



Seed mineral reserves and vigour of Bambara groundnut (*Vigna subterranea* L.) landraces differing in seed coat colour



T. Mandizvo^{*}, A.O. Odindo

Crop Science, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Pietermaritzburg, South Africa

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ABSTRACT

A newly emerged seedling, given light and water, but no external source of minerals, uses its internal mineral nutrient reserves effectively for an early establishment. This research sought to investigate the influence of seed coat colour on the abundance of mineral elements in Bambara groundnut. Four landraces (G340A, Kazai, Kazuma, and Mana) varying in seed coat colour were analysed for differences in seed mineral composition using energy dispersive x-ray (EDX) analysis and atomic absorption spectrometry (AAS). Seeds were germinated at 10 °C and 25 °C, and various indices including, (1) mean emergence time, (2) mean germination rate, (3) coefficient of velocity of germination, and (4) final germination percentage (FGP) were calculated. The importance of seed mineral elements in the establishment of Bambara groundnut was examined by measuring root length (RL), shoot length (SL), shoot dry mass (SDM), and root dry mass (RDM). Plant tissue elemental analysis was done using flame atomic emission spectrometry (FAES) for K and flame atomic absorption spectrometry (FAAS) for Mg, Cu, Mn, and Zn. There were significant differences ($P < 0.001$) in mineral element content of dry seeds. G340A and Kazai had the highest and the lowest K, P, Mg, Mn, and Zn (11.65 gkg^{-1} , 7.2 gkg^{-1} , 2.33 gkg^{-1} , 59.56 mgkg^{-1} , and 44.42 mgkg^{-1}), and (8.82 gkg^{-1} , 4.75 gkg^{-1} , 1.38 gkg^{-1} , 48.9 mgkg^{-1} , and 42.6 mgkg^{-1}), respectively. Cold test germination indices were significantly different, the highest FGP was 73.3% in G340A and the lowest was 57.8% in Kazai. There were strong positive correlations between seed mineral concentration and plant growth parameters ($p < 0.001$). We concluded that (1) seed mineral concentration has a significant impact on the early establishment of Bambara groundnut and (2) the dark-coloured landraces (hue 8°) used in this study have the highest concentration of macro and micro elements compared to light coloured seeds (hue 38°).

1. Introduction

There is a logical connection between the mineral elements content of seeds and the seedling quality. Plants used the 13 essential mineral elements for growth and development. While some mineral elements became limiting in natural settings, high-quality seeds that contained optimum levels of mineral elements should produce vigorous seedlings. Seeds store sufficient metabolic reserves to allow the successful establishment of seedlings (Bewley et al., 2013). The reserves of organic compounds, such as carbohydrates, lipids and proteins, when combined with mineral nutrients, ensure that energy needed for germination and seedling growth is sufficient. Before newly emerged seedlings shifted from being heterotrophs to autotrophs, they depended on food and mineral reserves of their parent seeds.

Improvements in seedling quality would only occur when both morphological and physiological attributes are considered (Grossnickle,

2012; Mandizvo and Odindo, 2019). Puttonen (1989) provided a list of measurement techniques to evaluate seedling quality. del Campo et al. (2009) proposed a more comprehensive list of morphological (shoot and root) and physiological (nutrient status, freezing tolerance, carbohydrates reserves, drought tolerance, and dormancy) attributes, which if present in seeds within the proper range of values, would enhance seedling performance after planting. Mineral elements of seeds constituted a significant source of essential elements to seedlings and developing individual vascular plants. In spite of their potentials as vigour propellers, studies of seed nutrient pools were not common.

Although the seed content of mineral elements constitutes a small proportion of the total demand, they are vital for plant establishment and growth. Bolland and Paynter (1990) reported that yields of annual pasture legumes were positively related to P concentrations of their seeds and the early development of wheat seedlings was clearly favoured by a high seed-P concentration when plants were grown at P deficiency (Liao

^{*} Corresponding author.

E-mail address: takudzmandizvo@gmail.com (T. Mandizvo).

et al., 2008). Adequate concentrations of cobalt in blue-lupin (*Lupinus angustifolius*) seeds were sufficient to prevent chlorosis in *Lupinus angustifolius* seedling cultivated without cobalt application (Robson and Mead, 1980). Plant growth and grain production in wheat grown with deficient Mn and Zn were positively related to seed contents of these elements (Ozturk et al., 2006). Altogether, these reports indicated that in the absence of an external mineral elements supply, seed-borne mineral elements acted as an important reservoir of minerals to ensure maximal growth and development in young seedlings of several crop species.

The fulcrum of this study originated from the study and suggestions by Chibarabada et al. (2014) who concluded that dark-coloured seeds have higher seed quality than light coloured seeds in Bambara groundnut. This allegation was based on the presence of high concentration of phenolics in dark-coloured seeds which gave them a drought tolerance ability over light coloured seeds. Although Modi (2002) suggested the use of seed mineral content as a rapid physiological seed quality test in Bambara groundnut, to date, no work has been done to address the suggestion. In particular, the attractive strategy of improving seed quality by developing lines with high micro and macro-element densities has not gained much attention. Among legume species, seed mineral elements' concentrations are variable, thus suggesting that the capacity for increasing nutrient concentration in legume seeds is greater (Garcia and Grusak, 2015).

Studies of possible tissue-to-tissue and cell to cell variations in mineral storage are important both for an understanding of how Bambara seeds developed initially and for an understanding of the events occurring during germination and seedling growth. The objectives of this research are to study: (1) the seed mineral content of Bambara groundnut; (2) influence of the seed coat colour on the abundance of mineral elements as well as; (3) the relationship between seed mineral concentrations and early seedling growth. Energy dispersive x-ray (EDX) analysis and atomic absorption spectrometry (AAS) were chosen as the investigative probe for our studies of mineral elements in Bambara groundnut landraces. EDX analysis was used for elemental mapping in Bambara groundnut seed regions and AAS was used to measure the elemental composition of Ca, Mg, Cu, Fe, Zn, Mn, and K remaining and accumulated in the cotyledon and shoot respectively at 14 and 21 days after sowing in nutrient-impooverished sand soil.

2. Materials and methods

2.1. Seed multiplication

Bambara groundnut landraces (G340A, Kazai, Kazuma, and Mana) with significant differences in seed coat lightness (Table 1) were sourced from the Department of Research and Specialist Services (DR&SS) (Harare, Zimbabwe). Seeds were multiplied in the field at Ukulinga research farm (29°40'1.65"S, 30°24'28.61"E, Pietermaritzburg) during the rainy season (November–April, 2017/2018). Planting was done on clay-loam soils in a completely randomized block design replicated three times. Blocking was done against moisture gradient; field was on a slope. Each plot measured 2.5 × 2 m, with in-row and inter-row spacing of 0.3 × 0.45 cm, respectively. Weeding and earthing were done by hand-hoe, irrigation was done by overhead sprinklers during periods of dry spells. Harvesting was done by hand-pulling, the moisture content of seeds was monitored at 12.2 ± 0.3% using the Intelligent Grain Moisture meter

Table 1

Hue, saturation and lightness for Bambara groundnut seed coat measured with a stereomicroscope.

