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Microsatellite Analysis of Common Bean (*Phaseolus vulgaris* L.) Genotypes in Tanzania for Diversity in Seed Iron and Zinc Micronutrients

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ABSTRACT

Common bean (*Phaseolus vulgaris* L.) grain has big dietary role in supplying protein, energy, vitamins, dietary fibre and micronutrients for millions of people worldwide. In Tanzania, reports have shown existence of Fe and Zn micronutrient variabilities among the common bean genotypes. Eighty-four (84) genotypes were collected to study such variability by seed biochemical and leaf molecular marker analysis. The analyses revealed significant ($p < 0.001$) genetic variability for seed Zn and Fe nutrient contents. Highest seed Fe content was 118 ppm for genotype *Kashiransoni* and for seed Zn was 51.81 ppm for *Imponzo* 5 genotype (both were collected from NPGRC). With seven (7) SSR primers associated with Fe and Zn traits, genetic diversity was evaluated. A marker BM154 scored PIC value 0.967 mean while marker BM160 had a lowest PIC score of 0.899. Using an estimated genetic similarity value, two main clusters with sub clusters in the dendrogram were developed. To corroborate the UPGMA analysis results, a Principal Coordinate Analysis (PCoA) was performed and displayed outputs into scattered plot presentation. For Fe micronutrient improvement purposes, Inula was proposed to be crossed with *Kashiransoni* while *Roba* with *Imponzo* 5 for Zn micronutrient improvement. Selection was based on both concentrations of nutrients for each genotype and their genetic similarity distances.

1.0 INTRODUCTION

Common bean (Phaseolus vulgaris L.) is one of the principal foods and cash crop legumes grown in the tropics and most of the production takes place in developing countries (Hillocks *et al.* 2006). Common bean grains have big dietary role in supplying protein, energy, dietary fiber and micronutrients for millions of people worldwide although its intake, in terms of nutrient content level, does not satisfy their nutrient requirements (Hillocks *et al.* 2006). Although common beans are grown largely by smallholder farmers for subsistence and mainly by women, about 40% of the total production from Africa is marketed (Wortmann *et al.* 1998). It is estimated that over 75% of rural households in Tanzania depend on common beans for daily sustenance (CIAT, 2008).

Micronutrients like zinc and iron are among the important nutrients required in small amounts in the diet to fulfill the nutritional needs of people. Both of these micronutrients are critical to human well-being and an adequate supply of iron and zinc helps to prevent iron deficiency which may lead to reduced oxygen transportation in blood, immune function and consequently to anemia (Blair *et al.* 2009). Zinc deficiency may lead to reduced immune function, fetal brain cell development and child's growth and cognitive development as well as other health problems of the developing world (Black, 2003). Zinc is also an anti-cancer element because a potential anti-oxidant. Identifying common bean with high level of these nutrients and integrating them in daily diet will help to improve the situation.

In Tanzania, Tryphone and Nchimbi-Msolla (2010) reported a large variability among common bean genotypes for Fe and Zn nutrient concentrations. Such variability is influenced by genotype environmental interaction and suggested improving climate adapted genotypes has greater potential than improving common bean based on the difference in content of Fe and Zn, for exploiting existing genetic diversity in Tanzania. Unfortunately, there is limited information on how the common bean genotypes being adopted in Tanzania vary genetically for iron and zinc. The knowledge of genetic diversity patterns increases the efficiency for conservation, utilization and genetic improvement of common beans (Beebe *et al.* 2000). Tremendous efforts have been made to delve deeper into the genetic diversity of these traits in several crop species, including common bean through the use of molecular markers (Talukder *et al.* 2010). Molecular markers used in estimating the genetic diversity and level of heterozygosity among plants and animals, thus have a great potential to help breeders develop new and improved varieties (Kumar et al., 2008). Therefore, this study will investigate the genetic diversity among germplasm collection of common bean to obtain suitable parental genotype (s) based on both genetic similarity distances and the nutrient levels of Fe and Zn.

2.0 MATERIALS AND METHOD

2.1 Location of the study

The study was conducted at Sokoine University of Agriculture (SUA) in Morogoro, Tanzania in the screen housed behind African seed building located at latitude 6.84795 S and 37.65904 E at 543 m above the sea level. The study was conducted for the period of December 2017 to October 2018.

2.2 Genotypes

Eighty-four (84) common bean genotypes seed were collected from National Plant Genetic Resource Center (NPGRC) at Arusha, Uyole National Research Institute (UNRI) at Mbeya and SUA at Morogoro. The seed was stored in a cold room (12˚C) until planting at Sokoine University of Agriculture, Department of Crop Science and Horticulture. Genotypes collected were diverse, representing a range of seed types, ranging from different seed coat colors, size and shape. During accessioning, there were no specific and strict criteria in choosing a genotype to collect. The collected accessions are as indicated in Table 1.

