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# DETERMINATION OF MICRO- AND MACRO-ELEMENT CONCENTRATIONS AND THEIR DIVERSITY FROM THE COMMON BEAN (*PHASEOLUS VULGARIS*, L) LANDRACES OF ZAMBIA

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## ABSTRACT

Bio-fortification of crops is one of those cheaper options to managed micronutrient deficiencies (MND), compared to supplementation and food fortification. For major crops, bio-fortification relies on the natural sources of variation in the mineral contents of the available genotypes including the landraces. Therefore, the main objective of this research was to determine the mineral content variation amongst the landraces and commercial varieties of common bean from Zambian alongside the CIAT reference lines using the Atomic Absorption Spectrophotometer (AAS) method. We observed significant differences ($p<0.05$) among the different sup-populations of the landraces, commercial varieties and CIAT reference lines for copper, sodium, iron, zinc, manganese, potassium, calcium and magnesium. On average, a 2-fold higher concentration was observed for the landraces compared to the commercial varieties and the CIAT reference lines for some minerals. Strong positive correlations ($p<0.05$) were revealed amongst these minerals. The sup-populations of these landraces with particularly high mineral contents for iron and zinc were identified and these remain very useful in the Zambian common bean improvement programmes for food and nutritional security.

## KEYWORDS

Common bean, Landraces, Bio-fortification, Macro and Micro elements, Zambia.

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## INTRODUCTION

Common bean (*Phaseolus vulgaris*, L) is grown for its fresh leaves and pods as vegetables, and dry grains in Latin America, Eastern and Southern Africa and South East Asia[1-5], with excellent nutritional properties linked to its high protein, carbohydrate, vitamin and mineral content. Despite the contribution from common bean and other food staples, the prevalence of micronutrient deficiencies (MND) is still high in developing countries and is caused mainly by lack of essential vitamins (Vitamin A) and minerals (Iron and Zinc)[6]. Iron deficiency anaemia (IDA) has been reported to be the most prevalent micronutrient condition globally[7,8]. Specifically, these authors presented that 65.5% of pre-school children suffering from anaemia, while 45.7% - 48.2% of women of reproductive age suffer from IDA, and that Vitamin A deficiency (VAD) affects 190 million children under the age of five. A total of 49 known essential nutrients needed to sustain human life together with some of their average energy allowance (AEA), recommended dietary allowance (RDA), estimated safe and adequate daily dietary intakes (ESADDI), minimum requirement (MR) and both the enhancing and decreasing anti-nutrient substances that promote/inhibit micronutrient bioavailability[9].

The roles of these minerals in the human diet have been elaborated upon by many authors[10-12]. For instance, calcium (Ca) and magnesium (Mg) play important roles in the development of bone and structural tissue formation, glucose and protein absorption and metabolism, regulation and dilation of blood vessels and regular heart beat[10,12]. The deficiency in Ca and Mg causes weak bone and structural connecting tissue formation, hypertension, and poor glucose absorption[13]. Ca deficiency is linked to some chronic diseases such as *osteoporosis*, and that Mg deficiency leads to energy production faltering and insufficient production[11]. Iron (Fe) is a crucial component of haem proteins, haemoglobin, and myoglobin required for oxygen transportation and vascular functions[14], zinc (Zn) serves as a cofactor in many enzymatic reactions[15], copper (Cu) is a coenzyme and a crucial cofactor in Fe utilisation and is required for cytochrome oxidase redox chemical reaction[16], manganese (Mn) is essential for immune system and effective food metabolism in addition to serving as cofactors in many enzymatic reactions[17].

Due to the prevalence of these MNDs, several approaches have been put in place to mitigate them through supplementation and food fortification. However, supplementation and fortification are cost ineffective and do not reach the rural poor[18] hence the development of bio-fortification. Bio-fortification has been defined differently but the key components are development of micronutrient-dense staple crops using the best traditional breeding practices and modern biotechnology[19] or improving the nutritional content of staple crops by breeding varieties that have a high content of the three limiting micronutrients (Vitamin A, Iron, Zinc) or their precursors than conventional ones[20], and a number of advantages associated with it are being advanced[21,22]. Iron content variations have been observed to occur between genotypes and wild and cultivated beans, although the wild beans had only a narrow advantage in iron content over cultivated ones[1,5]. The wide genetic variations amongst genotypes in iron and zinc contents in Tanzania between leaves (310.49 ppm of Fe, and 28.03 ppm of Zn) and seeds (55.01 ppm of Fe, and 31.4 ppm of Zn) has been reported[2].

Specific studies that rely on the mineral composition based on germplasm from African origin are few, as seen in the work of Typhone and Nchimbi-Msolla[2] and Mukamuhirwa *et al*[3] for Tanzania and Uganda respectively. This lack of information on the available common bean genotypes hampers a cheap source of food supplementation through bio-fortification of common beans for East and Southern African countries. The mineral composition and / or content of bean germplasm from major producing countries in these regions, including Zambia, remain unknown. Furthermore, how these minerals vary between landraces and commercial varieties has not been reported anywhere to the best of our knowledge.

Therefore, the main objective of this study to determine and present the macro- and micro-element concentrations of the sub-populations within the Zambian common bean landraces, and to compare how these vary with respect to the defined CIAT reference lines and Zambian commercial varieties. This is aimed at encouraging bio-fortification through breeding by identifying potential parental breeding lines among the sub-populations of these landraces with high iron and zinc content for food security and nutritional breeding programmes.