Bambara landraces	Hue (°)	Saturation (%)	Lightness (%)
Mana	8	81.47	14.77
Kazai	38	61.83	33.90
Kazuma	11	68.43	18.60
G340A	8	83.53	14.33

(KM-21G). Shelled seeds were stored in evacuated glass jars at 25 °C until mineral nutrient analysis and replanting.

2.2. Measure of macro and micro-elements

Measures of macro- and microelements in mature dry Bambara seeds were done using two methods: energy dispersive x-ray (EDX) analysis and atomic absorption spectrometry (AAS).

2.3. Energy dispersive x-ray analysis (EDX)

Three seeds of each selection were cryo-fractured in liquid nitrogen and split into halves using a scalpel blade. Seeds were mounted on stubs and secured using a double-sided insulating carbon tape (Fig. 1). Seed mineral composition was determined separately for the embryo, cotyledon, and seed coat regions.

2.4. Atomic absorption spectrometry

The chemical analysis involved the determination of P, K⁺, Ca²⁺, Mg²⁺, Zn²⁺, Cu²⁺, Fe²⁺, and Mn²⁺ in Bambara groundnut landraces. The contents of the elements were determined in the seed materials (2 g of Bambara seeds) after incineration in a muffle furnace at 650 °C. The resultant ash was solubilized on porcelain crucibles using 10 ml aqua regia (HNO₃ and HCl mixed in ratio 1:3) (Baldock and Schulte, 1996). Potassium was analyzed using flame atomic emission spectroscopy (FAES) with fast sequential absorption spectrometer (Varian AA280FS). Ca, Mg, Zn, Cu, Fe, and Mn concentrations were determined using flame atomic absorption spectroscopy (FAAS). The wavelength, recovery level and relative standard deviations (RSD) at which elements were analyzed are summarized in (Table 2). The phosphorus content was determined with a spectrometric method at 400 nm using a Varian Alpha UV-VIS (Spectronic Unicam, Berlin, Germany) (Sharifuddin et al., 2008).

2.5. Evaluation of seed and seedling vigour

2.5.1. Cold test

The factor cold test was a two-factorial experiment replicated three times to give 24 experimental units. Factor (1) was Bambara groundnut landraces (Mana, Kazai, Kazuma and G340A) and factor (2) temperatures at which seeds were exposed for the first seven days (10 °C and 25 °C). The cold test was done using the shoebox method according to the International Seed Testing Association (ISTA, 2015) with minor modifications. Germination plastic boxes measuring 17 cm × 11 cm × 10 cm (length × width × height) were used in this study. In each germination box, the base consisted of 4 cm nutrient impoverished river sand with 60% saturation moisture. Three replications of 15 seeds were placed on base sand that has been moistened and chilled in a cold room (Supra 750, Germany) at 10 °C for 24 hours. The seeds were covered with 5 cm sand soil and returned to 10 °C for seven days without light. The procedure was repeated but without chilling the seeds at 10 °C for seven days, instead, the seeds were left at room temperature (25 °C) for 7 days. The boxes were then moved to a growth chamber (Micro-Clima Arabidopsis Chamber, ECP01E, Snijders, Netherlands) for 7 days. Growth chamber conditions were set at 25 ± 1 °C, 75% relative humidity, illumination of 4000 lux for 12 hours and 350 ppm CO₂. Set values were controlled by the control unit (JUMO IMAGO 500).

The number of seeds germinated per day was recorded and the mean emergence time (MET), the mean germination rate (MGR), the coefficient of velocity of germination (CVG), and the final germination percentage (FGP) were calculated as follow: Matthews and Hosseini (2006)

$$MET = \frac{\sum (fx)}{\sum f} \quad (1)$$

Where: MET = mean emergence time, f = number of newly germinated



Fig. 1. Fixed and embedded seed of Bambara groundnut landraces mounted on stubs (1) Mana, (2) G340A, (3) Kazuma, and (4) Kazai. EDX analysis for the three seed regions of each Bambara landrace was done using a Zeiss EVO Ls15 scanning electron microscope (SEM) connected to a spectrometer (EDAX model 808A) detector, and an INCA microanalysis suit (ETAS Group). All analysis work took place at a speeding up voltage of 5 kV and working distance (WD) of 7.5 mm. Each analysis lasted 60 seconds in duration. To lessen equipment-caused variation, the EDX electronics were turned on 15 minutes before use, the filament of the SEM was kept aligned and the count rate was adjusted to 1200 counts/sec. Using the INCA window system, integrated counts under the Ca, K, Mg, P, Mn, Zn, Fe, and Cu peaks were determined. After peak values were determined the spectra were then automatically put through by INCA program.

Table 2

Wavelength, recovery level, and relative standard deviations used for elemental analysis in Bambara groundnut landraces.

Element	Wavelength (λ)	Recovery level (%)	RSD (%)
K	589.0 nm	97.7	6.5
Ca	422.7 nm	98.8	7.9
Mg	285.2 nm	99.5	5.1
Zn	213.9 nm	99.2	6.2
Cu	324.8 nm	96.1	5.8
Fe	248.3 nm	97.3	7.4
Mn	279.5 nm	98.6	7.7

seeds at a given time (day), and x = number of days from the date of sowing; Thomson and El-Kassaby (1993)

$$MGR = \frac{G1}{1} + \frac{G2}{2} + \dots + \frac{Gx}{x} \quad (2)$$

Where G1 = Germination percentage on the first day after sowing, G2 = Germination percentage on the second day after sowing; Mozumder and Hossain (2013)

$$CVG = 100 \times \frac{\sum Ni}{\sum NiTi} \quad (3)$$

Where Ni = Number of germinated seeds per day, Ti = Number of days from the start of the experiment; and

$$FGP = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds evaluated}} \times 100 \quad (4)$$

2.6. Measurement of germination axis

2.6.1. Root and shoot length

Measurement of the germinant axis was a two-factorial experiment replicated three times to give 24 experimental units. Factor (1) Bambara groundnut landraces (Mana, Kazai, Kazuma and G340A), and factor (2) days at which seedlings axis were measured (14 and 21 days after sowing). Seedlings that emerged through sand soil were uprooted and washed to remove sand particles. Measurements were done for root length and shoot length by using a computer-aided analysis of digital images. Digital images were acquired at a scanning resolution of 250 dpi using a flat-bed scanner (Hewlett Packard Scanjet 4c/t; Palo Alto, Calif.). Root and shoot length were quantified using Image software analysis.

