Journal of Food Research; Vol. 3, No. 3; 2014
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

Biochemical Composition Within *Coffea arabica* cv. Ruiru 11 and Its Relationship With Cup Quality

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Received: January 14, 2014 Accepted: February 25, 2014 Online Published: April 1, 2014

doi:10.5539/jfr.v3n3p31 URL: http://dx.doi.org/10.5539/jfr.v3n3p31

Abstract

Biochemical composition appears to be influenced by both genetic factors and plant growth conditions. The main objective of this study was to evaluate the biochemical composition of selected Ruiru 11 sibs and its relationship with cup quality. Thirty four (34) Ruiru 11 sibs grown in three different locations in Kenya were used in this study. The experiment was laid out in a Randomized Complete Block Design with three replications. Coffee cherries were picked during the peak harvesting period between 2009 and 2011. The cherries were wet processed and graded into different grades based on size, shape and density. Fifty (50) grams of the dry coffee beans per sib per replication were frozen at -80 ºC before grinding (< 0.5 mm particle size) in liquid nitrogen as specified by the Association of Official Analytical Chemists (AOAC). The samples were packed in small plastic bottles and stored at -80 ºC awaiting extraction of biochemical components. Caffeine, trigonelline and total chlorogenic acids were extracted and purified using classical methods and analysed using High Pressure Liquid Chromatography (HPLC). For the lipids, the sample was subjected to Soxhlet extraction using n-hexane. The study demonstrated the existence of high variation in biochemical composition among Ruiru 11 sibs. Significant correlations were observed between biochemical and cup quality traits indicating that biochemical composition plays a major role in determining the sensory quality of coffee. The growing environment was also found to have an effect on biochemical composition as portrayed by high locational variations.

Keywords: caffeine, trigonelline, lipids, total chlorogenic acid

1. Introduction

Biochemical compounds are metabolic products and they confer adaptive properties to plants. They participate in resistance to diseases as well as pests and give a characteristic odour or taste to edible plants (Dessalegn, 2005). Green coffee beans contain a wide range of different chemical compounds which react and interact at all stages of coffee processing to produce a final product with an even greater diversity and complexity of structure (Kathurima et al., 2010). These include Caffeine, Trigonelline, Chlorogenic acids (CGA), and lipids among others. Caffeine (1, 3, 7-trimetylxanthine), is one of the main alkaloid that is naturally found in leaves, seeds or fruits of 63 plant species (Belay, 2011). The most common sources of caffeine are coffee, cocoa beans, cola nuts and tea leaves (Mumin et al., 2006). The chemical formula for caffeine is $C_8H_{10}N_4O_2$ and the chemical structure is as shown in Figure 1.

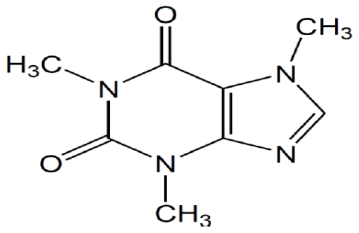

Figure 1. Chemical structure of caffeine

Caffeine content of green coffee varies widely among and within species (Ky et al., 2001; Silvarolla et al., 2004). The most common coffee species are *Coffea canephora* Pierre (Robusta coffee) and *Coffea arabica* L. (Arabica coffee). Robusta coffee in general has a higher caffeine content of about 2.2%, while that of Arabica is about 1.2% with a range of 0.6 to 1.9% (Clarke & Macarae, 1985; Franca et al., 2005; Belay et al., 2008; Belay, 2010). Intermediate values have also been reported for commercially less important species such as *Coffea liberica* and Arabusta (Arabica and Robusta crosses) with mean values of 1.35% and 1.72%, respectively (Clarke & Macarae, 1985). Other coffees in the genus Paracoffea are available in Africa and Asia with very low caffeine contents of about 0.2% (Clarke & Macarae, 1985).

The effects of environmental and agricultural factors are less important than genetic variation in controlling the caffeine contents of green coffee beans and it is also reported that fertilizers in particular potassium, phosphate, magnesium and calcium do not have a significant effect on caffeine (Clarke & Macarae, 1985). Similarly, roasting at some extent do not affect the content of caffeine, other than causing a slight relative increase due to the loss of other compounds (Clarke & Macarae, 1985; Farah et al., 2006).

Chlorogenic acids (CGA) are the main phenolic compounds found in green coffee beans (Belay, 2011). They belong to hydroxycinnamic acid classes and chiefly consists of caffeic acid (3, 4-dihydroxycinnamic acid), ferulic acid (3-methoxy-4-hydroxycinnamic acid), p-coumaric acid (4-hydroxycinnamic acid), and sinapic acid (3, 5-dimethoxy-4-hydroxycinnamic acid) (Manach et al., 2004; Zhu et al., 2006). The main groups of CGA compounds found in green coffee beans are caffeoylquinic acids with 3 isomers (3-, 4-, and 5-CQA); dicaffeoylquinic acids (diCQA) with 3 isomers (3-,4-diCQA; 3,5-diCQA; 4,5-diCQA); feruloquinic acids (FQA), with 3 isomers (3-, 4-, and 5-FQA); p-coumaroylquinic acids (pCoQA) with 3 isomers (3-, 4-, and 5-pCoQA) and six mixed diesters of caffeoylferuloy-quinic acids (CFQA) (Belay, 2011).

The total CGA content of green coffee beans vary according to species, degree of maturation and less importantly agricultural practices, climate and soil (Clifford, 1985; Farah et al., 2005b). In general, the percentage of CGA for regular green coffee beans on dry matter basis varies from 4 to 8.4% for Arabica and 7 to 14.4% for Robusta with some hybrids presenting intermediate levels (Farah et al., 2005a, 2005b). On the other hand, a low CGA content of 1.2% was reported in beans of *Coffea pseudozanquebariae*, a caffeine-free species native of East Africa. Such low contents have also been observed in some other low caffeine species from Africa (Clifford, 1985). Chlorogenic acids play a great role in the formation of pigments, taste and flavor of coffee beans, which determine the quality and acceptance of the beverages. They contribute to the final acidity of the beverages and the formation of lactones and other phenol derivatives responsible for flavor and aroma (Variyar et al., 2003).

Trigonelline (N-methyl-nicotinate) was named after the leguminous plant *Trigonella foenumgraecum* L. (Fenugreek) from which the compound was first isolated and characterized (Taguchi et al., 1985). The chemical formula for trigonelline is $C_7H_7NO_2$ and the chemical structure is as shown in Figure 2. It is a vitamin B6 derivative having a low, bitter taste in comparison to caffeine and probably the most substantial element which contributes to undue bitterness in coffee and is 100 percent water soluble (Anaparti, 2013). It is also known as Coffearin, Coffearine and Gynesine (Anaparti, 2013). It is widely distributed in plants within the subclass Dicotyledonae and is claimed to have anticarcinogenic, antimigraine, antiseptic, hypocholesterolemic, and hypoglycemic activities. Trigonelline is produced in green coffee beans by nicotinic acid (pyridinium-3-carboxylic acid) methylation using methionine, a kind of amino acid containing sulphur (Anaparti, 2013).

