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Multivariate analysis of nutritional diversity of selected macro and micro nutrients in pearl millet (*Pennisetum glaucum*) varieties

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Analysis on economically viable indigenous food cereals like pearl millet as alternative strategies to curb under nutrition and boost food security is of utmost importance to widen the essential nutrient sources for human beings. To contribute to this area, macro and micro nutrient analysis was carried out on 60 pearl millet genotypes. On each of the genotype, 7 biochemical parameters (starch, amylose, amylopectin, protein, K, Zn and P) were analyzed. Starch content of the genotypes ranged from 27 - 46.7% with a mean of 34.2%, while most of the genotypes had more amylopectin than amylose with exceptions of a few varieties with a ratio of 2:1. The protein content had a range of 4.6 - 9.9% with a mean of 7.1%. Zinc was among the highest level followed by phosphorous and finally potassium. The principal component analysis (PCA) showed that the first four PCA contributed to 79.8% of the variability among the pearl millet varieties. Cluster analysis grouped data into 6 clusters and a singleton with a genetic distance 0.37 – 8.73 showing great variability. Biochemical traits are useful tool for determining genetic variability in pearl millet and can contribute to breeding programs and enhance food security.

Key words: Nutritional contents, food security, breeding, principal component analysis, genetic distance, cluster analysis.

INTRODUCTION

Millet has been cultivated since the pre-historic ages in areas of North Africa and Central Asia. The whole grain is used in soups, stews or as a cooked cereal. Millet can also be popped; roasted or sprouted (Ronzio, 2004). Africa was the largest producer of millet in 2009 (20.6 million metric tonne), followed by Asia 12.4 million metric tons and India 10.5 million metric tons (FAO, 2009). Pearl millet is one of the most extensively cultivated cereals in the world, after rice, wheat and sorghum, and particularly in arid to semi-arid regions. Pearl millet is so important

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that it is estimated to be planted on around 14 million hectares in Africa and 14 million hectares in Asia (FAO, 2009).

Pearl millet (*Pennisetum typhoideum*) is the most widely grown type of millet because of its tolerance to difficult growing conditions such as drought, low soil fertility and high temperature, areas where other cereal crops, such as maize (*Zea mays*) or wheat (*Triticum aestivum*), would not survive (Maqbool et al., 2001). It is widely grown as a multi-purpose cereal grain crop principally for food, and also for feed, fodder, fuel and mulch on more than 26 million hectares, primarily in arid and semi-arid regions of India and Africa (FAO, 2000).

Indigenous foods like pearl millet are rich and inexpensive sources of protein, carbohydrates, dietary fibre, minerals and vitamins to millions of peoples in developed and developing countries, and are some of the basic foods of the indigenous populations of Africa (Luthria and Pastor-Corrales, 2006). Nutritionally, pearl millet is comparable and even superior to major cereals with respect to energy value, proteins, fat and minerals. It makes an important contribution to human diet due to high levels of calcium, iron, zinc, lipids and high quality proteins. Besides, it is also a rich source of dietary fiber and micro nutrients (Anu Sehgal and Kwatra, 2006; Malik et al., 2002). Carbohydrate components of pearl millet grains comprise of starch, dietary fiber and soluble sugars. Starch which consists of glucose in form of amylose and amylopectin is a predominant component of pearl millet endosperm. Pearl millet grains are all very high in calories- precisely the reason they do wonders for growing children and pregnant women (www.icrisat.org).

Micronutrient malnutrition can be defined as deficiency in one or more vitamins and minerals of importance for human health. It is an outcome of inappropriate dietary composition and disease (Nube and Voortman, 2006). Dietary micronutrient deficiencies affect a large part of the global population.

The World Health Organization estimates that globally some two billion people are affected by iron deficiency and that some 750 million people suffer from iodine deficiency (WHO, 2006; Unicef, 2006). Also, zinc deficiency is increasingly recognized as an important public health problem (Ramakrishnan, 2002; Black, 2003a, b). First, among poor populations, overall food intakes are often below minimum requirements and as a result not only the intake of macronutrients (carbohydrate, fat, protein), but also the consumption of micronutrients (minerals, trace elements and vitamins) can be inadequate (Nube and Voortman, 2006).

More than two billion people are reported to be iron deficient, which makes iron deficiency the most widespread human micronutrient deficiency in the world (Rengel et al., 1999). In recent years, interest in the occurrence of human zinc deficiency, in particular among children, has been growing strongly (Hotz and Brown, 2004).

Evaluating genetic diversity of germplasm can assist in differentiating varieties with the greatest novelty which as a result, is most desirable for the incorporation into crop improvement programs. Genetic diversity refers to the variation of heritable characteristics present among alleles of genes in different individuals of populations of species that serves as an important role in evolution by allowing a species to adapt to a new environment (Weir, 1996; Kremer et al., 1998).

The estimation of genetic distance using phenotypic and/or molecular markers can help determine suitable germplasm for incorporation into future plant breeding programs. Thus, assessment of genetic diversity in pearl millet germplasm and determination of their phenotypic and biochemical activities would help to know the breeding potential of a particular variety.

Quantitative assessment of genetic diversity is significantly important to determine the extent of genetic differences between and within crop species (Adugna, 2002). Genetic distances are measures of the average genetic divergence between two sequences, species or between populations within a species or taxa (Souza and Sorrells, 1991). Genetic similarity is the converse of genetic distances, that is, the extent of gene similarities among cultivars.

Genetically diverse parents produce high heterotic effects and yield desirable segregates. The pattern of genetic relationships between and within accessions can be shown by multivariate analyses. Cluster analysis on the other hand is a useful statistical tool for studying the relationships among closely related accessions. Therefore, the objective of this study was to evaluate and identify the quantities of particular macro and micro nutrients of pearl millet varieties and their suitability in food security and breeding.

MATERIALS AND METHODS

Sample preparation

The accessions evaluated were collection of open pollinated varieties (OPVs), commercially released varieties in East and Central Africa, local varieties and hybrids. These 60 pearl millet varieties sourced from ICRISAT, Kenya were grown in two sites Marigat (KARI –Perkerra) and Koibatek (Agricultural Training Centre, ATC-Koibatek) in Central Kenya region for two seasons. ATC- Koibatek lies at latitude 1° 35' S, and longitude 36° 66' E, altitude 1890 m a.s.l. in agro-ecological zone UM4, with low agricultural potential.

Average annual rainfall is 767 mm; mean annual minimum and maximum temperature are 10.9 and 28.8°C, respectively. KARI Perkerra-Marigat lies at a latitude of 1°45´ N and longitude 36°15´ E with an altitude 1067 m.a.s.l. The centre is situated in agro ecological zone 5 (LM5), soils are volcanic fluvisols of sandy/silty clay loam texture, slightly acidic to slightly alkaline. Annual rainfall mean is 654 mm. Mean annual minimum and maximum, temperatures are 32.4 and 16.8°C, respectively, and under field evaluation, the yield range from 3482 -1305 kg ha^{-1}. These varieties were powdered and analyzed in duplicate in their biochemical characteristics.