2.6.2. Fresh and dry mass

Plants were uprooted and washed to remove loose soil particles.

Paper towel was used to blot free surface moisture. Shoots, roots, and cotyledons were separated to measure fresh weight by cutting at the soil line using a scalpel blade (Fig. 2). An electronic balance (Presica 105A) was used to measure fresh weights separately.

Weighed fresh samples were heated in an air oven at 70 °C for 24 hours and then dried to a constant weight at 30 °C. Plant dry weight was equal to shoot dry weight plus root dry weight. The root-shoot ratio was calculated according to Agren and Ingestad (1987) (5).

$$\text{Root shoot ratio} = \frac{\text{Dry weight for roots}}{\text{Dry weight for top of plant}} \quad (5)$$

2.7. Plant tissue elemental analysis

2.7.1. Sample preparation

Sequentially harvested plant material (cotyledons and shoots) at 14 and 21 days after sowing were incubated at 70 °C to dryness for 24 hours. Dried samples were ground into a fine powder using a stainless-steel Wiley mill grinder. Ground samples (0.2 g) were put in quartz crucibles and ashed with Wild Barfield muffle furnace at 650 °C for 3 hours. Acid digestion was done according to the method of Huang et al. (2004) with minor modifications. The ash was dissolved in a mixture of 10 ml dilute aqua regia (HNO₃ and HCl mixed in ratio 1:3) in a 25 ml volumetric flask. Contents of the flask were brought to the volume by adding distilled H₂O and heated in a water bath for 1 hour at 85 °C. Samples were cooled to room temperature before analysis by atomic absorption spectrometry.

2.7.2. Atomic absorption spectrometry

Elemental analysis of Ca, Cu, Fe, K, Mg, Mn, and Zn was done using Fast Sequential Absorption Spectrometer (Varian AA280FS) hyphenated to a computer software (Spectr AA version 5.1 PRO). The AAS was calibrated using an ICP multi-element standard solution IV prepared within the normal operating range (0–100 ppm) of the flame absorbance and emission spectrophotometer (Hansen et al., 2009). The flame of the photometer was calibrated by adjusting the air flow (12 L/min) and gas flow (8 L/min). The flame hollow cathode lamp was allowed to stabilize for 5 minutes and the lamp was optimized to produce stable radiation. The readings of the galvanometer were adjusted to zero by spraying blank 5% HNO₃ into the flame. The sensitivity was adjusted by spraying the standard working solutions into the flame, to get a full-scale deflection of the galvanometer. Sample solutions were aspirated into the flame for three times and the readings of galvanometer were recorded. The concentration of elements in each sample was calculated from the graph of concentration against absorbance. The final concentration was calculated using the mass of the sample and the volume of the sample solution as shown in the equation: Kurilenko and Kostyreva (2016)

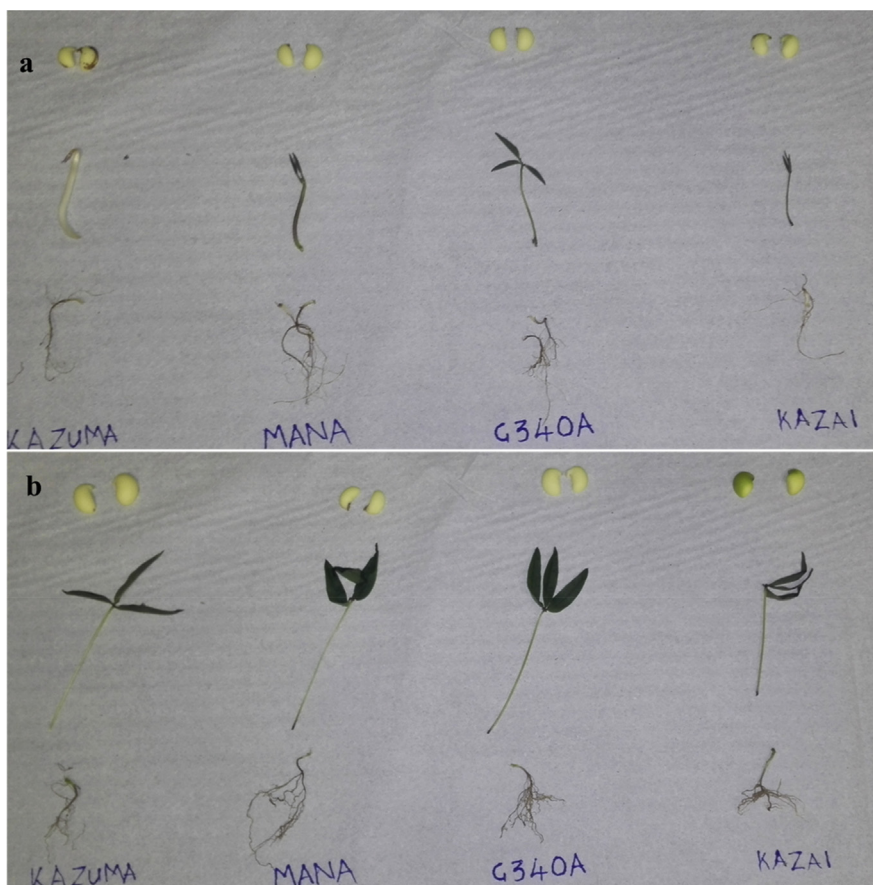


Fig. 2. Separate measurement of germinant axis cotyledons, shoots and roots at (a) 14 days after sowing and (b) 21 days after sowing.

$$\text{Final conc (ml/g)} = \text{Average} \times \frac{\text{Volume (25 ml)}}{\text{mass (0.2 g)}}$$

2.8. Statistical analysis

The experiments were executed in complete randomized design (CRD). Data collected were subjected to analysis of variance (ANOVA) using GenStat 18th edition (VSN International, United Kingdom) at the 5% level of significance. Means of significantly different variables were separated using Duncan's test in GenStat at the 5% level of significance.

3. Results

3.1. Energy dispersive x-ray (EDX) analysis

The EDX analysis in Bambara groundnut landraces revealed an elemental composition consistent with the mineral phases identified in

the SEM. The major elements identified were generally consistent.

