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Genetic Variability of Ethiopian Coffee (*Coffea arabica* L.) Accessions Collected from East Wollega Zone for Bean Biochemical Constituents

Getachew WeldeMichael[1], Sentayehu Alamerew[2], Leta Tulu[3] and Gezahegn Berecha[2]

[1]Jimma Agricultural Research Center, P.O. Box, 192, Jimma, Ethiopia, [2]Jimma University , College of Agriculture and Veterinary Medicine and [3] National Agricultural Biotechnology Research Center (NABRC), P. O. Box 249, Holetta, Ethiopia

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በቡና ዝርያዎቸ መካከል ያለው የባዮኬሚካል ውሁዶች ይዘት ተለያይነት የቡና ጥራት እና ጣዕምን በመረጣና በማዳቀል ለማሻሻል ከፍተኛ የሆነ ጠቀሜታ እንዳለው ይታወቃል። ነገር ግን በአገራችን የዚህ ዓይነቱ መረጃ በበቂ ሁኔታ አለመኖሩና በጥቅም ላይ አለመዋሉ የቡና ጥራት በተፈለገው ደረጃ ላለመድረሱ እንደ ምክንያት ከሚነሱት ችግሮች መካከል በዋናነት ይጠቀሳል። ስለዚህ ይህንን ችግር ከግምት ውስጥ በማስገባት አራት በብሔራዊ ደረጃ የተለቀቁ እንዲሁም 101 ከምስራቅ ወለጋ የቡና አብቃይ አካባቢዎች የተሰበሰቡ በድምሩ 105 የቡና ዝርያዎችን በፍሬያቸው ውስጥ የሚገኙትን የባዮኬሚካል ውሁዶች ተለያይነት ለመገምገም በማለም ይህ ሙከራ ተካሂዷል። ሙከራውም የተካሄደው በአግመንትድ ዲዛይን ሲሆን በእቅዱ መሰረት የትራይጎኖሊን፣ የክሎሮጀኒክ አሲድስ ፣ የካፊን፣ የጠቅላላ ፕሮቲን፣ የጠቅላላ ስብ፣ የጠቅላላ ሚኔራል/አሽ እንዲሁም የድራይ ማተር ይዘትን መረጃ በመውሰድ የትንተና ስራ ተከናውኗል። በቫሪያንስ ትንተና ውጤት ከድራይ ማተር ይዘት በስተቀር ሁሉም ባሕሪያት በዝርያዎች መካከል ከፍተኛ የሆነ ተለያይነት መኖሩን ለማወቅ ተችሏል። የፐሪንስጠል ኮምፖነንት ትንተና ውጤት የመጀመሪያው ኮምፖነንት ብቻ 47.9በመቶ የሚሆነውን ልዩነት ያሳየ ሲሆን የቶታል ክሎሮጀኒክ አሲድስ እና የጠቅላላ የስብ መጠን በከፍተኛ ደረጃ መለያየት በዝርያዎቹ መካከል ለታየው ተለያይነት የአንበሳውን ድርሻ ማበርከታቸውን በግልጽ አመልክቷል። የክላስተር ትንተና ውጤት ደግሞ በጥናቱ የተካተቱ 105 ዝርያዎችን በስድስት የክላስተር ቡድኖችና በሁለት ብቸኛ ቡድኖች ከፍሏቸዋል። ይህም ውጤት በዝርያዎች መካከል ልዩነት መኖሩን በተጨማሪ አረጋግጧል። የጀኔቲክ ዳይቨርጀንስ ትንተናም በአብዛኞቹ ቡድኖች መካከል ከፍተኛ የሆነ ርቀት መኖሩን ከማሳየቱም በላይ የተጠቀሱትን ውሁዶች ይዘት በማዳቀል ማሻሻል የሚቻል መሆኑን አመላክቷል። በአጠቃላይ በዚህ ጥናት ወጤት የታየው የባዮኬሚካል ውሁዶች ይዘት ተለያይነት ወደፊት የዝርያዎቹን የባዮኬሚካል ውሁዶች ይዘት በመረጣና በማዳቀል ለማሻሻል ከፍተኛ የሆነ እድል መኖሩን አረጋግጧል። ነገር ግን በዚህ ጥናት የታየውን ውጤት የበለጠ ለማጠናከርና ጠቅለል ያለ ድምዳሜ ላይ ለመድረስ እነዚህን ዝርያዎች በተለያዩ አካባቢዎች መገምገም እንዲሁም የተለያይነቱን ስራ በሞሎኪዩላር ደረጃ መስራት አስፈላጊ ይሆናል።

Abstract

Variability for coffee bean biochemical composition among the coffee accessions is vital for further quality improvement. However, lack of this information has been one of the major bottlenecks for any coffee quality improvement program. The current study was, therefore, conducted to evaluate the level of variability in green bean biochemical composition of coffee accessions collected from east Wollega coffee growing areas. Four standard checks and 101 Arabica coffee accessions were used for the study. The study was conducted using augmented design and data were collected on trigonelline, total chlorogenic acid, caffeine, crude protein, crude fat, crude ash and dry matter contents. Both univariate and multivariate analyses were employed to see the variability of biochemical constituents among the accessions. Analysis of variance showed significant differences ($P<0.05$) among the accessions for all biochemical attributes

except for bean dry matter content. Principal component analysis revealed that the first four principal components accounted for 96.9 % of the total variability. The first PC, with Eigenvalue greater than one, alone accounted for 47.9% of the total variation mainly due to the variation in total chlorogenic acid and crude fat content, suggesting that these traits are the major contributors for the observed variability. Besides, clustering grouped the accessions in to six distinct clusters and two solitary regardless of the collection sites indicating the existence of variability among the accessions. Genetic divergence analyses based on Mahalanobis statistics (D^2) showed significant inter cluster distance, implying that there is a chance to improve these biochemical compounds through hybridization. In general, the observed variability for bean biochemical compounds indicating a great opportunity for genetic improvement of east Wollega coffee for biochemical contents through selection and hybridization. However, the variability observed in this study should be further confirmed by conducting the experiment in different locations as well as using molecular techniques.

Keywords: Biochemical compounds, crude fat, crude protein, genetic variability and principal component

Introduction

Coffee beans are the seeds of a shrub belonging to the botanical family Rubiaceae and the genus *Coffea* (Clifford, 1991). They contain a complex mixture of biochemical components like caffeine, trigonelline, chlorogenic acids, sucrose and fat, which produce flavor and aroma during roasting (Pauline, 2013). The characteristic flavor and richness of coffee aroma makes it a special beverage (Yeretzian *et al*, 2003) and now coffee is the most commercialized and widely consumed beverage in the world. Besides, coffee is considered as a functional food, primarily owing to its high content of biochemical compounds that exert antioxidant and other beneficial biological properties (Farah, 2012).

The presence of these compounds in coffee green bean has various roles in coffee quality, production and new product development. Primarily, they impact quality in positive or negative way and are important factors in the determination of organoleptic cup quality. For example, high trigonelline and sucrose in green coffee beans contribute to the coffee cup quality whereas high caffeine and chlorogenic acid content affect the coffee quality by increasing bitterness (Cliford, 1985). Similarly, coffee oil carries the aroma and contributes to viscosity (Buffo and Cardelli- Freire, 2004) and protein is vital for coffee flavor since it is needed for the Maillard reaction (Farah, 2012). Apart from their contribution to coffee quality, these compounds play vital role in providing adaptive properties to plants as well as participate in imparting resistance to diseases and pests (Perez de la Vega, 1994). Furthermore, the presence of these biochemical compounds in coffee bean can also be used to develop other products like instant coffee and help to diversify the coffee products and can be used as a buffer especially at the time of price fall due to over production (Yigzaw, 2005).