Determination of protein content

Finely milled pearl millet grain of 0.1 g were weighed and transferred into a digestion tube. Selenium catalyst mixture weighing 1 g was mixed with the samples and 5 ml of concentrated sulphuric acid (96%) was added into the tube. The tubes were then heated cautiously in the digestion apparatus, at the fume cupboard until the digest was clear. The sample was transferred to a 100 ml volumetric flask, and distilled water was added into 100 ml graduated flask upto the mark. Boric acid indicator solution of 5 ml was then transferred to 100 ml conical flask containing 5 drops of mixed indicator and was placed under the condenser of the distillation apparatus. 10 ml of the clear supernatant liquid of the digest was then transferred into the apparatus, and then 10 ml of 46% sodium hydroxide was added and rinsed again with distilled water. Distillation was then commenced. After the first distillation, drops reached the boric acid indicator solution, colour changed from pink to green. A total of 150 ml of the distillate was collected. The solution was titrated with 0.0174 N sulphuric acids until the colour changed from green to pink.

Determination of starch content

Powdered sample of 0.25 g was homogenized in hot 80% ethanol to remove sugars. The residue was then centrifuged and retained. The residue was dried well over a water bath. To the residue, 5.0 ml of distilled water and 6.5 ml of 52% perchloric acid was added, and then extracted at 0°C for 20 min. The supernatants were centrifuged, pooled and made up to 100 ml. A quantity of 0.1 ml of the supernatant was pipetted out and made up to the volume of 1 ml with distilled water. The standards were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard and the volume made up to 1 ml in each tube with water. An amount of 4 ml of anthrone reagent was then added to each tube and sample heated for 8 min in a boiling water bath. Sample was cooled rapidly and the intensity of green to dark green colour was read using a spectrophotometer at 630 nm. The glucose content in the sample was determined using the standard calibration graph, and then the value was multiplied by a factor of 0.9 to arrive at the starch content.

Determination of amylose content

Powdered sample of 0.1 g was weighed, and 1 ml of distilled ethanol added followed by 10 ml of 1 N NaOH. The sample was heated for 10 min in a boiling water bath. The volume was made up to 100 ml. The extract taken was 2.5 ml and 20 ml of distilled water was added followed by three drops of 0.1% phenolphthalein. Drop wise HCl 0.1 N was then added until the pink colour just disappeared. 1 ml iodine reagent was added till the volume was 50 ml and the colour read at 590 nm using a spectrophotometer. Standard amylose solution 0.2, 0.4, 0.6, 0.8 and 1 ml was taken and the colour developed as in the case of the test samples. The amount of amylose present in the sample was calculated using the standard graph.

Determination of mineral content

A powdered sample of 0.1g was weighed and put into a dry, clean and labeled digestion tube. 5 ml of digestion mixture was added to each tube and also to 2 reagent blanks for each batch of samples. The sample was then digested at 360°C for 2 h after which the solution was clear. It was then allowed to cool. After cooling, 25 ml of distilled water was added and mixed well and left to cool again. The solution was then made up to 100 ml with distilled water and allowed to settle. Then, potassium and zinc was determined as follows, 4 ml of the wet digested sample solution was pipetted into a 100 ml volumetric flask, made to mark with distilled water and mixed well. The sample was then aspirated and directed into the atomic absorption spectrophotometer starting with the standards and blank solutions. The readings of the amount of the selected elements using their respective cathode lambs were recorded and the concentrations of the elements were determined by plotting a calibration curves.

Phosphorous was determined by taking 10 ml of the wet-ashed digestion solution into a 50 ml volumetric flask. 0.2 ml of 0.5% p-nitrophenol indicator solution was the added. The solution was made alkaline (yellow colour) by the addition of 6 N NH_3 solutions dropwise with gentle shaking followed by dilute 1 N HNO_3 dropwise until just colourless. Next, 5 ml of a mixture of ammonium molybdate/ammonium vanadate reagent was added. Finally, the solution was made to mark with distilled water and mixed well. It was then left for 30 min and the absorption measured using a U.V spectrophotometer and the concentration determined using a calibration curve.

Statistical analysis

All analyses were performed in duplicate (n = 2), the data was presented as means standard error of deviation (± SEM) and analysis of variance was determined at $p \leq 0.05$ level of significance. Correlations between different parameters were established using Pearson correlation coefficient. Multivariate analysis was undertaken using JMP statistical software, version 10. Principal component analysis (PCA) was used as a tool of data reduction to summarize the standardized data from biochemical composition analysis. The determination of genetic dissimilarity was Euclidean distance and the hierarchical agglomerative clustering method. Euclidean measure of distance was used for the estimation of genetic distance (GD) among varieties.

RESULTS AND DISCUSSION

Healthy diets contain an adequate and balanced combination of macronutrients (carbohydrates, fats and protein) and essential micronutrients (vitamins and minerals). Pearl millet is the major source of energy and protein for millions of people in Africa and has been reported that millet has many nutritious and medical functions (Obilana and Manyasa, 2002; Yang et al., 2012). In this study, starch content of the genotypes ranged from 27 - 46.7% with a mean of 34.2%. The first level of genotypes *Tsholotsho bearded*, *CIAKAUNGE-Vil3*, *ICMV 93771*, *KIRAKAORIGINAL, IP8772* and IP8773 had high amounts of starch contents that were not significantly different from 41.6 - 46.7%, *ICMA 00111 X SUDAN 11, ICMA 00888 X MIXTURE (KIRAKA/GACALIVIL2)*, *ICMA 00888 X HSD 2163* genotypes had the lowest starch of 27.6, 27.2, and 27.0 respectively. Foods with a low glycemic index like pear millet starch are useful to manage maturity onset diabetes, by improving metabolic control of blood pressure and plasma low density lipoprotein cholesterol levels due to less pronounced insulin response (Asp, 1996).

Most of the genotypes had less amylose than amylopectin with exceptions of a few varieties with a ratio of 1: 2. The amylase content ranged from 5.3 - 21.6% with a

mean of 11.5%. Genotypes *IP8766*, *ICMA 00111 X SUDAN 11*, *IP 10470*, *IP 8783*, *DEMBI YELLOW*, *SDMV 90031*, *SDMV 94014*, and *ICMV 221-1* had amylose levels of 16% and above, while *ICMA 93222 X HSD 2163*, *ICMA 00888 X SIUKU Vil 4b*, and *CIAKAUNGE – Vil 3* had the lowest amounts (Table 1). The first five genotypes above had more amylose than its amylopectin contents and these are favorable for baking, and preparing snacks. ANOVA showed that the first level of amylopectin *CIAKAUNGE-Vil3*, *ICMV 93771*, *KIRAKA ORIGINAL* and *Tsholotsho bearded* had amounts of 38.7, 36.2, 33.5 and 32.8% that were not significantly different. *ICMA 00111 X SUDAN 11* had the lowest content of 8.0% (Table 1).

The protein content had a range of 4.6 - 9.9% with a mean of 7.1% which is similar to studies done by Chethan and Malleshi (2007) and Singh and Raghuvanshi, (2012) who reported an average of 5 – 8 and 7%, respectively. *SDEA 4L-17 X HSD 7193*, *SDEA 4L-17 X CIAKAUNGE Vil3*, *MIXTURE (KIRAKA/GACALI Vil 2), IP 10471*, *ICMV 221-1*, *ICMA 00888 X HSD 7193*, *DEMBI YELLOW*, *ICMV 221*, *CIAKAUNGE-Vil 3* and *ICMV 91450* had amounts of 8% and above. Adeola et al. (1995) showed that the essential amino acid profile of pearl millet protein had lysine, threonine, methionine, tryptophan and cystine more than in proteins of sorghum and corn. These varieties have good protein and thus are good for human and livestock feeds.