## MATERIAL AND METHODS

### The plant materials

The sub-populations within the four Zambian landraces of Lusaka yellow, Lundazi, Mbala mixture and Solwezi; the CIAT control genotypes of 'Diacol Calima' (G4494, B and C) for the Andean genepool, and 'ICA Pijao' (G5773) for the Mesoamerican gene pool; and the Zambian commercial varieties as detailed here under (Table No.1) were used for this study. The landraces were collected by the Zambian Agricultural Research Institute's (ZARI) breeders, CIAT reference lines were sourced from the CIAT, Colombia, gene bank, and the commercial lines were collected from the Lusuntha border market between Zambia and Malawi. The seeds were kept in cool dry conditions and planted in the tropical glass house at the University of Bath under the following conditions: on a mixed compost of coarse, medium and fine in the ratio of 2:2:1 in a 5 litres pots that was supplemented with a slow releasing nutrients Osmocote Extract (Standard 12-14M, ICL, UK) at the rate of 5 grams per pot, Temperature of 22–28ºC, relative humidity (RH) of 40–80%, and a light period of 14 hours per day. These plants were maintained to maturity and their seeds harvested for this study. A total of 50 samples were used for this study, that is, Lusaka yellow had 8 sub-populations, Lundazi had 8 sub-populations, Mbala mixture had 7 sub-populations, and Solwezi had 8 sub-populations. Additionally, there were sub populations that overlapped between landraces: 1 overlap for Lusaka yellow and Mbala mixture, 2 for Lundazi, Mbala mixture, and Solwezi, and 8 for Mbala mixture and Solwezi, totalling to 42 sup-populations from all the landraces. In addition to these landrace sub-populations, there were four Zambian-Malawian varieties of Katwetwe, Kabulangeti, Sugar beans, and White bean that were collected from the Lusuntha border market between the two countries due to their market dominance, and finally the 4 CIAT reference varieties of G5773, G4494A, G4494C, G14470) were included in this study.

### Sample preparation and Acid digestion

The 100-seed weight of each of the 50 sub-population was determined prior to sample preparation as indicated in Table No.1 above. Six grams of sample were ground into a fine powder using the Andrew James wet and dry grinder (Andrew James, UK Ltd). Two grams of finely ground bean flour were stored in boiling tubes for subsequent acid digestion. Acid digestion was conducted as described by[2,12]. Briefly, to the fine ground bean flour in each of the boiling tube was added 20 ml of 6M 70% nitric acid for trace element analysis (Sigma-Aldrich, UK) and allowed to stand for an overnight. The boiling tubes were then arranged vertically in a glass beaker and placed in a Heraeus B6 Incubator (Fisher Scientific, UK) at a temperature $50^0$C for 3 hours. The digested samples were cooled to room temperature, filtered through Whatman filter paper, and diluted with trace element grade deionised water (Sigma-Aldrich, UK) in a 25 ml volumetric flask. The solution was thus ready for determination of macro and micro elements in the atomic absorption spectrophotometer (AAS) method of Perkin-Elmer 2380 (Fisher Scientific, UK).

### Determination of micro and macro elements in the samples

Calcium (Ca), magnesium (Mg), copper (Cu), potassium (K), manganese (Mn), phosphorus (P), iron (Fe) and zinc (Zn) were quantified using Atomic Absorption Spectroscopy (AAS) method of Perkin-Elmer 2380 (Fisher Scientific, UK), and their concentrations were converted and expressed in $mg100g^{-1}$ from the absorbance read in the (AAS) as described in[11,23,24]. For Iron and Zinc, the readings were evaluated against the standard curves prepared from the Iron diluted to a concentration of 100 mg $l^{-1}$ and Zinc diluted to 50 mg $l^{-1}$ as explained by[25].

### Data analysis

Data were collected in triplicate and the averages were computed in Microsoft Excel 2013. One-way ANOVA was applied to evaluate the variance of these micro and macro elements parameters; the Pearson coefficient was used to verify the existence of statistically significant correlations among the variables; the multivariate analysis of main PCA components was performed, with the aim to detect the existence of clusters grouping amongst the bean accessions according to their mineral concentrations. All these analyses were run using PAST software, Version 3.16[26] and R Software[27].

## RESULTS

### Micro- and macro-nutrient variation in common-bean landraces

A significant difference ($p < 0.05$) was recorded for the 100-seed weight (100SW), and micro and macro nutrients in common bean landraces from Zambia, CIAT reference lines and the Zambian commercial varieties (Table No.2). Specifically, the landrace of Lundazi was highest in calcium, a sub-population that overlaps between Lusaka yellow and Mbala mixture (LY+MM) was highest in iron, manganese, and zinc, a sub-population that overlaps between Mbala mixture and Solwezi (MM+SO) was highest in copper, potassium and magnesium, and the CIAT line (G4494B) was highest in sodium, It is important to pint here that 8 of the 9 highest values for the 100SW and macro- and micro-nutrients in common bean were from the Zambian landraces whereas 4 of the lowest values were from the Zambian commercial varieties, 2 from CIAT lines and 3 from the landraces.

### Micro and macro nutrient variation in common bean based on populations

Considering population averages as a whole, Lusaka yellow had three of the highest values for iron, potassium and calcium; Mbala mixture had two of the highest values for manganese and potassium; G5773 from CIAT lines had the highest values for copper and zinc; G4494B from a CIAT line had the highest values for sodium and magnesium; and the commercial variety of Kabulangeti had the highest value for seed weight (Table No.3). The landraces of Lundazi and Solwezi had neither the lowest nor highest values for the micro and macro nutrients. The six of the eight lowest values all came from the Zambian commercial varieties while the other two came from the CIAT lines. Thus, there were high values for these minerals in the landraces followed by CIAT lines and lower values were recorded among the Zambian commercial varieties. The Zambian landraces had 5 of the 8 highest values on average for copper, manganese, zinc, potassium, and calcium, CIAT reference lines had 3 of the 8 for iron, sodium and manganese, and the commercial varieties were high in 100 seed weight only. The Zambian commercial varieties had 7 of the 8 lowest average values for copper, iron, manganese, zinc, potassium, magnesium and calcium in common bean, and the other only lowest value from the landraces was for Sodium. At population and sub-population levels, analysis of variance showed there were significant differences ($p<0.05$) between these mineral contents among populations. Mann-Whitney pairwise significance analysis also showed that there were significant differences ($p<0.05$) between any pair of these micro and macro nutrients in the common bean germplasm studied. However, there was no correlation between any of the mineral content and 100SW.