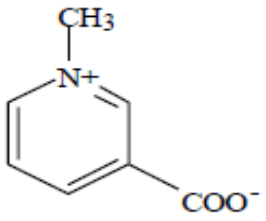

Figure 2. Chemical structure of trigonelline

Fenugreek and coffee are the commonly occurring and widely known substances containing trigonelline. Trigonelline comprises about 0.7% and 1.1% of the chemical composition of the green coffee of *C. canephora* and *C. arabica* respectively (Wasserman et al., 1993). Common foods containing trigonelline include barley,

cantaloupe, corn, onions, peas, soybeans, and tomatoes (Beckstrom-Sternberg & Duke, 1997). Trigonelline may be present in prepared coffee in concentrations as high as 1% (Taguchi et al., 1985, 1986). The average trigonelline content in a cup of coffee is 53 mg (0.39 mmol) (Clifford, 1985).

The lipid fraction of coffee is composed mainly of triacylglycerols, sterols and tocopherols, the typical components found in all common edible vegetable oils. Additionally, the so-called coffee oil contains diterpenes of the kaurene family in proportions of up to 20% of the total lipids (Speer & Kölling-Speer, 2006). Coffee contain between 7 and 17% fat. The lipid content of green Arabica coffee beans averages 15%, whilst Robusta coffee contains much less, averaging around 10%. Most of the coffee lipids are located in the endosperm of green coffee beans (Wilson et al., 1997). Only a small amount, the coffee wax, is located on the outer layer of the bean (Speer & Kölling-Speer, 2006). Green Arabica coffee oil reportedly contain 75% triglycerides with a high percentage of unsaponifiables, including about 19% total free and esterified diterpene alcohols, about 5% total free and esterified sterols, and the remainder, comprises other substances, such as tocopherols (Clarke & Vitzthum, 2001).

A lot of work has been done in attempting to understand the biochemical composition of green and roasted coffee beans and its effect on cup quality (Ky et al., 2001; Decazy et al., 2003; Bertrand et al., 2006; Farah et al., 2006; Kathurima et al., 2010; Tessema et al., 2011). Despite this, the specific contribution of evaluated biochemical traits to the final cup quality remains largely unknown. Kathurima et al. (2010) noted that there is missing link between biochemical assessment studies and the genetic improvement of coffee. They noted that more progress would be expected if biochemical studies are integrated at early stages of coffee improvement. Coffee quality is the result of complex interactions between the environment, the imposed management regime and the plant. In coffee beans, the biochemical composition appears to be influenced by both genetic factors (Montagnon et al., 1998) and plant growth conditions (Viani, 2001; Leroy et al., 2006). With all this realization, this study sought to evaluate the biochemical composition of Ruiru 11 sibs and to investigate the relationship between biochemical composition and the end result cup quality of different Ruiru 11 sibs grown in varying environments.

2. Materials and Methods

2.1 Description of Study Sites

The study was conducted in three different locations in Kenya namely Mariene in Meru county, Kisii near Kisii town in Kisii county and Koru in Kericho county.

2.1.1 Mariene

Mariene is located in the upper midland 2 agro-ecological zone at 0º N, 37º 35’ E, at an elevation of 1524 m above sea level. The area receives a mean annual rainfall of 1500 mm. The minimum average temperature is 11.0 ºC and maximum average temperature is 24.5 ºC. The soils are ando-humicacrisols, friable clays, strongly acidic, very low in bases and moderate in organic matter (Jaetzold et al., 2005).

2.1.2 Koru

Koru is located in the lower midland 3 agro-ecological zone at 0º 07’ S, 35º 16’ E and has an elevation of 1554 m above sea level. The annual average temperature ranges between 21 -24 ºC. The soils are eutricnitosols, friable clays, and weakly acidic to neutral, rich in bases, available phosphorous and moderate inorganic matter (Jaetzold et al., 2005).

2.1.3 Kisii

Kisii is found in the upper midland 1 at 0º 41’S, 34º 47’E at 1680 m above sea level. The annual average temperature is 19.4 ºC. The soils are molicnitosols, friable clays with acidic pH, low to moderate bases and are high in organic matter (Jaetzold et al., 2005).

2.2 Experimental Layout and Design

The experiment was set up in existing coffee experimental plots that were established in April 1990 in Koru and Kisii and in April 1991 in Meru. The plots were laid out in a Randomized Complete Block Design (RCBD) with three replications. Each replication consisted of 12 trees of each sib planted at a spacing of 2 m by 1.5 m. All the plots had undergone change of cycle twice. Other agronomic practices including weeding, pruning, desuckering and fertilizer application were carried out as recommended.

2.3 Test Genotypes

Thirty four (34) Ruiru 11 sibs (Table 1) were evaluated in this study alongside two entries of SL28 used as

checks. One entry of SL28 was sprayed with copper fungicides to control CBD and CLR. The fungicides were applied using a 20 litres knapsack sprayer at recommended rates as provided in CRF technical circular number 804 (Coffee Research Foundation, 2013). The spray was programmed to start just before flowering and continue up to just before berry ripening at a spray interval of 21 days. The other entry of SL28 was not sprayed.

Table 1. The pedigree of the 34 Ruiru 11 sibs evaluated

	Female Parent						
Male Parent	Cat.86	Cat.88	Cat.90	Cat.124	Cat.127	Cat.128	Cat.134
SL34 × [(SL34 × RS) HT]	-	-	-	135	-	137	-
SL28 × [(SL28 × RS) (B × HT)]	1,11,41	22,42	3,23	5	6	7	50
SL28 × [(N39 × HT) (SL4 × RS)]	71	72	-	-	-	-	80
SL28 × [(K7 × RS) (SL34 × HT)]	-	52	-	-	-	-	-
SL28 × [(SL34 × RS) HT]	91,111, 121,131	112,14	93,103, 123,143	105,115, 125	106	107,117	100

Key: RS = Rume sudan, HT = Hibrido de Timor, B = Bourbon.