Pearl millet contains various essential micro nutrients needed by the body. There are wide fluctuations in the total mineral and trace elements contained in pearl millet, the biggest factor determining this is the nature of the soil it is grown in. These minerals are required in the human body for numerous functions in the body. The genotypes *ICMV 221-1*, *GACAATIVIL-6 (ICRISAT), DEMBI YELLOW*, *SDMV 90031*, *KIRAKA ORIGINAL* and *SOSATC 88* exhibited the highest levels of phosphorous that were not significantly different. The phosphorous content ranged from 28.3 -1593.0 ppm with a mean of 362.1 ppm. This is slightly higher than those found by Singh and Srivastava (2006) who reported that the finger millet phosphorus content ranged from 130 to 295 mg% with a mean value of 180.43 mg%. Phosphorus is an essential component of adenosine triphosphate (ATP), a precursor to energy in the body and also they are precursors of nucleic acids that make up the genetic code of organisms (Liang et al., 2010; Devi et al., 2011).

Zn deficiency is common in underdeveloped countries and is mainly associated with malnutrition, affecting the immune system, wound healing, the senses of taste and smell, and impairing DNA synthesis (Zago and Oteiza, 2001). Zn seems to support normal growth and development in pregnancy, childhood and adolescence. Zn has been recognized to act as an antioxidant by replacing metals that are active in catalyzing free radical reactions, such as Fe (Oteiza et al., 2004). *ICMA 00888 X HSD 7193* had significantly high zinc content of 1345.5 ppm while *GACAATIVIL-6 (ICRISAT)* exhibited the lowest amount of 57.0 ppm. The other genotypes had zinc contents below 378 ppm. The range was from 57.1 - 1345.5 ppm with a mean of 193.4 ppm. This particular variety can be explored in breeding programs so as to develop varieties with high zinc content.

Potassium is important to keep the body parts running smoothly and is involved in maintaining water and electrolyte balance and regulating nerve and muscle functions (Oniango et al., 2003). The potassium levels in most genotypes had no big significant difference. The range was from 13.6–432 ppm with a mean of 160.4 ppm. *Tsholotsho bearded*, *ICMA 00888 X HSD 7193, 863 A X HSD 3508*, and *ICMA 93222 X DEMBI YELLOW* are some of the genotypes with high potassium while *GACAATIVIL-6 (ICRISAT), MIXTURE (KIRAKA/GACALI Vil 2)* and *CMA 93222 X ICMV 221* had the lowest potassium levels. Determining the amounts of various micro and micro nutrients in pearl millet is essential to ascertaining its importance in food security.

Correlation studies showed a significant positive correlation of 0.78, $p \leq 0.05$ between starch and amylopectin. Amylose also exhibited a positive association with phosphorous 0.28, $p \leq 0.05$. The other parameters had no significant relationship with each other. These associations hint on the possible genetic associations. The macro and micro nutrient analysis showed significant variations between the genotypes analyzed and this was also reported by Singh and Raghuvanshi (2012). Hence, finger millet has shown good potential to supply these much needed nutrients to help curd food insecurity.

Principal component analysis

The genetic diversity of 60 pearl millet varieties was observed for their biochemical makeup which is a requirement for the pre-selection of varieties for future breeding programs for better varieties to enhance food security. The principal component analysis grouped the characteristics into starch, amylose, amylopectin, Zn, Ph and K that accounted for the entire (100%) variability, however only four principal components were significant. According to Hair et al. (1998) Eigen value greater than 1 are considered significant and component loadings greater than ± 0.3 were deemed meaningful. As a result, only the first four principal components were used for the study and traits with loadings greater than ±0.3 were taken to represent the corresponding principal axis.

PC1 (principal component 1) alone explained 28.4% of the total variety among the varieties and was mainly due to the influence of the carbohydrates that is starch, amylose and amylopectin with amylose having a negative loading. The sign indicates the direction of the relationship between the components and the variables (Johnson, 1998). The 2nd principal component accounted for 20.4% of the total variation was predominantly a function of starch, amylose, potassium, zinc and phosphorous all with

Table 1. Proximate analysis of macro and micro nutrients of 60 pearl millet genotypes.

