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Green bean biochemical attributes of Arabusta coffee hybrids from Kenya using HPLC and soxhlet extraction methods

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Abstract

Robusta coffee yields higher than Arabica coffee. However, it is limited by the inferior cup quality. The biochemical compounds found in coffee interact and determines the final cup quality. The objective of the study was to characterize the biochemical compounds found in Arabusta coffee hybrids. Twenty coffee genotypes including the Arabusta hybrids, backcrosses, Robusta and Arabica coffee were established at KALRO-Alupe (Busia) and Siaya -ATC in the year 2015. Coffee cherry was harvested and processed in the year 2018 and the green beans were analysed for sucrose, oil, trigonelline, caffeine and chlorogenic acids using the HPLC and soxhlet method. There were significant differences amongst the genotypes for these biochemical compounds across the two different environments (Busia and Siaya counties). Robusta recorded higher levels of caffeine and chlorogenic acids while the Arabusta hybrids recorded intermediate levels of the biochemical compounds between the Robusta and Arabica coffee species. The Genotype by Environment (G x E) interaction effect was only significant for chlorogenic acids. Caffeine, sucrose, oil and trigonelline levels were significantly high for genotypes evaluated in Siaya when compared to Busia. Chlorogenic acid had a positive significant association with caffeine, but was negatively correlated with coffee oil and sucrose. Coffee oil indicated a positive significant association with sucrose and Trigonelline. The Principal Component Analysis (PCA) differentiated the genotypes based on the levels of biochemical compounds indicating high genetic variation amongst the genotypes. Arabusta hybrids exceeded Robusta coffee in performance of biochemical compounds which implies that there was a successful introgression of quality genes.

Keywords: Aroma, breeding, environment, flavor quality, variety.

Introduction

Coffee is normally traded as green coffee before it is roasted for consumption. Green coffee beans contain various chemical composites, which are complex in structures interacting at all stages of coffee growth determining the final cup quality (Kathurima et al., 2010; Gichimu et al., 2014a). Factors that affect the quality and biochemical compounds present in coffee are altitude, genetics, shade, harvesting period and processing practices (Tolessa et al., 2018; Duarte et al., 2010; Worku et al., 2018). The flavor and aroma of roasted coffee depend on the metabolites that accumulate within the coffee bean after roasting acting as precursors (George et al., 2008). These attributes depend also on the degree of roasting and the presence of defects in the coffee beans (Franca et al., 2005). Maillard reactions and caramelisation which occurs during roasting affects the interaction of the chemical composition of the green bean, which is responsible for the aroma that develops during roasting (Liu et al., 2019).

The major biochemical compounds which compose of chlorogenic acids, caffeine, trigonelline sucrose and oil have been used for discrimination of coffee varieties within and across species (Clifford et al., 1989; Ky et al., 2001b). The presence of these compounds helps to discriminate between the different coffee genotypes making them key factors in the determination of organoleptic cup quality (Aluka et al., 2016). Characterization of varieties for the biochemical compounds analysis is crucial in development of varieties with desirable quality. The presence of trigonelline sugars and oils could have a positive influence on liquor quality while chlorogenic acids and caffeine could be unfavorable (Kathurima et al., 2010).

Caffeine may exists in different plant species. It is normally found in different parts of plant including fruits, seed and even the leaves. Coffee, cocoa beans and tea leaves are the known major sources of caffeine (Mumin et al., 2006). Caffeine, which is partially accountable for the bitterness in coffee is one of the highest occurring purines in green coffee (Farah, 2012). The levels of caffeine vary between and even within species (Silvarolla et al., 2004; Ky et al., 2001). Robusta generally has a higher value of caffeine (2.2%) while Arabica has an average value of 1.2% ranging from 0.6 to 1.9 %(Belay et al., 2008; Franca et al., 2005). The less commercialized species of Liberica and Arabusta have 1.35 and 1.72% caffeine content, respectively (Clarke and Macarae, 1985).

