https://doi.org/10.1002/leg3.156>.
- Dhull, S. B., K. S. Sandhu, S. Punia, M. Kaur, P. Chawla, and A. Malik. 2020. "Functional, Thermal and Rheological Behavior of Fenugreek (*Trigonella foenum-graecum* L.) Gums From Different Cultivars: A Comparative Study." *International Journal of Biological Macromolecules* 159: 406–414. <https://doi.org/10.1016/j.ijbiomac.2020.05.094>.
- Dhull, S. B., S. Punia, M. K. Kidwai, et al. 2020. "Solid-State Fermentation of Lentil (*Lens culinaris* L.) With *Aspergillus awamori*: Effect on Phenolic Compounds, Mineral Content, and Their Bioavailability." *Legume Science* 2: e37. <https://doi.org/10.1002/leg3.37>.
- Dhull, S. B., S. Punia, R. Kumar, et al. 2021. "Solid State Fermentation of Fenugreek (*Trigonella foenum-graecum*): Implications on Bioactive Compounds, Mineral Content and In Vitro Bioavailability." *Journal of Food Science and Technology* 58, no. 5: 1927–1936. <https://doi.org/10.1007/s13197-020-04704-y>.
- Dhull, S. B., S. Punia, K. S. Sandhu, P. Chawla, R. Kaur, and A. Singh. 2020. "Effect of Debittered Fenugreek (*Trigonella foenum-graecum* L.) Flour Addition on Physical, Nutritional, Antioxidant, and Sensory Properties of Wheat Flour Rusk." *Legume Science* 2: e21. <https://doi.org/10.1002/leg3.21>.
- Dong, Q., and H. Saneoka. 2020. "Physiological Characteristics, Phytase Activity, and Mineral Bioavailability of a Low-Phytate Soybean Line During Germination." *Plant Foods for Human Nutrition* 75, no. 3: 383–389. <https://doi.org/10.1007/s11130-020-00827-x>.
- Donkor, E. F., R. R. Adjei, B. Amadu, and A. S. Boateng. 2022. "Genetic Variability, Heritability and Association Among Yield Components and Proximate Composition of Neglected and Underutilized Bambara Groundnut [*Vigna subterranea* (L) Verdc] Accessions for Varietal Development in Ghana." *Heliyon* 8, no. 6: e09691. <https://doi.org/10.1016/j.heliyon.2022.e09691>.
- Hlanga, N. C., A. T. Modi, and I. Mathew. 2021. "Evaluating Nutritional Content Among Bambara Groundnut Lines." *Journal of Food Composition and Analysis* 102: 104053. <https://doi.org/10.1016/j.jfca.2021.104053>.
- Horwitz, W., and G. W. Latimer Jr., eds. 2012. *Official Methods of Analysis of the AOAC*. 19th ed. Rockville, MD: AOAC International.
- Huang, J., X. Hao, Y. Jin, et al. 2018. "Temporal Transcriptome Profiling of Developing Seeds Reveals a Concerted Gene Regulation in Relation to Oil Accumulation in Pongamia (*Millettia pinnata*)." *BMC Plant Biology* 18, no. 1: 140. <https://doi.org/10.1186/s12870-018-1356-8>.
- Kanmaz, E. O., and G. Ova. 2015. "Genotypic Variation on Oil Content, Fatty Acid Composition and Phenolic Compounds in Linseed (*Linum usitatissimum*)." *Materials Science* 52, no. 3: 249–255. <https://doi.org/10.20289/EUZF.35393>.
- Karmakar, A., S. Bhattacharya, S. Sengupta, et al. 2020. "RNAi-Mediated Silencing of ITPK Gene Reduces Phytic Acid Content, Alters Transcripts of Phytic Acid Biosynthetic Genes, and Modulates Mineral Distribution in Rice Seeds." *Rice Science* 27: 315–328. <https://doi.org/10.1016/j.rsci.2020.05.007>.
- Kermode, A. R. 2011. "Plant Storage Products (Carbohydrates, Oils and Proteins)." In *Encyclopedia of Life Sciences (ELS)*. Chichester, UK: John Wiley & Sons, Ltd. <https://doi.org/10.1002/9780470015902.a0001325.pub2>.