Genotype	Starch (%)	Amylose (%)	Amylopectin (%)	Protein (%)	P - ppm	Zn- ppm	K - ppm
Tsholotsho bearded	46.6^{a}	13.8^{defghijkl}	32.8^{abcd}	6.1^{cde}	341.3^{fghijkl}	61.0^{gh}	432.1^{a}
CIAKAUNGE - Vil 3	45.3^{ab}	6.5^{qr}	38.7 ^{a}	9.9^{a}	119.4^{ijklm}	161.8^{defgh}	34.7^{qrstu}
ICMV 93771	44.4^{ab}	8.2^{nopqr}	36.2^{ab}	7.6^{abcd}	285.8^{ghijklm}	135.2^{defgh}	148.1^{fghijklmnopqrstu}
KIRAKA ORIGINAL	41.7^{bc}	8.2^{nopqr}	33.5^{abc}	7.6^{abcd}	1181.2^{bc}	195.9^{bcdefgh}	72.6^{opqrstu}
IP 8773	41.6^{bc}	12.8^{efghijklmn}	28.7^{cdefg}	5.3^{de}	135.2^{ijklm}	178.9^{cdefgh}	124.1^{fghijklmnopqrstu}
IP 8772	39.9^{cd}	10.1^{ijklmnopqr}	29.7^{bcdef}	6.1^{cde}	293.7^{fghijklm}	288.5^{bcd}	124.2^{fghijklmnopqrstu}
OKOA	39.8^{cd}	9.8^{jklmnopqr}	29.9^{bcde}	4.5^{e}	935.6^{cd}	284.1^{bce}	213.4^{cdefghijklm}
ICMV 96603	39.5^{cde}	11.5^{ghijklmnop}	28.0^{cdefgh}	6.1^{cde}	48.1^{lm}	113.4^{defgh}	35.0^{qrstu}
ICMV 91450	39.2^{cdef}	9.7^{klmnopqr}	29.4^{bcdef}	9.1^{ab}	182.8^{hijklm}	107.3^{defgh}	299.6^{abcd}
IP 8767	38.6^{cdefg}	14.7^{bcdefghij}	23.8^{efghijklmnop}	5.3^{de}	400.7^{fghi}	259.6^{bcdef}	44.0^{qrstu}
IP 8766	38.4^{cdefg}	21.6^{a}	16.8^{opqrst}	7.6^{abcd}	159.0^{hijklm}	147.4^{defgh}	226.5^{bcdefghij}
SDMV 96063	38.4^{cdefg}	11.0^{ghijklmnopq}	27.4^{cdefghi}	6.1^{cde}	135.2^{ijklm}	285.0^{bcde}	234.8^{bcdefghi}
IP 10470	38.4^{cdefg}	19.5^{ab}	18.8^{lmnopqrst}	7.6^{abcd}	376.9^{fghijk}	161.0^{defgh}	220.9^{cdefghijk}
IP 8764	38.2^{cdefg}	11.3^{ghijklmnopq}	26.9^{cdefghijk}	6.1^{cde}	151.1^{hijklm}	219.0^{bcdefgh}	248.7^{bcdefg}
SIUKU Vil 4B	38.0^{cdefgh}	11.2^{ghijklmnopq}	26.7^{cdefghijk}	7.6^{abcd}	270.0^{ghijklm}	250.0^{bcdefg}	82.1^{mnopqrstu}
IP 10471	38.0^{cdefgh}	12.9^{efghijklm}	25.0^{efghijklm}	8.3^{abc}	309.6^{fghijklm}	65.3^{fgh}	254.7^{bcdef}
KAT PM 2	37.2^{defghi}	10.8^{ghijklmnopq}	27.0^{cdefghij}	6.8^{bcde}	258.1^{ghijklm}	367.0^{bc}	233.1^{bcdefghi}
Okashani 2	37.2^{defghi}	10.1^{ijklmnopqr}	26.3^{cdefghijkl}	6.8^{bcde}	115.4^{ijklm}	119.5^{defgh}	208.0^{cdefghijklmn}
Tsholotsho	36.5^{defghij}	12.4^{efghijklmno}	24.0^{efghijklmnop}	6.1^{cde}	285.8^{ghijklm}	245.7^{bcdefgh}	94.0^{jklmnopqrstu}
ICMV 221 BRISTILED	36.1^{defghijk}	10.6^{ghijklmnopq}	25.5^{defghijklm}	7.6^{abcd}	198.6^{hijklm}	233.0^{bcdefgh}	65.0^{pqrstu}
GACAATI VIL -6 (ICRISAT)	35.2^{efghijkl}	10.7^{ghijklmnopq}	24.4^{efghijklmn}	6.8^{bcde}	238.3^{hijklm}	378.4^{b}	225.0^{bcdefghij}
IP 8783	35.2^{efghijkl}	18.6^{abcd}	16.6^{pqrst}	7.6^{abcd}	1391.2^{ab}	57.0^{h}	23.7^{tu}
KIRAKA Vil -b Vil - 1 (Irunduni)	35.1^{fghijkl}	11.9^{fghijklmnop}	23.2^{efghijklmnopq}	7.6^{abcd}	36.2^{m}	136.5^{defgh}	156.2^{efghijklmnopqrst}
IP 7390	35.1^{fghijkl}	14.2^{cdefghijk}	20.8^{ijklmnopqrst}	5.3^{de}	289.8^{fghijklm}	63.1^{gh}	235.2^{bcdefghi}
SDMV 94014	34.9^{fghijkl}	16.5^{bcdef}	18.4^{mnopqrst}	7.6^{abcd}	373.0^{fghijk}	246.1^{bcdefgh}	110.0^{hijklmnopqrstu}
SDMV 90031	34.8^{ghijkl}	16.6^{bcdef}	18.1^{mnopqrst}	6.1^{cde}	1359.5^{ab}	165.8^{defgh}	191.3^{defghijklmnop}
SHIBE	34.6^{ghijklm}	14.8^{bcdefghi}	19.7^{jklmnopqrst}	7.6^{abcd}	218.4^{hijklm}	173.6^{defgh}	32.6^{rstu}
IP 6791	33.7^{hijklmn}	13.6^{efghijklm}	20.1^{ijklmnopqrst}	6.8^{bcde}	87.7^{jklm}	160.1^{defgh}	86.5^{klmnopqrstu}
NKIRIGACHA Vil 8	33.2^{ijklmno}	8.8^{mnopqr}	24.4^{efghijklmn}	7.6^{abcd}	60.0^{lm}	139.6^{defgh}	64.3^{pqrstu}
ICMA 00888 X HSD 7193	33.2^{ijklmnop}	11.8^{fghijklmnop}	21.3^{ghijklmnopqrs}	8.3^{abc}	590.9^{ef}	1345.5^{a}	356.4^{ab}
DEMBI YELLOW	32.9^{ijklmnop}	17.3^{abcde}	15.5^{rstu}	8.3^{abc}	1343.7^{ab}	244.4^{bcdefgh}	138.2^{fghijklmnopqrstu}
SOSAT C 88	32.8^{jklmno}	9.8^{klmnopqr}	23.0^{efghijklmnopqr}	6.8^{bcde}	1173.3^{bc}	172.7^{defgh}	243.1^{bcdefh}
ICMV 221-1	32.6^{jklmno}	16.4^{bcdef}	16.1^{qrstu}	8.3^{abc}	1593.3^{a}	234.8^{bcdefgh}	203.4^{cdefghijklmno}
KITHARAKA Vil 9	32.5^{jklmno}	9.5^{klmnopqr}	23.0^{efghijklmnopqr}	7.6^{abcd}	163.0^{hijklm}	226.0^{bcdefgh}	196.2^{defghijklmnop}
ICMV 221	32.3^{jklmno}	8.8^{mnopqr}	23.5^{efghijklmnopq}	9.1^{ab}	107.5^{ijklm}	161.0^{defgh}	28.9^{stu}
ICMA 93222 X HSD 2163	32.1^{jklmno}	7.0^{pqr}	25.1^{efghijklm}	6.1^{cde}	83.7^{jklm}	127.8^{defgh}	219.0^{cdefghijkl}
KAIGONGI	31.8^{klmnopq}	7.5^{pqr}	24.3^{efghijklmno}	6.1^{cde}	123.4^{ijklm}	107.7^{defgh}	84.0^{lmnopqrstu}
ICMA 00888 X SERERE - IRAMBA	31.3^{lmnopqr}	8.9^{lmnopqr}	22.4^{fghijklmnopqrs}	6.8^{bcde}	277.9^{ghijklm}	203.8^{bcdefgh}	168.9^{defghijklmnopq}
SIUKU Vil 4a	31.3^{lmnopqr}	9.00^{lmnopqr}	22.3^{fghijklmnopqrs}	7.6^{abcd}	210.5^{hijklm}	147.4^{defgh}	128.4^{fghijklmnopqrstu}
ICMA 93222 X KIRAKA Vil 1	31.3^{lmnopqr}	11.0^{ghiklmno}	20.2^{ijklmnopqrst}	6.8^{bcde}	198.6^{hijklm}	121.7^{defgh}	288.0^{bcde}
MIXTURE (KIRAKA / GACALI Vil 2)	31.2^{lmnopqr}	7.8^{opqr}	23.4^{efghijklmnopq}	8.3^{abc}	147.1^{hijklm}	186.3^{cdefgh}	28.0^{stu}
863 A X FS VARIETY	31.2^{lmnopqr}	10.9^{ghijklmnopq}	20.2^{ijklmnopqrst}	4.5^{e}	230.3^{hijklm}	234.3^{bcdefgh}	142.9^{fghijklmnopqrstu}
ICMA 93222 X DEMBI YELLOW	30.9^{lmnopqr}	10.0^{ijklmnopqr}	20.9h^{ijklmnopqrst}	6.8^{bcde}	91.7^{jklm}	128.7^{defgh}	332.8^{abc}
KIRAKA Vil 1	30.9^{lmnopqr}	7.5^{pqr}	23.4^{efghijklmnopq}	7.6^{abcd}	325.4^{fghijklm}	251.8^{bcdefg}	67.6^{pqrstu}
SDEA 4L - 17 X HSD 7193	30.3^{mnopqr}	10.6^{ghijklmnopq}	19.7^{jklmnopqrst}	8.3^{abc}	186.8^{hijklm}	169.3^{defgh}	74.6^{nopqrstu}
KAT PM 1	30.3^{mnopqr}	15.1^{bcdefgh}	20.2^{ijklmnopqrst}	8.3^{abc}	135.2^{hijklm}	120.8^{defgh}	106.5^{ijklmnopqrstu}
ICMA 93222 X ICMV 221	30.3^{mnopqr}	10.0^{ijklmnopqr}	20.2^{ijklmnopqrst}	6.8^{bcde}	262.0^{ghijklm}	104.6^{defgh}	67.5^{pqrstu}
SDEA 4L - 17 X CIAKAUNGE Vil 3	30.3^{mnopqr}	10.0^{ijklmnopqr}	15.1^{stu}	6.8^{bcde}	115.4^{ijklm}	61.0^{gh}	13.6^{u}
863 A X HSD 3508	30.2^{mnopqr}	13.0^{efghijklmn}	17.1^{nopqrst}	6.8^{bcde}	210.5^{hijklm}	123.0^{defgh}	300.6^{abcd}
KIMBEERE	30.2^{nopqr}	8.6^{mnopqr}	21.5^{ghijklmnopqrs}	7.6^{abcd}	75.8^{jklm}	95.0^{defgh}	159.1^{efghijklmnopqrs}
ICMA 93222 X KAT PM 1	30.0^{nopqr}	10.9^{ghijklmnopq}	19.1^{lmnopqrst}	7.6^{abcd}	75.8^{klm}	370.6^{bc}	142.7^{fghijklmnopqrstu}