### Micro and macro nutrient variation in common bean based on seed colour

When the seeds were classified according to seed colour there was no statistical difference ($p<0.05$) between the macro and micro mineral contents for the common bean germplasm from Zambia. For the results presented here, there was a very minor separation between dark red and maroon, and they could be treated interchangeably. 100 seed weight was highest in green and lowest in yellow; copper was highest in maroon and lowest in white; Iron was highest in maroon and lowest in white; manganese was highest in yellow and lowest in pink; sodium was highest in red and lowest in white; zinc was highest in maroon and lowest in white; potassium was highest in red and lowest in purple; magnesium was highest in red and lowest in green; and calcium was highest in red and lowest in green (Table No.4). Red and maroon took most high values for the mineral concentrations measured, and white look most of lowest values for these minerals, with green colour taking two and purple and pink taking one each of these low values of the mineral concentration. Black, brown, and grey did not take any of the highest or lowest values and were all close to the top higher values.

### Pair wise correlation analyses of the micro and macro nutrients in common bean

Pairwise correlation analyses produced strong significant ($p<0.05$) positive relationships between some of the minerals in common beans germplasm from Zambia (Table No.5). These strong positive significant relationships were observed between copper and iron and zinc; iron and manganese and zinc; sodium and copper and iron; potassium and magnesium and calcium; magnesium and calcium; and calcium and sodium. Frequently, strong positive correlations occurred between micro-nutrients themselves and between macro-nutrients themselves and less frequently between a pair involving micro- and macro-elements relationships. The negative significant correlations were observed between sodium and zinc and calcium, whereas the strongest positive significant correlation was observed between Sodium and Copper.

**Principal component analysis (PCA) and Neighbor joining clustering based on the mineral concentrations in common beans**

The multivariate analysis using principal component analysis (PCoA) generated the partial distribution of all the sub-populations based on the micro and macro element concentrations (Figures No.1). The results indicated that the macro and micro element concentrations can be explained by 8 axes, of which only 4 had significant contributions to the spatial separation of the sub-populations explaining 70.54 % of the total variability. The first axis explained 22.85% of the total variability with elements of Cu, Fe, Na, Zn, K, and Ca being positively significant; the second axis explained 18.41% with all the elements being positively significant except K; the third axis explained 16.53% with Cu, Fe, Mg, and Ca being positively significant; and the fourth axis explained 12.76% with all the elements except Ca and Fe being significant, with the eigenvalues of 1.96, 1.58, 1.42, and 1.10 respectively. However, there were no clear clustering patterns for all the sub-populations based on the macro and micro mineral concentrations in the neighbour joining.

## DISCUSSION

The main objective of this study was to determine the micro and macro nutrient concentrations in dry seeds of common bean germplasm from Zambia. This study focused on the micro nutrients in common beans as these have been reported to cause the highest global health risks, particularly iron and zinc deficiencies[7,9]. Among the macro elements, sodium, calcium, potassium and magnesium were included because of their nutritional importance in the human diet as major components of bones and teeth, and proper functioning of muscles and central nervous system[24].

The results from this study showed that, there are significant differences ($p<0.05$) in the concentrations of these micro and macro elements in common bean landraces from Zambia. These results agree with previous studies for example, the variations of iron and zinc between leaves and seeds of Tanzanian common bean genotypes, and plant parts[2] was reported. They showed that from leaves, the average values were 310.49 ppm of Fe, and 28.03 ppm of Zn whereas from seeds were 55.01 ppm (5.5 mg) of Fe, and 31.4 ppm (3.14 mg) of Zn. The average Fe and Zn concentration of 5.5 mg/100g and 3.5 mg/100g respectively in the core collection of CIAT genotypes[1] was presented. The work of[24] on the germplasm of Madeira Island of Portugal and showed that on average the mineral concentrations were 1890 mg/100g for K, 150 mg/100g for Mg, 6.01 mg/100g for Fe, 1.01 mg/100g for Cu, 3.01 mg/100g for Zn, and 1.45 mg/100g for Mn. The mineral contents of common bean genotypes from India were found to be on average as 1.81 mg/100g Fe, 0.78 mg/100g Zn and 20.30% protein content. The mineral concentrations in the 29 genotypes from USA were studied and reported that Zn ranged from 3.4 to 6.4 mg/100g and Fe was from 0.89 to 11.29 mg/100g by[11].

It is apparent from the above that there has been no specific study directed towards the mineral concentrations in the landraces of common bean from many major bean-growing regions of the world. This could be the probable reason why the values of the micro and macro elements reported in this study are higher than those that had previously been reported. However, several factors have been suggested that effect the mineral concentrations of common beans: common bean plant parts[2]; growing environment and genotypes variations[11,23]; origin, genotypes, environmental conditions (temperature, soils, and fertilization), growing conditions[24]; and the weeding regimes that affect both mineral nutrient uptake and retention in the plant as well as soil moisture for zinc[29]. When the results of this study are broken down in to landraces, CIAT lines and Zambian commercial lines (Table No.3) there are higher values on average for landraces, followed by CIAT lines and lowest in the commercial varieties. It is important to note further here that, the averages of the mineral content of the Zambian commercial varieties fall within most of the values reported earlier. This implies that, the presence of landraces in this study is the reason for the higher values than the average values for mineral content observed, and it demonstrates how important these landraces can be used in improving nutritional component of commercial varieties already in production.

Akond[11] studied the mineral compositions of USA genotypes and identified 7 genotypes with high iron and zinc concentrations which were recommended to be used as parental material for mineral content breeding in USA. In this current study we identified 23 sub-populations with iron concentrations above average (21.38 mg/100g): 6 in Lusaka yellow, 3 in Lundazi, 3 in Mbala mixture, 6 in Solwezi, one in an overlap between Lusaka yellow and Mbala mixture, 3 in an overlap between Mbala mixture and Solwezi and 2 in CIAT lines of G5573 and G4494C. This study further identified 26 sub-populations with zinc concentration above average (8.08 mg/100g): 7 in Lusaka yellow, 4 in Lundazi, 4 in Mbala mixture, 4 in Solwezi, one in an overlap between Lusaka yellow and Mbala mixture, 5 in overlap between Mbala mixture and Solwezi, and 2 in CIAT lines of G5573 and G4494C. It is important to point out here that the CIAT line G4494 had two plant types: a dwarf type with white flowers and a semi climber with a pink flower colour; therefore, G4494C refers to the latter plant type. CIAT line G4494B can also be considered for future breeding work since it had the highest values for sodium. The overlap between Lusaka yellow and Mbala mixture, mentioned above, is the dominant composition in Lusaka yellow, and had the highest average values for Fe, Zn and Mn with elongate/kidney seed shape with dark blue colour around its helium. This could partly explain why the landrace of Lusaka yellow beans have a higher preference in the markets within Lusaka and other parts of Zambia.