Ethiopia is currently one of the major coffee producing countries in Africa and supply large volume of coffee to the world market annually. However, production of the crop is challenged by many factors such as the effects of newly emerging diseases and adaptation problems, which are expected to happen as a result of climate changes. Moreover, apart from production problems, coffee export is also constrained by the change in consumption pattern to caffeine free coffee types, even if composition of caffeine could reduce a risk of Parkinson's disease development (Higdon and Frei, 2006). Hence, countries like Ethiopia, of which economy is mainly dependent on the revenues obtained from coffee export, should be proactive in supplying good quality and caffeine free coffee to the world market as these characteristics determine the attractiveness of coffee for consumption purposes and serves as standards for price determination (Agwanda *et al*., 2003; Katharina *et al*., 2009; Gichimu *et al*., 2012).

Biochemical compounds that determine coffee cup quality arise from precursors in green beans during development (Davrieux *et al*., 2003) and are genetically and environmentally influenced (Montagnon *et al*., 1998; Leroy *et al*., 2006). Biochemical composition and quality attributes of green coffee beans may be affected by genotypes (Leroy *et al*., 2006; Farah, 2012), altitude, edaphic and climatic conditions (Harding *et al*., 1987; Decazy et *al*., 2003), and post-harvest practices (Bytof *et al*., 2007).

Therefore, as knowledge and information on variability for bean biochemical composition is a prerequisite to develop varieties possessing good quality, disease resistance, adaptive and that can satisfy the ever growing demand of consumers in the world, it is essential to characterize coffee accessions based on biochemical attributes. In view of this, several researchers reported the genetic effects on biochemical composition of coffee beans. Anthony *et al.* (1993) have used biochemical compounds to assess the diversity of the genus *Coffea.* Similarly, Martin *et al.* (1998) differentiated Arabica and Robusta coffee varieties using chlorogenic acids, caffeine, trigonelline, amino acids and polyphenol content of green beans. Furthermore, Ky *et al.* (2001) have assessed the diversity of caffeine, trigonelline, chlorogenic acids and sucrose contents in wild Arabica and Robusta accessions and confirmed the presence of high levels of polymorphism for these biochemical compounds among the accessions within a species as well as between species, where the level of polymorphism has been found to be high as compared to that observed using molecular markers. The presence of biochemical variation among coffee beans has also been reported for coffee accessions collected from southwest (Abeyot *et al*., 2011) and northwest Ethiopia (Ygzaw *et al*., 2007). Although such variations are believed to be important to determine organoleptic cup quality of coffee and select pest resistant, adaptable and consumers preferred coffee varieties, accessions collected from east Wollega, which has a special demand in the world market for its peculiar fruity flavor, have not been studied for

variations in bean biochemical composition. The current study was therefore, conducted to evaluate the variability among east Wollega coffee accessions for bean biochemical composition.

Materials and Methods

Description of the sample collection area

The study was conducted using coffee samples collected from trees grown at Haru Agricultural Research Sub-Center, which is located 468 km away from the capital, Addis Ababa, at 8^0 59' N longitudes and 35^0 47'E latitude at an altitude of 1752 masl. Haru receives mean annual rainfall of 1727mm, with uni-modal pattern, the peak being in July. Usually the rainfall starts in March and may extend up to September or November. The area has an average minimum and maximum temperatures of 16^0C and 27^0C, respectively and it has Acrisol soils type (Tsegaye and Taye, 2000).

Experimental materials

The experimental materials consisted of four stand check varieties and 101 coffee accessions, which were collected from east Wollega coffee growing areas and conserved at Haru research sub center (Table 1). Coffee from this area is well known by the name of 'Gimbi Coffee' and Nekempte coffee' and fetches very high price on the world market and have been given the highest priority in local land race development program.

Research design

The experiment was conducted using augmented design, where only the controls were replicated, while the treatments were not replicated. The accessions were planted in five blocks and the checks were planted after five treatments in each block. Each plot had 10 trees and the samples were prepared from these trees.

Coffee sample preparation

Coffee samples were prepared by wet processing method (Behailu *et al*, 2008). After drying to standard moisture content (11-12%), the parchment was removed by huller, the beans were carefully cleaned by hand and 300 mechanically undamaged and defect free green beans were taken from each of 105 accessions and ground to a fine powder using a coffee green bean grinder

Table.1. Geographical origin of coffee (*Coffea ararica* L.*)* germplasm accessions collected from East Wollega, Western Ethiopia

Accession	Altitude (masl)	Specific site	Woreda	Total no collected
EW1/09,EW106- 108/09	1706 -1732	Tsige	Sasiga	4
EW2 - 3/09	1700	Legagorba	Sasiga	2
EW4 /09	1660	Migna	Sasiga	1
EW6 – 9/09	1891 -1940	Babuserte	Sasiga	4
EW11 -13/09	1882 – 1900	Gabajimata	Sasiga	3
EW14/09	1724	Tufisa	Sasiga	1
EW15 -17/09	1711	Werasayo	Sasiga	3
EW24-35/09	1518 -1695	Gidugalesa	Sasiga	12
EW36, 38-40/09	1579 -1599	Weligalte	Sasiga	4
EW18 -19/09	1695	Lelistu	Sasiga	2
EW21 – 23/09	1600 -1630	Gidu	Sasiga	3
EW41 – 48, 50-51/09	1921 -1968	Ayeru	Sasiga	10
EW53 – 59, 61/09	1866 -1871	Arya	Sasiga	8
EW62, 64 –66, 68- 74/09	1782 – 1832	Hadiya	Sasiga	11
EW75 -80/09	1715 -1735	Gallo	Sasiga	6
EW81 – 82/09	1670	Kumburo	Sasiga	2
EW104/09	1750	Senbetadure	Sasiga	1
EW83 – 90/09	1664 -1708	Ayra	Sasiga	8
EW91 – 96/09	1683 – 1688	Bata	Sasiga	6
EW97 – 102/09	1635 – 1649	Bosoka	Sasiga	6
EW103/09&EW105/09	2100	Sasiga	Sasiga	2
EW109	1615	Mender misreta	Dega	1
EW111/09	1888	Haro	Dega	1
Standard checks (*Haru1*, *Chala*, S*ende* and *Menesibu*)				4
Total				**105**

Extraction and determination of trigonelline, chlorogenic acid and caffeine contents

Caffeine, chlorogenic acids and trigonelline were extracted and purified according to the method of Ky *et al.* (2001). Half a gram of each ground coffee sample was accurately weighed in 100 ml Erlenmeyer flask and 50 ml of double distilled water boiled at 95^0c was added, followed by heating at 121^oC in heating plate for 20 min. Extracts were filtered using whatman filter paper and then finally filtered with micro filter size of 0.45 mm and were maintained for caffeine, chlorogenic acids and trigonelline determination.