Table 1. Contd.

ICMA 93222 X CIAKAUNGE Vil - 3	29.9^{nopqr}	10.4^{ijklmnopqr}	19.5^{klmnopqrst}	6.1^{cde}	83.7^{jklm}	83.2^{efgh}	231.9^{bcdefghi}
253 X 254/KOG X NKIRIGACHA VIL 8	29.8^{nopqr}	9.5^{klmnopqr}	20.3^{ijklmnopqrst}	6.8^{bcde}	440.3^{fgh}	63.6^{gh}	166.7^{defghijklmnopqr}
ICMA 00888 X SIUKU Vil 4b	29.6^{nopqr}	5.32^{r}	24.3^{efghijklmno}	6.8^{bcde}	182.8^{hijklm}	109.0^{defgh}	225.2^{bcdefghij}
ICMA 93222 X OKOA	29.4^{opqr}	9.1^{lmnopqr}	20.3^{ijklmnopqrst}	6.8^{bcde}	28.3^{m}	161.8^{defgh}	257.0^{bcdef}
253 X 254/KOG X PMV 3	28.9^{opqr}	15.3^{bcdefg}	13.65^{tu}	6.8^{bcde}	547.3^{fg}	152.2^{defgh}	208.7^{cdefghijklmn}
ICMA 93222 XICMV 221-1	28.9^{pqr}	10.2^{hijklmnopqr}	18.6^{mnopqrst}	5.3^{de}	384.9^{fghij}	241.7^{bcdefgh}	117.4^{ghijklmnopqrstu}
ICMA 00111 X SUDAN 11	27.6^{qr}	18.9^{abc}	8.6^{u}	7.6^{abcd}	880.1^{cde}	161.4^{defgh}	168.7^{defghijklmnopq}
ICMA 00888 X MIXTURE (KIRAKA /GACALI VIL 2)	27.2^{r}	9.6^{klmnopqr}	17.5^{nopqrst}	7.6^{abcd}	856.3^{de}	190.7^{bcdefgh}	94.1^{jklmnopqrstu}
ICMA 00888 X HSD 2163	27.0^{r}	10.2^{ijklmnopqr}	16.8^{pqrst}	7.6^{abcd}	365.0^{fghijk}	102.0^{defgh}	143.0^{fghijklmnopqrstu}

Means with the same letter in the same column are not significantly different.

Table 2. Principal component analysis of starch, amylose, amylopectin, proteins, phosphorous, zinc and potassium in pearl millet varieties showing Eigen vectors, Eigen value and their percentage contribution to the total variations explained in the first two principal component axes.

Variables	PC1	PC2	PC3	PC4
Starch	0.50	0.50	0.13	-0.29
Amylose	-0.44	0.44	0.02	-0.36
Amylopectin	0.69	0.15	0.10	-0.02
Protein	-0.02	-0.22	0.74	0.16
Phosphorous	-0.27	0.30	0.44	-0.37
Zinc	-0.09	0.36	0.33	0.68
Potassium	-0.09	0.52	-0.35	0.40
Eigenvalue	1.99	1.43	1.14	1.02
Individual (%)	28.43	20.47	16.30	14.62
Cumulative (%)	28.43	48.90	65.19	79.81

positive loadings. Therefore varieties with high PC2 scores would have high amounts of these parameters. The 3rd principal component with 16.2% variance separated these varieties on protein, phosphorous, zinc, potassium with potassium having a negative loading. PC4 accounted for 14.6% of the variation that was attributed to amylose, phosphorous, zinc and potassium with positive and negative loadings. Protein concentration was important in only PC3 showing that it had little contribution to the variation among varieties unlike amylose that contributed to variation in PC1, PC2 and PC4 (Table 2). The micronutrients contributed to the variation in the 2nd, 3rd and 4th principal components, they contributed to a larger percentage in the variation of these varieties

A plot of the PC1 and PC2 showed that *CIAKAUNGE Vil 3 (#E)*, *ICMA 00888 X HBD 7193* (#J), *ICMA 00111 X SUDAN 11* (#14), *ICMA 93771* (#Z) and *Tsholotsho bearded (#7)* were the most divergent from the majority group centered on zero. The bi-plot would give a breeder the ability to visualize the distances between the varieties and point out the best varieties to be selected depending on several variables compressed in the two major principal components. Their divergence was attributed to their high contents of starch and amylose for *ICMA 93771* (#Z) and *CIAKAUNGE Vil 3 (#E)*, high zinc for *ICMA 00888 X HBD 7193* (#J), high phosphorous content for *ICMA 00111 X SUDAN 11 (#14)*, and high potassium for *Tsholotsho bearded (#7)*. Varieties with close proximity in the score plot are similar; those near the origin are distinctive while those far from the origin are extremes. The varieties which overlap in the principal component axis had some relationships in the concentration of the used traits. These extremes varieties are favorable for breeding programs due to their biochemical difference from the rest which makes it unique. In Figure 1, the loading plot shows the similarities and differences between the biochemical parameters.

Principal component loadings plots classified the varieties into four quadrants based on the concentration of the minerals, proteins and carbohydrates analyzed in this study. The varieties scattered in 3 quadrants

Table 3. Summary of cluster means of 60 pearl millet varieties biochemical characteristics.

