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Potential Use of Kenyan Tea Cultivars in Development of High Value Diversified Products

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Abstract: Tea industry in Kenya contributes to the economy by being the largest agribusiness and a major foreign exchange earner. However, significant revenue is lost when tea is sold in undiversified form. This has created a need to characterize the available clones for suitability in high value tea product development. Samples were obtained from 204 tea accessions conserved in Kericho and Kangaita Centres of the Tea Research Institute (TRI) and assayed for total polyphenols, catechins, caffeine, anthocyanins, chlorogenic acid and theanine. There were significant cultivar differences ($P \leq 0.05$) in total polyphenols (16.4%- 30.9%) and total catechins (11.03%-25.42%). Sixteen (16) new clones recorded significantly ($P \leq 0.05$) higher polyphenol contents (mean value of 28.11%) than the standard reference clone, TRFK 6/8 (27.4%) indicating their suitability in the development of high quality black teas. Fifteen clones were suitable for the manufacture of theaflavin-3, 3'-digallate rich black tea based on their high ECG and EGCG levels. Clones TRFK 301/5 and TRFK 301/4 had a high EGC/EC and low EGCG/ECG ratios and were found suitable for manufacture of less astringent green orthodox teas, while clones TRFK 687/1 and 73/7 had the least caffeine contents at 1.96% and 2.04%, respectively, implying their amenability for manufacture of low-caffeine tea beverages. Clones assayed for chlorogenic acid and theanine showed that AHP SC 31/37 and TRFK 6/8 had the highest contents at 0.13% and 1.7%, respectively, and are suitable for chlorogenic and theanine rich teas. The observed chemical and therefore quality differences based on clones and regions show that these Kenyan tea cultivars have high diversity in biochemical attributes and maybe suitable for development of diversified tea products with geographical indications.

Keywords: Catechins; total polyphenols; chlorogenic acid; anthocyanins; theanine; caffeine, Kenya.

Running title: Kenyan tea cultivars in the development of diversified tea products

Introduction

Tea, a non-alcoholic beverage that is widely consumed worldwide is produced from *Camellia sinensis*. The popularity of tea is attributed to its attractive aroma, refreshing taste and also its potential health benefits[1]. In Kenya, tea cultivation is the largest agribusiness contributing over 26% of total foreign exchange earnings, over 4% of GDP[2] and is also a source of livelihood for 4 million Kenyans[3]. Kenya is the third largest producer of tea supplying 22% of the world's black tea[2]. About 95% of Kenyan tea is exported but with little or no product diversification and only 5% is consumed locally[2]. Because tea farming is one of the profitable agro-enterprises in Kenya, the tea industry in Kenya has expanded greatly over the years from 36,000 metric tons in 1971 to 445,105 metric tons in 2014[2]. The increase in tea acreage and the consequent increase in production per unit area has created a

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problem of overproduction as the conventional market outlets are not expanding. This situation has been made worse since Kenyan tea is processed almost entirely as black Cut, Tear and Curl (CTC) tea and is sold in bulk leading to a decrease in demand. This has caused a glut in the market with tea prices either stagnating or decreasing[4]. For example; annual average unit prices for processed black tea at the Mombasa auction decreased from an average high of US$ 3.18 per Kg in 2012 to a low of US$ 2.54 in 2013 and 2.14 in 2014[5].

In the face of increasing production costs and dwindling returns to producers, the future of the Kenyan tea industry maybe gleam. There is therefore a need for appropriate interventions to enable market outlets expansion through product diversification, value addition and novel marketing strategies[6], all in line with Kenya's vision 2030. This could entail a replication of the Asian success story in the production and branding of tea products and extracts marked with high levels of functional components which can then be marketed as "functional foods" or used as raw material for fast moving consumer and environmentally friendly home and industrial cleaning agents, deodorizers, antimicrobial agents among others[7]. Such an endeavour must however be preceded by the extensive characterization of Kenyan tea cultivars to identify biochemical properties that can putatively enhance their nutritional potential and ultimately enhance intrinsic value for local and export markets.With the increasing quest for plant derived health-enhancing phytochemicals by consumer's worldwide, tea beverage could be marketed more based on its level of bioactivity.

Emerging scientific data from pharmacological and physiological studies continue to show that regular consumption of tea has beneficial effects on human health. In particular, tea has been shown to protect against cancer[8-10] and cardiac diseases[11-12], modulate activity of detoxification enzymes such as glutathione peroxidase and glutathione reductase[13], stimulate immune function and decrease platelet aggregation[14,15], promote oral health[16] as well as exhibit antioxidant , anti-viral[17], anti-bacterial[18], anti-fungal[18] and anti-inflammatory properties[19]. These health benefits are related to the levels and composition of bioactive molecules in tea such as polyphenols (catechins, anthocyanins, theaflavins, thearubigins, and chlorogenic acid among others), alkaloids (caffeine, theophylline and theobromine), amino acids (mainly theanine), vitamins and minerals. Despite this support of potential positive effects on health, the state of research on these aspects is limited particularly for Kenyan tea products. Research on health aspects of tea have been carried out mainly on green tea. There is need to screen suitable clones that can to be used as raw materials in the manufacture of high value teas and diversified tea products with an aim of increasing their use as health promoting compounds.