Biochemical analysis was conducted in the food science and nutrition laboratory at the Ethiopian Institute of Agricultural Research (EIAR), Addis Ababa. Simultaneous analysis of trigonelline, chlorogenic acids and caffeine was carried out according to the modified method of Vignoli *et al.* (2014) using the HPLC machine consisting of solvent reservoir, a quaternary pump, auto sampler, a 250 x 4.6 mm column with a 5 μm pore size . Compounds were eluted with a mobile phase consisting of 5% acetic acid and acetonitrile and elution was carried out at

0.7 ml/min.by injecting 10µl of sample in to the chromatographic system. Calibration curves for standards of trigonelline, chlorogenic acid and caffeine were obtained by injecting increasing concentrations of mixed standards and by plotting the peak area measured at wavelength of 272nm for trigonelline and caffeine and at 320nm for total chlorogenic acids. All chromatographic results were acquired and processed using software connected with the HPLC machine. The slope obtained from the linear fit for each calibration curve was used to estimate the concentration of the three coffee bioactive compounds in all samples. Figures (1, 2 & 3) show the calibration curve for trigonelline, chlorogencic acid and caffeine.

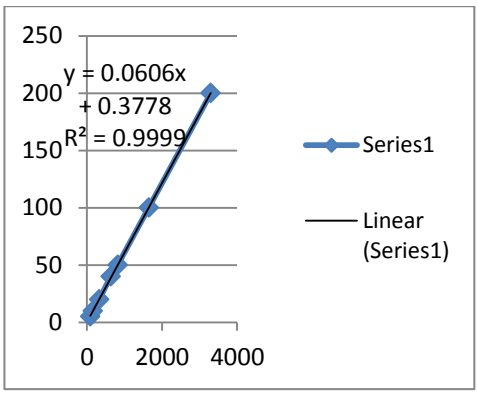

Figure 1. Calibration curve for trigonelline

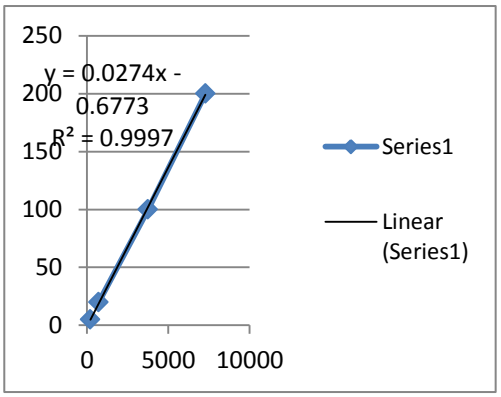

Figure 2. Calibration curve for total chromogenic acid

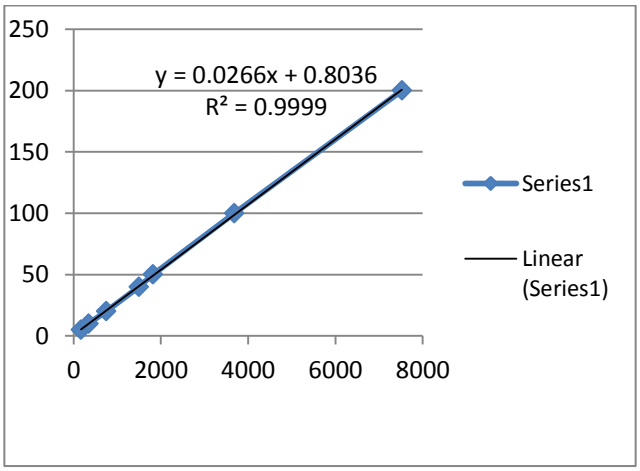

Figure.3.Calibration curve for caffeine

Determination of crude protein, crude fat and ash contents

Crude protein, crude fat and crude ash contents were also determined at EIAR, food science and nutrition laboratory, according to the method developed by AOAC (2001).

Crude protein (%)

Crude protein content of the samples was determined according to AOAC (2001) using (Foss kjeltech 8400, Sweden) analysis unit. Homogenous sample of 2gm of green coffee powder was weighed and added in to digestion tube. Then, two kejeltab (3.5gm K_2So4, 0.4 gm of CuSo4) and 15ml of concentrated H_2SO_4 were added in to the digestion tube and the tube was gently shaken uniformly to wet the sample with the acid. The digestion tube contained rack with the exhaust system loaded on preheated digestion block for 45 min. and then heated to 420^0C for 1hr to allow digestion. All samples were digested until the solutions turned green and clear.

After samples were cooled, mixture of distilled water of 80 ml and 50 ml of 40 % NaOH (w/v), respectively, were added in to the digestion tubes. Then the digestion tube was placed in distillation unit and the distillation cycle was completed approximately in 4-7 min. Finally, a receiver solution, 2% boric acid (30 ml) with mixed indicators, was added in to the tubes for titration. Percent of total nitrogen and crude protein were calculated as follows.

$$\text{N}\ (\%) = \frac{(\text{V}-\text{B}) \times \text{N of acid} \times 1.4007}{\text{Weight of sample in gm}} \times 100$$

$$(\%)\text{Crude protein} = \text{N}\ (\%) \text{ x } 6.25$$

Where, N (%) = Percent total nitrogen, V= Volume of H_2SO_4 consumed, B = Blank titration, N = Normality of H_2SO_4 (0.1M) and 1.4007= Mill-equivalent weigh to nitrogen (14mg)

Crude fat (%)

Crude fat content of the samples was measured as per the AOAC (2001) method by using Soxtech extraction system (Foss Soxtech TM 8000 extraction unit, Sweden). About 2 gm of ground green coffee bean sample was weighed (W1) in to each extraction thimbles covered with absorbent cotton and pre-dried at 103^0C for 2 hrs in fan assisted oven and weight of cooled cups was measured (W2). The thimbles with their samples were placed into fat determination machine (Soxtech Tm 8000 extraction system) to continue digestion of samples by adding 50 ml petroleum ether in to each cup using dispenser. The extraction process was carried out for 20, 40 and 10 min. for boiling, rinsing and recovery, respectively. Then, the cups with their residue were removed from the Soxtech system and placed in drying oven at 105^0C for 30 min and the cups were then allowed to cool in desiccators for an hour. The mass of each cooled cup together with its fat content was weighted (W3) and finally the crude fat content was calculated using the following formula.

$$\%\ (\text{Crude fat}) = \frac{(W3 - W2)}{W1} \times 100$$

W1= Weight of the sample
W2= Weight of the extraction cup
W3= Weight of fat residue and extraction cup

Ash content (%)

Ash content was determined by high temperature incineration in electric muffle furnace according to AOAC (2001) method. Three grams of sample was weighed (M1) and placed in previously dried and weighed ash cup (crucible) (M2). Then, the crucible with the sample was placed in muffle furnace set at 530^{o}c and heated overnight until the samples color turned in to grey color. The crucible with its residue was removed from the furnace, cooled in desiccators and then weighted (M3). The crude ash content was then expressed in percent as,

$$\%\ (\text{Crude ash}) = \frac{(M3 - M2)}{M1} \times 100$$

M1= Weight of the sample
M2= Weight of crucible
M3= Weight of crucible and sample

Moisture content and dry matter determination:

To express all biochemical data on dry weight basis, the moisture content of the coffee samples was determined according to procedure suggested by (AOAC, 2000). Dry matter content of was determined by oven drying the bean samples of each accession overnight at 105 ^{0}C to a constant weight (Clifford, 1985). Then, the moisture content was determined as initial weight of the sample minus its oven dry weight divided by its initial weight. On the other hand, dry matter content was taken as the inverse of moisture content of the sample.