Cluster	Starch	Amylose	Amylopectin	Protein	Phosphorous	Zinc	Potassium	Mean
1.0	38.4	11.6	26.8	6.3	439.9	212.3	89.5	103.2
2.0	46.7	13.8	32.9	6.1	341.3	61.0	432.2	117.0
3.0	33.7	17.1	16.6	7.2	636.8	186.1	172.3	134.1
4.0	43.8	7.6	36.2	8.4	528.9	164.3	85.2	109.8
5.0	37.6	11.1	26.5	7.3	169.8	185.7	233.6	84.6
6.0	30.8	9.6	21.2	7.1	237.9	157.8	150.9	77.7
7.0	33.2	11.9	21.3	8.4	590.9	1345.6	356.4	296.8
Mean	37.7	11.8	25.9	7.3	420.8	330.4	217.2	

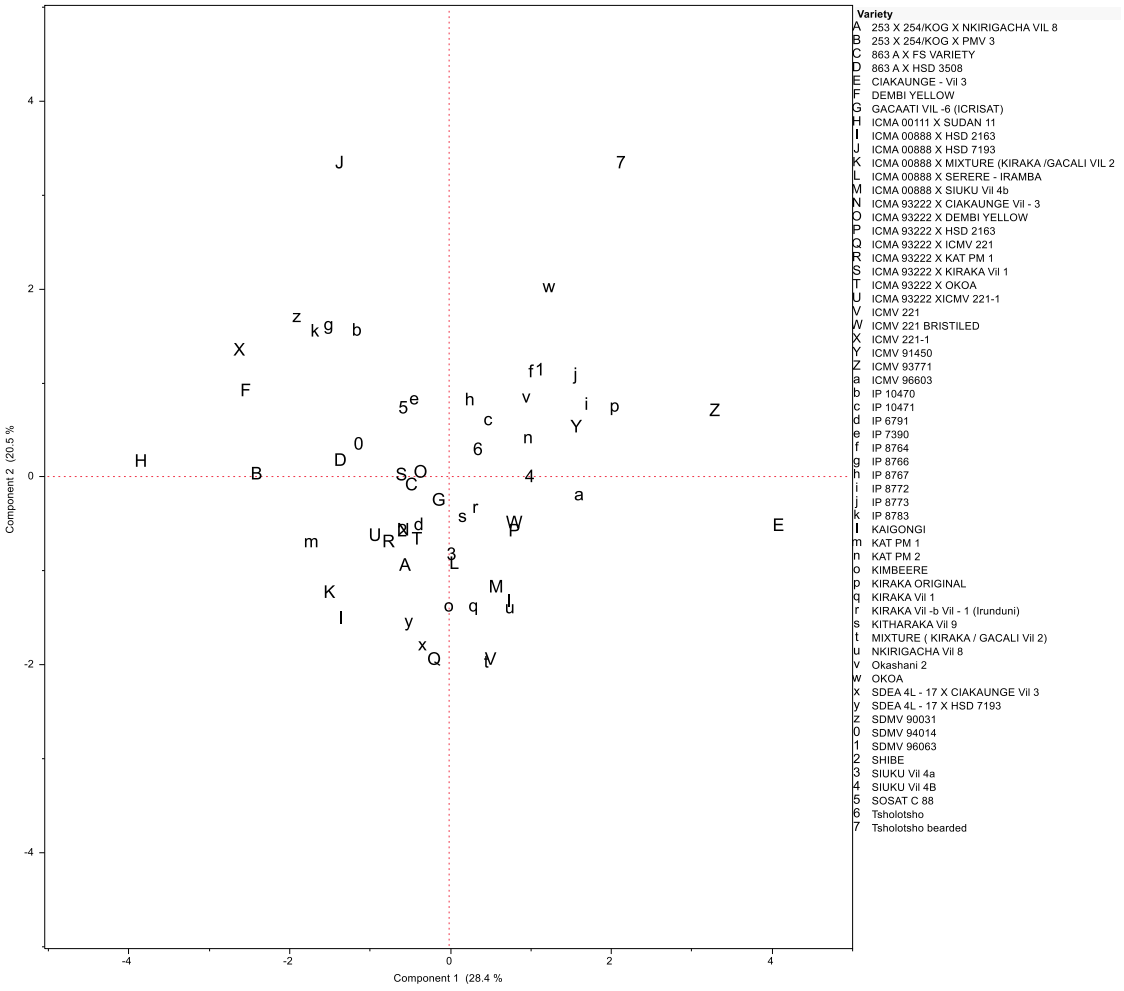

Figure 1. Principal component score plot of PC1 and PC2 describing the overall variation among pearl millet varieties estimated using biochemical character data.

demonstrating genetic variability in their composition. The varieties on the top left quadrant were related in amylose, phosphorous, zinc and potassium. The right top quadrant varieties were related in starch and amylopectin while the right bottom varieties did not show any associations in the measured traits. The left bottom varieties showed portrayed relations in their protein content. The distance between the locations of any two varieties on the score plot is directly proportional to the degree of similarity or difference between them in terms of their analyzed traits