3.2. Macro-elements

Seed mineral composition of P, K, and Mg did not differ significantly ($P > 0.05$) among Bambara groundnut landraces (Table 3). The mean percentage composition of P, K and Mg in the seed coat region of the landraces were lower than that of the embryo region composition (Table 3). There were differences between P, K, and Mg concentrations in either landraces. The mean composition of P, K, and Mg were higher in embryo and cotyledon region than in seed coat region.

3.3. Micro-elements

There were significant differences ($P < 0.05$) among landraces with respect to calcium. The highest calcium content (2.03%) was in the seed coat region of G340A, while the lowest (0.46%) was recorded in Kazai's

Table 3
Seed mineral nutrient (macro-nutrients) composition of studied Bambara groundnut landraces measured using EDX analysis.

Bambara landraces	Phosphorus (%)			Potassium (%)			Magnesium (%)		
	P ₁	P ₂	P ₃	K ₁	K ₂	K ₃	Mg ₁	Mg ₂	Mg ₃
Kazai	53.100	13.240	2.300	28.780	7.460	0.840	16.120	4.030	1.070
Mana	52.730	14.320	2.290	29.380	6.760	0.850	15.730	3.930	1.050
Kazuma	53.030	13.260	2.297	29.220	6.510	0.870	15.700	3.930	1.050
G340A	53.130	13.500	2.300	29.110	6.920	1.120	16.350	4.090	1.090
Mean	53.000	13.58	2.296	29.120	6.910	0.920	15.98	3.996	1.064
P (0.05)	0.920	0.891	0.958	0.930	0.681	0.209	0.421	0.412	0.399
l.s.d	1.486	3.653	0.065	2.143	1.810	0.322	1.006	0.250	0.065
cv %	1.500	14.300	1.500	3.900	13.900	18.600	3.300	3.300	3.300

P1-Phosphorus in embryo region; P2-Phosphorus in cotyledon region; P3-Phosphorus in seed coat region K1-Potassium in embryo region; K2-Potassium in cotyledon region; K3-Potassium in seed coat region; Mg1-Magnesium in embryo region; Mg2-Magnesium in cotyledon region; Mg3-Magnesium in seed coat region.

embryonic region (Table 4). With respect to zinc, G340A recorded the highest percentage in embryo region (1.53%), while Kazai had the lowest percent in the seed coat region (0.09%). For copper, G340A recorded the highest percent (0.37%) in the embryonic region, while Kazuma recorded the least percent (0.02%) in the seed coat region. The percentage for manganese showed significant differences, with G340A recording highest in embryo region (0.29%), while Kazai had the least recording in the seed coat region (0.01%).

3.4. Seed macro- and microelement composition, atomic absorption spectrometry

The content of macro- and microelements, except for sodium, were significantly different ($P < 0.001$) (Table 5). All the landraces were characterized by high potassium (8.82–11.65 g kg⁻¹ DM) and low sodium content (0.16–0.17 g kg⁻¹ DM). The highest level of potassium was detected in G340A and Mana (11.65 and 10.49 g kg⁻¹ DM), whereas the lowest content of this element was recorded in Kazai (8.82 g kg⁻¹ DM) ($P < 0.05$). The greatest amount of phosphorus in 1 kg (DM basis) was found in G340A (7.2 g) and Mana (6.26 g), whereas its lowest quantity (4.75 g) was detected in Kazai ($P < 0.05$).

The highest content of calcium (2.83 g kg⁻¹) was noted in Mana and the smallest quantity was noted in Kazai (1.86 g kg⁻¹ DM) ($P < 0.05$). There were no significant differences in seed mass of Bambara groundnut landraces ($P = 0.184$) (Table 5).

Evaluated landraces accumulated significant amounts of manganese. The greatest levels of this element were noted in Mana (60.45 mg kg⁻¹ DM), compared with 48.9 mg kg⁻¹ DM in Kazai ($P < 0.05$) (Table 5). The highest iron content was noted in Kazai (52.2 mg kg⁻¹) and Mana recorded the lowest content (37.16 mg kg⁻¹). Among the analyzed landraces, G340A and Kazuma contained the highest levels of zinc, while the lowest content of this element was noted in Mana ($P = 0.001$). The concentration of copper ranged from 6.32 to 9.1 mg kg⁻¹ DM, with the lowest values noted in Kazai and the highest in G340A, respectively.

3.5. Seed vigour

3.5.1. Germination indices

Seed germination parameters recorded 14 days after sowing are presented in (Table 6). Temperature significantly affected the germination of Bambara groundnut. At 25 °C, FGP ranged from 82.2% to 93.3% compared with 60%–73.3% at 10 °C. There was a significant difference in the mean emergence time (MET), with the landrace G340A having the lowest MET, (9.74 days and 10.34 days at 25 °C and 10 °C respectively).

Table 4

Seed mineral nutrient (micro-nutrients) composition of studied Bambara groundnut landraces measured using EDX analysis.

Region	Bambara landrace	Ca (%)	Zn (%)	Cu (%)	Mn (%)
Embryo	G340A	1.010 ^a	1.527 ^a	0.373 ^a	0.290 ^a
	Kazai	0.457 ^d	0.247 ^d	0.103 ^d	0.063 ^d
	Kazuma	0.743 ^c	0.457 ^c	0.303 ^c	0.103 ^c
	Mana	0.823 ^b	0.803 ^b	0.343 ^b	0.167 ^b
	L.s.d ($P=0.05$)	0.169	0.169	0.017	0.041
Cotyledon	G340A	1.060 ^a	0.810 ^a	0.19 ^b	0.077 ^a
	Kazai	0.507 ^c	0.130 ^d	0.09 ^d	0.030 ^c
	Kazuma	0.793 ^b	0.240 ^c	0.1233 ^c	0.050 ^{ab}
	Mana	0.873 ^b	0.427 ^b	0.2167 ^a	0.053 ^a
	L.s.d ($P=0.05$)	0.085	0.091	0.012	0.024
Seed Coat	G340A	2.027 ^a	0.603 ^a	0.170 ^a	0.053 ^a
	Kazai	0.747 ^d	0.093 ^d	0.100 ^c	0.010 ^c
	Kazuma	0.957 ^c	0.183 ^c	0.017 ^d	0.040 ^b
	Mana	1.303 ^b	0.320 ^b	0.157 ^b	0.037 ^b
	L.s.d ($P=0.05$)	0.085	0.064	0.018	0.028

Note: Means were separated using Duncan's test. Means with same letters within a column are not significantly different and means with different letters within a column are significantly different.

The coefficient of velocity of germination (CVG) and mean germination rate (MGR) showed significant differences among landraces and temperature treatments ($P = 0.001$).