Statistical analysis

Analysis of variance

The analysis of variance (ANOVA) was performed using Macro-SAS program developed for the augmented design analysis with SAS 9.3 software (SAS institute, 2011). Fisher's protected least significant difference (LSD) test at 5% level of significance was employed for mean comparisons, whenever treatment differences were significant

Multivariate analysis

Principal component analysis was performed by employing Minitab statistical software (Minitab, 2010). Besides, the data were also subjected to cluster analysis by employing the method of average linkage clustering strategy of the observation using proc cluster procedure of SAS version 9.3 software (SAS, 2011).The

numbers of clusters were determined by following the approach suggested by Copper and Miligan (1988) by looking into three statistics namely, Pseudo F, Pseudo t^2 and cubic clustering criteria. Cluster mean was calculated by taking the mean value of each trait in each cluster. Finally, genetic divergence between the clusters was determined using the generalized Mahalanobis's D^2 statistics (Mahalanobis, 1936). The D^2 values obtained for pairs of clusters were tested for significance at 5% and 1 % levels of significance against the tabulated values of p degrees of freedom, where p is the number of variables considered (Singh and Chaudhary, 1987).

Results and Discussion

Analyses of variance revealed statistically significant ($P<0.05$) differences among the accessions for all traits except dry matter content (Table 2) indicating the presence of variability which can be exploited to improve the biochemical constituents of east Wollega coffee collections through selection and hybridization.

The range, mean performance, coefficient of variation and LSD for seven attributes are presented in Table 3 and Figure 4-6. Coffee bean trigonelline content varied from 0.573 to 2.25 % with a mean value of 1.06 % on dry matter basis (dmb). Accessions such as EW73/09, EW12/09 and EW87/09 had the higher trigonelline content with respective values of 2.25, 2.07 and 1.84% while EW 22/09, EW56/09 and EW 103/09 had relatively lower trigonelline content with values of 0.573, 0.593 and 0.65%, respectively.

Table 2. Mean squares values for five biochemical and two bean physical attributes evaluated in 101 east Wollega coffee accessions and four standard checks in 2016/17

Character	B lock (df= 4)	Treat/ Adjusted. (df= 104)	Test (df=100)	Control (df=3)	Tests vs Controls (df=1)	Error (df=12)
Trigonelline	0.005^{ns}	0.058**	0.055**	0.0053^{ns}	0.498**	0.002
Total chlorogenic acid	0.241^{ns}	0.813*	0.809*	1.119*	0.267 ^{ns}	0.297
Caffeine	0.003*	0.03**	0.024**	0.211^{ns}	0.211**	0.004
Crude protein	0.083 ^{ns}	0.317**	0.275**	0.312*	4.544**	0.062
Crude fat	0.417 ^{ns}	0.974*	0.874*	1.313*	9.883**	0.365
Crude ash	0.136 **	0.1205**	0.115**	0.332**	0.03^{ns}	0.024
Dry matter content	0.621*	0.229 ^{ns}	0.218 ^{ns}	0.059 ^{ns}	1.93**	0.174

Note: df= degrees of freedom

The accessions were also diverse in total chlorogenic acids content of coffee beans that ranged from 2.77% to 7.86 % with mean value of 5.05%. Among the tested coffee accessions, EW 99/09, EW 09/09 and EW31/09 showed higher total chlorogenic acids content values of 7.86, 7.65 and 7.10%, respectively. On the other hand, EW 94/09, EW 70/09 and EW57/09 had lower chlorogenic acids content, with values of 3.11, 3.05 and 2.77%, respectively.

Caffeine content of coffee beans also showed variation among the accessions ranging from 0.93 to 1.77 % with an average value of 1.44%. Accessions, EW 51/09, EW 46/09 and EW61/09 showed higher caffeine content values of 1.77, 1.73 and 1.69 %, respectively. While, EW 76/09, EW 31/09 and EW32/09 had lower caffeine content of 1.034, 1.03 and 0.93%, respectively.

Crude protein content of coffee beans also showed variation among the accessions ranging from 12.95 to 16.1% with an average value of 14.69 %. Among the tested 105 coffee accessions, accessions, EW 91/09, EW 94/09 and EW61/09 were the best for crude protein content with values of 16.1, 15.98 and 15.80%, respectively. On contrary, accessions, EW 24/09, EW 64/09and EW42/09 showed lower crude protein content of 13.33, 13.14 and 12.95%, respectively.

Crude fat content of the coffee beans was another important biochemical attributes considered and it also showed variation among the tested coffee accessions ranging from 12.74 to 18.71% with average values of 16.01%. Accessions, EW09/09, EW 24/09 and EW 92/09 showed higher crude fat content values of 18.71, 17.98 and 17.63 %, respectively. Conversely, accessions, EW39/09, EW 44/09 and EW 58/09 showed the lower values for this trait. Similarly, ash content of coffee beans showed variation among the accessions and ranged from 3.16 to 5.09 % with a mean of 3.84 %. Although there was no significant difference among the accessions, bean dry matter content ranged from 90.46 to 92.86%, with an average value of 91.8%.

The levels of trigonelline, chlorogenic acid, caffeine, crude protein, crude fat, crude ash and dry matter content exhibited in the present work were within the ranges observed in other similar studies. For example, Silvaroa *et al.* (2000) have reported the existence of variation in caffeine content that ranged between of 0.5 and 2.8% with mean values 1.2% for 68 progenies collected from Kaffa province and from 0.4 to 2.9% with mean values of 1.1 % for 22 progenies collected from Illu-Ababora province. Besides, Key *et al.* (2001) also evaluated caffeine, trigonelline, chlorogenic acids and sucrose contents in wild *C. arabia* and *C. robusta* accessions and reported the presence of significant variations between the accessions of the two major coffee species as well as among the accessions of each species for these biochemical compounds. They have also reported that the variations in trigonelline, total chlorogenic acids and caffeine content ranged between 0.9 and 1.8%, 3.4 and 4.8% and 1.0 and 1.6%, respectively. Similarly, in a study conducted on 24 coffee Arabica promising genotypes in Ethiopia, Abeyot *et al*. (2011) reported highly significant variation for bean caffeine, fat, protein, ash and dry matter content that ranged between 1.00-1.51%, 12.10-14.98%, 3.69– 5.24%, 1.99- 4.22% and 90.55-95.06%, respectively. However, the range of protein content reported by the authors was much smaller than the current report and this could probably attributed to variations in methods of determination,

number and type of genotypes used for the studies. Furthermore, Gimase (2014) reported highly significant variation among 17 coffee genotypes for caffeine, oils and sucrose, but non-significant difference for chlorogenic acid and trigonelline. In another similar work conducted in Uganda, Khapre *et al*. (2017) reported that trigonelline, total chlorogenic acid and caffeine contents of coffee Arabica bean were in the ranges of 0.92 to 1.25%, 7.11 to 7.94 % and 1.05 to 1.34%, respectively, on dry weight basis.