2.4 Cherry Picking, Processing and Grinding of Coffee Samples

Coffee cherries were picked during the peak harvesting period of May to July at Mariene and September to November at Koru and Kisii between the years 2009 and 2011. The cherries were picked from all the 12 trees of each test genotype and bulked per replication. The cherries were then pulped, fermented, washed and the beans dried to final moisture content of 10.5 to 11% following the standard processing procedures outlined in CRF technical circular number 204 (Coffee Research Foundation, 2010). The parchment was then hulled and graded to seven grades based on size, shape and density as follows: AA – beans retained by 7.15 mm screen; AB – beans retained by 5.95 mm screen; TT – light beans separated from AA and AB using Pneumatic separator; PB – beans retained by a piano wire screen with 4.43 mm spaces; C – beans retained by a piano wire screen with 2.90 mm spaces; T – very small beans and broken bits; E – elephant beans which are the largest coffee beans resulting from two coffee seeds in one cherry joining together (a genetic defect). Only the premium grades (AA and AB) were used for biochemical and cup quality (sensory) evaluation because they form the biggest proportion of the coffee beans and are the most desired in the market.

2.5 Extraction and Determination of Biochemical Compounds

Fifty (50) grams of dry coffee beans per sib per replication were frozen at -80 ºC before grinding (<0.5 mm particle size) in liquid nitrogen using Ultra Centrifugal ZM 200 Mill (Retsch, Germany) as specified by the Association of Official Analytical Chemists (AOAC, 1990). The samples were packed in small plastic bottles and stored at -80 ºC awaiting extraction of biochemical components. Different extraction and purification methods were used according to the specific compounds as described in section 2.5.1 – 2.5.4 below. The lipids were extracted using the standard soxhlet extraction method developed by the Association of Official Analytical Chemists (AOAC, 1990). Caffeine, trigonelline and CGA were extracted and purified according to the method of Ky et al. (2001).

2.5.1 Extraction and Determination of Lipids

Each sample of the ground coffee powder from section 2.4 above was taken and its moisture content (M) determined using infrared moisture analyzer (OHAUS-MB45). Five (5) grams of the powder was weighed in a thimble, marked as W and dried for 2 hours at 100ºC. An empty round bottomed flask was dried at 105 ºC for 1 hour, cooled in a dessicator, weighed and recorded as W1. The thimble (with green coffee sample) was placed in a soxhlet extraction apparatus and lipids extracted with n-hexane solvent for 8 hours. The extract was left to evaporate to near dryness using rotavapor before further drying in an oven for 1 hour at 105 ºC after which it was cooled and weighed. The drying and weighing was alternated at 30 minutes intervals until no more loss in weight between two successive intervals was observed. The lowest attainable weight was recorded as W2 and percent of lipids in dry weight basis (DWB) calculated using the following formula:

$$\% \text{ Lipids (DWB)} = \frac{10000(W2-W1)}{W(100-M)}$$

2.5.2 Extraction of Caffeine

Five (5) gms of the ground coffee powder was weighed in a 250 ml Erlenmeyer flask before adding 3.5 gms of magnesium oxide and 200 ml of double distilled water. The mixture was refluxed by boiling while continually cooling the vapor to liquid and returning it back to the flask for 25 minutes and then left to cool. It was then filtered under vacuum on celite and the filtrate recovered in a 250 ml flask topping up the volume with distilled water. Twenty (20) ml of this preparation was then pipetted into 100 ml flask adjusting to volume with mobile phase consisting of 35% v/v methanol, 65% v/v distilled water and 0.1% v/v glacial acetic acid. The extracts were then filtered using millex (0.45 μm) filters and injected into HPLC.

2.5.3 Extraction of Trigonelline

Six (6) gms of the ground coffee powder was weighed in a 250 ml Erlenmeyer flask before adding 0.20 gms of magnesium oxide and 40 ml of double distilled water. The mixture was refluxed for 10 minutes and left to cool. It was then filtered under vacuum on celite and the filtrate recovered in a 50 ml flask topping up the volume with distilled water. Twenty (20) ml of this preparation was then pipetted into 100 ml flask adjusting to volume with mobile phase. The extracts were then filtered using millex (0.45 μm) filters and injected into HPLC.

2.5.4 Extraction of Total Chlorogenic Acids (CGA)

Seven (7) gms of the ground coffee powder was weighed in a 250 ml Erlenmeyer flask before adding 40 ml of double distilled water. The mixture was refluxed for 15 minutes and then left to cool. It was then filtered under vacuum on celite and the filtrate recovered in a 50 ml flask topping up the volume with distilled water. Twenty (20) ml of this preparation was then pipetted into 100 ml flask adjusting to volume with mobile phase. The extracts were then filtered using millex (0.45 μm) filters and injected into HPLC.

2.6 Quantification of Biochemical Composition

Caffeine, trigonelline and CGA chromatography were carried out on a Hewlett Packard system HPLC consisting of Discovery C 18 or Eurospher 100-5 C18 column with Isocratic flow of 1 ml/min. Working standards of 10, 20, 40, 60 and 80 mg per 100 ml of mobile phase were injected into the HPLC. A calibration curve was made using the standard concentration and area of sample and subsequently used to calculate the composition of the respective biochemical component using the area generated after the retention time. The detection was carried out at 278 nm (caffeine), 266 nm (trigonelline) and 324 nm (CGA).

2.7 Cup Quality (Sensory) Evaluation

Roasting of the dry beans was done to attain a medium roast using a probat laboratory roaster manufactured by PROBAT-Werke, Germany. The samples were weighed before and after roasting to determine the uniformity of roasting. The samples were ground immediately after roasting using a probat laboratory grinder (Type 55 LM 1500) manufactured by PROBAT-Werke, Germany. A rinsing quantity of every sample was run through the grinder before grinding the test sample. Each sib was ground individually and deposited into the cupping cups, ensuring that the whole and consistent quantity of sample gets deposited into each cup with five cups per sample. The ground samples were then infused in hot water using a predetermined ratio of 8.25 gms per 150 ml of water prior to cupping. Sensory evaluation procedure described by Lingle (2001) was followed. Seven sensory variables namely: fragrance, flavour, aftertaste, acidity, body, balance and preference, were assessed by a trained panel of seven and rated on a 10-point scale as follows: 1 = very poor and 10 = outstanding for the attributes fragrance/aroma, flavor, aftertaste, balance and preference; 1 = very flat and 10 = very bright for acidity; and 1 = very thin and 10 = very heavy for body. An overall score (total score) was calculated as the sum of all the seven variables plus 30 points that are normally added to adjust the final score to a 100-point basis.

2.8 Data Analysis

The biochemical data were subjected to Analysis of Variance (ANOVA) at 5% level of significance using XLSTAT Version 2012 statistical software. Separate as well as combined analysis of variance was performed on data from all locations. Least significance difference (LSD5%) was used to separate the means. To assess the diversity among sibs based on their biochemical composition, the data was organized into a matrix and subjected to cluster analysis using XLSTAT Version 2012. A dendrogram was constructed using the unweighted pair-group method with arithmetic average [UPGMA]. In order to determine the association between the biochemical

components and the relationship between biochemical composition and cup quality, correlation tests were done to compare different variables.