Table 1: Common bean (*Phaseolus vulgarism* L.) genotype accessions from different regions of Tanzania

S/N	Accession number	Genotype	Classification	Seed Zn (ppm)	Seed Fe (ppm)	Origin
1	SUA222	Beti 10	Improved	34.37m-x	43.3d	Morogoro
2	NPGRC2154	Biliomunyungu	Landrace	34.65n-y	53.56l-p	Kagera
3	SUA501	Cal 143	Improved	31.1h-s	58.48r-u	Morogoro
4	UYL5011	CalimaUyole	Improved	36.69o-B	54.8m-q	Mbeya
5	SUA180	Canadian Wonder	Improved	34.17m-x	66.12A-E	Morogoro
6	SUA6301	Cheupe	Improved	43B-I	64.61z-B	Morogoro
7	NPGRC 286	Chilanda 6	Landrace	27.94d-m	53.04j-o	Rukwa
8	NPGRC 287	Chilanda 7	Landrace	34.1m-x	71.29G-I	Rukwa
9	NPGRC 134	Chilemba 4	Landrace	33.97m-x	44.29de	Rukwa
10	NPGRC 135	Chilemba 5	Landrace	31.86i-t	58.77s-v	Rukwa
11	NPGRC 306	Chilemba 6	Landrace	26.9c-j	61.68w-y	Rukwa
12	NPGRC 307	Chilemba 7	Landrace	25.75b-i	84.08PQ	Rukwa
13	NPGRC 133	Chilemba3	Landrace	42.91A-I	75.02K-M	Rukwa
14	NPGRC 401	Fibea	Improved	35.66o-z	57.55q-t	Morogoro
15	NPGRC 4312	FukamaOkole	Landrace	41.73z-I	53.26k-o	Kagera
16	NPGRC 3153	Gwezamenyo	Landrace	38.77u-E	61.7w-y	Kigoma
17	NPGRC 147	Ilanda / Kalinso	Landrace	38.12t-D	69.76F-H	Rukwa
18	NPGRC 334	Imponzo 4	Landrace	37.89t-D	64.13y-A	Mbeya
19	NPGRC 335	Imponzo 5	Landrace	47.89IJ	66.72A-E	Mbeya
20	NPGRC 337	Imponzo 7	Landrace	51.81J	72.4H-K	Mbeya
21	NPGRC 331	Imponzo1	Landrace	46.08G-H	55n-q	Mbeya
22	NPGRC 188	Imponzo8	Landrace	27.29d-l	90.57R	Mbeya
23	NPGRC 198	Imponzo9	Landrace	43.16C-I	76.57LM	Mbeya
24	NPGRC 4258	Inula	Landrace	20.5ab	32.36a	Kagera
25	SUA10	Jesca	Improved	25.97b-i	66.64A-E	Morogoro
26	NPGRC 70	Kablanketi	Landrace	30.84h-r	56.57q-t	Rukwa
27	NPGRC 3157	Kalambi	Landrace	42.71A-I	107.29T	Kigoma
28	NPGRC 2158	Kanyamunywa	Landrace	39.26v-F	52.46i-m	Kagera
29	NPGRC 3175	Kashiransoni	Landrace	45.93G-I	118.24U	Kigoma
30	NPGRC 69	Kasukanywele	Landrace	25.57b-i	65.05z-D	Rukwa
31	NPGRC 2190	Kibeho	Landrace	24.03a-f	68.83E-G	Kagera
32	SUA1400	Kigoma	Improved	32.45j-u	48.22fg	Morogoro
33	NPGRC 4336	Kiisiki	Landrace	24.72b-h	66.44A-E	Kagera
34	NPGRC 4265	Kisapuli	Landrace	28.89d-n	50.21g-j	Kagera
35	NPGRC 4259	Kya Karagwe	Landrace	34.77n-y	52.82j-o	Kagera
36	SUA444	Lyamungo 90	Improved	35.9n-z	65.94A-E	Morogoro
37	SUA333	Lyamungu 85	Improved	24.19a-g	55.7o-r	Morogoro
38	NPGRC 3511	Maharage - kienyeji	Landrace	23.9a-e	49.74g-i	Kigoma
39	NPGRC 3816	Maharage karanga	Landrace	33.53k-w	36.92b	Kigoma
40	SAU1300	Maini	Improved	34.36m-x	46.71ef	Morogoro
41	NPGRC 218	Malima / Ndondo	Landrace	44.78E-I	67.79C-F	Mbeya
42	NPGRC 4269	Maliwalinda	Landrace	32.43j-u	93.63S	Kagera
43	NPGRC 3164	Mamesa	Landrace	30.68h-q	74.1J-L	Kigoma
44	NPGRC 3141	Mbuvamutwe	Landrace	34.04m-x	76.37LM	Kigoma
45	SUA808	Mkanamna	Improved	37.11q-C	68.37EF	Morogoro
46	SUA601	Msafiri	Improved	22.67a-d	52.02h-m	Morogoro
47	SUA1003	Mshindi	Improved	41.86z-I	59.25t-w	Morogoro
48	SUA16	Msolin	Improved	40.04w-G	50.86g-l	Morogoro
49	NPGRC 3120	Mulembegwa	Landrace	34.64n-y	85.44Q	Kigoma
50	NPGRC 3150	Mutsinga	Landrace	40.26x-G	67.91D-F	Kigoma
51	NPGRC 2178	Mwanamwana	Landrace	35.97o-z	81.93OP	Kagera
52	NPGRC 3155	Mwanja	Landrace	45.44F-I	66.22A-E	Kigoma
53	NPGRC 3119	Mwolo -yellow	Landrace	41.96z-I	62.93x-z	Kigoma