Additional result from this study showed that there was no direct correlation ($p < 0.05$) between mineral contents and 100SW. This agrees with the findings of Akond[11] and Maraghan[23] when they reported a similar result from their earlier studies. Therefore, seed size is mainly an attribute for seed yields as explained by Agung[30] in Faba beans (*Vicia faba* L) and not for mineral content estimation. However, this study disagrees with the finding of Beebe[1] that Fe content tends to be present at higher values in the Andean genepool than the Mesoamerican genepool. Here, we report Fe in CIAT G5773 – Mesoamerican at 25.0 mg/100g, and in CIAT G4494C – Andean at 24.5 mg/100g that agrees with the results of Akond[11] where they also observe no direct relation with the genepools in relation to the mineral contents for the genotypes from USA.

Positive and negative correlations between the micro and macro elements have been reported in earlier studies[1, 2, 11, 12, 24, 28]. This study also reported positive and significant correlations ($p< 0.05$) between micro elements as presented in Table No.5. The strong and significant correlations between micro and macro elements have been explained to mean that the genetic factors for increasing one mineral content co-segregate with the genetic factor for increasing the other mineral content with which they share a significant correlation[2,9]. Therefore, deducing from these results, increasing the content of iron would increase the content for zinc, manganese, and sodium while increasing the zinc content would increase the content for iron, copper, and sodium. A similar observation was made for macro elements such that increasing the potassium content would increase the content for magnesium and calcium, increasing calcium would increase potassium, sodium and magnesium, and increasing magnesium would increase calcium and potassium.

To explore further the observed correlations among the mineral contents in common bean for breeding purposes, a focus paper by Welch and Graham[9] observed that there is significant variability to increase the concentrations of iron and zinc, and that the traits required for the genetic improvement of iron and zinc concentrations are stable across different bean growing environments, although their concentrations are affected by GXE interactions. Beebe[1] had confirmed that, there is an environmental and seasonal stability in the iron concentrations for the CIAT collections. The genetic parameters of iron and zinc concentrations in Andean bean seeds by looking at their concentrations from crosses between two sets parents (IAC Boreal x Light Red Kidney and Ouro Branco x Light Red Kidney), their $F_1$ plants, $F_1$ reciprocals, $F_2$ plants, $F_2$ reciprocals, and backcrosses ($BC_{11}$ and $BC_{12}$) were studied[31]. Zemolin and the co-authors[31] concluded that there are no maternal effects, and that the seeds of the $F_1$ generation will represent fertilisation between the parents in both hybrids combinations. Therefore, these results mean that there is no time that needs to be wasted in undertaking reciprocal crosses with $F_1$ plants before backcrossing hence allowing segregating populations of common beans with high mineral contents to be generated and deployed within a shorter period of time. On the other hand, although Possobom *et al*[32] observed a significant expression of maternal effects in the Mesoamerican genepool, they linked this expression of maternal effects to iron accumulation in the seed coat in Mesoamerica beans or seed embryo in Andean beans.

Possobom and his colleagues[32] then suggested that the selection of superior common bean recombinants for iron concentration should begin at $F_3$ generation if iron accumulates more in the seed coat (Mesoamerican) and at $F_2$ if the iron accumulates more in the seed embryo (Andean).

Finally, on the breeding and inheritance of seed iron and zinc concentrations in common bean, for example, 6 QTLs for zinc and 5 QTLs for iron that are clustered on the upper half of the linkage group B11 were identified[25]. Other QTLs for Zn were identified on linkage groups B3, B6, B7 and B9, and B4, B6, B7 and B8 for Fe. This information means that the scientists are getting so close to identifying the candidate gene(s) for Fe and Zn concentrations in common bean, which will be very useful in the era of marker assisted breeding that will shorten the breeding cycle by allowing screening for these minerals at seedling stage of growth for common bean.

**Table No.1: Details of the sub-populations of common bean with their seed coat colour and 100-seed weight (100SW) used for the macro and micro mineral concentration determination**

| S.No | Landrace | Sub-population | Seed Colour | 100SW |
|---|---|---|---|---|
| 1 | Lusaka Yellow (LY) | LY1 | Yellow | 27.05 |
| | | LY2 | Creamy yellow | 29.47 |
| | | LY3 | Deep yellow | 34.52 |
| | | LY4 | Olive green | 48.00 |
| | | LY5 | Yellow | 31.26 |
| | | LY6 | Creamy yellow | 24.31 |
| | | LY7 | Brownish yellow | 29.51 |
| | | LY8 | Yellow | 26.91 |
| 2 | Lundazi (LU) | LU1 | Dark red | 42.54 |
| | | LU2 | Maroon | 20.75 |
| | | LU3 | Red | 22.51 |
| | | LU4 | Dark brown | 33.58 |
| | | LU5 | Black | 24.27 |
| | | LU6 | Purple | 41.17 |
| | | LU7 | Red mottled | 27.94 |
| | | LU8 | Purple | 25.19 |