3. Results

Ruiru 11 sibs recorded significant ($P < 0.05$) differences among them for the four biochemical components namely caffeine, trigonelline, CGA and lipids across the different locations and seasons. Analysis of variance of the individual years with the locations combined revealed that the locational effect was significant ($P < 0.05$) except for caffeine in 2010. Location by genotype ($G \times E$) interactions were also significant ($p > 0.05$) for all the traits (Table 2).

Table 2. P values of multi-locational analysis of variance for biochemical composition of Ruiru 11 sibs at different locations and seasons

	Sib Variations											Location Variations			Location x Sib Interactions		
	Mariene				Koru				Kisii								
Traits	2009	2010	2011	Combined	2009	2010	2011	Combined	2010	2011	Combined	2009	2010	2011	2009	2010	2011
CGA	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***
Caffeine	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.565^{ns}	0.000***	0.000***	0.000***	0.000***
Trigonelline	0.000***	0.000***	0.001***	0.004**	0.000***	0.021*	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.009**	0.001***	0.000***	0.000***
Lipids	0.000***	0.022*	0.000***	0.000***	0.000***	0.001***	0.000***	0.000***	0.001***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***
DF	35	35	35	35	35	35	35	35	35	35	35	1	2	2	35	70	70

Key: * = Significant at $p < 0.05$; ** =Significant at $p < 0.01$; *** =Significant at $p < 0.001$; ^{ns} =Not Significant.

Table 3 shows the diversity among the Ruiru 11 sibs at the three locations as determined by biochemical levels of CGA, caffeine, trigonelline and lipids. At Kisii, R11-111 recorded the highest level of CGA (7.95%) which was not significantly different ($P > 0.05$) from that of six other sibs namely R11-103, R11-100, R11-23, R11-107, R11-115 and R11-71. R11-131 recorded the lowest level of CGA (6.207%) which was not significantly different ($P > 0.05$) from that of nine other Ruiru 11 sibs and both sprayed and unsprayed SL28. Caffeine levels at Kisii ranged from 1.501% (R11-112) to 1.124% (R11-121) while trigonelline varied from 1.285% (R11-23) to 1.004% (R11-121). The level of lipids varied from 16.477% (R11-142) to 12.543% (R11-93) (Table 3).

At Koru, R11-123 recorded the highest CGA content (6.716%) which was not significantly different ($P > 0.05$) from R11-143 (6.412%). R11-41 recorded the lowest CGA content (5.242%). Caffeine levels at Koru ranged from 1.538% (R11-23) to 1.073% (R11-52) while trigonelline levels varied from 1.339% (R11-106) to 1.124% (R11-112). The lipid levels ranged between 18.405% (R11-72) to 14.878% (R11-41) (Table 3).

At Mariene, R11-22 recorded the highest levels of CGA (6.253%) which was statistically similar to that of ten other sibs and sprayed SL28. R11-117 recorded the lowest CGA content (5.370%). Caffeine levels at Mariene varied from 1.250% (R11-91) to 1.011% (R11-72) while trigonelline ranged from 1.177% (R11-105) to 0.949% (R11-71). Unsprayed SL28 recorded the highest lipid content (16.447%) which was statistically similar to that of R11-52 (16.235). R11-117 recorded the lowest lipid content (13.859%) at Mariene (Table 3).