- Kurdyukov, S., Y. Song, M. B. Sheahan, and R. J. Rose. 2014. "Transcriptional Regulation of Early Embryo Development in the Model Legume *Medicago truncatula*." *Plant Cell Reports* 33, no. 2: 349–362. <https://doi.org/10.1007/s00299-013-1535-x>.
- Lazali, M., L. Louadj, G. Ounane, et al. 2014. "Localization of Phytase Transcripts in Germinating Seeds of the Common Bean (*Phaseolus vulgaris* L.)." *Planta* 240, no. 3: 471–478. <http://www.jstor.org/stable/43565804>.
- Mabhaudhi, T., and A. T. Modi. 2013. "Growth, Phenological and Yield Responses of a Bambara Nut (*Vigna subterranea* (L.) Verdc.) Landrace to Imposed Water Stress Under Field Conditions." *South African Journal of Plant and Soil* 30, no. 2: 69–79.
- Maguire, J. D. 1962. "Speed of Germination-Aid in Selection and Evaluation for Seedling Emergence and Vigor." *Crop Science* 2: 176–177.
- Mandizvo, T., and A. O. Odindo. 2020. "Spectrophotometric Quantification of Phytic Acid During Embryogenesis in Bambara Groundnut (*Vigna subterranea* L.) Through Phosphomolybdenum Complex Formation." *Emirates Journal of Food and Agriculture* 32, no. 11: 778–785. <https://doi.org/10.9755/ejfa.2020.v32.i11.2178>.
- Meis, S. J., W. R. Fehr, and S. R. Schnebly. 2003. "Seed Source Effect on Field Emergence of Soybean Lines With Reduced Phytate and Raffinose Saccharides." *Crop Science* 43: 1336–1339.
- Mohan, V. R., P. S. Tresina, and E. D. Daffodil. 2016. "Antinutritional Factors in Legume Seeds: Characteristics and Determination." In *Encyclopedia of Food and Health*, edited by B. Caballero, P. M. Finglas, and F. Toldrá, 211–220. Cambridge, Massachusetts, USA: Academic Press. <https://doi.org/10.1016/B978-0-12-384947-2.00036-2>.
- Nsubuga, F. W. N., O. J. Botai, J. M. Olwoch, C. J. DeW Rautenbach, Y. Bevis, and A. O. Adetunji. 2014. "The Nature of Rainfall in the Main Drainage Sub-Basins of Uganda." *Hydrological Sciences Journal* 59, no. 2: 278–299. <https://doi.org/10.1080/02626667.2013.804188>.
- Oballim, G., M. Obura, R. W. Opile, and J. O. Ochuodho. 2023. "Changes in Seed Quality During Seed Development and Maturation of Bambara Nut (*Vigna subterranea* (L.) Verdc.) Landraces." *International Journal of Agronomy* 2023: 5953877. <https://doi.org/10.1155/2023/5953877>.
- Pandey, V., V. Krishnan, N. Basak, et al. 2016. "Phytic Acid Dynamics During Seed Development and It's Composition in Yellow and Black Indian Soybean (*Glycine max* L.) Genotypes Through a Modified Extraction and HPLC Method." *Journal of Plant Biochemistry and Biotechnology* 25: 367–374. <https://doi.org/10.1007/s13562-015-0348-0>.
- Pilu, R., D. Panzeri, G. Gavazzi, S. K. Rasmussen, G. Consonni, and E. Nielsen. 2005. "Phenotypic, Genetic and Molecular Characterization of a Maize Low Phytic Acid Mutant (*lpa241*)." *Theoretical and Applied Genetics* 107: 980–987.
- Pramitha, J. L., S. Rana, P. R. Aggarwal, R. Ravikesavan, A. J. Joel, and M. Muthamilarasan. 2020. "Diverse Role of Phytic Acid in Plants and Approaches to Develop Low-Phytate Grains to Enhance Bioavailability of Micronutrients." *Advances in Genetics* 107: 89–120. <https://doi.org/10.1016/bs.adgen.2020.11.003>.