Methodology

Sample preparation

Approximately 300g of two leaves and a bud were sampled in triplicates from each of 204 tea clones conserved at the Timbilil Estate (0°26′S, 37°15′ E) and Kangaita Centre (0°30′S, 37°16′E) of the Tea Research Institute (TRI). A total of 164 clones were obtained from Timbilil while 40 clones were obtained from Kangaita centre. The clones comprised 98 clones that were earlier released for commercial cultivation based on high yielding properties and those 106 clones under test for various attributes.The leaf samples were steamed for 1-2 minutes immediately after plucking, dried in a microwave at 600-800 watts and pulverized with a grinder into fine powder to be used in assaying various components namely; total polyphenols, catechins, caffeine and anthocyanins. Chlorogenic and theanine levels of 15 clones selected from the 204 clones based on their suitability for development of high quality black tea were also determined. The analysis was done using the TRI established and published wet chemistry procedures, HPLC and spectrophotometric protocols.

Determination of catechin levels

Catechin extraction and HPLC analysis was carried out as described by the International Organization for Standardization (ISO) 14502 procedure[20].

Determination of total polyphenolic content

The Folin-Ciocalteu phenol reagent method was used to determine total polyphenols as described in the British Standard ISO document BS ISO 14502-1:2005E[21].

Determination of anthocyanins content

Extraction and quantitative analysis of the anthocyanins and their profiles in the tea extracts was carried out by

a high-performance liquid chromatography method as described by Kerio *et al*.[22].

Chlorogenic acid

Five grams of each sample was weighed into a conical flask and 45ml of aqueous methanol (50:50 v/v) added and magnetically stirred for 40 minutes. The resulting mixture was filtered and the residue washed with 10ml of 50% methanol. The first filtrate and the washed residue were combined and condensed to approximately 25ml by a rotavapor at 50°C. After cooling, the liquid was transferred into a 50ml volumetric flask and diluted with 50% aqueous methanol to the volume. An aliquot of the diluted sample was filtered through a 0.45 µm Millipore filter and 10µl of the filtrates were assayed by HPLC.

The analysis was carried out on a chromatography system equipped with a high precision pump (Shimadzu model LC-10-ATVP) operating at 327nm, and a phoenix Luna C18 (2504.6 mm i.d.; 5µm particle size; 100 Å pore size). A mixture of acetonitrile and 0.5% aqueous phosphoric acid (11.5:88.5 v/v) was used as the mobile phase with the flow rate at 1.0 ml/minute and injection volume of 20 µl. A stock solution was prepared by accurately weighing 25mg/ml of HPLC grade methanol. From the stock, five additional standards with concentrations of 0.0784, 0.05488, 0.0392, 0.02352, 0.00784 and 0.00392 mg/ml were prepared by appropriate dilution of the stock solution. The standard solutions were injected twice into the chromatograph to obtain the peak values. A calibration curve was drawn by plotting the peak areas against the concentration of the chlorogenic acid standard. The chlorogenic acid content was determined by the corresponding calibration curve and expressed as milligram of chlorogenic acid per gram of the respective tea clone.

Theanine

One gram of a finely ground sample was weighed in a 200 ml beaker and 100 ml of boiling double distilled water added. The mixture was allowed to brew for 5 minutes on a magnetic stirrer after which it was filtered. The tea brew was allowed to cool then made up to volume and filtered using a 0.45 µm membrane before HPLC analysis. A phenomenex column Aqua 250 x 4.6 internal diameter was used. A gradient elution was employed with a thermostatically controlled column compartment and an ultraviolet detector set at 210nm. An injection volume of 20 µl and a flow rate of 1 ml/minute were used with the following solvent system; mobile phase A composed of double distilled water and mobile phase B composed of 100% acetonitrile. Analysis time was 10 minutes with 100% mobile phase A, and then the next 8 minutes was wash time with 20% mobile phase A and 80% mobile phase B. The next 20 minutes was conditioning with 100% mobile phase A before the next injection. To calculate theanine content, a theanine calibration graph was drawn, using concentration of theanine in the working solutions against theanine peak area of the sample.