| | | | | |
|---|---|---|---|---|
| 3 | Mbala Mixture (MM) | MM1 | Pinkish mottled | 52.68 |
| | | MM2 | Purplish mottled | 30.67 |
| | | MM3 | Grey to brownish green | 38.29 |
| | | MM4 | Yellow | 31.45 |
| | | MM5 | Yellow | 26.90 |
| | | MM6 | Pinkish with yellow speckles | 50.15 |
| | | MM7 | Brownish yellow | 30.44 |
| 4 | Solwezi (SO) | SO1 | Pinkish mottled | 51.94 |
| | | SO2 | Purple mottled | 32.16 |
| | | SO3 | Dark Red/Maroon | 40.16 |
| | | SO4 | Pink | 20.55 |
| | | SO5 | Red | 30.01 |
| | | SO6 | Red mottled | 37.40 |
| | | SO7 | Pink with black mottled | 38.50 |
| | | SO8 | Pink with black speckles | 30.07 |
| 5 | LY and MM overlap | LYMM | Yellow | 37.51 |
| 6 | LU, MM and SO overlap | LMS1 | Purplish with speckles | 39.21 |
| | | LMS2 | Grey to brownish green | 29.23 |
| 7 | MM and SO overlap | MS1 | White | 31.83 |
| | | MS2 | Pinkish mottled | 31.57 |
| | | MS3 | Purplish mottled | 29.41 |
| | | MS4 | Brownish to Pinkish mottled | 27.02 |
| | | MS5 | Dark grey with speckles | 37.72 |
| | | MS6 | Blackish with brown mottling | 38.52 |
| | | MS7 | Dark brownish mottled | 49.74 |
| | | MS8 | Black with Cream mottling | 33.16 |
| 8 | CIAT Reference Lines | G5773 | Black | 19.76 |
| | | G4494B | Red mottled | 39.70 |
| | | G4494C | Red mottled | 31.89 |
| | | G14470 | Cream with purple stripped | 36.91 |
| 9 | Zambian/Malawian commercial Lines | Long White | White with purple dotted helium | 36.35 |
| | | Kabulangeti | Dark grey speckled | 46.16 |
| | | Katwetwe | Purple mottled | 30.81 |
| | | Sugar Bean | Cream purple stripped | 37.56 |

**Table No.2: Variations in the mineral content: copper (Cu), iron (Fe), manganese (Mn), sodium (Na), zinc (Zn), potassium (K), magnesium (Mg), and calcium (Ca) in the landraces of Lusaka yellow (LY), Lundazi (LU), Mbala mixture (MM), and Solwezi (SO), four CIAT Reference Lines, and four Zambian commercial Varieties**