Sucrose is a major sugar in the coffee beans ranging from 5% to 9.5% in *Coffea arabica* and from 4% to 7% in *Coffea canephora* contributing mostly to reducing sugars which are involved in Maillard reactions, occurring during roasting (Grosch, 2001; Kathurima 2013; Ky et al., 2001). Sucrose occurs most and is a precursor affecting the aroma and taste of the coffee beverage during roasting (Maria et al., 2017; Farah, 2012). Trigonelline on the other hand is by nicotinic acid (pyridinium-3-carboxylic acid) methylation using methionine, a kind of amino acid containing Sulphur. Sucrose levels in *C. arabica*, have been reported to range from 0.88% to 1.77% and *C. canephora* in ranges between 0.75% to 1.24% (Ky et al., 2001). Trigonelline has a low bitter taste, when compared to caffeine being 100% water soluble and it is a vitamin B6 derivative (Gichimu et al., 2014).

Triacylglycerols with fatty acids are major coffee oils in the green bean found in equal portions as those found in vegetable oils (Speer and Kölling-Speer, 2006). The coffee oil levels of green Arabica coffee averages 15%, while it is much lower in Robusta coffee (10%) (Gichimu et al., 2014). The oil has diterpenes of the kaurene family in proportions of up to 20% of the total lipids and carries most of the coffee aroma contributing to the viscosity of the coffee beverage (Buffo and Freire, 2004). The percentage of chlorogenic acids (CGA) varies among the species. For example, *C. Arabica* contains 4 to 8.4% and *C. Canephora* about 7 to 14.4%, while some hybrids have medium levels of the biochemical compounds (Farah et al., 2005a, 2005b). Chlorogenic acids is critical in the formation of pigments, taste and flavor of coffee beans, acidity and defines cup quality and preference of the brew (Gichimu et al., 2014, Variyar et al., 2003).

The relationship between the sensory performance and the biochemical compounds is used in determining the liquor quality (Farah et al., 2006). The biochemical compounds act as Aroma and flavor precursors affecting the quality of the coffee beverage (Cheng at el., 2016). Assessment of diversity of biochemical attributes is key in development of varieties that have necessary biochemical compounds as another important aspect of secondary metabolites is their involvement in quality (Gichimu et al., 2014; Kathurima, 2013; Granati et al., 2003; Moufida and Marzouk, 2003). Determining the elements that influence the coffee quality remains an important area of study in coffee breeding. This is because the biochemical components in coffee influence the organoleptic properties that contribute to the final cup quality. This is key in determining its market value and use. This study aimed at assessing the biochemical content of the Arabusta hybrids and their backcrosses grown in two separate locations in Western Kenya with the focus of selecting the best performing genotypes for the breeding program.

Results

Biochemical composition of coffee genotypes

There were significant differences on the biochemical composition which included oil, chlorogenic acid, trigonelline, caffeine and sucrose among the coffee genotypes established in Busia and Siaya. Chlorogenic acid varied among the genotypes, where Robusta recorded significantly higher levels of CGA, and genotype BC03 and ARH2 recorded the lowest levels. Robusta coffee also recorded significantly higher percentages of caffeine, while SL28, an Arabica coffee, recorded the lowest level. Genotype CV1 recorded significantly lower quantity of oil while SL28 recorded the highest quantity. Genotypes ARH3 and SL28 recorded significantly higher levels of sucrose, while genotype CV1 recorded significant lower levels. Batian recorded higher levels of trigonelline, whereas genotypes ARH2, ARH4, ARH7, BC01, BC02 and Robusta recorded the least levels of trigonelline (Table 2).

Environmental effect on biochemical composition

The environmental effects were significant for all the biochemical components except for the chlorogenic acids. Chlorogenic acids recorded a significant G x E interaction while the interaction for oil, trigonelline, sucrose and caffeine was not significant (Table 3). The chlorogenic acid contents were scored high in Busia compared to Siaya and its effects were not significant (Fig 2, a). The quantities of caffeine, sucrose, oil and trigonelline were significantly higher in Siaya when compared to those recorded in Busia. The biochemical compound means were either placed above or below the median. The inter quartile range differed amongst the environments for each measured attribute, indicating the variation within the coffee genotypes in each environment.