3.6. Plant growth parameters

Root length (RL), shoot length (SL), root dry mass (RDM) and shoot dry mass (SDM) were all significantly different ($P < 0.01$) among landraces at both 14 and 21 days after sowing (DAS) Fig. 3(a) - (d), respectively. At 14 DAS, G340A and Kazai recorded the highest and lowest RL (52.3 mm) and (29 mm), respectively [Fig. 3 (a)]. G340A had the highest SL of 34 mm and 38.67 mm at 14 and 21 DAS respectively. The lowest SL was observed on Kazai at both sampling intervals [Fig. 3 (b)]. The highest (0.50 mg plant⁻¹) and the lowest (0.22 mg plant⁻¹) root biomass at 14 DAS was recorded in G340A and Kazai, respectively. Values of RDM increased significantly at 21 DAS with Kazai recording the highest biomass of 1.27 mg plant⁻¹ and G340A the lowest of 0.76 mg plant⁻¹ Fig. 3(c). The shoot dry mass significantly differed among treatments and ranged from 0.32 to 0.6 mg plant⁻¹ and from 2.57 to 4.01 mg plant⁻¹ after 14 and 21 days, respectively. Kazai recorded the lowest SDM while G340A showed the highest one [Fig. 3 (d)].

3.7. Nutrient content of seeds, growth parameters and seed coat colour correlation

There was a strong and positive correlation of Fe and Zn with seed coat lightness ($r^2 = 0.92$) ($P < 0.001$) and Zn ($r^2 = 0.70$) ($P < 0.001$) respectively. Potassium, P, Mg and Mn were positively correlated with shoot length ($r^2 = 0.95$, $r^2 = 0.97$, $r^2 = 0.98$ and $r^2 = 0.92$), shoot dry mass ($r^2 = 0.93$, $r^2 = 0.95$, $r^2 = 0.95$ and $r^2 = 0.82$) and root dry mass ($r^2 = 0.92$, $r^2 = 0.94$, $r^2 = 0.95$ and $r^2 = 0.81$). Calcium and Zn were positively correlated to seedling growth parameters with correlation values ranging from $r^2 = 0.33$ to $r^2 = 0.72$ ($P > 0.005$) (Table 7).

A strong negative correlation was observed between the seed coat lightness and the Ca content ($r^2 = -0.56$). Potassium, P, Mg, and Mn were negatively correlated with Fe; only Ca was positively correlated to Fe ($r^2 = 0.83$). Iron and growth parameters (SL, RL, and SDM) were negatively correlated (weak negative correlations) $r^2 = -0.26$, $r^2 = -0.04$, and $r^2 = -0.14$ respectively (Table 7).

3.8. Plant tissue elemental analysis

The analyses showed that the K, Ca, Mg, Mn, and Zn concentrations in shoots of different landraces at a particular date were significantly different ($P < 0.005$). For example, on 14 DAS the potassium and calcium concentrations in G340A were 7249 mg/L and 7389 mg/L, respectively; while in Kazai the concentrations were 5286 mg/L and 4742 mg/L, respectively. Similar variations were encountered in other mineral elements (Table 8), undoubtedly reflecting differences both in landraces' capacity to accumulate the mineral elements and in dry matter accumulation [Fig. 3(d)]. The mean concentrations for mineral elements in the cotyledons obtained on the first sampling date (14 DAS), compared to second sampling date (21 DAS) show that in general, the mineral element concentration in cotyledons reserves declined with time (Table 9).

4. Discussion

4.1. Energy dispersive x-ray (EDX) analysis

Results for EDX are semi-qualitative estimates based on standard analysis and theoretical intensity corrections (Fantoni et al., 2008). From Table 3 it is evident that P, K, and Mg are present in all the regions examined with the highest proportion in the embryonic region. In legume seeds, it has been observed that phytin globoids are included in protein bodies of embryonic cells and that P, Mg, and K are accumulated in the globoids. Elemental mapping analysis in this study supported the

Table 5

Average mass and mineral element content in dry Bambara groundnut landrace seeds estimated from bulk samples by AAS method.

Bambara landraces	Seed mass (g) (n = 25)	Macroelements g kg ⁻¹ DM					Microelements mg kg ⁻¹ DM				
		K	Na	P	Ca	Mg	Mn	Fe	Zn	Cu	
G340A	21.380	11.650 ^a	0.166	7.200 ^a	2.590 ^c	2.230 ^a	59.560 ^b	49.670 ^b	44.420 ^a	9.100 ^a	
Kazai	21.730	8.820 ^d	0.158	4.750 ^d	1.860 ^d	1.380 ^c	48.900 ^d	52.200 ^a	42.600 ^c	6.320 ^d	
Kazuma	21.310	9.400 ^c	0.166	5.430 ^c	2.810 ^b	1.950 ^b	54.880 ^c	39.660 ^c	43.490 ^b	7.260 ^c	
Mana	21.740	10.490 ^b	0.170	6.260 ^b	2.830 ^a	2.030 ^a	60.450 ^a	37.160 ^d	40.960 ^d	8.440 ^b	
P value	0.184	<.001	0.988	<.001	<.001	<.001	<.001	<.001	<.001	<.001	
s.e.d	0.223	0.178	0.008	0.187	0.069	0.089	0.124	0.535	0.093	0.073	
l.s.d	0.513	0.410	0.012	0.431	0.161	0.206	0.287	1.233	0.215	0.167	
cv %	1.300	2.200	8.400	3.900	3.400	5.800	0.300	1.500	0.700	1.100	

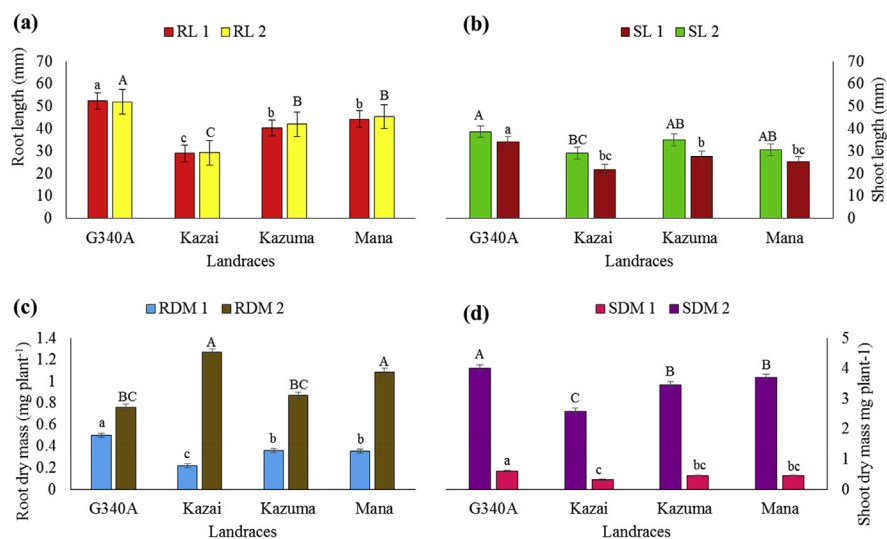
Values are means of triplicate analyses, means followed by different letters within a column are statistically different (P<0.05).