54	SUA909	Nanavala	Improved	39.94w-G	49.77g-i	Morogoro
55	SUA800	Nanka	Improved	31.03h-s	56.25p-s	Morogoro
56	NPGRC 2213	Ndimila enkobe	Landrace	37.52s-D	88.09R	Kagera
57	NPGRC 3005	Njano ndefu	Landrace	36.52o-A	66.82A-F	Kigoma
58	UYL5010	Njano Uyole	Improved	41y-H	50.13g-j	Mbeya
59	NPGRC 3154	Nyamanza	Landrace	34.88n-y	73.49l-k	Kigoma
60	UYL5015	Nyeupe Uyole	Improved	33.16j-v	67.98D-E	Mbeya
61	UYL5018	Pasi	Improved	25.36b-h	52.99j-o	Mbeya
62	SAU1007	Pesa	Improved	33.35k-v	56.75q-t	Morogoro
63	SUA200	Roba	Improved	18.23a	58.7s-v	Morogoro
64	SUA1009	Rojo	Improved	37.42s-D	77.76MN	Morogoro
65	NPGRC 4248	Ruhondela	Landrace	28.5d-n	50.33g-k	Kagera
66	NPGRC 2220	Rukurulana	Landrace	34.06m-x	80.1NO	Kagera
67	NPGRC 4352	Ruvunja	Landrace	34.83n-y	67.18B-F	Kagera
68	SAU500	Selian 05	Improved	37p-C	64.86z-C	Morogoro
69	SUA777	Selian 06	Improved	30.28f-o	61.54v-y	Morogoro
70	SUA11	Selian 94	Improved	36.67o-B	48.23fg	Morogoro
71	NPGRC 3156	Seredi	Landrace	45.4F-I	51.89h-m	Kigoma
72	NPGRC 4221	Shona	Landrace	43.58D-I	76.88LM	Kagera
73	NPGRC 4322	Shona egunia	Landrace	29.15e-n	48.01fg	Kagera
74	NPGRC 111	Soya nano	Landrace	23.96a-f	60.7u-x	Morogoro
75	SUA1010	Sua 90	Improved	33.64k-w	49.15f-h	Morogoro
76	NPGRC 1604	Tichakuronza	Landrace	47.23H-J	72.14H-J	Kagera
77	NPGRC 3182	Ugweza	Landrace	33.68l-w	40.15c	Kigoma
78	UYL5017	Uyole 03	Improved	27.26d-k	57.3q-t	Mbeya
79	UYL5016	Uyole 04	Improved	37.27r-D	67.85C-F	Mbeya
80	UYL5012	Uyole 16	Improved	37.15q-C	67.26B-F	Mbeya
81	UYL5020	Uyole 94	Improved	33.21j-v	60.81u-x	Mbeya
82	UYL5013	Uyole 96	Improved	25.63b-i	38.35bc	Mbeya
83	UYL5009	Uyole84	Improved	20.68a-c	34.37a	Mbeya
84	SUA1001	Zawadi	Improved	30.47g-p	53.37l-o	Morogoro
		Mean		34.44	62.533	
		SEM		1.845	0.898	
		%CV		9.3	2.5	
		P-value		<.001	<.001	

Key: *UYL – Uyole, SUA - Sokoine University of Agriculture, NPGRC- National Plant Genetic Resource Center, SEM-Standard error of means and %CV- Percentage coefficient of variation*

2.3 Soil sampling and chemical analysis

Composite soil samples obtained were analysed as described by Carter (1993). Bulk soil samples were taken at a depth of 0 - 20 cm on an area of 2 × 2 m^2. Composite soil constituted nine sub-samples randomly collected from forestry area covering 1.0 ha. For pot experimentation, sub-samples were thoroughly mixed, sterilized, air dried and ground to pass through an 8.0 mm mesh. Further preparations for soil analysis were carried whereby, the 2.0 mm sieved composite soil samples were used for laboratory physical and chemical analyses. Composite soil samples obtained were analysed for pH, cation exchange capacity, exchangeable bases (Ca, K, Mg and Na), micronutrients (Fe, Zn, Mn and Cu), Nitrogen (N), available phosphorus, particle size distribution and organic carbon (OC) as described by Carter (1993). The soil pH was determined in water at a soil: water ratio of 1:2.5 suspension using pH meter. Electrical conductivity was measured in 1:2.5 soil: water using the electric conductivity meter (Thomas, 1996). Cation exchange capacity (CEC) was determined by the ammonium-acetate saturation method and quantification of exchangeable bases: K, Ca, Na and Mg were determined from the ammonium-acetate filtrates following the Lindsay and Norvell (1978) methods. Exchangeable calcium (Ca) and magnesium (Mg) were determined by atomic absorption spectrophotometry whereas K and Na were extracted using ammonium acetate and analysed by flame spectrophotometry. The DTPA extractable Cu, Fe, Mn and Zn were determined by atomic absorption spectrophotometry (Lindsay and Norvell, 1978). Total nitrogen was determined by the micro-Kjeldahl digestion distillation method (Bremner and Mulvaney, 1982). Soil extractable P was determined by using the Bray-1-P method by Kuo, (1996) and colour was

developed by the ascorbic acid-molybdate blue method (Murphy and Rilley, 1962). Particle size analysis was determined by the hydrometer method after dispersing the soil samples with sodium hexametaphosphate solution (Gee and Bauder, 1986). Soil textural classes were determined using the USDA textural class triangle (USDA, 1975).