Correlation and PCA analysis

Chlorogenic acid and caffeine showed negative significant correlation to oil (r=-0.49) and sucrose (r=-0.43), respectively but had a positive significant association with caffeine (r=0.77) (Table 4). Coffee oil showed positive significant associations with sucrose (r=0.81) and trigonelline (r=0.48). Trigonelline associated positively with all the biochemical components being significant for oil (r=0.48) and sucrose (r=0.43). The PC1 (15.35%) and PC2 (74.76%) were sufficient to discriminate the coffee genotypes based on the biochemical compounds. Oils, sucrose and trigonelline recorded high positive values, while caffeine and trigonelline had low values (Figure 3). The genotypes were grouped together based on their biochemical composition. Genotypes, SL28, BC03 and ARH3 were grouped closely together based on oil and sucrose, while Batian was discriminated solely based on trigonelline levels. Genotypes which include Robusta, CV1 and CV2 were grouped together based on chlorogenic acid and caffeine levels (Figure 3).

Discussion

There were significant differences in biochemical composition among the coffee genotypes evaluated within and between the two different environments (Busia and Siaya). The differences showed a high genetic variation within the genotypes that led to different performances in terms of the lipid oils, sucrose, caffeine, chlorogenic acids and the trigonelline. This indicated that there is a possibility of improving the selection efficiency for biochemical composition within the breeding program. The difference in the biochemical levels is due to the fact that biochemical composition within coffee genotypes varies depending on the species, the maturation time and environmental factors (Farah et al., 2005b, Belay 2011). Dessalegn, (2005) reported significant variations for biochemical composition on Ethiopian coffee evaluated in different environments. Chlorogenic acid levels were high in Robusta coffee (11.2%) while Arabusta hybrids had lower levels (7.3 to 9.1%) which was comparable to Arabica coffee genotypes. Bicho et al. (2013b) and Upadhyay and Mohan Rao, (2013) reported a range of 7.0-14.4% for Robusta coffee and 4.0-8.4% for Arabica coffee. In Robusta species, the cholorogenic acid content is influenced more by genetics. It is well-known that it produces higher levels than the Arabica species, affecting the cup quality negatively since the chlorogenic acids adds to the astringency or unwanted bitterness. Consequently, chlorogenic acids affect the flavor and aroma which is responsible for the differences in cup quality between Arabica and Robusta coffee (Variyar et al., 2003; Upadhyay and Mohan Rao, 2013). Caffeine levels varied from 1.3 to 1.5% in Arabica coffee, 1.3 to 2% in Arabusta hybrids, 1.6 to 2.2% in backcrosses and 2.6% in Robusta. Gimase et al. (2014) reported ranges similar to the findings of this study, with caffeine content of 1.1-1.6%, 2.4% and 1.8 -2.2% in Arabica, Robusta and Arabusta coffee, respectively. The caffeine levels vary within species being high in *C canephora* and low in *C arabica*. From the total produced caffeine, 94% is attributed to the genetics of the species and the rest to the environment effects (Montagnon, 2000). From the sequencing conducted by Kumar et al., (2015), the expression of CaXMT1, CaMXMT1 and CaDXMT2 transcripts were low in *C. arabica* than *C. canephora* elucidating the lower levels of caffeine in Arabica. In addition, the hybridization of *C. arabica* to reduced *C. eugenioides* sub-genome (maternal genome to *C. arabica*) contributed to low caffeine level in Arabica (Perrois et al., 2015). Trigonelline also varied among the coffee genotypes of the different species with Arabica coffee having 1.2 to 1.5%, Robusta (1%), backcrosses 1 to 1.3% while the levels in Arabusta hybrids ranged from 1.0 to 1.3%. Bicho et al. (2013a) carried out a study to discriminate the different levels of biochemical compounds in Arabica and Robusta coffee and reported higher levels of trigonelline in Arabica, when compared to Robusta. Arabica coffee is known to have high levels of trigonelline, which is an important factor in determining the cup quality of coffee since it's an aroma precursor contributing to the desirable flavor. Sucrose levels varied amongst the coffee genotypes evaluated ranging from 7.7% to 8.8% for Arabica, Robusta (5.9%), backcrosses (5.8 to 8%) and Arabusta hybrids (5.4 to 8.3%). The sucrose levels are within the ranges reported by Tran et al., (2016) who reported ranges of 7.4 to 11.1% in Arabica and 4.05 to 7.05% for Robusta coffee. Sucrose is an aroma precursor contributing immensely to cup quality during coffee roasting. Joe et al. (2009) studied the synthesis within coffee plant and found that during the endoperm development, the sucrose levels are increased up to the berry's ripening stage, after which it slows down in Robusta. However, for Arabica the accumulation is continuous throughout the fruit development. Coffee oil contributes to the final flavor during roasting by adding to the final texture and mouth feel, since they carry fat soluble vitamins (Oestreich-Janzen, 2010). From this study, the levels varied from 14.7 to 17.8% for Arabica coffee, 12.3 to 16.7 % in Arabusta hybrids, 12.1 to 16.8% for backcrosses while for Robusta it was 12.5%. Gimase et al. (2014) reported ranges of 13.4 to 15.25% for Arabusta coffee, 12.5 to 18.4% for Arabica coffee and 13.4% for Robusta coffee. Odeny et al. (2016) also reported oil content of 15.79-18.99% for Arabica coffee genotypes. Study by Simkin et al., (2006) revealed that the synthesis and storage of oil varies between species based on a study of gene profiling for the five oleosin genes that encode the oil storage proteins in *C arabica* and *C. canephora* species. He reported that Arabica coffee starts to store its oil from the start of berry development unlike the Robusta coffee. This also explains why the oil concentration levels is high in Arabica than in Robusta.