Table 6

Germination indices of seed from 4 Bambara groundnut landraces under different temperatures.

Bambara landraces	Germination indices							
	MET (day)		CVG		MGR		FGP	
	10 °C	25 °C	10 °C	25 °C	10 °C	25 °C	10 °C	25 °C
G340A	10.340 ^a	9.740 ^a	13.760 ^b	13.340 ^a	0.050 ^a	0.046 ^b	73.300 ^a	93.300 ^a
Kazai	12.650 ^b	11.940 ^d	10.610 ^a	14.380 ^{ab}	0.076 ^b	0.030 ^a	57.800 ^{bc}	82.200 ^{bc}
Kazuma	12.680 ^b	11.310 ^c	10.270 ^a	17.550 ^c	0.078 ^b	0.020 ^a	60.000 ^b	88.900 ^{ab}
Mana	10.580 ^a	8.810 ^b	14.520 ^c	12.660 ^a	0.047 ^a	0.071 ^c	68.900 ^b	86.700 ^b
l.s.d	0.262	0.371	1.891	1.337	0.021	0.015	8.330	5.890

Note: Values followed by different letters within a column differ significantly (LSD, P= 0.005) MET: Mean Emergence Time, CVG: Coefficient of Velocity of Germination, MGR: Mean Germination Rate, FGP: Final Germination Percentage.

**Fig. 3.** Growth response of Bambara landraces to the nutrient deficient soil at 14 and 21 days after sowing. (a) Root length (RL); (b) Shoot length (SL); (c) Root dry mass (RDM) and (d) Shoot dry mass (SDM). RL 1 (root length at 14 DAS), RL 2 (root length at 21 DAS).

observations by Blair et al. (2009) who indicated that P was abundant element in phytic acid, and K and Mg were conceived to form complexes with phytic acid. Vadivel and Janardhanan (2001) have recognized that K is located in the pericarp of wild jack bean. We also found that K occurred in the seed coat, in contrast to wild jack bean seeds. Since pericarp and seed coat are composed of cell wall layers, we suggested that K is associated with the carboxyl group of pectin molecules in the cell wall of the seed coat.

Calcium was scarcely found in embryonic tissues of Bambara seeds. Similar observations were previously reported (Kyriacou et al., 2014; Lombi et al., 2011; Lu et al., 2013). In this study, we found that the percentage of Ca was greater in the seed coat than in the embryonic and cotyledon regions. From the data reported here, it is likely that Ca is associated with the carboxyl groups of pectin molecules in cell wall to

form Ca-pectin complexes. While there is a lot of Zn, Cu, and Mn inside the embryonic region, these cations are widespread in Bambara cotyledon tissue and could be detected in seed coats. The correlations of Zn, Cu and Mn percentage with that P in the seed embryonic region suggested their association with phytate. No specific pattern for the calcium distribution was found. Well-defined elemental distribution occurred with Zn, Cu, and Mn. Manganese, Zn, and Cu were found in embryo region and seed coat at highest and lowest concentrations respectively. Iwai et al. (2012) reported that, there are some specific distribution patterns of minerals in seeds dependent upon cell type. However, this report was based on single spot analysis which involves analyzing areas of the size of the electron beam. In the aspect of mineral nutrition, we need the information of the overall distribution of mineral nutrients in whole Bambara groundnut seeds.

Table 7

Correlation table for plant growth parameters of Bambara groundnut landraces and mineral element content.

	Hue	K	P	Ca	Mg	Mn	Fe	Zn	Cu	SL	RL	SDM	RDM
Hue	1												
K	0.32 ^{NS}	1											
P	0.27 ^{NS}	0.99 ^{***}	1										
Ca	-0.56 ^{NS}	0.51 ^{NS}	0.57 [*]	1									
Mg	-0.05 ^{NS}	0.87 ^{***}	0.91 ^{***}	0.85 ^{**}	1								
Mn	-0.2 ^{NS}	0.86 ^{**}	0.89 ^{**}	0.83 ^{**}	0.93 ^{***}	1							
Fe	0.92 ^{***}	-0.05 ^{NS}	-0.11 ^{NS}	0.83 ^{NS}	-0.43 ^{NS}	-0.54 ^{NS}	1						
Zn	0.7 ^{**}	0.28 ^{NS}	0.29 ^{NS}	0.06 ^{NS}	0.27 ^{NS}	-0.08 ^{NS}	0.52 ^{NS}	1					
Cu	0.12 ^{NS}	0.98 ^{***}	0.98 ^{***}	0.66 [*]	0.93 ^{***}	0.95 ^{***}	0.25 ^{NS}	0.16 ^{NS}	1				
SL	0.14 ^{NS}	0.95 ^{***}	0.97 ^{***}	0.72 ^{**}	0.98 ^{***}	0.92 ^{**}	-0.26 ^{NS}	0.33 ^{NS}	0.97 ^{***}	1			
RL	0.42 ^{NS}	0.85 ^{**}	0.87 ^{**}	0.52 [*]	0.86 ^{**}	0.66 [*]	-0.04 ^{NS}	0.69 ^{**}	0.81 ^{***}	0.91 ^{***}	1		
SDM	0.26 ^{NS}	0.93 ^{***}	0.95 ^{***}	0.65 [*]	0.95 ^{***}	0.82 ^{**}	-0.14 ^{NS}	0.51 [*]	0.92 ^{***}	0.98 ^{***}	0.97 ^{***}	1	
RDM	0.28 ^{NS}	0.92 ^{***}	0.94 ^{***}	0.64 [*]	0.95 ^{***}	0.81 ^{**}	= 0.12 ^{NS}	0.53 [*]	0.91 ^{***}	0.98 ^{***}	0.98 ^{***}	0.99 ^{***}	1

*SL-shoot length; RL-root length; SDM-shoot dry mass; RDM-root dry mass.

Table 8

Average concentrations for mineral elements accumulated in shoots of Bambara groundnut landraces at 14 and 21 days after sowing (DAS) in the nutrient deficient soil.