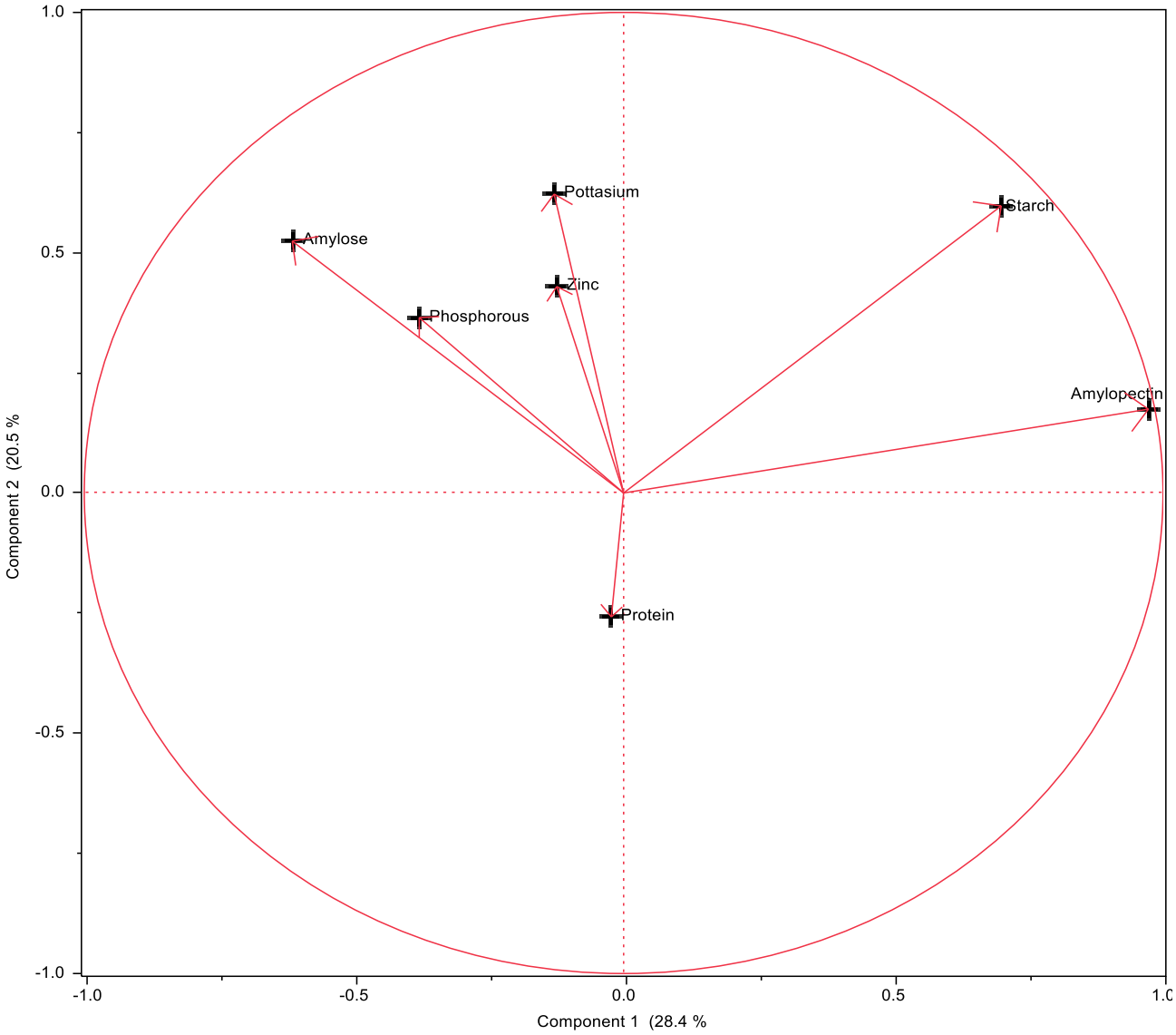

Figure 2. Principal component analysis loading plot for seven biochemical traits of 60 pearl millet varieties.

(Figure 2).

Cluster analysis and genetic distance

Estimates of genetic distance matrix was based on the nutritional traits for all pair wise combinations of (60×59)/2 =1770 for the 60 pearl millet varieties (data not shown). The observed genetic distance was from 0.37 - 8.73 pair wise combinations showing the diversity of the varieties in terms of their nutritional composition. The lowest genetic distance of 0.37 and 0.44 were recorded between *SDEA 4L 17 X HSD 7193* and *SDEA 4L – 17 X CIAKAUNGE Vil 3*, and between *IP 8764* and *SDMV 96063*, respectively. The highest genetic distance of 9.1 and 8.73 was between *CIAKAUNGE Vil 3* and *ICMA 00888 X HSD 7193* and between *Tsholotsho bearded* and *ICMA 00888 X HSD 7193*. The low genetic distance within the varieties points towards relatedness and thus confirms that there is enough genetic diversity in the measured mineral elements, carbohydrates and protein among the varieties despite the relatedness. The varieties with high genetic distance can be adopted for breeding programs.

Cluster analysis portrayed a clear differentiation between sorghum varieties. Table 3 reveal the difference among clusters by summarizing cluster means for the seven biochemical parameters. The highest cluster mean was recorded in phosphorous (420.8) and the lowest was in protein (7.3). Maximum cluster mean was recorded in cluster VII (296.8) and III (134.1). This showed the existence of maximum genetic divergence among the

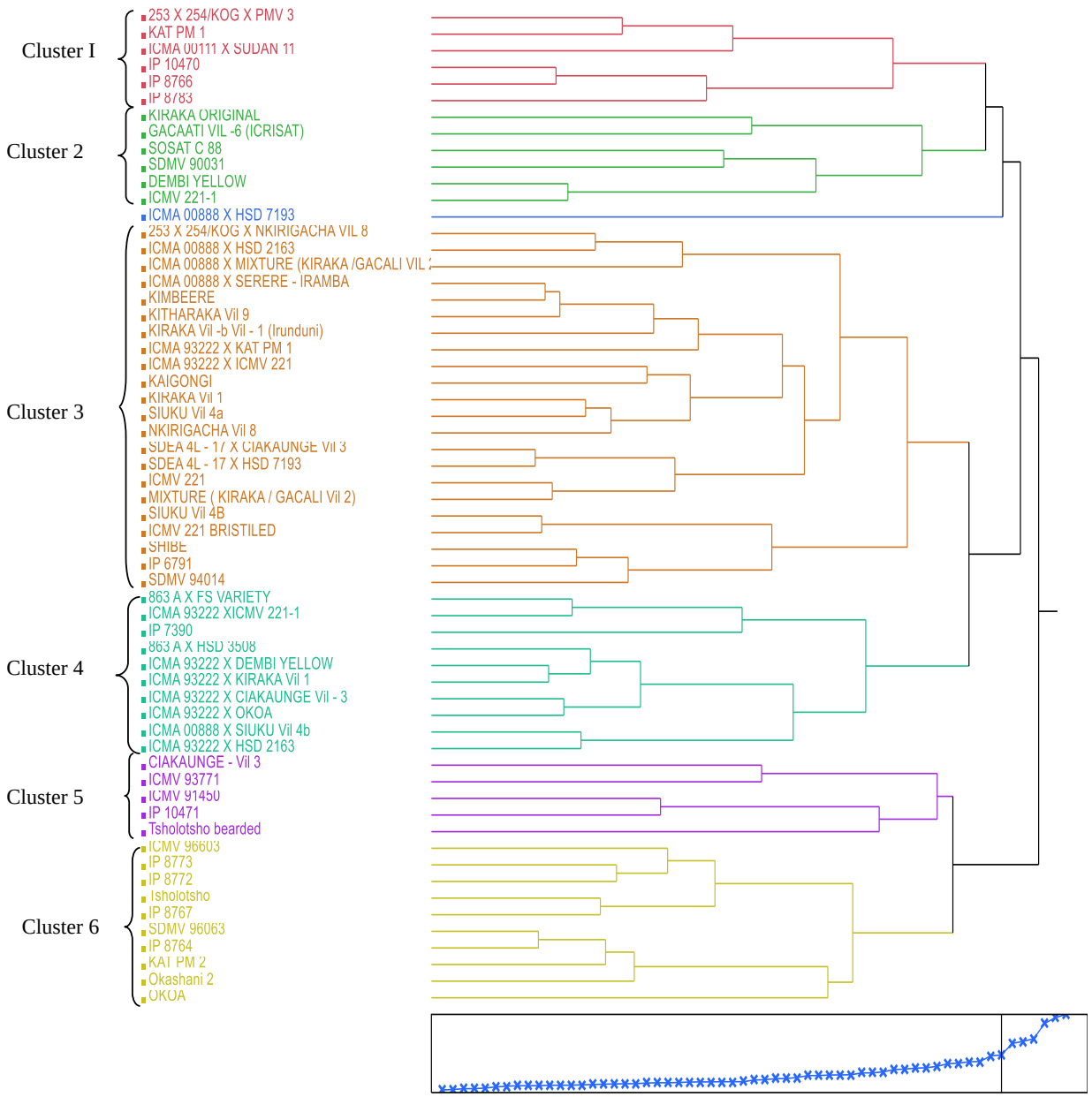

Figure 3. Hierarchical cluster dendrogram showing clusters 1 - 4 of 60 biochemical and morphological characteristics of pearl millet varieties.

varieties in these clusters. Based on these parameters, the varieties were grouped into clusters shown on the dendrogram. The dendrogram divided the varieties into 7 clusters and a singleton as shown (Figure 3). Cluster I was characterized by varieties with good amylose and phosphorous contents with other parameters in moderate amounts. Cluster II varieties were characterized by highest phosphorous and high amylose moderate amounts of starch and proteins. Cluster III had low starch, good protein and the lowest phosphorous amounts. Cluster IV had the high potassium and amylopectin, low starch and protein. Cluster V had the highest starch, protein, amylopectin and potassium. Cluster VI had the highest zinc, amylopectin and protein, medium phosphorous and low protein. Variety *ICMA 00888 X HSD 7193* was grouped as a singleton and this showed that it was dissimilar from other varieties in terms of its nutritional composition. As a result, this pearl millet has the capability of being adopted in plant breeding programs. The crossing of pearl millet varieties in different clusters will provide higher heterotic groups in breeding. Various authors including Shergo (2010)

demonstrated genetic diversity among sorghum varieties on the basis of their nutritional composition. In pearl millet quality improvement programs, it is vital to critically identify and quantify varieties to enhance their nutritional quality like minerals, proteins and carbohydrates.