Table 3. Mean, range, LSD and coefficient of variations for five biochemical and two bean physical attribute of 101 east Wollega coffee accessions and four standard checks in 2016/17

Character	Mean ± SE	Range	CV (%)	(LSD at 5%)
Trigonelline	1.06 (± 0.05)	0.573-2.25	4.28	0.11
Total chlorogenic acids	5.05 (± 0.64)	2.77- 7.86	10.08	1.39
Caffeine	1.44(± 0.07)	0.93-1.77	4.43	0.15
Crude protein	14.69(± 0.29)	12.95-16.10	1.69	0.63
Crude fat	16.01(± 0.71)	12.74-18.71	3.77	1.55
Crude ash	3.84(± 0.18)	3.16-5.09	4.04	0.39
Dry matter content	91.8(± 0.49)	90.46-92.86	0.45	ns

Note: CV= coefficient of variation, LSD= least significant difference and ns= non-significant

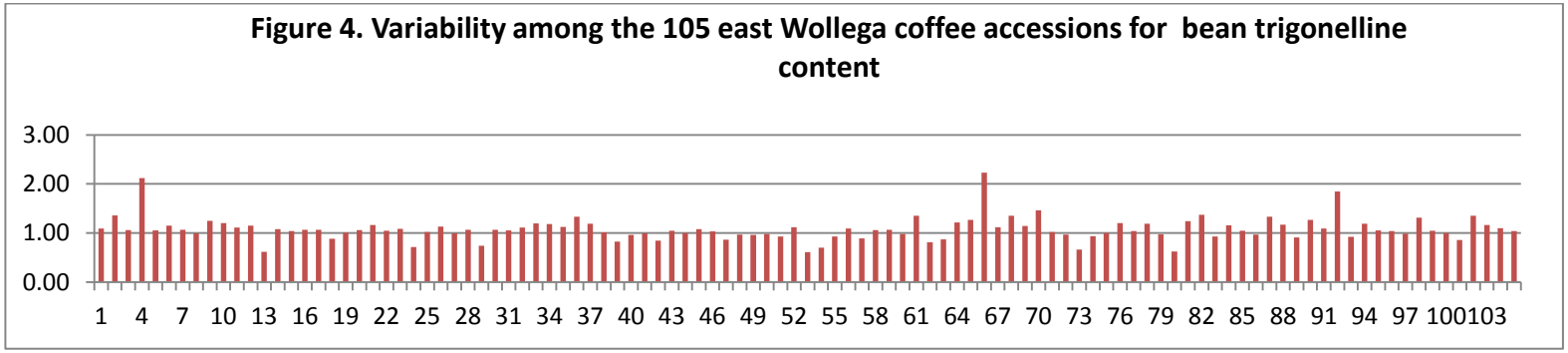
Figure 4. Variability among the 105 east Wollega coffee accessions for bean trigonelline content
3.00
2.00
1.00
0.00
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58 61 64 67 70 73 76 79 82 85 88 91 94 97 100103

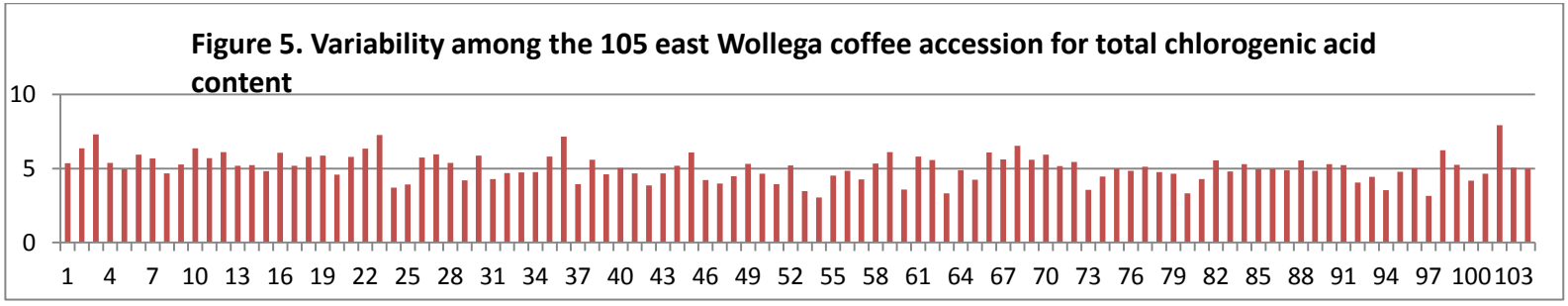
Figure 5. Variability among the 105 east Wollega coffee accession for total chlorogenic acid content
10
5
0
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58 61 64 67 70 73 76 79 82 85 88 91 94 97 100 103

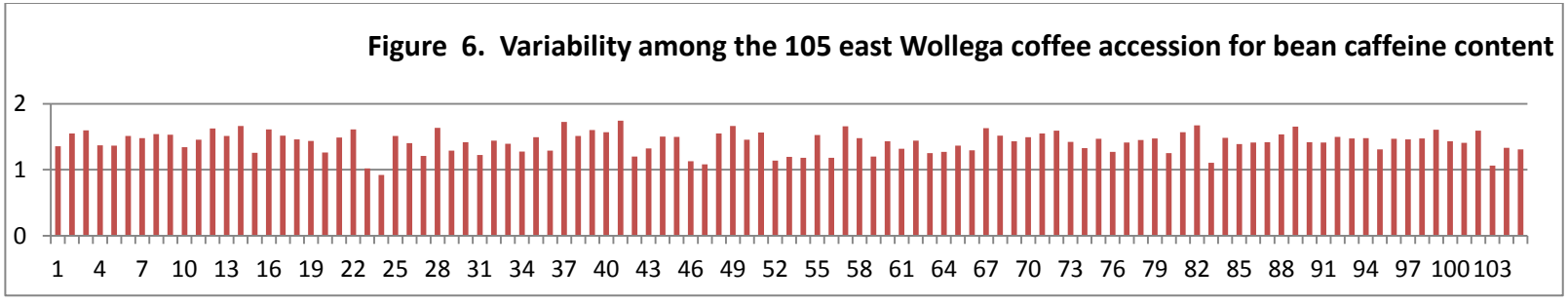
Figure 6. Variability among the 105 east Wollega coffee accession for bean caffeine content
2
1
0
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58 61 64 67 70 73 76 79 82 85 88 91 94 97 100 103

Principal component analysis

To evaluate the relative contribution of each attributes to the observed variability, principal component analysis was performed and the result is presented in Table 4. It was observed that the first four principal components cumulatively accounted for 96.9 % the total variability, where each explained 47.9, 33.7, 9.9 and 5.4 %, respectively. Moreover, in the current study, the above four PCs had Eigenvalues of 1.022, 0.645, 0.190 and 0.103, respectively.

Table 4. Eigenvectors and eigenvalues of six principal components for five bean biochemical compounds and crude ash content

Variable	PC1	PC2	PC3	PC4	PC5	PC6
Trigonelline	0.075	-0.078	-0.085	0.121	0.002	**-0.979**
Total chlorogenic acids	**0.627**	**-0.741**	0.179	0.109	0.028	0.109
Caffeine	0.016	-0.030	0.089	0.062	0.012	-0.082
Crude protein	-0.216	0.090	**0.694**	**0.673**	-0.016	0.007
Crude fat	**0.741**	**0.660**	0.112	0.033	0.025	-0.001
Crude ash	-0.035	0.007	-0.043	0.054	**0.997**	0.010
Eigenvalue	**1.022**	**0.645**	**0.190**	**0.103**	**0.040**	**0.019**
Proportion	**0.479**	**0.337**	**0.099**	**0.054**	**0.021**	**0.010**
Cumulative	**0.479**	**0.816**	**0.915**	**0.969**	**0.990**	**1.00**

Note: PC= principal component

According to Chatfield and Collins (1980), principal components with Eigenvalues of greater than one were significant and should be considered for subsequent analysis. Therefore, according to this cut point, only the first principal component was considered for identifying the contributing traits (Table 4). In addition to Eigenvalues, Eigenvectors cut of limit in each principal component is also equally important in identifying the traits responsible for the variation. In this regard, Hair *et al*. (2009) suggested that Eigenvector values greater than the absolute value of 0.3 was considered as an important contributor for the variation. Besides, Chahal and Gosal (2002) recommended that characters with the largest absolute value closer to unity within the first principal component influence the clustering more than those with lower absolute value closer to zero. Therefore, according to this principle, chlorogenic acid and crude fat contents, which had Eigenvector values closer to unity in the first and second PCs, are important traits and played a vital role in classifying the accessions into different groups and should be considered while selecting diverse parents for further crossing program.