Bambara landraces	Shoot mineral element concentration													
	K (mg/L)		Ca (mg/L)		Mg (mg/L)		Cu (mg/L)		Fe (mg/L)		Mn (mg/L)		Zn (mg/L)	
	14	21	14	21	14	21	14	21	14	21	14	21	14	21
G340A	7249	8325	7389	7577	406.600	940.600	9.900	16.500	1.063	1.537	0.147	0.378	29.860	36.540
Kazai	5286	6871	4742	6237	315.200	427.200	5.060	11.02	0.673	1.013	0.098	0.102	24.120	28.440
Kazuma	5861	6393	6616	6727	387.200	720.300	6.240	7.650	0.952	1.578	0.236	0.280	26.940	36.080
Mana	6444	7521	6832	7125	375.900	682.400	8.160	11.150	1.5167	1.738	0.272	0.308	28.910	36.280
P-value	<.001	<.001	<.001	<.001	0.001	<.001	0.074	<0.001	<.001	0.024	<.001	<.001	<.001	<.001
s.e.d	95.900	40.800	163	81.600	14.770	24.550	1.634	0.898	0.003	0.190	0.016	0.02	0.004	0.875
I.s.d	221.200	94.100	376	188.300	34.060	56.610	3.767	2.072	0.008	0.438	0.037	0.046	0.009	2.018

Note* s.e.d = standard error of difference, I.s.d = least significant difference.

Table 9

Mean concentrations for mineral elements extracted from cotyledons of Bambara groundnut landraces at 14 and 21 days after sowing in the nutrient deficient soil.

Bambara landraces	Cotyledon mineral element concentration													
	K (mg/L)		Ca (mg/L)		Mg (mg/L)		Cu (mg/L)		Fe (mg/L)		Mn (mg/L)		Zn (mg/L)	
	14	21	14	21	14	21	14	21	14	21	14	21	14	21
G340A	7976	3210	5738	2885	420	783	12.950	6.300	0.814	0.852	0.089	0.102	20.760	15.580
Kazai	7100	4711	4529	3567	416	476	5.990	5.430	0.466	0.789	0.045	0.054	19.030	16.270
Kazuma	7761	4258	4824	1988	246	542	6.620	2.330	0.590	0.416	0.051	0.029	21.070	19.100
Mana	7401	4167	4424	2017	504	637	7.730	5.740	0.496	0.501	0.065	0.037	20.970	18.700
P-value	<.001	<.001	<.001	<.001	<.001	0.056	<.001	<.001	0.126	<.001	<.001	0.035	0.364	0.253
s.e.d	46.000	62.800	40.800	40.900	29.600	37.100	0.289	0.345	0.138	0.053	0.003	0.021	1.227	1.924
I.s.d	106.100	144.800	94.100	94.300	68.300	85.600	0.667	0.796	0.319	0.121	0.007	0.049	2.830	4.436

Note* s.e.d = standard error of difference, I.s.d = least significant difference.

4.2. Seed vigour

The percent germination was maximal in G340A at 25 °C but was affected by cold temperature. Emergence occurred earlier at 25 °C, but was delayed as temperature decreased. The MET values were low at 25 °C and high at 10 °C, and might be explained by the slowing down of metabolic reactions due to cold stress, causing metabolic disequilibrium due to different Q10 of enzymatic reactions that occurred in the seed during germination. Enzyme activities and their metabolic pools in plants favouring warm conditions are particularly sensitive to cold (Gill and Tuteja, 2010; Rivero et al., 2001). Cold temperature caused a clear reduction in FGP. Temperature below the optimum results in progressively poor germination as a result of cold stress.

Cold stress occurs only in tropical and subtropical plants that are adapted to a temperature range of 15 °C–50 °C (Allen and Ort, 2001). In this study, the actual amount of cold stress on Bambara groundnut was a product of intensity (10 °C) and duration (7 days) which caused metabolic imbalances to germinating seeds. When cells that are not cold resistant are cooled, the membrane lipid change from fluid aggregation

state to crystalline inflexible state (Vigh et al., 2005). This is accompanied by thickening of membranes and inactivation of integral membrane proteins (thermotrophic phase transition). Ion pumps became inactivated by cold, resulting in the progressive breakdown of compartmentalization within cells (Korner, 2016; Rihan et al., 2017; Zhu, 2016). This applies to proton gradients required for the supply of ATP to regenerating cells of the seed.

In studies with clover and alfalfa, germination response varied greatly among species at temperatures lower than 10 °C (Zaka et al., 2017). Successful germination at low temperature is closely associated with (1) genotype (Sincik et al., 2004) (2), fatty acids profile of seeds (Thomashow, 1998) and (3) membrane lipid composition (Liu et al., 2013; Uemura et al., 2006). Based on our findings, landraces with dark seed coat colour exhibited a higher degree of tolerance to cold stress by recording high values of final germination. This can be related to macro and microelement composition in landraces evaluated. Ozcan et al. (2013) reported that legume seeds with high organometallic elements exhibited significant phytochemical compounds and antioxidant activities. The same reports were published in experiments with *Pisum sativum*

(Troszyńska and Ciska, 2011) and *Phaseolus vulgaris* (And and Shahidi, 2005). The identity of plant cell sensors to cold remain unknown (Chinnusamy et al., 2006; Xiong et al., 2002). Multiple primary sensors are involved, with each perceiving a specific aspect of stress. The proportion of calcium in seeds is important in determining the cold hardness of seeds. The membrane protection function of Ca is more pronounced under conditions of low-temperature stress. Calcium participates in intracellular signaling system by acting as a diffusible second messenger to the initial stimuli. Increased calcium concentration leads to calcium binding by regulatory proteins, which turn calcium signal into a biological response.

4.3. Mineral content of seeds and seedling vigour

In seeds, phytates are synthesized to store phosphates and cations which are released when the phytates are broken down during germination (Brinch-Pedersen et al., 2002). The present study revealed that seeds might be a major source of nutrients for satisfying seedling growth requirements. This was pronounced under nutrient-impooverished soil conditions, plant dry mass, root, and shoot elongation were sensitive to seed mineral element composition. They were strong positive correlations between seed-borne K, P, Mg, Mn and Zn with shoot length, root length and dry matter (Table 7). High seed P content improves plant establishment and increases dry matter accumulation. This is a corollary of faster initial root growth, which gives seedlings an earlier access to growth-limiting resources (water and mineral elements). According to Grant et al. (2001), seed P reserves sustain a maximal growth of seedlings for several weeks after germination, till the plant has three or more leaves and a substantial root system.