2.4 Screen house experimentation and agronomical practices

Eighty-four (84) genotypes were arranged in a completely randomized design (CRD) and replicated three times. Before sowing, the 4 kg potted soil was watered and allowed to stay for one day. Four seeds were sown; thinning was done at age of 10 days after emergence. Irrigation by re-introducing trapped water (infiltrates) on bottomed trays was carried out regularly to maintain the moisture content.

2.5 Chemical analysis for iron and zinc nutrients in common bean seeds

After harvesting, bean seeds from each pod of individual plant were mixed thoroughly and taken for chemical analysis. Dry ash method of extraction was undertaken to determine iron and zinc content (Jorhem, 1993). Digested plant samples were taken for reading absorbance values using the Atomic Absorption Spectrophotometer (AAS) method. A standard curve was computed and used to determine the concentration of each sample using Microsoft excel program (version 2016).

2.6 Genomic DNA extraction

Total genomic DNA was extracted from a trifoliate leaf tissue sample of 14 days old plants by using the Quick-DNA Plant/Seed Mini-Prep Kit protocols. DNA quality and quantity were assessed on gel electrophoresis stained with safe view DNA loading dye (EZ-vision) EZ vision. Seven simple sequence repeat (SSR) markers were used to detect the polymorphisms and assess genetic diversity of 84 common bean genotypes collected from two organizations, with several sources of their origin and marker diversity as shown in Table 1and 3 respectively.

2.7 SSR markers and PCR amplification

SSR primer pairs were selected on the basis of the published genetic diversity analysis on common bean by (Talukder *et al.* 2010). Seven microsatellite primers with high polymorphism levels (≥0.5) were selected and used for final polymerase chain reaction (PCR) amplification (Table 10) for investigating the genetic diversity on Fe and Zn micronutrients in the dry bean accession. The DNA amplification was carried out according Talukder et al. (2010) protocol with minor modifications. A final volume of 25 µl for the PCR reaction mix included 1.5 µl genomic DNA, 0.1 µl of forward and reverse primers, 12.5 µl of 2X× TaqMaster mix and 10 µl of nucleotide free sterile double distilled water. The temperature profile in the thermocycler was of one cycle of 95 0C for 3 min; 40 cycles of 95 ^{0}C for 1 min, from 55 to 57 ^{0}C for 1 min, and 72 ^{0}C for 2 min; and one cycle of 72 ^{0}C for 10 min.

2.8 Electrophoretic separation and visualization of amplicons

Superfine agarose (2 %) gel preparation involved the addition of 150 ml of chilled 1x TBE buffer (pH 8.0) to a flask with 3g of superfine agarose powder. Twenty microlitres (20µl) of safe view DNA loading dye (EZ-vision) was added to the gel and cast on a tray to set at room temperature. A gel was then carefully placed in a tank with plenty of 1x TBE buffer and into each well, 3 µl of the samples were carefully loaded with the first well having 1 µl of DNA ladder (100 bp). Gel electrophoresis was run for 200 min at 120 volts. The bands were visualized under an ultra violet trans-illuminator and photo-shopped using the Picasa software.

2.9 Data analysis

2.9.1 Chemical analysis

Obtained absorbance values were processed using MS excel (2018) to determine the concentrations through R square value by establishment of standard curve. The treatment effects were analyzed by analysis of variance (ANOVA) using GenStat V. 25 software. In additional, Fisher’s least significant difference (LSD) was used for mean separation at 5% level of probability.

2.9.2 Genetic diversity

Gel images obtained after electrophoresis were processed using IMAGE J software to determine the molecular weight of the SSR marker bands in reference to the loaded DNA ladder. The determined molecular weights of the SSR marker bands were analyzed by POWERMARKER V.3.25 for estimating the mean number of observable alleles (Na), major allele frequency, allele frequency, observed heterozygosity, expected heterozygosity, gene diversity and the polymorphic information content (PIC).

2.9.3 Cluster analysis

Genetic diversity analyses were conducted using numerical taxonomy and the multivariate analysis system, NTSYSpc V. 2.2. Genetic similarity values were computed between all possible pairs with the SIMQUAL option and ordered in a similarity matrix. The similarity matrix was run by sequential, agglomerative,

hierarchical, nested (SAHN) clustering with the unweighted pair group with arithmetic averaging (UPGMA) method as an option. The dendrogram and cluster groupings were constructed by the UPGMA clustering algorithm from the SAHN option of NTSYSpc v.2.2.

2.9.4 Principal coordinate analysis

To corroborate the interrelationships between the common bean genotypes, a scatter plot representation was built using principal coordinate analysis (PCoA) with the Nei's similarity coefficient. The analyses were performed using GenAlEx statistical software V 6.5 (Peakall and Smouse, 2012).

3.0 RESULTS

3.1 Soil chemical analysis

The experimental forestry soils had medium to high chemical and sandy clay loam textural class as physical characteristics (Table 2). The analysed composed forestry soil based on the selected soil parameters, showed optimal condition that favour growth of common bean as described by Landon, (1991). Therefore, the forestry soils were suitable for production of common beans and other field crops like cereals.