Conclusion

Similar to many other cereals, pearl millets have high carbohydrate and other nutrients, making them useful components of dietary and nutritional balance in foods. The genotypes of the pearl millet analyzed, presented a broad variability in the studied contents and most of them are comparable to the contents found in the pearl millets cultivated worldwide. Dietary deficiencies, can be dealt with by encouraging the population to consume traditional foods like pearl millet especially women and children. Including these readily available cereals in the diet will improve nutrition status. Based on the observed variation for both qualitative and quantitative characteristics, it can be concluded that phenotypic diversity of pearl millet varieties is important to classify the genetic potential of varieties and increase the efficiency of the pearl millet breeding programs.

Conflict of interest

The authors did not declare any conflict of interest.

REFERENCES

Adeola O, Orban JI (1995). Chemical composition and nutrient digestibility of pearl millet (Pennisetum glaucum) fed to growing pigs. J. Cereal Sci. 22(2):177-184.

Adugna W (2002). Genetic diversity analysis of linseed (Linum usitatissmum L.) in different environments, PhD Thesis, University of the Free State, Bloemfontein, South Africa.

Asp NG (1996). Dietary Carbohydrate: Classification by Chemistry and Physiology. J. Food Chem. 7:9-14.

Anu SS, Kwatra A (2006). Nutritional evaluation of pearl millet based sponge cake. J. Food Sci. and Technol. 43(3):312-313.

Black RE (2003a). Micronutrient deficiency - an underlying cause of morbidity and mortality. Bulletin of the World Health Organization, pp 81-79.

Black RE (2003b). Zinc deficiency infectious disease and mortality in the developing world. J. Nutritional Suppliments, 1,1484S-1489S. Chethan S, Malleshi NG (2007). Finger millet polyphenols:optimization of extraction and the effect of pH on their stability. J. Food Chem. 105:862-870.

Devi PB, Vijayabharathi R, Sathyabama N, Malleshi G, Priyadarisini VB (2011). Health benefits of finger millet (Eleusine coracana L.) polyphenols and dietary fiber: review. J. Food Sci. and Technol. DOI, 10.1007/s13197-011-0584-9.

FAO (2009). FAOSTAT. Food and Agriculture Organisation of the United Nations. FAOSTAT, http://faostat.fao.org/site/339/.

FAO (2000). Bulletin of statistics. Vol. 1, FAO, Rome, pp.16-36.

Hair JF, Andrson JR, Tatham RE, Black WC (1998). Multivariate data analysis, 5th Edn, Prentice-Hall international, Inc, London.

Hotz C, Brown KH (2004). International Zinc Nutrition Consultative Group (IZiNCG) technical document. Boston, Massachusetts: International Nutrition Foundation for UN University Press.Food and Nutrition Bulletin 25, Supplement 2.

Johnson DE (1998). Applied multivariate method for data analysis. Duxbury press, Pacific Grove, California.

Kremer A, Petit RG, Pons O (1998). Measures of polymorphism within and among populations. In: Karp A, Issac PG, Ingram DS. (Eds.). Molecular tools for screening biodiversity plants and animals, Chapman and Hall. London. 301-311.

Liang S, Yang G, Ma Y (2010). Chemical characteristics and fatty acid profile of foxtail millet bran oil. J. Am. Oil Chemists Society, 87:63-67.

Luthria DL, Pastor-Corrales MA (2006). Phenolic acids content of fifteen dry edible bean (Phaseolus vulgaris L.) varieties. J. Food Composition and Analysis, 19:205-211.

Maqbool SB, Devi P, Sticklen M (2001). Biotechnology: genetic improvement of sorghum (Sorghum bicolour (L.) Moench). J. In vitro Cell Developmental Biology, 37, 504-515.

Malik M, Singh U, Dahiya S (2002). Nutrient composition of pearl millet as influenced by genotypes and cooking methods. Journal of Food Science and Technology, 39(5):463-468.

Nube M, Voortman RL (2006). Simultaneously addressing micronutrient deficiencies in soils, crops, animals and human nutrition: opportunities for higher yields and better health. Centre for World Food Studies. Amsterdam.

Obilana AB, Manyasa E (2002). Millets. In: Belton, P.S and Taylor, J. R. N. (Eds.). Pseudo cereals and less common cereals: Grain properties and utilization potential. Springer-Verlag, New York. pp, 177–217.

Oteiza PI, Mackenzie GG, Verstraeten SV (2004). Metals in neurodegeneration involvement of oxidants and oxidant-sensitive transcription factors. Molecular Aspects and Medicine, 25:103–115.

Oniang'o RK, Mutuku MJ, Malaba SJ (2003). Contemporary African foods habits and their nutritional and their nutritional health implications. Asian Pacific J. Clin. Nutr., 12(3):231-236.

Ramakrishnan U (2002). Prevalence of micronutrient malnutrition worldwide. J.Nutritional Reviews, 60:S46-S52.

Rengel Z, Batten GD, Crowley DE (1999). Agronomic approaches for improving the micronutrient density in edible portions of field crops. J. Field Crop Research, 60:27-40.

Robert R (2004). The encyclopaedia of nutrition and good health, 2nd Edn. Viva books Pvt, Ltd, New Delhi.

Singh P, Raghuvanshi RS (2012). Finger millet for food and nutritional security. Afric. J. Food Sci. 6(4):77-84.

Singh P, Srivastava S (2006). Nutritional composition of sixteen new varieties of fingermillet. Journal Community Mobilization Sustainable Development 1(2):81-84.

Shergo A (2010). Biodiversity in plant grain and nutritional characteristics of sorghum [Sorghum bicolour] (L) Moench accessions from Ethiopia and South Africa, Ph.D. Thesis, University of the Free State, Bloemfontein, South Africa.

Souza E, Sorrels ME (1991). Prediction of progeny variation in oat from parental genetic relationships. Theoretical and Applied Genetics, 82:233-241.

UNICEF, (2006). Iodine Deficiency Disorders (IDD) (http://childinfo.org/areas/idd/).

WHO (2006). Preventing and controlling micronutrient deficiencies in populations affected by an emergency, WHO, WFP, Unicef, WHO internet accessed on 26 April 2006.

www.icrisat.org. Icrisat-researchprograms.htm, Accessed on 20/10/2014.

Weir BS (1996). Genetic Data Analysis II: Methods for Discrete Population Genetic Data. Sunderland. MA, Sinauer Associates. Inc.

Yang X, Wan Z, Perry L, Lu, H, Wang Q, Hao C, Li J, Xie F, Yu J, Cui T, Wang T, Li M, Ge GH (2012). Early millet use in northern China. Proceedings of the National Academy of Science USA, pp.1-5.

Zago, MP, Oteiza PI (2001). The antioxidant properties of zinc interactions with iron and antioxidants. Free Radical Biology and Medicine, 31:266-274.