Data analysis

All determinations for the levels of total polyphenols, total and individual catechin fractions, caffeine and anthocyanins were done and the data subjected to analysis of variance using GENSTAT-C statistical software packages. The least significant differences Test (LSD) was used to separate the means.

Results And Discussion

Total polyphenols

Clones screened for phytochemicals showed significant ($p \leq 0.05$) variations in total polyphenol content (**Figure 2**). The average polyphenolic content was 25.5%, with clone BBK 152 recording the highest polyphenol content of 30.9%, while TRFK 833/1 had the lowest polyphenols composition at 16.4%. The exhibited variation (Figure 2) was generally continuous, an indication of polygenic influence on total polyphenol contents and presence of wide range of selection for cultivars with varying potential for different products formulations. From the PCA plot (Figure 1), most of the cultivars with low polyphenolic content were largely of the Chinary type (sinensis) and clustred in one group, while the medium and high polyphenolic content cultivars were predominantly assamica with a few chinary and cambod types clustering in another group. The clones in low polyphenolic content cluster would be highly suitable for processing high quality green orthodox or CTC teas[4, 23, 24], while those in medium and high polyphenolic cluster could be exploited for oolong and black orthodox/CTC teas[25]. Indeed, the low polyphenolic content cluster include TRFK St 543 (cv. Yutakamidori) and TRFK St 536 (cv. Yabukita), the most popular green tea cultivars in Japan, which were introduced in Kenya in 2001[26, 27].

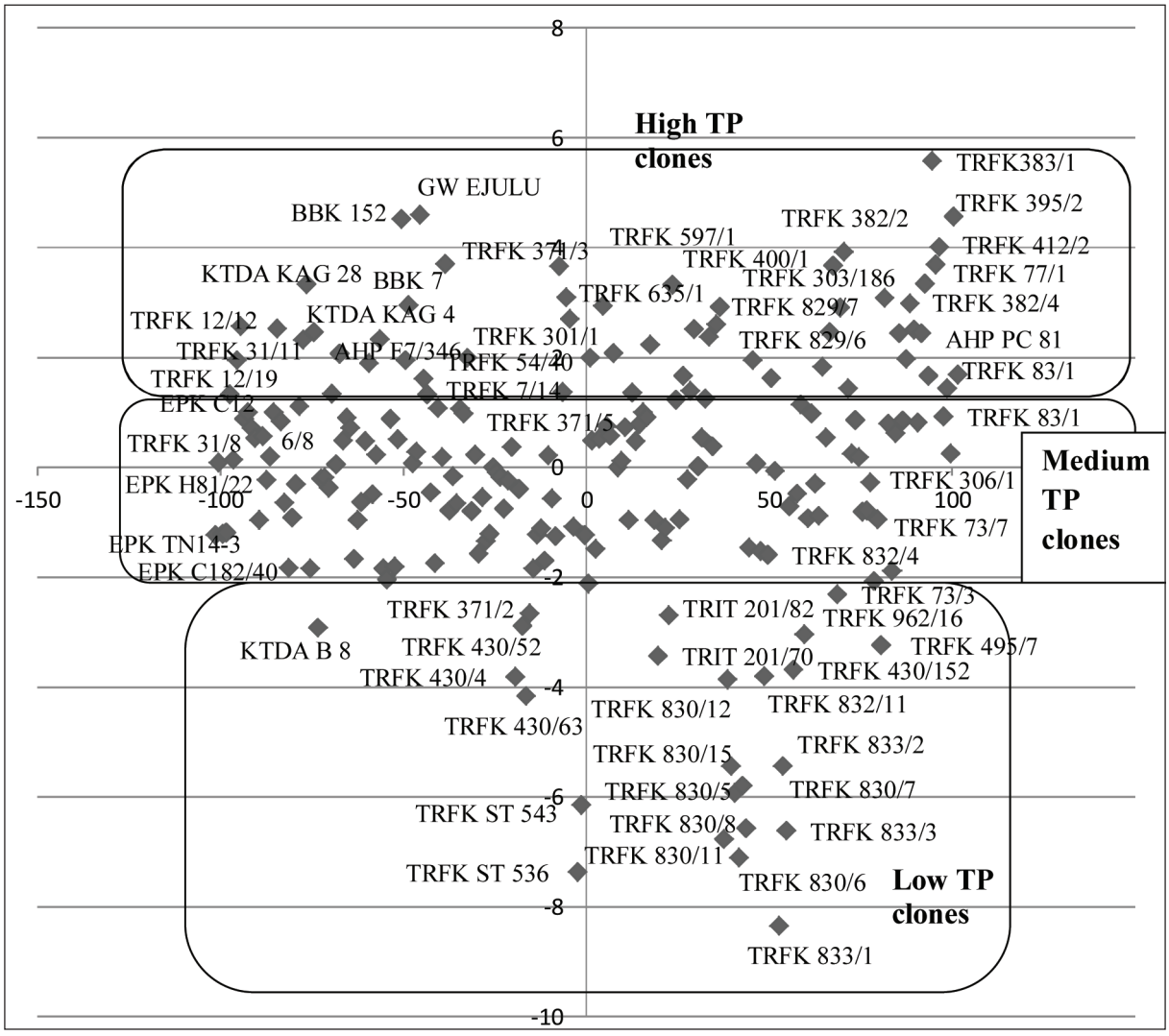

Figure 1: Principle component analysis on variation in Total polyphenols (TP) among the 204 clones.