| Populations | Sub-Pops. | 100SW | Cu | Fe | Mn | Na | Zn | K | Mg | Ca |
|---|---|---|---|---|---|---|---|---|---|---|
| Lusaka Yellow | LY1 | 27.05 | 2.40 | 21.50 | 3.50 | 0.60 | 7.75 | 3900.00 | 570.00 | 650.00 |
| | LY2 | 29.47 | 2.50 | 22.00 | 3.00 | 0.70 | 7.75 | 5100.00 | 590.00 | 950.00 |
| | LY3 | 34.52 | 2.20 | 26.00 | 3.50 | 3.40 | 9.25 | 4700.00 | 540.00 | 800.00 |
| | LY4 | 48.00 | 2.55 | 21.50 | 3.00 | 1.00 | 8.25 | 4500.00 | 480.00 | 375.00 |
| | LY5 | 31.26 | 1.50 | 18.50 | 3.00 | 0.65 | 8.50 | 4800.00 | 650.00 | 675.00 |
| | LY6 | 24.31 | 2.95 | 28.50 | 4.50 | 1.20 | 9.75 | 5700.00 | 650.00 | 775.00 |
| | LY7 | 29.51 | 2.80 | 31.00 | 4.00 | 0.70 | 10.00 | 5800.00 | 590.00 | 650.00 |
| | LY8 | 26.91 | 2.10 | 23.50 | 4.00 | 1.70 | 9.25 | 5900.00 | 600.00 | 950.00 |
| Lundazi | LU1 | 42.54 | 2.65 | 18.50 | 3.50 | 1.85 | 9.00 | 5000.00 | 540.00 | 600.00 |
| | LU2 | 20.75 | 3.00 | 29.50 | 3.50 | 0.75 | 9.50 | 5200.00 | 620.00 | 550.00 |
| | LU3 | 22.51 | 2.50 | 25.50 | 3.50 | 5.35 | 7.00 | 5300.00 | 620.00 | 525.00 |
| | LU4 | 33.58 | 2.50 | 23.00 | 3.50 | 0.90 | 9.00 | 5200.00 | 600.00 | 600.00 |
| | LU5 | 24.27 | 2.10 | 19.50 | 2.50 | 1.05 | 6.00 | 5000.00 | 670.00 | 525.00 |
| | LU6 | 41.17 | 2.20 | 20.50 | 3.50 | 4.20 | 6.75 | 5000.00 | 510.00 | 550.00 |
| | LU7 | 27.94 | 2.10 | 17.50 | 4.00 | 2.30 | 8.75 | 5200.00 | 600.00 | 1275.00 |
| | LU8 | 25.19 | 1.80 | 16.50 | 3.50 | 0.80 | 6.50 | 4400.00 | 620.00 | 875.00 |
| Mbala Mixture | MM1 | 52.68 | 2.40 | 26.50 | 2.75 | 0.80 | 9.00 | 5900.00 | 640.00 | 725.00 |
| | MM2 | 30.67 | 2.10 | 20.00 | 2.50 | 0.50 | 8.25 | 5200.00 | 550.00 | 600.00 |
| | MM3 | 38.29 | 1.75 | 21.00 | 2.00 | 0.45 | 10.00 | 4900.00 | 540.00 | 650.00 |
| | MM4 | 31.45 | 2.45 | 21.50 | 3.00 | 0.75 | 7.25 | 5400.00 | 590.00 | 900.00 |
| | MM5 | 26.90 | 1.80 | 19.50 | 3.00 | 0.60 | 6.50 | 3600.00 | 480.00 | 575.00 |
| | MM6 | 50.15 | 0.85* | 9.50 | 2.00 | 1.40 | 5.50 | 3900.00 | 460.00 | 225.00 |
| | MM7 | 30.44 | 2.65 | 25.50 | 2.50 | 0.80 | 8.50 | 4400.00 | 480.00 | 425.00 |
| Solwezi | SO1 | 51.94 | 2.55 | 21.50 | 2.50 | 0.90 | 9.25 | 4000.00 | 510.00 | 575.00 |
| | SO2 | 32.16 | 2.65 | 25.00 | 3.50 | 0.70 | 7.00 | 4300.00 | 510.00 | 500.00 |
| | SO3 | 40.16 | 2.90 | 24.00 | 3.25 | 0.90 | 11.25 | 4500.00 | 550.00 | 400.00 |
| | SO4 | 20.55 | 2.50 | 26.50 | 3.00 | 0.90 | 8.00 | 4400.00 | 550.00 | 450.00 |
| | SO5 | 30.01 | 2.15 | 19.00 | 2.50 | 1.20 | 6.50 | 5000.00 | 630.00 | 450.00 |
| | SO6 | 37.40 | 2.30 | 17.50 | 2.25* | 0.65 | 7.00 | 4800.00 | 510.00 | 425.00 |
| | SO7 | 38.50 | 2.30 | 23.00 | 2.50 | 1.10 | 9.00 | 4900.00 | 510.00 | 400.00 |
| | SO8 | 30.07 | 2.40 | 26.50 | 3.50 | 0.85 | 8.75 | 4800.00 | 590.00 | 525.00 |
| LY + MM | LYMM | 37.51 | 2.65 | 34.00 | 4.50 | 1.35 | 12.00 | 5000.00 | 540.00 | 925.00 |
| LU+MM+SO | LMS1 | 39.21 | 1.60 | 16.00 | 2.50 | 0.60 | 6.50 | 4400.00 | 510.00 | 425.00 |
| | LMS2 | 29.23 | 2.35 | 19.50 | 3.50 | 0.70 | 7.00 | 5400.00 | 550.00 | 725.00 |
| MM + SO | MS1 | 31.83 | 2.15 | 17.00 | 2.50 | 0.60 | 6.50 | 5600.00 | 650.00 | 700.00 |
| | MS2 | 31.57 | 2.25 | 21.00 | 2.75 | 0.60 | 8.25 | 4700.00 | 600.00 | 475.00 |
| | MS3 | 29.41 | 2.50 | 27.50 | 4.00 | 0.90 | 9.50 | 6100.00 | 690.00 | 725.00 |
| | MS4 | 27.02 | 2.05 | 7.00* | 2.50 | 0.90 | 7.00 | 6000.00 | 620.00 | 650.00 |
| | MS5 | 37.72 | 2.75 | 22.00 | 3.50 | 0.70 | 9.25 | 5700.00 | 610.00 | 950.00 |
| | MS6 | 38.52 | 3.10 | 29.50 | 3.50 | 0.80 | 10.50 | 5500.00 | 620.00 | 875.00 |
| | MS7 | 49.74 | 3.15 | 27.00 | 3.00 | 0.55 | 10.50 | 4400.00 | 510.00 | 625.00 |
| | MS8 | 33.16 | 2.40 | 20.50 | 2.50 | 0.70 | 8.00 | 4700.00 | 570.00 | 650.00 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| CIAT Lines | G5773 | 19.76* | 2.50 | 25.00 | 4.00 | 2.50 | 9.50 | 5000.00 | 540.00 | 575.00 |
| | G4494B | 39.70 | 2.45 | 19.50 | 3.50 | 9.30 | 7.25 | 5900.00 | 680.00 | 450.00 |
| | G4494C | 31.89 | 2.30 | 24.50 | 3.50 | 0.75 | 8.25 | 5500.00 | 680.00 | 625.00 |
| | G14470 | 36.91 | 2.15 | 20.00 | 2.50 | 0.50* | 7.25 | 430.00 | 490.00 | 300.00 |
| Zambian Commercial Lines | Long White | 36.35 | 1.60 | 14.00 | 3.00 | 0.80 | 6.50 | 3500.00* | 500.00 | 250.00* |
| | Kabulangeti | 46.16 | 1.65 | 13.00 | 2.75 | 2.90 | 5.50 | 3600.00 | 430.00 | 400.00 |
| | Katwetwe | 30.81 | 1.35 | 11.00 | 3.00 | 1.50 | 6.50 | 3600.00 | 380.00* | 325.00 |
| | Sugar Bean | 37.56 | 1.10 | 11.50 | 3.50 | 0.75 | 4.00* | 3600.00 | 440.00 | 325.00 |
| Mean | | 33.89 | 2.27 | 21.38 | 3.15 | 1.34 | 8.09 | 4786.60 | 563.20 | 602.50 |
| Std Err | | 1.15 | 0.07 | 0.79 | 0.09 | 0.21 | 0.23 | 132.51 | 10.00 | 29.79 |
| Coeff of var | | 24.01 | 21.40 | 25.99 | 19.28 | 111.99 | 19.76 | 19.58 | 12.55 | 35.17 |

*lowest values, the bold values are the highest values for each mineral concentration in mg/100g and 100SW

**Table No.3: Variation in the mineral content by populations: copper (Cu), iron (Fe), manganese (Mn), sodium (Na), zinc (Zn), potassium (K), magnesium (Mg), and calcium (Ca) among the Zambian Landraces, CIAT reference lines and Zambian Commercial varieties**