The findings from the current study were partly in agreement with the findings of Abeyot *et al.* (2011) who reported that the first four principal components accounted for 84.2% of the total variability, mainly due to variations in caffeine, crude protein, crude fat, crude ash and dry matter contents. Yigzaw (2005) also reported that the four PCs accounted for 100% of the variability and caffeine and chlorogenic acids were more important. In another similar work conducted in

Kenya, Gimase *et.al.* (2014) also reported that the first three PCs accounted for 84.17% of the total variability, where caffeine and fat in the first PC and total chlorogenic acid and sucrose in the second PC were the most discriminating biochemical among the coffee genotypes.

Cluster analysis

The genetic relationship of 105 coffee accessions based on six attributes was assessed using Hierarchical clustering method and is presented in Table 5 and Figure 7. The coffee accessions were classified into six distinct groups comprising of two to 65 accessions, and two solitary remained ungrouped. Cluster I comprised the highest number of accessions (65) followed by cluster II and cluster IV comprising 29 and 3 accessions, respectively. Cluster III, V and VI each comprised of two accessions. On the other hand, the lowest number with single accession was observed for cluster VII and VIII (Table 5 and Figure 7). This indicated the existence of considerable variability among the accessions for those attributes. Therefore, these accessions could be used as sources of genes for developing varieties with desirable biochemical composition in the future.

Besides, it was also observed that accessions collected from the same site fell in different cluster groups while those collected from different sites grouped in the same cluster, suggesting that geographic and genetic diversity are not necessarily correlated and this could be due to gene flow as a result of human interference. In agreement with the present finding, Yigzaw (2007) has grouped 42 Ethiopian coffee genotypes in to eight distinct clusters regardless of the collection area. Similarly, Ky *et al.* (2001) reported the presence of high biochemical diversity in Arabica coffee. Gichimu, *et al.* (2014) also reported variation in biochemical composition among coffee genotypes using cluster analysis which grouped 34 Ruiru 11 sibs in three clusters. On the other hand the four check varieties namely, *Sende, Menesibu, Haru* 1 and *Chala* were grouped in the same cluster (cluster I), suggesting that the varieties were similar one another for these attributes and they were also similar to the accessions in the cluster.

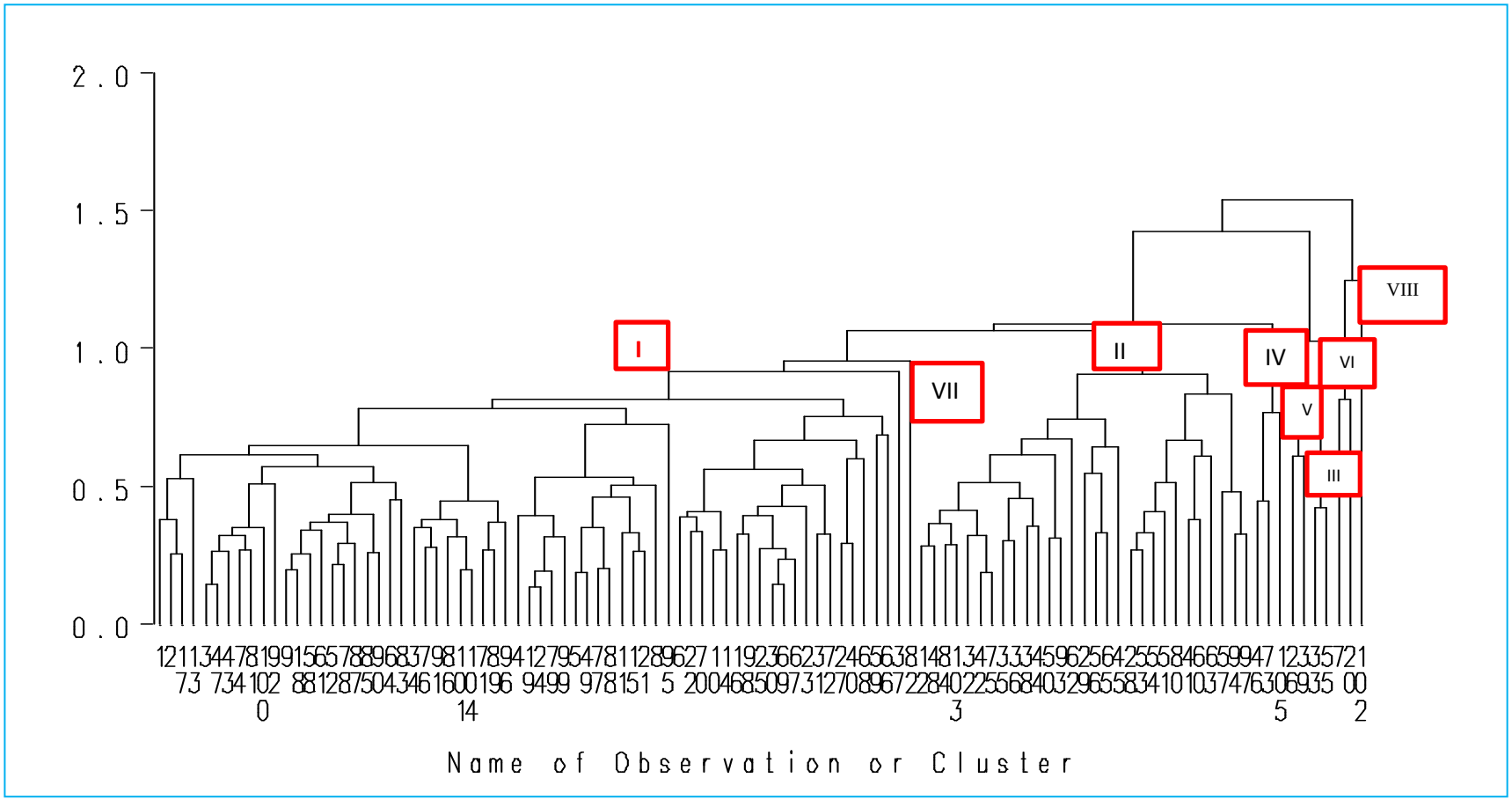

Figure 7. Dendrogram showing hierarchical clustering patterns of 105 *Coffea arabica* L. accessions (UPGMA) based on five biochemical attributes and crude ash content

Cluster mean performance

The mean values of six attributes for each of the clusters are presented in Table 6. Accessions EW/106/99 and EW99/99, which were grouped singly in cluster VII and VIII, respectively, had the highest values for trigonelline and crude protein content. Two clusters also had higher values of caffeine and chlorogenic acid, respectively, suggesting that the accessions could be used as parental lines in crossing program because high level of chlorogenic acid and caffeine in coffee bean strongly associated with disease resistance (Ky *et al.*, 1999)

On the other hand, cluster IV had the lowest mean values of caffeine and chlorogenic acid implying that this cluster is desirable for selection of parents having low chlorogenic acid and caffeine content. According to Ky *et al*. (1999), accessions with low values for these traits would result in better cup quality. Therefore, by using accessions from cluster IV, there is a possibility of improving coffee quality. Besides, the coffee accessions grouped under cluster VI had the highest mean values for crude fat content, suggesting that accessions classified in this particular cluster can be used as desirable sources of gene to improve the aroma and flavor of coffee as crude fat content coffee bean is strongly correlated with cup quality traits (Gichimu, *et al.*, 2014).