Table 2: Physical-chemical properties of the experimental forestry soils

Soil parameter	Values	Remark (Landon, 1991).
pH in water	6.79	Neutral
Electrical Conductivity (EC) (µS /cm)	451	Medium
Cationic Exchange Capacity (CEC)	32.6	High
Organic Carbon (% OC)	2.62	High
Organic matter (% OM)	4.52	High
Nitrogen (%)	0.50	Medium
Carbon: Nitrogen ratio	9.04	Good quality of the OM
Phosphorous ($mgkg^{-1}$)	9.33	Medium
Extractable K (Cmol (+) kg^{-1})	1.90	High
Extractable Na (Cmol (+) kg^{-1})	0.14	Low
Extractable Mg (Cmol (+) kg^{-1})	0.41	Low
Extractable Ca (Cmol (+) kg-1)	16.85	High
DTPA Extractable micronutrients (mg kg-1)		
Fe	34.96	High
Zn	4.08	High
Particle size analysis (PSA)		
%Clay	33.56	
%Silt	9.64	
%Sand	56.8	
Textural class	Sandy clay loam (USDA, 1975)	

3.2 Chemical analysis for iron and zinc contents on common bean seeds

The iron content in seed for collected genotypes of Common bean differed significantly (P<0.001) (Table 1). It varied from 32.36 to 118.24 ppm, with a mean of 62.533ppm. Genotypes of *Kashiransoni, Kalambi* and *Maliwalinda* were observed with the highest seed iron (Fe) contents of 118.24 ppm, 107.29 ppm and 93.63 ppm respectively. On the other hand, 32.36 ppm, 34.37 ppm and 36.92 ppm were the lowest contents of iron observed in genotypes of *Inula, Uyole84* and *Maharage-karanga* respectively. The genotypes differed significantly (P<0.001) in seed zinc contents (Table 1). Values varied from 18.23 to 51.81 ppm with a mean of 34.44 ppm. Genotypes *Imponzo5, Imponzo7* and *Tichakuronza* were observed with the highest seed zinc (Zn) contents of 51.81 ppm, 47.89 ppm and 47.23 ppm respectively meanwhile 18.23 ppm, 20.5 ppm and 20.68 ppm were the lowest contents of zinc observed in genotypes *Roba, Inula* and *Uyole 84* respectively.

3.3 Allelic diversity of common bean accessions

The key parameters used to define genetic diversity among the common bean accessions from Tanzania are presented in Table 3. There was a high polymorphism with a mean of 24.857 alleles per locus and a range of 17 to 40 alleles in the germplasm. The frequency for the major allele ranged from 0.064 for primer BM154 to 0.208 for the primer BMd16 with mean of 0.139. In total, the 7 markers detected 174 alleles and PIC ranging from 0.892 for the primer BMd16 to 0.967 for the primer BM154 with the mean of 0.923. The overall mean for the expected heterozygosity ranged from 0.899 for BMd16 primer to 0.967 for BM154 primer. The observed heterozygosity was

observed on the two primers of BM160 and BM181 with value 0.025 and 0.027 respectively.

Table 3: Genetic information for the markers used to detect the genetic diversity of 84 Common bean genotypes collected from different regions of Tanzania for the Fe and Zn traits.

S/N	Marker	Sample amplified	Major allele frequency	Allele number	Gene Diversity	He	Ho	PIC
1	BM154	78	0.064	40	0.929	0.967	0.000	0.967
2	BM160	79	0.120	24	0.940	0.939	0.025	0.936
3	BM170	75	0.107	24	0.937	0.937	0.000	0.934
4	BM181	73	0.192	23	0.901	0.900	0.027	0.893
5	BM211	68	0.147	23	0.922	0.921	0.000	0.917
6	BMd16	72	0.208	17	0.899	0.899	0.000	0.892
7	BMd33	66	0.136	23	0.927	0.927	0.000	0.922
Mean			0.139	24.86	0.922	0.927	0.007	0.923

3.4 Cluster analysis

For a better understanding of the genetic relationship among common bean genotypes, the genetic similarity (GS) values were submitted to hierarchical clustering by UPGMA. The dendrogram based on Nei's coefficient of genetic distance suggested the existence of two clusters with sub clusters (Figure 1). The Cluster I was further subdivided into two sub clusters of A and B in total comprised of 31 and 2 genotypes of common bean respectively. Cluster II was also divided into two sub cluster designated as A and B with a total of 28 and 23 genotypes respectively (Table 4).