Table 3. Average levels of different biochemical components per sib per location

	Kisii				Koru				Mariene			
Genotypes	CGA (%)	Caffeine (%)	Trigon (%)	Lipids (%)	CGA (%)	Caffeine (%)	Trigon (%)	Lipids (%)	CGA (%)	Caffeine (%)	Trigon (%)	Lipids (%)
R11-1	6.755 g-l	1.340 c-g	1.127 i-o	15.279 a-f	5.688 h-m	1.262 f-k	1.260 a-g	15.626 g-l	5.790 d-i	1.179 b-e	1.087 a-e	14.158 no
R11-3	7.361 b-e	1.321 d-h	1.177 e-i	13.326 hi	6.358 b	1.314 d-h	1.217 c-i	16.795 b-h	5.754 d-j	1.143 d-j	1.074 b-g	14.611 h-o
R11-5	6.937 d-k	1.289 e-i	1.181 d-h	14.265 c-i	5.410 l-n	1.294 d-i	1.180 g-j	16.441 d-j	5.729 e-j	1.192 b-d	1.027 d-h	15.848 a-c
R11-6	6.913 e-k	1.304 d-i	1.131 h-o	14.840 b-h	5.897 d-j	1.194 i-l	1.209 d-i	15.760 f-l	5.764 d-j	1.088 k-p	1.060 b-g	14.465 k-o
R11-7	7.045 c-j	1.416 a-c	1.196 c-g	14.330 c-h	5.691 h-m	1.281 d-j	1.158 h-j	16.947 b-f	6.073 a-d	1.133 e-l	1.130 a-c	15.215 c-k
R11-11	6.769 f-k	1.174 lm	1.152 f-k	14.865 a-h	5.895 d-j	1.281 d-j	1.203 d-j	14.878 l	6.223 ab	1.149 c-i	1.047 b-g	14.813 g-n
R11-22	6.637 h-l	1.268 f-k	1.161 f-j	15.409 a-e	5.535 k-n	1.476 ab	1.319 ab	15.283 i-l	6.253 a	1.075 m-p	1.069 b-g	15.231 c-j
R11-23	7.535 a-c	1.388 b-d	1.285 a	13.792 f-i	5.803 f-k	1.538 a	1.296 a-c	16.891 b-g	6.188 a-c	1.158 b-g	1.044 b-g	15.016 e-m
R11-41	6.654 h-l	1.193 j-m	1.175 e-i	14.478 b-h	5.242 n	1.265 f-k	1.195 e-j	15.094 kl	5.560 i-k	1.153 b-h	1.033 d-h	15.512 b-g
R11-42	6.876 e-k	1.373 b-e	1.255 ab	13.261 hi	5.679 h-m	1.260 f-k	1.266 a-f	15.640 g-l	5.907 b-g	1.049 pq	1.082 b-e	14.695 h-n
R11-50	7.046 c-j	1.484 a	1.092 m-p	16.074 ab	5.954 c-h	1.262 f-k	1.239 b-h	17.836 a-c	5.902 b-h	1.127 e-m	1.077 b-g	15.754 a-e
R11-52	7.157 c-h	1.321 d-h	1.236 a-c	15.556 a-d	6.182 b-e	1.073 m	1.241 b-h	17.606 a-d	6.143 a-c	1.057 n-q	1.044 b-g	16.230 ab
R11-71	7.471 a-d	1.300 d-i	1.099 l-p	15.323 a-f	5.806 f-k	1.274 e-k	1.194 e-j	18.405 a	5.945 a-g	1.047 pq	0.949 h	14.572 h-o
R11-72	6.970 d-k	1.334 c-g	1.143 g-m	15.785 a-c	5.635 h-m	1.317 d-g	1.228 c-i	16.772 b-h	5.693 f-k	1.011 q	0.988 g-h	14.938 f-m
R11-80	6.928 d-k	1.198 j-m	1.107 k-p	15.427 a-e	5.601 i-m	1.300 d-h	1.187 e-j	16.431 d-j	5.896 b-h	1.109 g-n	1.063 b-g	15.106 c-l
R11-91	6.835 e-k	1.236 h-l	1.086 n-p	15.740 a-c	6.194 b-e	1.177 kl	1.200 d-j	16.325 d-k	5.771 d-j	1.250 a	1.094 a-e	15.159 c-k
R11-93	7.122 c-i	1.375 b-e	1.190 c-g	12.543 i	5.908 d-i	1.214 h-l	1.213 c-i	15.538 h-l	5.807 d-i	1.157 b-h	1.078 b-f	15.481 b-g
R11-100	7.536 a-c	1.257 g-l	1.021 qr	16.001 ab	5.866 e-k	1.233 g-l	1.164 h-j	15.845 f-l	5.863 c-i	1.104 h-o	1.023 d-h	14.666 h-n
R11-103	7.726 ab	1.448 ab	1.168 f-j	13.762 f-i	5.763 f-k	1.181 j-l	1.213 c-i	15.222 j-l	5.756 d-j	1.014 q	0.991 f-h	14.839 g-n
R11-105	6.987 c-j	1.418 a-c	1.101 k-p	15.676 a-c	5.382 mn	1.321 d-g	1.219 c-i	16.561 c-i	5.803 d-i	1.169 b-f	1.177 a	15.263 c-j
R11-106	7.029 c-j	1.370 b-e	1.136 h-n	13.857 e-i	5.554 j-n	1.288 d-i	1.339 a	15.994 f-l	5.733 e-j	1.171 b-f	1.014 e-h	15.535 b-g
R11-107	7.528 a-c	1.273 f-j	1.132 h-o	13.816 e-i	6.079 b-g	1.263 f-k	1.208 d-j	15.672 f-l	5.970 a-g	1.081 l-p	1.073 b-g	14.711 h-n
R11-111	7.945 a	1.500 a	1.131 h-o	13.626 g-i	6.354 b	1.382 b-d	1.230 c-i	17.461 a-d	5.760 d-j	1.138 e-k	1.133 ab	14.533 j-o
R11-112	7.275 b-g	1.501 a	1.117 j-o	15.001 a-g	5.661 h-m	1.226 g-l	1.124 j	15.818 f-l	6.044 a-e	1.204 ab	1.027 d-h	14.553 i-o
R11-115	7.472 a-d	1.358 c-f	1.118 j-o	15.589 a-d	6.179 b-e	1.308 d-h	1.251 b-g	17.427 a-e	6.084 a-d	1.115 g-m	1.077 b-g	14.698 h-n
R11-117	6.504 j-l	1.418 a-c	1.191 c-g	15.054 a-g	5.729 h-l	1.353 c-f	1.149 ij	16.028 f-l	5.370 k	1.178 b-e	1.067 b-g	13.859 o
R11-121	6.594 i-l	1.124 m	1.004 r	14.021 d-i	5.916 d-i	1.220 g-l	1.182 f-j	16.107 f-l	5.663 g-k	1.092 j-p	1.059 b-g	14.268 m-o
R11-123	7.322 b-f	1.365 b-e	1.232 b-d	14.713 b-h	6.716 a	1.374 b-e	1.223 c-i	15.854 f-l	5.711 f-j	1.200 a-c	1.075 b-g	15.298 c-i
R11-125	7.148 c-h	1.302 d-i	1.089 n-p	15.662 a-c	6.272 bc	1.299 d-h	1.220 c-i	18.060 ab	5.444 jk	1.135 e-k	1.082 b-e	15.817 a-d
R11-131	6.207 l	1.189 j-m	1.145 f-l	14.013 d-i	5.744 g-k	1.286 d-i	1.209 d-i	16.130 e-l	5.576 h-k	1.098 i-p	1.105 a-d	15.065 d-l
R11-135	6.547 j-l	1.369 b-e	1.183 d-h	14.847 b-h	5.704 h-m	1.219 g-l	1.224 c-i	17.538 a-d	6.010 a-f	1.107 g-o	1.089 a-e	14.365 l-o
R11-137	6.662 h-l	1.270 f-k	1.083 op	14.299 c-h	6.089 b-f	1.361 c-f	1.182 f-j	15.246 j-l	5.792 d-i	1.145 d-j	1.042 c-g	15.198 c-k
R11-142	6.425 kl	1.300 d-i	1.168 f-j	16.477 a	5.929 c-i	1.450 a-c	1.283 a-d	16.026 f-l	6.081 a-d	1.054 o-q	0.990 f-h	15.247 c-j
R11-143	7.153 c-h	1.229 i-l	1.225 b-e	14.809 b-h	6.412 ab	1.433 bc	1.215 c-i	16.440 d-j	5.895 b-h	1.153 b-h	1.066 b-g	15.326 c-h
SL28(NS)	6.530 j-l	1.181 k-m	1.149 f-l	15.814 a-c	5.588 i-m	1.290 d-i	1.153 ij	15.848 f-l	5.774 d-j	1.111 g-m	1.004 e-h	16.447 a
SL28(S)	6.682 h-l	1.218 i-l	1.062 pq	15.340 a-f	6.235 b-d	1.154 lm	1.269 a-e	17.796 a-c	6.222 ab	1.122 f-m	1.052 b-g	15.693 a-f

Means followed by the same letter(s) within the column are not significantly different at P≤0.05. Trigon = Trigonelline

Key: The hyphen (-) represents the alphabetical range between the letters

Combined analysis showed significant ($P \leq 0.05$) differences between the locations. Location by genotype interactions were also significant ($P \leq 0.05$). Locational effect on biochemical composition for specific Ruiru 11 sibs is shown in Table 4. The highest levels of caffeine and CGA were observed at Kisii followed by Koru while the highest levels of trigonelline were observed at Koru. Mariene and Kisii recorded the lowest levels of trigonelline and lipids respectively.