Moreover, coffee accessions grouped under cluster II and V had the highest mean values for crude ash content. In agreement with the current findings, Yigzaw (2005) and Abeyot, *et al*. (2011) observed variation among different clusters for coffee bean biochemical contents.

Table 5.The distribution of 101 east Wollega coffee accessions and four standard checks into eight clusters based on multivariate analysis for five bean biochemical attributes and crude ash content

Clusters	No.gen.	Percent	Coffee accessions
I	65	61.904	Chala , Haru, Menesibu, Sende, EW 01/09, EW 02/09, EW 04/09, EW 06/09, EW 08/09, EW 09/09, EW 11/09, EW 13/09, EW 14/09, EW 15/09, EW 16/09, EW 17/09, EW 18/09, EW 21/09, EW 22/09, EW 23/09, EW 24/09, EW 25/09, EW 27/09, EW 30/09, EW 31/09, EW 34/09, EW 39/09, EW 41/09, EW 44/09, EW 48/09, EW 51/09, EW 55/09, EW 62/09, EW 64/09, EW 66/09, EW 68/09, EW 71/09, EW 73/09, EW 74/09, EW 75/09, EW 77/09, EW 78/09, EW 79/09, EW 80/09, EW 81/09, EW 83/09, EW 84/09, EW 85/09, EW 86/09, EW 87/09, EW 90/09, EW 92/09, EW 93/09, EW 95/09, EW 96/09, EW 97/09, EW 98/09, EW 100/09, EW 101/09, EW 102/09, EW 104/09, EW 105/09, EW 107/09, EW 109/09, EW 111/09
II	29	27.62	EW 12/09, EW 28/09, EW 29/09, EW 32/09 EW 36/09, EW 38/09, EW 40/09, EW 42/09, EW 43/09, EW45/09, EW 46/09, EW 50/09, EW 53/09, EW 54/09, EW 56/09, EW 57/09, EW 59/09, EW 61/09, EW 65/09, EW 69/09, EW 70/09, EW 72/09, EW 76/09, EW 88/09, EW 89/09, EW 91/09, EW 94/09, EW 103/09, EW 108/09
III	2	1.904	EW 33/09, EW 58/09
IV	3	2.86	EW 07/09, EW 47/09, EW 82/09
V	2	1.904	EW 26/09, EW 35/09
VI	2	1.904	EW 03/09, EW 19/09
VII	1	0.952	EW 106/09
VIII	1	0.952	EW 99/09
Total	**105**	**100**	

Table 6. Cluster means for coffee bean biochemical constituents and crude ash content (%) of 101 East Wollega coffee accessions and four standard checks

Variable	C.I	C. II	C.III	C. IV	C.V	C.VI	C.VII	C.VIII
Trigonelline	1.13	0.99	0.85	0.85	1.12	1.02	1.39	1.35
Chlorogenic acids	5.4	4.22	4.14	3.55	5.91	7.03	5.69	7.86
Caffeine	1.42	1.40	1.43	1.32	1.56	1.58	1.69	1.56
Crude protein	14.51	14.85	15.00	14.95	13.80	13.97	15.53	15.29
Crude fat	16.34	15.06	13.00	16.99	13.51	18.34	15.60	16.07
Crude ash	3.75	4.07	3.68	3.74	4.07	3.81	3.84	3.26

Note: C= cluster

Genetic divergence

The Mahalanobis square distance (D^2) between clusters of the 105 coffee accessions for bean biochemical constituents and crude ash content is given in Table 7. The inter -cluster distance analysis showed significant ($P<0.05$) differences between cluster I and VII, II and III, II and V and III and V and highly significant differences between other paired clusters except for cluster I and II, I and IV and II and IV , which showed non-significant difference. The highest inter cluster distance was observed between cluster III and VI (D^2=124.62), while the minimum distance was between cluster I and IV (D^2=8.63). The significant inter-cluster distances observed in the current study, may imply that there is a very good opportunity to bring about improvement in coffee biochemical constituents by crossing accessions from different clusters. In line with this, Sharma (1998); Singh (2003) and Gemechu (2006) reported that crosses involving parents belonging to the divergent clusters are expected to manifest maximum recombination and segregation.

Table.7. Average inter-cluster divergence (D^2) values for coffee bean biochemical compounds and crude ash contents of 101 of east Wollega coffee accessions and four standard checks

	I	II	III	IV	V	VI	VII	VIII
I	0	12.06 ns	44.39**	8.63ns	26.67**	26.21**	16.34*	18.57**
II		0	14.45*	11.89 ns	15.82*	68.35**	18.91**	45.38**
III			0	46.31**	14.73*	124.62**	39.60**	76.00**
IV				0	44.27**	42.53**	23.79**	43.94**
V					0	78.75**	31.18**	49.17**
VI						0	48.07**	28.17**
VII							0	22.30**
VIII								0

Note: *= significant at $P< 0.05(\chi 2) = 12.59$, **=highly significant at $P<0.01(\chi 2) = 16.81$

Conclusion

Evaluation of biochemical constituents of green coffee beans is fundamental as it provides information on cup quality. Therefore, the current study was carried out to estimate the variability among 101 east Wollega coffee accessions and four standard checks for bean biochemical compounds and physical attributes (ash and dry matter content). The results showed the existence of considerable variation among the accessions for green bean caffeine, chlorogenic acids, trigonelline, crude protein, crude fat and crude ash. Principal component analysis also revealed that crude fat and total chlorogenic acids were the major contributors to the observed variability among the tested accessions and can serve as criteria for selecting diverse parents for targeted crossing program. The diversity of the genotypes was further confirmed by cluster analysis which grouped the accessions in to six distinct clusters and two solitary with significant inter cluster distances. Therefore, the observed variability among the accessions for these biochemical attributes could be used to develop coffee varieties with desirable green bean biochemical compositions. Nevertheless, further detailed study on coffee sensory evaluation and molecular analysis may be required to come up with more comprehensive conclusion.

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References

Abeyot Tessema, Sentayehu Alamerew, Taye Kufa, and Weyessa Garedew. 2011. Variability and association of quality and biochemical attributes in some promising *Coffea arabica* germplasm collections in southwestern Ethiopia. *International Journal of Plant Breeding and Genetics*, **5(**4):302-316

Agwanda CO, Baradat P, Eskes AB, Cilas C and Charrier A. 2003.Selection for bean and liquor qualities within related hybrids of Arabica coffee in multi-local field trials. *Euphytica*, **131**(1):1-14.

Anthony, F., Clifford, M.N.and Noirot, M. 1993. Biochemical diversity in the genus *Coffea* L. chlorogenic acids, caffeine and mozambioside contents. *Genetic Resources and Crop Evolution*, **40**(2):.61-70.

AOAC.2000. Association of official analytical chemists international, Maryland, USA.

AOAC.2001. Association of official analytical chemists international, Maryland, USA.

Behailu Weldesenbet, Abrar Sualeh, Negussie Mekonnen and Solomon Endris. 2008.Coffee processing and quality research in Ethiopia. pp. 307-328. *In*: Girma Adugna, Bayetta Belachew, Tesfaye Shimber, Endale Taye and Taye Kufa (eds.).coffee diversity and knowledge. Proceedings of a national workshop four decades of coffee research and development in Ethiopia, 14-17 August 2007, Addis Ababa, Ethiopia.

Buffo RA and Cardelli Freire C. 2004. Coffee flavour: an overview, *flavor and fragrance journal*, **19**(2):99-104.