Corresponding to the findings, there were significant differences in seed P content among landraces (Table 5), with the dark-coloured seeds (G340A) recording the highest P content (7.2 g kg⁻¹ DM). A variation of seed P concentration amounts from (1) phytoavailability of mineral elements in the soil, (2) environmental factors affecting plant growth and (3) variation between plant genotypes of the same plant species grown in the same environment. The Bambara groundnut landraces used in this study have the same soil nutritional history and produced under same environmental conditions. Therefore, the seed P concentration variation was attributable to the variation in landraces used. This can be supported by previous findings in which chromosomal loci (QTL) affecting seed P concentration have been identified in legumes (1) *Phaseolus vulgaris* (Blair et al., 2009; Cichy et al., 2009) and (2) *Lotus japonicas* (Klein and Grusak, 2009).

Seed P is the only source available to support the inceptive growth of seedlings, upon germination, and its reserves are rapidly mobilized and translocated to emerging root and shoot tissues. According to White and Broadley (2009), 30%–80% of seed P occurs in the form of phytate. Phytate accumulates in seeds as mixed salts of cations (K, Mg, Ca, Mn, Fe and Zn). Activities of phosphatases and phytases in dry seeds, upshot phytate hydrolysis to provide P source for developing seedlings. The positive correlation between P and bio-metallic cations (K, Mn, Mg, Zn, Cu, and Fe) (Table 7) is in line with observations by Rukma Reddy (2001) who reported direct proportion between seed P and mineral cations.

From our findings, it appeared that landrace seeds with the high concentration of K (Table 5) outgrew landraces with low mineral concentration (Fig. 3). This can be explained by the coupled plant growth parameters with physiological roles of mineral elements. Requirements for cell extension are (1) softening of the cell wall induced by indole acetic acid (IAA) and (2) solute accumulation within the cell to create internal osmotic potential (Zhao et al., 2012). Accumulation of K⁺ in expanding cells reduces their water potential and stabilize their pH. The IAA-stimulated H⁺ efflux is electrochemically balanced by a stoichiometric K⁺ influx, in the absence of K⁺, IAA induced elongation declines and ceases within a few hours. Therefore, root and shoot elongation may have a direct proportion with seed K concentration.

It is possible to make a comparison between the concentrations of

mineral nutrients of the four landraces because of same soil nutritional history. It was found that mature seeds of evaluated landraces did not differ significantly in seed size ($P = 0.184$), but mineral elements significantly differ ($P < 0.05$) (Table 5). Seeds contain food reserves of organic compounds and mineral nutrients for nurturing the young seedling (Lamont and Groom, 2013). Prior to becoming photosynthetically active and developing root hairs, all the nutrient requirements for the germinant must be met from seed stores (Nadeem et al., 2014). Mineral nutrients of seeds constitute a significant source of essential element for seedlings and developing individuals of plants. The seed content in macronutrients and micronutrients, although constituting only a small proportion of the total demand, may also be essential for plant establishment and growth, thereby supporting the early stages of plant development under conditions of nutrient shortage or limited acquisition rates.

4.4. Cotyledons and seedlings mineral content

All other mineral elements in seed cotyledons decreased in concentration from 14 to 21 DAS except the magnesium. Substantial amounts, often >50%, of K, Ca, Cu, Fe, Mn, and Zn were translocated from the initial cotyledons into the seedling. All landraces showed a relationship between the loss of each element and dry matter from the cotyledons throughout the experiment, and a similar proportion between stem and leaves (shoots) of the amount of specific nutrients mobilized from cotyledons of parent seeds. The loss in mineral element concentration and dry matter of cotyledons are related to phytic acid degradation. Phytic acid accounts for 1%–8% of the dry weights of legume seeds (Averitt et al., 2017), during germination, it is broken down by enzyme phytase in a hydrolysis reaction, releasing Pi, metal ions, and myo-inositol for use by the growing individual.

However, a puzzling increase in cotyledon Mg concentration was observed in all landraces (Table 9). Interestingly, the cotyledons changed in colour from white to green, this will concur with a report by Shaul (2002), Mg is the central atom in chlorophyll molecule. The total proportion of total Mg localized in the chloroplast is between 10–20%, less than half of which is bound in chlorophyll. The spiking increase of Mg concentration in cotyledons may be a gauge to the photosynthetic potential of cotyledons. Other work revealed that shading the *Hakea* spp. cotyledons from light had little effect on seedling growth (Watt and Evans, 1999). Hence in a competitive situation, seed cotyledons, as a proxy of nutrient support for growth rather than an energy source, could be vital in a nutrient-poor environment, particularly in nutrient impoverished soils and for genotypes with little inherent drought tolerance, where rapid root growth is required to ensure that contact with soil moisture is maintained. If light coloured landraces endemic to nutrient-poor soils are slower to develop a vertical root, and hence less likely to reach permanent moisture, it is possible that they are physiologically more drought-prone than dark-coloured seeds.

Large amounts of K, Ca, Mn, Cu, Fe, and Zn were translocated to the growing shoot. However, at 14 and 21 DAS, the dark-coloured landrace G340A had the significantly highest concentration of bio-metals in the shoot (Table 8). A review by Clark (1983) indicated that differences in mineral concentration in seedlings might be due to different plant genotypes and translocation coefficient of mineral elements for plant use. Diversity among genotypes, cultivars, varieties, lines and inbreds within a plant species for mineral element translocation, distribution and use have been recognized. Numerous reviews have been written on this subject (Antonovics et al., 1971; Clark and Brown, 1980; Gerloff, 1963; Rengel, 2001; Safaya, 1976), significant differences among landraces for mineral elements should be recognized. Many differences are under genetic control, but their expression may be altered when plants are grown in different environments. These landraces differences help explain plant adaptations to many mineral stress conditions noted throughout the world. These landrace differences provide the basis for better adaptation and survival under unique mineral stress conditions.

5. Conclusion

Although the mineral elements contained in a seed contributes little to the final mineral content of a mature plant, it significantly contributes to the mineral nutrition of a young seedling. Greater seed mineral reserves allow seedlings to establish faster and ultimately produce plants with higher vigour and performance. Our research findings suggest that dark-coloured seeds landraces (G340A) warrant further study as potential sources of seedling vigour in nutrient impoverished soils. Any comparison between landraces and varieties must be treated with caution until more is known about optimal regimes for the different lines. For plant nutrient values to be purposeful, they must be compared to standard values. Unfortunately, published mineral element values for Bambara groundnut are not available. Therefore, this work can be considered as a pilot study in order to generate data for elemental composition in Bambara groundnut seedlings for future comparisons. The limitation with using generic nutrient standards is that they may not be sensitive enough to reveal significant differences. Until more specific data can be accumulated, these general nutrient standards are the currently available. More work is required to gather specific mineral element values for Bambara groundnut seeds and seedlings to improve seed quality selection decisions.

Declarations

Author contribution statement

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

Alfred Odour Odindo: Contributed reagents, materials, analysis tools or data; Wrote the paper.

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