Table 4. Locational effect on biochemical composition of Ruiru 11 sibs

	Caffeine (%)			CGA (%)			Trigonelline (%)			Lipids (%)		
Genotypes	Kisii	Koru	Mariene	Kisii	Koru	Mariene	Kisii	Koru	Mariene	Kisii	Koru	Mariene
R11-1	1.340 a	1.262 a	1.179 b	6.755 a	5.688 b	5.790 b	1.127 ab	1.260 a	1.087 b	15.279 a	15.626 a	14.158 a
R11-3	1.321 a	1.314 a	1.143 b	7.361 a	6.358 b	5.754 c	1.177 a	1.217 a	1.074 b	13.326 c	16.795 a	14.611 b
R11-5	1.289 a	1.294 a	1.192 b	6.937 a	5.410 b	5.729 b	1.181 a	1.180 a	1.027 b	14.265 b	16.441 a	15.848 a
R11-6	1.304 a	1.194 b	1.088 c	6.913 a	5.897 b	5.764 b	1.131 b	1.209 a	1.060 c	14.840 a	15.760 a	14.465 a
R11-7	1.416 a	1.281 b	1.133 c	7.045 a	5.691 c	6.073 b	1.196 a	1.158 a	1.130 a	14.330 b	16.947 a	15.215 b
R11-11	1.174 b	1.281 a	1.149 b	6.769 a	5.895 b	6.223 ab	1.152 a	1.203 a	1.047 b	13.792 a	15.538 a	14.813 a
R11-22	1.268 b	1.476 a	1.075 c	6.637 a	5.535 b	6.253 a	1.161 b	1.319 a	1.069 b	14.865 b	16.891 a	15.231 b
R11-23	1.388 b	1.538 a	1.158 c	7.535 a	5.803 c	6.188 b	1.285 a	1.296 a	1.044 b	15.409 a	15.094 a	15.016 a
R11-41	1.193 b	1.265 a	1.153 b	6.654 a	5.242 b	5.560 b	1.175 a	1.195 a	1.033 b	14.478 a	14.878 a	15.512 a
R11-42	1.373 a	1.260 b	1.049 c	6.876 a	5.679 b	5.907 b	1.266 a	1.255 a	1.082 b	13.261 b	15.283 a	14.695 a
R11-50	1.484 a	1.262 b	1.127 b	7.046 a	5.954 b	5.902 b	1.092 b	1.239 a	1.077 b	16.074 a	15.640 a	15.754 a
R11-52	1.321 a	1.073 b	1.057 b	7.157 a	6.182 b	6.143 b	1.236 a	1.241 a	1.044 b	15.556 a	17.836 a	16.230 a
R11-71	1.300 a	1.274 a	1.047 b	7.471 a	5.806 b	5.945 b	1.099 ab	1.194 a	0.949 b	15.323 ab	17.606 a	14.572 b
R11-72	1.334 a	1.317 a	1.011 b	6.970 a	5.635 b	5.693 b	1.143 b	1.228 a	0.988 c	15.785 b	18.405 a	14.938 b
R11-80	1.198 b	1.300 a	1.109 c	6.928 a	5.601 c	5.896 b	1.107 b	1.187 a	1.063 b	15.427 b	16.772 a	15.106 b
R11-91	1.236 a	1.177 a	1.250 a	6.835 a	6.194 ab	5.771 b	1.086 b	1.200 a	1.094 b	15.740 ab	16.431 a	15.159 b
R11-93	1.375 a	1.214 b	1.157 b	7.122 a	5.908 b	5.807 b	1.190 a	1.213 a	1.078 b	12.543 c	16.325 a	15.481 b
R11-100	1.257 a	1.233 a	1.104 b	7.536 a	5.866 b	5.863 b	1.021 b	1.164 a	1.023 b	16.001 a	15.845 a	14.666 b
R11-103	1.448 a	1.181 b	1.014 c	7.726 a	5.763 b	5.756 b	1.168 a	1.213 a	0.991 b	13.762 b	15.222 a	14.839 a
R11-105	1.418 a	1.321 a	1.169 b	6.987 a	5.382 c	5.803 b	1.101 a	1.219 a	1.177 a	15.676 a	16.561 a	15.263 b
R11-106	1.370 a	1.288 a	1.171 b	7.029 a	5.554 b	5.733 b	1.136 b	1.339 a	1.014 c	13.857 b	15.994 a	15.535 a
R11-107	1.273 a	1.263 a	1.081 b	7.528 a	6.079 b	5.970 b	1.132 ab	1.208 a	1.073 b	13.816 b	15.672 a	14.711 a
R11-111	1.500 a	1.382 b	1.138 c	7.945 a	6.354 b	5.760 c	1.131 b	1.230 a	1.133 b	13.626 b	17.461 a	14.533 b
R11-112	1.501 a	1.226 b	1.204 b	7.275 a	5.661 b	6.044 b	1.117 a	1.124 a	1.027 b	15.001 a	15.818 a	14.553 a
R11-115	1.358 a	1.308 a	1.115 b	7.472 a	6.179 b	6.084 b	1.118 b	1.251 a	1.077 b	15.589 b	17.427 a	14.698 b
R11-117	1.418 a	1.353 a	1.178 b	6.504 a	5.729 b	5.370 b	1.191 a	1.149 ab	1.067 b	15.054 b	16.028 a	13.859 c
R11-121	1.220 a	1.124 b	1.092 b	6.594 a	5.916 b	5.663 c	1.004 c	1.182 a	1.059 b	14.021 b	16.107 a	14.268 b
R11-123	1.365 a	1.374 a	1.200 b	7.322 a	6.716 a	5.711 b	1.232 a	1.223 a	1.075 b	14.713 b	15.854 a	15.298 ab
R11-125	1.302 a	1.299 a	1.135 b	7.148 a	6.272 b	5.444 c	1.089 b	1.220 a	1.082 b	15.662 b	18.060 a	15.817 b
R11-131	1.189 ab	1.286 a	1.098 b	6.207 a	5.744 b	5.576 b	1.145 b	1.209 a	1.105 b	14.013 c	16.130 a	15.065 b
R11-135	1.369 a	1.219 b	1.107 c	6.547 a	5.704 b	6.010 ab	1.183 a	1.224 a	1.089 b	14.847 b	17.538 a	14.365 b
R11-137	1.270 a	1.361 a	1.145 b	6.662 a	6.089 b	5.792 b	1.083 b	1.182 a	1.042 c	14.299 b	15.246 a	15.198 a
R11-142	1.300 a	1.450 a	1.054 b	6.425 a	5.929 a	6.081 a	1.168 a	1.283 a	0.990 b	16.477 a	16.026 a	15.247 b
R11-143	1.229 b	1.433 a	1.153 b	7.153 a	6.412 b	5.895 b	1.225 a	1.215 a	1.066 b	14.809 a	16.440 a	15.326 a
SL28(NS)	1.181 b	1.290 a	1.111 c	6.530 a	5.588 c	5.774 b	1.149 a	1.153 a	1.004 b	15.814 a	15.848 a	16.447 a
SL28(S)	1.218 a	1.154 b	1.122 c	6.682 a	6.235 b	6.222 b	1.062 b	1.269 a	1.052 b	15.340 b	17.796 a	15.693 b