Bytof G, Knopp SE, Kramer D, Breitenstein B, Bergervoet JHW, Groot SPC and Selmar SD. 2007. Transient occurrence of seed germination processes during coffee postharvest treatment. *Annals of Botany*, **100**: 61–66

Chahal GS and Gosal SS. 2002. Principles and Procedures of Plant Breeding: Biotechnology and conventional approaches. Arosa Publishing House, New Delhi.

Chatfield C and Collin AJ. 1980. Introduction to multivariate analysis, 3rd edition. Chapman and Hall in association with Methuen, New York, USA

Clifford MN. 1985. Chlorogenic acids. *In*: R.J. Clarke, R. Macrae (eds). Coffee: Chemistry. *Elsevier Applied Science Publishers, London.* **1:** 153-202.

Clifford MN, Gibson CL, Rakotomalala, JJ, Cros E and Charrier A. 1991. Caffeine from green beans of Mascaro coffea. *Phytochemistry,* **30**(12):4039-4040.

Copper MC and Milligan GW.1988. The effect of error on determining the number of clusters. pp: 319-328. Proceeding of international workshop on data analysis, decision support and expert knowledge representation in marketing and related areas of research.

Davrieux F, Bertrand B, Bastianelli D and Guyot B. 2003. Authentication of three green coffee cultivars from Costa Rica by near infrared spectroscopy. 11th International conference on Near Infrared Spectroscopy, Cordoba, Spain.

Decazy F, Avelino J, Guyot B, Perriot JJ, Pineda C and Cilas C. 2003. Quality of different Honduran coffees in relation to several environments. *J. of Food Sci.,* **68**:2356-2361

Farah A. 2012. Coffee constituents: *In*: Chu, Y.F (ed), Coffee: Emerging health effects and disease prevention, **1:**22-58.

Gemechu Keneni. 2006. Variablity and interrelationship of some metric characters in groundnut (*Arachis hypgea* L.). MSc. Thesis, Alemaya University

Gichimu BM, Gichuru EK, Mamati GE and Nyende AB. 2014. Biochemical composition within *Coffea arabica* cv. Ruiru 11 and its relationship with cup quality. *Journal of Food Research*, **3** (3):31-44.

Gichimu BM, Gichuru EK, Mamati GE and Nyende AB. 2012. Selection within *Coffea arabica* cv. Ruiru 11 for high cup quality. *Afr. J. Food Sci.* **6**(18):456-464.

Gimase JM, Thagan WM, Kirubi DT, Gichuru EK and Kathurima CW. 2014. Genetic diversity of Arabusta coffee (*Coffea arabica* L) X coffea Canephora Pierre) and their parental genotypes. MSc thesis, Keyatta University

Hair JF, Babin BJ, Black WC and Anderson RE. 2009. Multivariate Data Analysis. London, UK

Harding PE, Bleeker P and Freyne DF. 1987. Land Suitability Evaluation for Rained Arabica Coffee Production: Western Highlands Province, Papua New Guinea. *Coffee Research Report*, **4**: 39-45.

Hidgon JV and Frei B. 2006. Coffee and health: A review of recent human research. *Critical reviews in food science and nutrition,* **46**(2):101-123

Kathurima CW, Gichimu BM, Kenji GM, Muhoho SM and Boulanger R. 2009. Evaluation of beverage quality and green bean physical characteristics of selected Arabica coffee genotypes in Kenya, Afr. *J. Food Sci.* **3**(11):365-371.

Khapre Y, Kyamuhangire W, Njoroge EK and Kathurima CW. 2017. Analysis of the diversity of some Arabica and Robusta coffee from Kenya and Uganda by sensory and biochemical components and their correlation to taste. *IOSR Journal of environmental science, toxicology and food technology*, **11**(10):39-43.

Ky CL, Louarn J, Dussert S, Guyot B, Hamon S and Noirot M. 2001. Caffeine, trigonelline, chlorogenic acids and sucrose diversity in wild *Coffea arabica* L.and C. *canephora* P. accessions. *Food chemistry*, **75(**2):223-230.

Ky CL, Louarn J, Guyot B, Charrier A, Hamon S and Noirot M. 1999. Relations between and inheritance of chlorogenic acid contents in an interspecific cross between Coffea pseudozanguebariae and Coffea liberica var. dewevrei. Theoretical and Applied Genetics 98: 628-637.

Leroy T, Ribeyre F, Bertrand B, Charmetant P, Dufour M, Montagnon C, Marraccini P, and Pot D, 2006. Genetics of coffee quality. *Braz. J. Plant Physiol*., **18**(1):229-242

Mahalanobis PC. 1936. On the generalized distance in statistics. *J. Genet*. **41:** 159-193.

Martín MJ, Pablos F and González AG. 1998. Discrimination between Arabica and Robusta green coffee varieties according to their chemical composition. *Talanta*, **46**(6):1259-1264.

Minitab. 2010. Minitab Statistical Software Packages Version 17, Minitab Inc. USA.

Montagnon C, Guyot B, Cilas C, & Leroy T. 1998. Genetic parameters of several

biochemical compounds from green coffee, *Coffea canephora*. *Plant Breed*., **117**: 576-578

Pauline A. 2013. Genetic and phenotypic diversity of cultivated Robusta coffee (*Coffea canephora* Pierre) in Uganda and effect of environmental factors on quality. Doctoral dissertation, University of Nairobi-Kenya.

Perez de la Vega M. 1994. Biochemical characterization of populations. *In:* M.D. Hayward, N.O. Bosemark and I. Romagosa (Eds.), Plant breeding: principles and prospects. pp.184-200. Chapman and Hall, London.

SAS (Statistical Analysis System) software . 2011, SAS Institute, Cary, NC, USA.

Sharma JQ. 1998. Statistical and biometrical techniques in Plant Breeding: New Age. Intl. Pvt. Ltd., India, pp. 67.

Silvarolla MB, Mazzafera P and Alves de Lima MM. 2000. Caffeine content of Ethiopian *Coffea arabica* beans. *Genet Mol Biol*, **23**:213–215

Singh BD. 2003. Plant breeding, 5th (ED), Kalyani, New, and Delhi, India

Singh RK and Chaudhary BD. 1987. Biometrical methods in quantitative genetic analysis. Kalyani publishers, New Delhi-Ludhiana, India. 318p.

Tsegaye Yilma and Taye Kufa. 2002. Characterization of the farming systems of *Haru* wereda west Wollega zone, Oromiya region. *Research Report (Ethiopia)*.

Vignoli JA, Viegas MC, Bassoli DG and Benass MT. 2014. Roasting process affects differently the bioactive compounds and the antioxidant activity of Arabica and Robusta coffees, food research international, **61**:279-285

Yeretzian C, Jordan A and Lindinger W. 2003. Analyzing the head space of coffee by proton-transfer- reaction mass-spectrometry. *International Journal of Mass Spectrometry*, **223**:.115-139.

Yigzaw Desalegn. 2005. Assessment of genetic diversity of Ethiopian Arabica coffee genotypes using morphological, biochemical and molecular markers. A PhD Dissertation, University of the Free State, South Africa. 197p.

Yigzaw D, Labuscagne MT, Osthoff G and Herselman L. 2007. Variation of green bean caffeine, chlorogenic acid, sucrose and trigolline contents among Ethiopian Arabica coffee accessions., *SINET:Ethiop.j.sci.*,**30**(1):77-82 *an Journal of Science, 30(1):.77-82.*