| Origin | Populations | No of sub-pops | 100SW | Cu | Fe | Mn | Na | Zn | K | Mg | Ca |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Zambian Landraces | Lusaka Yellow | 9.00 | 32.06 | 2.41 | 25.17** | 3.67 | 1.26 | 9.17 | 5044.44** | 578.89 | 750.00** |
| | Std Error | | 2.40 | 0.14 | 1.70 | 0.20 | 0.30 | 0.45 | 18.14 | 18.14 | 62.36 |
| | Lundazi | 10.00 | 30.64 | 2.28 | 20.60 | 3.35 | 1.85 | 7.60 | 5010.00 | 584.00 | 665.00 |
| | Std Error | | 2.53 | 0.41 | 1.35 | 0.15 | 0.52 | 0.41 | 110.00 | 16.94 | 78.28 |
| | Mbala Mixture | 18.00 | 35.86 | 2.28 | 21.36 | 9.92** | 0.76 | 8.33 | 5044.44** | 567.22 | 656.94 |
| | Std Error | | 1.87 | 0.13 | 1.56 | 0.16 | 0.06 | 0.41 | 170.70 | 15.29 | 44.96 |
| | Solwezi | 18.00 | 34.90 | 2.45 | 21.67 | 2.96 | 0.79 | 8.32 | 4955.56 | 571.67 | 584.72 |
| | Std Error | | 1.81 | 0.09 | 1.26 | 0.12 | 0.04 | 0.36 | 146.02 | 13.41 | 38.73 |
| | Mean for Landraces | | 33.36 | 2.36[b] | 22.20 | 4.98[b] | 1.17[a] | 8.36[b] | 5013.61[b] | 575.45 | 664.17[b] |
| | Standard Error | | 2.15 | 0.19 | 1.47 | 0.16 | 0.23 | 0.41 | 111.22 | 15.95 | 56.08 |
| CIAT Reference Lines | G5773 | 1.00 | 19.76* | 2.50** | 25.00 | 4.00 | 2.50 | 9.50** | 5000.00 | 540.00 | 575.00 |
| | G4494B | 1.00 | 39.70 | 2.45 | 19.50 | 3.50 | 9.30** | 7.25 | 5900.00 | 680.00** | 450.00 |
| | G4494C | 1.00 | 31.89 | 2.30 | 24.50 | 3.50 | 0.75 | 8.25 | 5500.00 | 680.00** | 625.00 |
| | G14470 | 1.00 | 36.91 | 2.15 | 20.00 | 2.50* | 0.50* | 7.25 | 430.00 | 490.00 | 300.00 |
| | Mean | | 32.07[a] | 2.35 | 22.25[b] | 3.38 | 3.26[b] | 8.06 | 4207.50 | 597.50[b] | 487.50 |
| | Standard Error | | 4.41 | 0.08 | 1.45 | 0.31 | 2.06 | 0.53 | 1272.55 | 48.71 | 72.53 |
| Zambian Commercial Varieties | Long White | 1.00 | 36.35 | 1.60 | 14.00 | 3.00 | 0.80 | 6.50 | 3500.00* | 500.00 | 250.00* |
| | Kabulangeti | 1.00 | 46.16** | 1.65 | 13.00 | 2.75 | 2.90 | 5.50 | 3600.00 | 430.00 | 400.00 |
| | Katwetwe | 1.00 | 30.81 | 1.35 | 11.00* | 3.00 | 1.50 | 6.50 | 3600.00 | 380.00* | 325.00 |
| | Sugar Bean | 1.00 | 37.56 | 1.10* | 11.50 | 3.50 | 0.75 | 4.00* | 3600.00 | 440.00 | 325.00 |
| | Mean | | 37.72[b] | 1.43[a] | 12.38[a] | 3.06[a] | 1.49 | 5.63[a] | 3575.00[a] | 437.50[a] | 325.00[a] |
| | Standard Error | | 3.17 | 0.13 | 0.69 | 0.16 | 0.50 | 0.59 | 25.00 | 24.62 | 30.62 |

*lowest population value, **highest population value, [a]lowest mean value, [b]highest mean value, and all concentrations were reported in mg/100g

**Table No.4: Variation in the mineral contents by seed colours: Zambian landraces, CIAT lines and Zambian commercial varieties based on seed colours. The number in bracket after seed colour represents the number of sub-populations in that seed colour**

| Seed Colour | Mean/Std Err | 100SW | Cu | Fe | Mn | Na | Zn | K | Mg | Ca |
|---|---|---|---|---|---|---|---|---|---|---|
| Black (5) | Mean | 30.84 | 2.48 | 23.50 | 3.00 | 1.23 | 8.60 | 5020.00 | 582.00 | 605.00 |
| | Standard Error | 3.80 | 0.17 | 1.78 | 0.32 | 0.32 | 0.76 | 131.91 | 28.53 | 78.82 |
| Brown (3) | Mean | 36.78 | 2.57 | 19.00 | 3.00 | 0.78 | 8.83 | 5200.00 | 576.67 | 625.00 |
| | Standard Error | 6.75 | 0.32 | 6.11 | 0.29 | 0.11 | 1.01 | 461.88 | 33.83 | 14.43 |
| Green (1) | Mean | 48.00 | 2.55 | 21.50 | 3.00 | 1.00 | 8.25 | 4500.00 | 480.00* | 375.00* |
| Grey (4) | Mean | 37.85 | 2.13 | 18.88 | 2.94 | 1.19 | 7.94 | 4900.00 | 532.50 | 681.25 |
| | Standard Error | 3.46 | 0.26 | 2.02 | 0.36 | 0.57 | 1.03 | 463.68 | 37.50 | 113.36 |
| Maroon (2) | Mean | 30.46 | 2.95 | 26.75 | 3.38 | 0.83 | 10.38 | 4850.00 | 585.00 | 475.00 |
| | Standard Error | 9.71 | 0.05 | 2.75 | 0.13 | 0.08 | 0.88 | 350.00 | 35.00 | 75.00 |
| Pink (7) | Mean | 38.23 | 2.15 | 21.64 | 2.71* | 0.85 | 8.14 | 4700.00 | 557.14 | 510.71 |
| | Standard Error | 4.93 | 0.22 | 2.30 | 0.18 | 0.12 | 0.47 | 263.67 | 22.64 | 58.72 |
| Purple (8) | Mean | 34.05 | 1.92 | 18.50 | 3.25 | 1.24 | 6.75 | 3978.75* | 518.75 | 503.13 |
| | Standard Error | 1.95 | 0.19 | 2.09 | 0.19 | 0.44 | 0.53 | 580.66 | 34.35 | 73.25 |
| Red (07) | Mean | 33.14 | 2.35 | 20.29 | 3.25 | 3.06 | 7.68 | 5242.86 | 608.57 | 621.43 |
| | Standard Error | 2.68 | 0.07 | 1.25 | 0.24 | 1.20 | 0.38 | 139.48 | 24.54 | 112.78 |
| White (2) | Mean | 34.09 | 1.88* | 15.50* | 2.75 | 0.70* | 6.50* | 4550.00 | 575.00 | 475.00 |
| | Standard Error | 2.26 | 0.28 | 1.50 | 0.25 | 0.10 | 0.00 | 1050.00 | 75.00 | 225.00 |
| Yellow (11) | Mean | 29.94* | 2.36 | 24.68 | 3.50 | 1.13 | 8.77 | 4936.36 | 570.91 | 752.27 |
| | Standard Error | 1.13 | 0.13 | 1.47 | 0.20 | 0.25 | 0.46 | 228.14 | 17.29 | 52.05 |

*lowest value, bold is the highest value for the mineral content in mg/100g.