There was no genotype by environment interactions for all biochemical attributes measured in the study except for cholorogenic acids while the effects of the environments were significant for all the biochemical attributes except for cholorogenic acid. The lack of environmental effect on chlorogenic acids could be an indication that its synthesis is more influenced by the genetics of the genotypes than environment since the *C canephora* is known to produce more chlorogenic acids than the *C arabica* species. Kathurima et al. (2010) and Gichimu et al. (2014) evaluated Arabica coffee in different environments and reported significant environmental and genotype by environment effects for the biochemical compounds. There were negative relationships between the sucrose, oils with caffeine and cholorogenic acids indicating a close but competing linkage between the two pathways (Baumann, 2006). This also implies that selection for increased levels of oil and sucrose levels will definitely lead to lowered levels of cholorogenic acids and caffeine that negatively affects the cup quality. Trigonelline was the only biochemical component that showed positive relationships with all the other components measured with significant association with oil and sucrose. The positive correlations between the trigonelline and oil, sugars and indicate that trigonelline attribute can be used in the direct selection for sucrose and oil to improve the cup quality. Odeny et al. (2016) evaluated coffee under shade in different environments and found a positive correlation between oil and sucrose and trigonelline contents. Caporaso et al. (2018) also reported a positive correlation between trigonelline and sucrose and a negative relationship between caffeine and sucrose which agrees to the findings of this study. The different groupings of the genotypes indicated that it is possible to differentiate genotype based on their biochemical attributes. The PCA clearly illustrates the genetic variation amongst the coffee genotypes that were evaluated.

Table 1. Description of coffee genotypes used in the experiment.

Genotype	Genotype description
ARH1	CATURRA X (SL 28 X UT 6)
ARH2	CATURRA X (SL 28 X UT 6))
ARH3	CATURRA X (SL 28 X UT 6)
ARH4	CATURRA X (SL 34 X UT 6)
ARH5	CATURRA X (SL 34 X UT 6))
ARH6	SL 28 X (SL 34 X UT 6)
ARH7	SL 28 (SL 34 X UT 8)
BC01	SL 34 X (SL 34 X UT 6)
BC02	SL 28 X (SL 28 X UT 6)
BC03	SL 28 X (SL 28 X UT 6)
BC04	SL 34 X (SL 34 X UT 6)
BC05	SL 34 X (SL 34 X UT 6)
BC06	SL 28 x (SL 28 X UT 8)
CV1	PL 4 CONGUSTA 161
CV2	PL 4 CONGENSIS 263
ARV	PL 4 169, 177, 178 ARABUSTA
Robusta	Commercial variety
Ruiru 11	Commercial variety
Batian	Commercial variety
SL28	Commercial variety