Table 4: Distribution of genotypes to different clusters based on the UPGMA methods

Cluster	Sub cluster	Number of genotypes	Name of genotypes
I	A	31	Jesca, Kibego, Uyole 90, Sua 90, Kanyamunywa, Mshindi, NyeupeUyole, Uyole 94, Maharage-kienyeji, Zawadi, Shona-Egunia, Chilemba 4, Uyole 03, Rojo, Rukurulana, Kasukanywele, Chilemba 7, Biliomunyungu, Mwolo-Yellow, Maini, Msolin, Imponzo 9, Imponzo 1, Malima/Ndondo, Calima-Uyole, Fibea, Pasi, Msafiri, Kigoma, NjanoUyole and Njanondefu
	B	2	Kablanketi and Uyole 04
II	A	28	Selian 94, Ruvunja, FukamaOkole, Ilanda/kalinso, Canadian wonder, Uyole 16, Maharagekaranga, Lyamungu 85, Cal 143, Inula, Ruhondela, Chilemba 6, Imponzo 8, Kiisiki, Ugweza, Imponzo 7, NdimilaEnkobe, Nyamanzi, Chilemba 3, Mwanamwana, Maliwalinda, Mamesa, Mbuvamutwe, Mwanja, Mulembegwa, Seredi and Kashiransoni
	B	23	Soya Njano, Beti 10, Nanka, Roba, Lyamungu 90, Imponzo 5, Chilanda 7, Selian 06, Tichakuronza, Uyole 84, Mkanamna, Mutsinga, Shona, Selian 05, Kalambi, Chilemba 5, Chilanda 6, Kyakaragwe, Cheupe, Gwezamenyo, Nanayala, Pesa and Kisapuli.

3.5 Principal co-ordinate analysis

The common bean genotypes were represented by a scatter plot in 2-dimensions using the results of the principal coordinate analysis (PCoA) obtained by estimating the Nei's genetic similarity distance. It revealed the global structure similar to the dendrogram analysis, but the distribution of these accessions was shown more clearly in scatter plot (Figure 2). Overall, the clustering pattern of the genotypes in the principal coordinates analysis corresponds with the dendrogram derived from UPGMA (Figure 1).

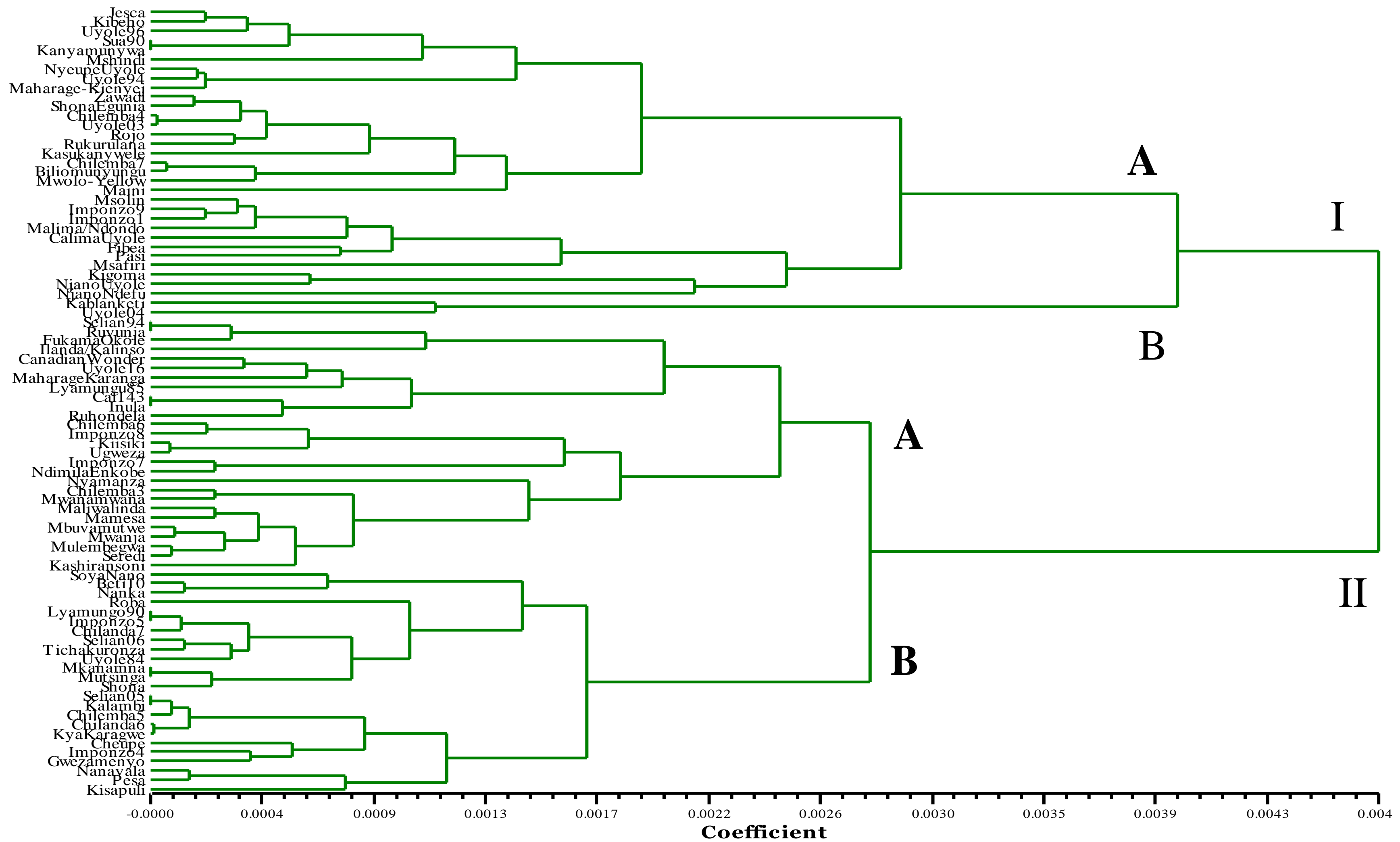

Figure 1: Dendrogram representing the genetic diversity among the 84 accessions of Common bean of Tanzania based on Dice coefficient of similarity matrix data using UPGMA cluster analysis