**Table No.5: Pair wise correlations coefficients for the mineral content of Coper (Cu), Iron (Fe), Manganese (Mn), Sodium (Na), Zinc (Zn), Potassium (K), Magnesium (Mg), and Calcium (Ca) in common beans**

| S.No | | Cu | Fe | Mn | Na | Zn | K | Mg | Ca |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Cu | | 0.000 | 0.003 | 0.927* | 0.000 | 0.007 | 0.006 | 0.017 |
| 2 | Fe | 0.785* | | 0.000 | 0.866* | 0.000 | 0.026 | 0.013 | 0.016 |
| 3 | Mn | 0.403 | 0.518* | | 0.091 | 0.003 | 0.016 | 0.039 | 0.000 |
| 4 | Na | 0.013 | -0.025[a] | 0.242 | | 0.422 | 0.199 | 0.381 | 0.720* |
| 5 | Zn | 0.699* | 0.764* | 0.407 | -0.116[a] | | 0.013 | 0.053 | 0.002 |
| 6 | K | 0.380 | 0.315 | 0.338 | 0.185 | 0.350 | | 0.000 | 0.000 |
| 7 | Mg | 0.381 | 0.350 | 0.291 | 0.127 | 0.275 | 0.674* | | 0.000 |
| 8 | Ca | 0.335 | 0.338 | 0.489 | -0.052[a] | 0.431 | 0.535* | 0.509* | |

*strong positive significant correlations, and [a]negative and significant correlations ($p < 0.05$)

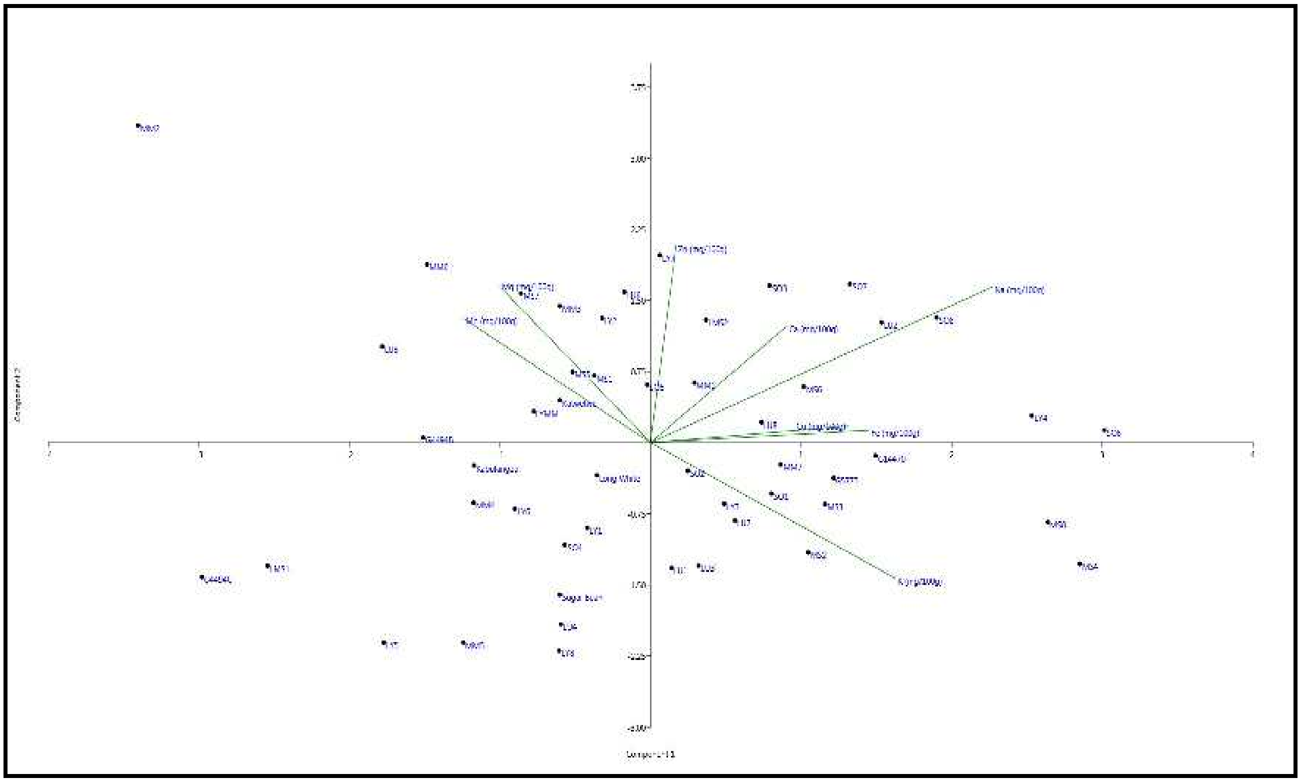

**Figure No.1: Representation of the Euclidean bi-plot by principal component analysis (PCA) with transformed data for all the variables in the analysis. Legend: Ca – calcium; Cu – Copper; Fe – Iron; K – Potassium; Mg – Magnesium; Mn- Manganese; Na – Sodium; and Zn – Zinc**

## CONCLUSION

The PCoA distributions result in this research supports the initial assumption that there is a wide natural variation in the concentrations of macro and micro elements among these common bean landraces from Zambia that can be exploited for common bean improvement programmes in Zambia, and or provide for a short term material exchange between Zambia and her neighbouring/regional countries for common bean breeding programmes in Southern and Eastern Africa. The identified sub-populations with high amount of iron and zinc present an opportunity to breed new varieties of common bean with enhanced iron and zinc content based on the available common bean germplasm within Zambia. Participatory common bean breeding of these identified sub-populations of landraces and commercial varieties within Zambia and the neighbouring countries and evaluating their progenies for iron and zinc contents remain the next course of action towards food and nutritional security using common beans.

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## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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