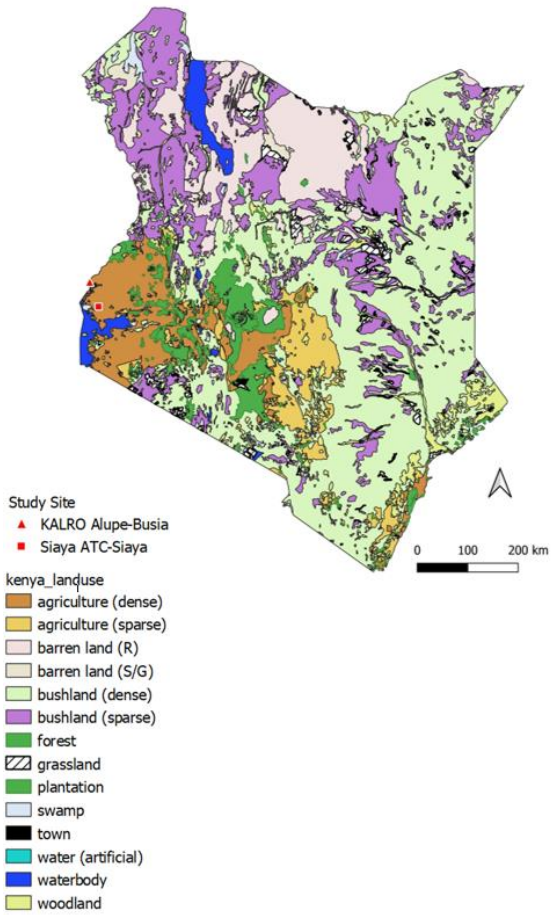

Fig 1. Map of Kenya showing different land uses and the two environments (KALRO Alupe and Siaya ATC) where the experiment was established.

Table 2. Mean and Standard Errors of biochemical components of green beans for coffee genotypes from KALRO-Alupe (Busia) and Siaya ATC.

Genotype	CGA	Caffeine	Oil	Sucrose	Trigonelline
ARH1	8.72^{abcd}	1.62^{abcd}	15.44^{ccdefg}	7.74^{cdef}	1.15^{ab}
ARH2	7.32^{a}	1.37^{ab}	14.55^{bcdefg}	7.16^{abcdef}	1.11^{a}
ARH3	8.3^{abc}	1.46^{abc}	16.67^{efg}	8.3^{f}	1.29^{ab}
ARH4	9.11^{abcd}	1.98^{cde}	12.29^{abc}	5.44^{ab}	0.98^{a}
ARH5	9.09^{abcd}	1.85^{abcde}	13.6a^{bcde}	6.15^{abcde}	1.15^{ab}
ARH6	8.77^{abcd}	1.54^{abc}	15.42^{cdefg}	8.12^{f}	1.17ab
ARH7	7.89^{abc}	1.32^{a}	14.35^{abcdef}	7.6^{cdef}	1.1^{a}
BC01	9.28^{abcd}	1.96^{bcde}	12.15^{abc}	5.8^{abc}	1.02^{a}
BC02	8.96^{abcd}	1.65^{abcd}	14.75^{bcdefg}	7.39^{bcdef}	1.05^{a}
BC03	7.15^{a}	1.62^{abcd}	15.68^{defg}	8.05^{ef}	1.27^{ab}
BC04	10.54^{cd}	2.2^{def}	13.67^{abcde}	7.09^{abcdef}	1.19^{ab}
BC05	8.56^{abcd}	1.68^{abcd}	14.19^{abcdef}	7.9^{def}	1.23^{ab}
BC06	7.84^{abc}	1.59^{abc}	16.84^{efg}	7.47^{cdef}	1.23^{ab}
CV1	10.49^{bcd}	2.29^{ef}	11.17^{a}	5.5^{ab}	1.17^{ab}
CV2	11.25^{d}	2.35^{ef}	11.6^{ab}	5.4^{a}	1.23^{ab}
ARV	9.45^{abcd}	1.92^{bcde}	14.16^{abcdef}	7.56^{cdef}	1.12^{ab}
Robusta	11.21^{d}	2.62^{f}	12.45^{abcd}	5.94^{abcd}	1.01^{a}
Ruiru	8.32^{abc}	1.52^{abc}	14.72^{bcdefg}	7.74^{cdef}	1.22^{ab}
Batian	7.73^{ab}	1.51^{abc}	17.05^{fg}	8.08^{ef}	1.52^{b}
SL28	8.07^{abc}	1.32^{a}	17.8^{g}	8.82^{f}	1.26^{ab}
%CV	**1.5**	**0.4**	**1.4**	**1.2**	**3.2**
Mean	**8.9**	**1.77**	**14.42**	**7.155**	**1.17**
SE	**0.52**	**0.11**	**0.65**	**0.37**	**0.07**