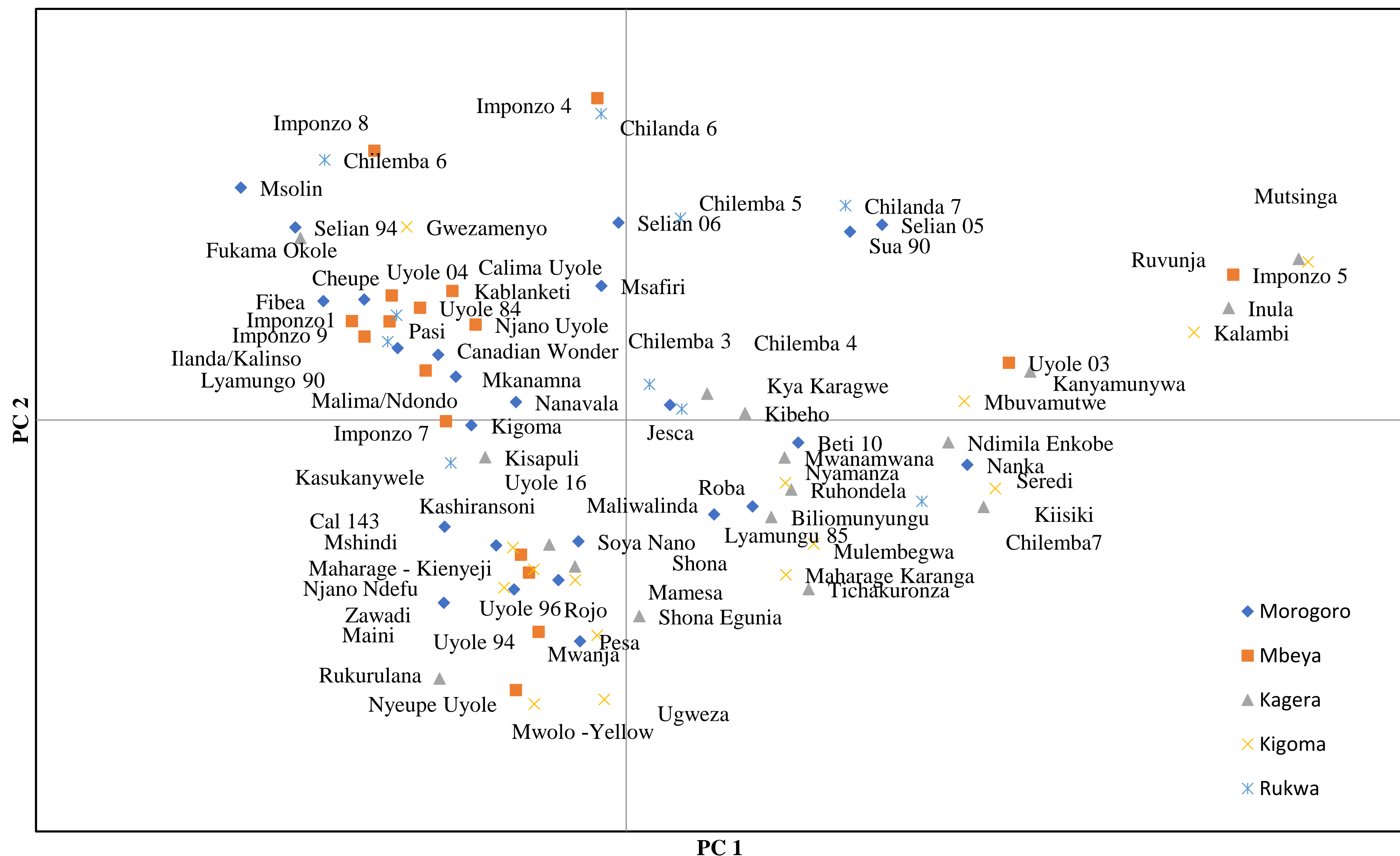

Figure 2: Principal coordinate analysis (PCoA) of common bean accessions from the microsatellite diversity based on molecular weight (band sizes) of alleles.

4.0 DISCUSSION

4.1 Iron and zinc contents in common bean

The high seed Fe and Zn content were expressed with genotypes of *Kashiransoni* and *Imponzo5*. Both genotypes were sourced at the gene bank of Tanzania (NPGRC) being collected from Kigoma (*Kashiransoni*) and Mbeya (*Imponzo 5*) regions. There was no environmental contribution to the significantly ($p<0.001$) variability of both seed Fe and Zn content on the experimented genotypes of common bean because the study was conducted in screen-house conditions hence differences in their genetic makeup could have resulted into such variability.