- Punjabi, M., N. Bharadvaja, M. Jolly, A. Dahuja, and A. Sachdev. 2018. "Development and Evaluation of Low Phytic Acid Soybean by siRNA Triggered Seed Specific Silencing of Inositol Polyphosphate 6-/3-/5-Kinase Gene." *Frontiers in Plant Science* 9: 804. <https://doi.org/10.3389/fpls.2018.00804>.
- Puwastien, P., T. E. Siong, J. Kantasubrata, G. Craven, R. R. Feliciano, and K. Judprasong, eds. 2011. *ASEAN Manual of Food Analysis*. Salaya, Thailand: Regional Centre of ASEAN Network of Food Data System, Institute of Nutrition, Mahidol University.
- Raboy, V. 2009. "Approaches and Challenges to Engineering Seed Phytate and Total Phosphorus." *Plant Science* 177: 281–296. <https://doi.org/10.1016/j.plantsci.2009.06.012>.
- Raboy, V. 2020. "Low Phytic Acid Crops: Observations Based on Four Decades of Research." *Plants* 9, no. 2: 140. <https://doi.org/10.3390/plant9020140>.
- Raboy, V., and D. B. Dickinson. 1987. "The Timing and Rate of Phytic Acid Accumulation in Developing Soybean Seeds." *Plant Physiology* 85: 0841–0844.

- Ren, S. C., Z. L. Liu, and P. Wang. 2012. "Proximate Composition and Flavonoids Content and In Vitro Antioxidant Activity of 10 Varieties of Legume Seeds Grown in China." *Journal of Medicinal Plant Research* 6, no. 2: 301–308. <https://doi.org/10.5897/JMPR11.1408>.
- Sangronis, E., and C. J. Machado. 2007. "Influence of Germination on the Nutritional Quality of *Phaseolus vulgaris* and *Cajanus cajan*." *LWT Food Science and Technology* 40: 116–120.
- Semba, R. D., R. Ramsing, N. Rahman, K. Kraemer, and M. W. Bloem. 2021. "Legumes as a Sustainable Source of Protein in Human Diets." *Global Food Security* 28: 100520. <https://doi.org/10.1016/j.gfs.2021.100520>.
- Shunmugam, A. S. K., C. Bock, G. C. Arganosa, F. Georges, G. R. Gray, and T. D. Warkentin. 2015. "Accumulation of Phosphorus-Containing Compounds in Developing Seeds of Low-Phytate Pea (*Pisum sativum* L.) Mutants." *Plants* 4: 1–26. <https://doi.org/10.3390/plants4010001>.
- Silva, V. M., E. H. M. Boleta, J. T. Martins, et al. 2019. "Agronomic Bio-Fortification of Cowpea With Selenium: Effects of Selenate and Selenite Applications on Selenium and Phytate Concentrations in Seeds." *Journal of the Science of Food and Agriculture* 99: 5969–5983. <https://doi.org/10.1002/jsfa.9872>.
- Silva, V. M., F. F. Putti, P. J. White, and A. R. dos Reis. 2021. "Phytic Acid Accumulation in Plants: Biosynthesis Pathway, Regulation and Role in Human Diet." *Plant Physiology and Biochemistry* 164: 132–146. <https://doi.org/10.1016/j.plaphy.2021.04.035>.
- Singh, N., P. Jain, M. Ujainwal, and S. Langyan. 2022. "Escalate Protein Plates From Legumes for Sustainable Human Nutrition." *Frontiers in Nutrition* 9: 977986. <https://doi.org/10.3389/fnut.2022.977986>.
- Soares, M. M., L. J. Silva, G. L. Oliveira, M. C. Sekita, and D. C. F. S. Dias. 2014. "Endogenous Level of Phosphorous in Soya Bean Seeds and the Relationship With Physiological Quality, Oil and Protein Content." *Seed Science and Technology* 42: 433–443. <https://doi.org/10.15258/sst.2014.42.3.09>.
- Soriano, D., A. Orozco-Segovia, J. Marquez-Guzman, K. Kitajima, A. Gamboa-de Buen, and P. Huante. 2011. "Seed Reserve Composition in 19 Tree Species of a Tropical Deciduous Forest in Mexico and Its Relationship to Seed Germination and Seedling Growth." *Annals of Botany* 107, no. 6: 939–951. <https://doi.org/10.1093/aob/mcr041>.