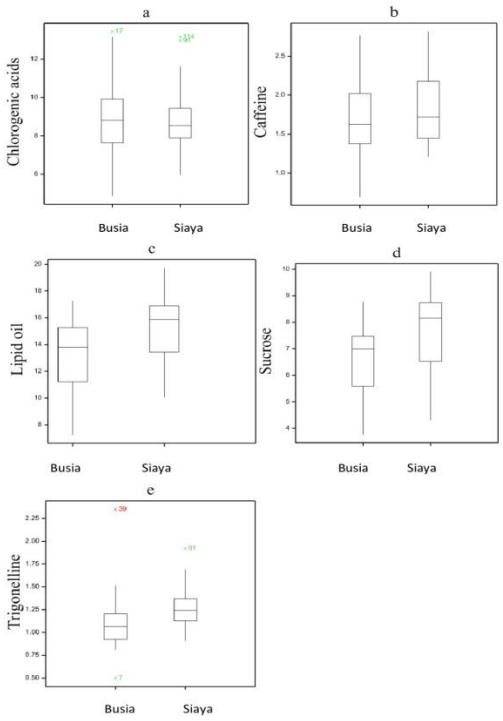

Fig 2. Box plots showing the variation in performance of the biochemical attributes for coffee genotypes across the two environments (a-e) (Busia and Siaya).

Table 3. Mean squares for biochemical components of green bean for coffee genotypes evaluated at Siaya ATC and KALRO-Alupe (Busia) during 2018 season.

Source	Rep	Gen (G)	Envt (E)	G x E	Error
Df	2	19	1	19	9
Chlorogenic acid	0.744	8.591*	4.994 (NS)	2.316(S)	1.704
Caffeine	0.002	0.81***	0.382*	0.081(NS)	0.079
Oil	1.566	21.01***	120.453***	2.631(NS)	2.524
Sucrose	0.271	6.796***	29.46***	1.321(NS)	0.865
Trigonelline	0.056	0.088**	0.935***	0.065 (NS)	0.036

Key: *, ** and *** NS represent, (*P*<0.05), (*P*<0.001), (*P*<0.001) and non-significant respectively

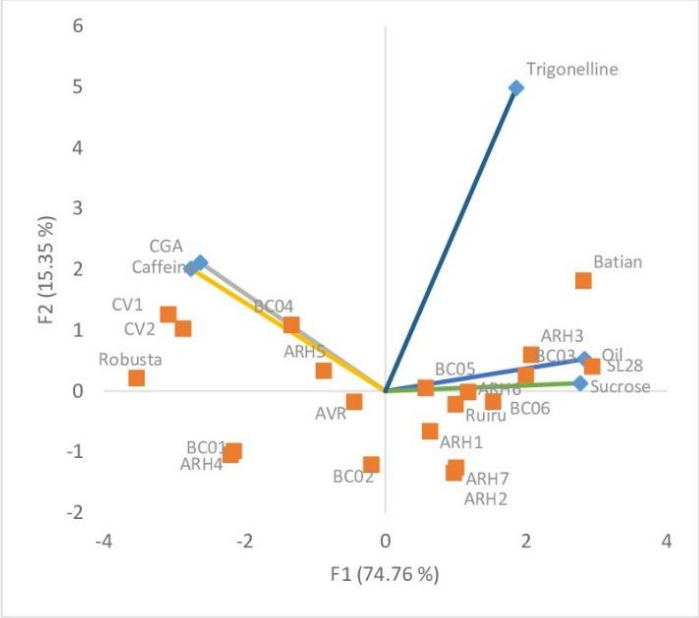

Fig 3. Variations amongst the coffee genotypes based on their biochemical composition as displayed by the PCA analysis.

Table 4. Correlation coefficients for the different biochemical attributes of coffee green beans for coffee genotypes in Busia and Siaya environments for the year 2018.