Means followed by the same letter(s) within the row are not significantly different at $P \leq 0.05$

Key: The hyphen (-) represents the alphabetical range between the letters

Variation in biochemical composition among the genotypes was further demonstrated by the cluster dendrogram developed using the four biochemical components (Figure 3). Three main classes labeled 1, 2 and 3 in the figure were formed when the similarity index was considered for clustering. Eighteen (18) of Ruiru 11 sibs were clustered in Class 1. Class 2 contained only three individuals namely R11-125, R11-72 and unsprayed SL28. The sprayed SL28 clustered with 14 Ruiru 11 sibs in cluster 3 but was much closer to R11-115, R11-50 and R11-71. Within class diversity of 25.73% was recorded alongside a between classes diversity of 74.27%. The highest between class diversity was observed between classes 1 and 3 while classes 1 and 2 were the most closely related. The parentage of these sibs did not appear to play significant role in modifying their diversity.

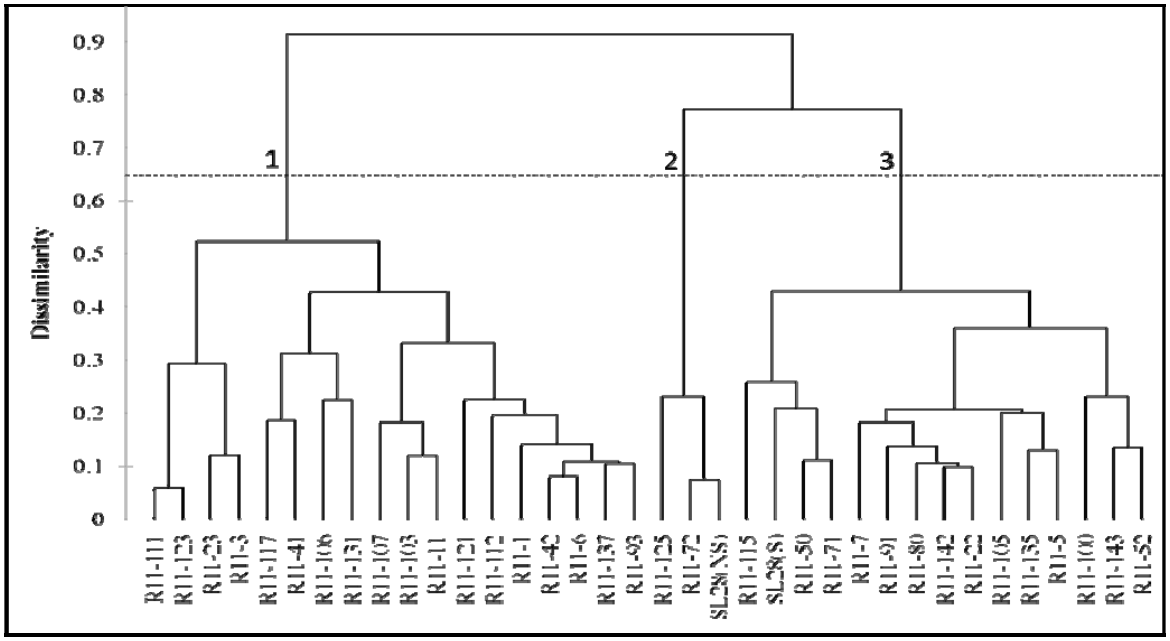

Figure 3. Dendrogram depicting diversity between sibs based on biochemical components. The broken line shows the point at which the dendrogram was truncated to define distinct sub-clusters

Correlation coefficients portrayed negative association between caffeine and all the other three biochemical components namely CGA, trigonelline and lipids which recorded positive correlations between them. CGA and caffeine recorded negative correlations with all cup quality traits except at Kisii where positive correlations were observed between them and body and balance respectively (Table 5). On the other hand, significant positive correlations were observed between trigonelline and most of the cup quality traits except balance at Kisii. The lipids were also positively correlated to all cup quality traits except flavor at Koru and balance at Kisii and Koru.

Table 5.Correlation between biochemical components of Ruiru 11 sibs at different locations

	Variables	CGA			
Kisii	Caffeine	**-0.206**			
Koru	Caffeine	-0.058			
Mariene	Caffeine	**-0.551**	Caffeine		
Koru	Trigonelline	-0.032	**-0.354**		
Kisii	Trigonelline	**0.269**	**-0.320**		
Mariene	Trigonelline	**0.357**	-0.071	Trigonelline	
Kisii	Lipids	**0.286**	**-0.416**	**0.172**	
Koru	Lipids	**0.368**	**-0.300**	**0.229**	
Mariene	Lipids	**0.346**	**-0.178**	-0.087	Lipids
Kisii	Fragrance	0.051	**-0.238**	**0.145**	0.085
Koru	Fragrance	**-0.371**	**-0.148**	**0.458**	-0.063
Mariene	Fragrance	-0.008	0.017	0.064	**0.116**
Kisii	Flavor	-0.119	-0.004	-0.114	-0.009
Koru	Flavour	**-0.137**	-0.005	0.100	**-0.119**
Mariene	Flavor	-0.011	0.011	0.101	**0.131**
Kisii	Aftertaste	0.054	**-0.283**	0.067	0.095
Koru	Aftertaste	**-0.113**	-0.102	**0.286**	-0.051
Mariene	Aftertaste	-0.005	0.012	0.095	0.100
Kisii	Acidity	0.032	**-0.243**	**0.126**	**0.134**
Koru	Acidity	-0.092	**-0.126**	**0.297**	-0.038
Mariene	Acidity	0.015	0.033	0.080	**0.163**
Kisii	Body	**0.206**	**-0.477**	**0.462**	**0.280**
Koru	Body	**-0.223**	**-0.161**	**0.460**	-0.023
Mariene	Body	0.064	-0.005	0.107	**0.155**
Kisii	Balance	**-0.226**	**0.209**	**-0.298**	**-0.232**
Koru	Balance	**-0.258**	0.001	**0.174**	**-0.147**
Mariene	Balance	-0.010	0.013	0.015	0.080
Kisii	Preference	-0.013	-0.126	0.001	0.059
Koru	Preference	**-0.146**	-0.069	**0.251**	-0.074
Mariene	Preference	0.000	0.001	0.062	**0.143**
Kisii	Total Score	-0.016	**-0.178**	0.039	0.061
Koru	Total Score	**-0.195**	-0.096	**0.308**	-0.075
Mariene	Total Score	0.004	0.014	0.083	**0.140**

Values in bold are different from 0 with a significance level alpha=0.05.