4.2 Genetic diversity of common bean genotypes

The employed UPGMA clustering method divided the common bean genotypes into two main clusters. The main two branches of the dendrogram have showed a considerable range of genetic diversity of genotypes in respect to the genetic similarity distance being less than 9e-04, suggesting existence of low genetic diversity based on Fe and Zn traits. This existence could be due to either the genotypes in equation comes from the similar origin or the SSR markers used could not clearly detect large range of diversity. Similar results for the diversity of common bean genotypes for iron and zinc nutrients of Tanzania corresponds with numerous and deliberate efforts done by other researchers worldwide by using various molecular techniques including SSR marker. Talukder *et al*. (2010) observed low level of genetic diversity because since the GS for the common bean genotypes was more than 50%, hence suggesting low genetic variability. Low genetic variability between the two main clusters does hinder selection of parental genotypes for cross breeding programs due to significant ($p<0.001$) difference in variabilities of seed Fe and Zn content. Thus, it can be expected that the introgression of a desired trait would be easier for this cross without sacrificing valuable trait(s) and/or adding unwanted trait(s) as a consequence of linkage drag (Talukder *et al*. 2010).

4.3 Selection of genotypes for breeding programs

Selection of genotypes for breeding purpose based on micronutrient variability and genetic diversity has been suggested in many crop species including common bean by Talukder *et al*. (2010) and rice (Gregorio, 2000). From the current results of genetic diversity analysis, several breeding strategies can be derived; however, the interest at present is to develop populations to map Zn and Fe content traits and to breed common bean cultivars with enhanced Zn and Fe contents.

Based on seed Iron (Fe) and Zinc (Zn) contents of the genotypes and genotypic variability at molecular level, the cross-breeding program for seed Fe content was designed to cross the genotypes with high Fe content namely *Kashiransoni, Kalambi* and *Maliwalind*a while low Fe content genotypes were Inula, Uyole84 and *Maharage-karanga*. The high Fe contents genotypes *Kashiransoni, Kalambi* and *Maliwalinda* belonged to clusters II-A, II-B and II-A respectively and those of low Fe contents viz. *Inula*, Uyole 84 and *Maharage-karanga* belonged to clusters; II-A, II-B and II-A respectively. The genetic similarity distance values of genotypes Inula with *Kashiransoni, Kalambi* and *Maliwalinda* are $1.704e^{-4}$, 0 and $1.0116e^{-3}$ respectively and those of Uyole 84 with *Kashiransoni, Kalambi* and *Maliwalinda* are $3.91e^{-3}$, $3.31e^{-3}$ and $3.20e^{-3}$ respectively and those of *Maharage-karanga* with *Kashiransoni, Kalambi* and *Maliwalinda* are $8.89e^{-4}$, $2.79e^{-3}$ and $1.03e^{-3}$ respectively. These values indicate that the selected genotypes are divergent at the molecular level with significant differences in seed Fe content and could result in better segregation and recombination of the desired alleles in successive generations during population development.

For the crossing program to enhance seed Zn content, genotype *Imponzo 5* with high Zn content (ppm) was selected against the genotypes of *Roba* and Inula with low zinc contents. The high Zn content genotype of *Imponzo 5* belonged to clusters II-A and the low Zn content genotypes of *Roba* and *Inula* belonged to clusters II-B and II-A respectively. The genetic similarity distances value of genotype *Roba* with *Imponzo 5* was $8.72e^{-4}$ and that of Inula with *Imponzo 5* was $5.28e^{-4}$. These values indicate that the selected genotypes are divergent at the molecular level with significant differences in seed Zn content and could result in better segregation and recombination of the desired alleles in successive generations during population development. Crossing combinations with genotypes between and within cluster having high genetic diversity and mineral content would be expected to accumulate positive alleles derived from unique sources and generate breeding lines with even higher seed mineral contents.

Also, crossing of the selected genotypes would be of significant important for genetic studies because they're contrasting for the observed traits. Genetic studies offer an opportunity of utilizing the genetic information like inheritance patterns of Fe and Zn, additive effect and dominance effects of gene can be determined as knowledge of heritability is crucial for decisions making concerning screening and breeding methodologies and the scale of breeding.

5.0 CONCLUSION

The variability in iron and zinc content was significant ($p<0.001$) across all experimented genotypes. The range of seed iron content was 32.36 for Inula to 118.24 ppm for *Kashiransoni* genotype with mean of 62.533 ppm. For seed zinc content it ranged from 18.23 ppm for *Roba* to 51.81 ppm for *Imponzo 5* with a mean of 34.44 ppm. The SSR primers used in this study showed to be effective on detecting the alleles bonded to the trait of iron and zinc. The high PIC (0.967) value was shown by BM154 primer which detected 40 alleles for 78 samples of genotypes that were amplified. The overall results obtained by SSR analysis of the common bean genotypes in the present study has shown that there is genetic divergence among the collected genotypes based on the traits. The UPGMA analysis together with the scattered plot presentation (PCoA) has successfully grouped the genotypes into sense of similarity by using the estimated genetic similarity distance. It is recommended that the genotypes *Kashiransoni* and Inula for iron and *Roba* and *Imponzo 5* for zinc as breeding materials as sources Fe and Zn. Introgression of gene for Fe and Zn enrichment should rely on consumer preferred common bean genotypes as a key group during participatory plant breeding (PPB) programs. Further, the inheritance patterns of Fe and Zn traits has to be studied since the knowledge of heritability is crucial for decisions making concerning screening and breeding methodologies and the scale of breeding.

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