- Sparvoli, F., and E. Cominelli. 2015. "Seed Bio-Fortification and Phytic Acid Reduction: A Conflict of Interest for the Plant?" *Plants* 4: 728–755. <https://doi.org/10.3390/plants4040728>.
- Taahir, H., V. Jideani, and M. L. R. Hill. 2018. "Flavonoids and Tannin Composition of Bambara Groundnut (*Vigna subterranea*) of Mpumalanga, South Africa." *Heliyon* 4: e00833. <https://doi.org/10.1016/j.heliyon.2018.e00833>.
- Tan, X. L., S. Azam-Ali, E. V. Goh, et al. 2020. "Bambara Groundnut: An Underutilized Leguminous Crop for Global Food Security and Nutrition." *Frontiers in Nutrition* 7: 601496. <https://doi.org/10.3389/fnut.2020.601496>.
- Urbano, G., M. Lopez-Jurado, P. Aranda, C. Vidal-Valverde, E. Tenorio, and J. Porres. 2000. "The Role of Phytic Acid in Legumes: Anti-Nutrient or Beneficial Function?" *Journal of Physiology and Biochemistry* 56, no. 3: 283–294.
- Vaz Patto, M. M., R. Amarowicz, A. N. A. Aryee, et al. 2015. "Achievements and Challenges in Improving the Nutritional Quality of Food Legumes." *Critical Reviews in Plant Sciences* 34, no. 1–3: 105–143. <https://doi.org/10.1080/07352689.2014.897907>.
- Warsame, A. O., N. Michael, D. M. O'Sullivan, and P. Tosi. 2022. "Seed Development and Protein Accumulation Patterns in Faba Bean (*Vicia faba*, L.)." *Journal of Agricultural and Food Chemistry* 70: 9295–9304. <https://doi.org/10.1021/acs.jafc.2c02061>.
- Wink, M. 2010. *Biochemistry of Plant Secondary Metabolism*. 2nd ed. Chichester, United Kingdom: Wiley Publishers. <https://doi.org/10.1002/9781444320503.ch1>.
- Wortmann, C. S., and C. A. Eledu. 1999. *Uganda's Agro-Ecological Zones: A Guide for Planners and Policy Makers*. Kampala, Uganda: Centro Internacional de Agricultura Tropical (CIAT).
- Xu, Y., A. Cartier, D. Kibet, et al. 2016. "Physical and Nutritional Properties of Edamame Seeds as Influenced by Stage of Development." *Journal of Food Measurement and Characterization* 10, no. 2: 193–200. <https://doi.org/10.1007/s11694-015-9293-9>.
- Yao, D. N., K. N. Kouassi, D. Erba, F. Scazzina, N. Pellegrini, and M. C. Casiraghi. 2015. "Nutritive Evaluation of the Bambara Nut Ci12 Landrace [*Vigna subterranea* (L.) Verdc. (Fabaceae)] Produced in Côte d'Ivoire." *International Journal of Molecular Sciences* 16, no. 9: 21428–21441. <https://doi.org/10.3390/ijms160921428>.
- Zhao, M., H. Zhang, H. Yan, L. Qiu, and C. C. Baskin. 2018. "Mobilization and Role of Starch, Protein, and Fat Reserves during Seed Germination of Six Wild Grassland Species." *Frontiers in Plant Science* 9: 234. <https://doi.org/10.3389/fpls.2018.00234>.
- Zhao, P., L. Chu, K. Wang, et al. 2022. "Analyses on the Pigment Composition of Different Seed Coat Colors in Adzuki Bean." *Food Science and Nutrition* 10, no. 8: 2611–2619. <https://doi.org/10.1002/fsn3.2866>.
- Zhou, L., Y. Ye, Q. Zhao, et al. 2018. "Suppression of ROS Generation Mediated by Higher InsP3 Level Is Critical for the Delay of Seed Germination in *lpa* Rice." *Plant Growth Regulation* 85: 411–424. <https://doi.org/10.1007/s10725-018-0402-8>.