	Chlorogenic acid	Caffeine	oil	Sucrose	Trigonelline
Chlorogenic acid	-	0.7741***	-0.4893***	-0.4302***	0.0519
Caffeine		-	-0.4926***	-0.4833***	0.1019
Oil			-	0.8119***	0.4889***
Sucrose				-	0.4354***
Trigonelline					-

Key: *** represent (*P*<0.001)

Materials and methods

Experimental materials

Twenty genotypes including seven Arabusta hybrids (ARH1-ARH7), six different backcross derivatives of Arabica to Arabusta hybrids (BCO1-BCO6), Congensis 263 cramer (CV1), Congusta 161 Cramer (CV2), Arabusta coffee, Robusta and three arabica genotypes (Batian, Ruiru 11 and SL28) were evaluated during the study (Table 1)

Description of the experimental sites

Siaya: Siaya resides between 0º 30 N' and 0º 45' E with an altitude that varies from 1,135m to 1,500m above sea level with mean annual rainfall of 1,500mm (Fig 1). The annual mean temperatures ranged from 20.9º C and 22.7º C. The soils are well-drained to very deep (chromic/orthic acrisols and ferrasols) (Jaetzold et al., 2009).

Busia: Busia is located between 0º 30 N' and 34º 30' SE with an altitude that varies from 1241m to 1343m above sea level (Fig 1). The mean annual rainfall is 1400mm with an annual maximum temperature range from 26º C and 29º C. The soils are developed on basic and intermediate rocks (dolerites and andesites) (Rachilo and Michieka, 1991).

Experimental design

Five coffee trees were planted per genotype per plot with a spacing of 3m by 3m per plot. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications at KALRO-Alupe (Busia) and Siaya ATC. All other management practices including weeding, pruning and general maintenance were carried out as recommended.

Harvesting and processing

The ripe coffee cherries were harvested in the field and processed by removing the pulp and mucilage and then dried to a moisture content of 11%. The parchment was then dehulled to generate clean coffee beans, which were then graded into different grades (AA, AB, PB, C, E, TT, T). Fifty grams of AA and AB bean grades were weighed and used in the analysis of the biochemical composition after being crushed to powder using liquid Nitrogen. Genotype SL28 was used as a standard in measuring the biochemical compounds.

Extraction and quantification of crude oil

The coffee bean crude oil was extracted and quantified using the Soxhlet extraction method (AOAC, 1995) as described by Kathurima (2013).

Extraction of caffeine, trigonelline and total chlorogenic acids (CGA)

Determination of caffeine, trigonelline and chlorogenic acids was done using the protocols as provided by CIRAD (2003a) and CIRAD (2003b).

Extraction and analysis of sucrose

The extraction and analysis of sucrose was done according to the method of Osborne and Voogt (1978) used by Kathurima (2013).

Statistical analysis

The biochemical data were subjected to Analysis of Variance (ANOVA) using GENSTAT statistical software version 18 and effects declared significant at 5%. The General Linear Model (GLM) was used (Jansen, 1993). Least Significance Difference was used to do combined analysis of variance. It was performed on data from the two sites, while Tukey's test was used to separate the means. Correlation of the biochemical attributes were computed using GENSTAT statistical software using the Pearson Correlation Coefficient. The Principle Component Analysis of the biochemical were plotted based on the important principle components together with cluster analysis using the unweighted pair-group method with arithmetic average (UPGMA) to create a dendrogram based on Euclidean distances using XLSTAT software.

Conclusion

Arabica has high levels of trigonelline, sucrose and oil which contributes to the improved flavor and aroma with reduced bitterness in coffee. Therefore, it is imperative to select for coffee genotypes that have high levels of oil, sucrose and trigonelline in order to satisfy the market needs. The Arabusta hybrids on average scored high for oil, sucrose and trigonelline, and low for chlorogenic acids and caffeine when compared to Robusta. Some of the hybrids competed well with Arabica coffee meaning that the interspecific hybridization was successful in introgressing of good quality traits from Arabica to Robusta. The correlation of the biochemical attributes indicated that it is possible to use oil and sucrose for selection due to its positive relationship to improved cup quality since it would lead to indirect selection for low levels of caffeine and chlorogenic acids.

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