4. Discussion

Significant variation in CGA, caffeine, trigonelline and lipids was observed among Ruiru 11 sibs. The sibs were well differentiated in all the three locations that were evaluated and in all the seasons. This variability was in agreement with several other findings (Silvarolla et al., 2000; Ky et al., 2001; Tessema et al., 2011) that reported presence of significant variations among genotypes of Arabica coffee in biochemical compositions. Such levels of variation were not expected among varietal sibs and more so considering the narrow genetic diversity

associated with Arabica coffee (Agwanda et al., 2003; Gichimu & Omondi, 2010). This finding partly concurred with Kathurima et al. (2010) who reported significant differences in CGA and caffeine among ten Ruiru 11 sibs. They however found no significant differences between the sibs for trigonelline and lipids. Although biochemical composition of Arabica coffee is reportedly different from that of Robusta coffee (*Coffeaca nephora*) (Wasserman et al., 1993; Farah et al., 2005a, 2005b; Speer & Kölling-Speer, 2006) the biochemical composition of canephoroid genome-introgressed Ruiru 11 sibs was found to be comparatively close to that of pure non-introgressed Arabica SL28. This was attributed to the process of backcrossing during the breeding process which restores most of the desirable traits of pure Arabica into the introgressed cultivars.

The biochemical components of the Ruiru 11 sibs were within the levels reported in the literature for Arabica coffee (Bertrand et al., 2003; Franca et al., 2005; Farah et al., 2005a; Farah et al., 2005b; Belay et al., 2008; Kathurima et al., 2010; Belay, 2010; Tessema et al., 2011). The average level of CGA varied from 5.37% to 7.95%. Similar findings were reported by Kathurima et al. (2010). In general, the percentage of CGA for regular green coffee beans on the dry matter varies from 4 to 8.4% for Arabica and 7 to 14.4% for Robusta with some hybrids presenting intermediate levels (Farah et al., 2005a, 2005b). Caffeine content was ranging from 1.01% to 1.54%. This was within the range reported in literature that the caffeine content of Arabica coffee is about 1.2% with a range of 0.6 to 1.9% (Franca et al., 2005; Belay et al., 2008; Belay, 2010). The average level of trigonelline varied from 0.95% to 1.34%. According to Wasserman et al. (1993) Arabica and Robusta coffee comprises about 1.1% and 0.7% trigonelline respectively. The observed level of trigonelline was therefore within acceptable range. Coffee contain between 7 and 17% fat. Green Arabica coffee beans averages 15% lipid content, whilst Robusta coffees contain much less, averaging around10% (Wilson et al., 1997; Speer & Kölling-Speer, 2006). In this study, the average lipid content varied from 12.45% to 18.41% which was close to the reported figures.

Environmental factors, such as altitude and rainfall, have been highlighted as contributing to the quality of the coffee beverage (Decazy et al., 2003; Avelino et al., 2005; Rodrigues et al., 2009). The observed variations in biochemical components at different locations indicated that the growing environment has a strong effect on biochemical composition. The differences were attributed to differences in edaphic and climatic conditions of the three locations. Similar results were reported by Bertrand et al. (2006), Kathurima et al. (2010) and Tessema et al. (2011). This study also found that the environment made a greater contribution to the total variation than genetic variation. This contradicted the findings of other authors who reported that the effects of environmental and agricultural factors are less important than genetic variation in controlling the biochemical contents of green coffee beans (Farah et al., 2005b; Belay, 2011). The highest levels of CGA were observed at Kisii whose elevation is relatively high (1680 m ASL) compared to Koru (1554 m ASL) and Mariene (1524 m ASL). There was also positive correlation between caffeine content and elevation but a negative correlation between the level of lipids and elevation. Tessema et al. (2011) also found a positive correlation between elevation and the levels of caffeine and acidity while Bertrand et al. (2006) reported that CGA and fat concentrations increased with increasing elevation.

Genotype by environment is the phenotypic effect of interactions between genes and the environment. In this study, significant G × E interactions was observed in all biochemical components. This was an indication that different Ruiru 11 sibs responded differently to different environments. This also concurred with the observations made by Kathurima et al. (2010). High G × E interactions for quality traits have been reported as a major setback in achieving faster progress in selection (Agwanda et al., 2003). These significant interactions might be to a large extent attributable to the low precision in balancing the growing conditions in the multi-locational trials and may also be partly explained by trial characteristics. Apart from biochemical traits, significant G × E interactions has also been reported on other quality related traits in Arabica coffee. For example, Wamatu et al. (2003) reported significant G × E interactions on coffee yields. Mawardi and Hulip (1995), Agwanda et al. (2003) and Omondi (2008) also observed highly significant G × E interactions in cup quality and bean characteristics of Arabica coffee.

Association between biochemical components and cup quality has been studied by several researchers (Ky et al., 2001; Decazy et al., 2003; Farah et al., 2006; Bertrand et al., 2006; Tessema et al., 2011). In this study, correlation coefficients portrayed negative associations between caffeine and the other three biochemical components namely CGA, trigonelline and lipids all of which recorded positive correlations between them. CGA and caffeine recorded negative correlations with most of the cup quality traits. High levels of these two biochemical components would therefore lower the cup quality. Farah et al. (2006) also observed a strong association between the level of CGA and low cup quality. The lower levels of CGA in Arabica coffee as compared to Robusta coffee also appear to explain this situation (Ky et al., 2001). However, Tessema et al. (2011)

observed a positive correlation between acidity and sensory quality but a negative correlation between caffeine and sensory quality in Arabica coffee. Unlike CGA and caffeine, the lipids were positively correlated to most of the cup quality traits. Decazy et al. (2003) also observed that preference is positively linked to fat concentration. Significant positive correlations were observed between trigonelline and most of the cup quality traits. Similar observation was made by Farah et al. (2006). Trigonelline give rise to flavour products, including furans, pyrazine, alkyl-pyridines and pyrroles (Ky et al., 2001; Dessalegn, 2005). High levels of trigonelline and lipids would therefore improve the cup quality.

5. Conclusion

The study demonstrated the existence of a high variation in biochemical composition among Ruiru 11 sibs. There is therefore high potential for intra-cultivar selection for further improvement of its quality. The growing environment was found to have a strong effect on biochemical composition as portrayed by high locational variations. The occurrence of significant G × E interactions in most of the biochemical traits evaluated was an indication that the best improvement strategy should be a multi-locational selection. Significant correlations observed between biochemical and cup quality traits indicate that biochemical composition plays a major role in determining the sensory quality of coffee. It further indicates that chemical analysis of green beans may be used as an additional tool for coffee quality evaluation. Germplasm characterization on basis of their biochemical composition is therefore imperative during quality improvement.

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