The observed differences in the polyphenolic composition among the tea clones can be attributed to several factors such as the cultivars genotype, geographical origin of the clone, soil composition, harvesting time, post-harvest treatment and physical structure of the leaves[28 29]. Clones with high polyphenolic content such as BBK 152 were of Assamica origin, while those with low polyphenol content such as TRFK 833/1 are of Chinary origin. Previous studies have shown the superiority of the Assam teas compared to the Chinary and Cambod teas which is attributed to differences in their ecological origins thereby the influence by geographical status of the clones to their polyphenol composition[30,31,32]. In addition, the individual clone's genotype is of great importance in contributing to the observed polyphenolic composition. Usually, clonal performance in terms of yield and levels of synthesized polyphenols as well as the other tea constituents are genetically linked. In Kenya, clones with high phenolic content and high yielding properties such as TRFK 6/8 are therefore used as parents in breeding programmes. Consequently, the resulting progenies usually possess higher phenolic composition as evidenced in this current study. Notably, a total of 46 clones had phenolic composition higher than TRFK 6/8 (27.4%), a clone used as an internal reference standard because of its proven high quality. However, 16 clones had not been released to farmers for commercial exploitation mainly due to their low yields. The clones include TRFK 301/1, TRFK 381/5, TRFK 381/1, TRFK 635/1, TRFK 400/1, TRFK 382/2, TRFK383/1, TRFK 11/52, TRFK 11/26, TRFK 655/1, TRFK 829/3, TRFK 829/6, TRFK 829/7, TRFK 77/1, TRFK 412/2, TRFK 395/2 and TRFK 395/2. The TRFK 830 and TRFK 833 clonal series were of relatively low total phenolic

composition of less than 22% and should not be used in production of phenolic rich tea products. Their suitability for processing of green teas could be explored.

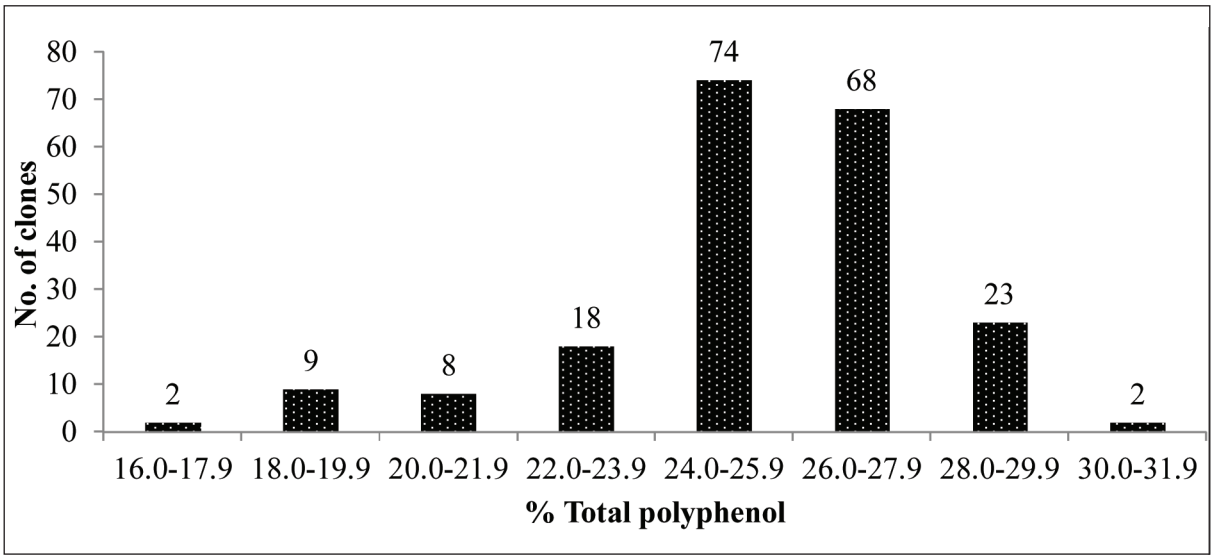

Figure 2: Variation in total polyphenols among the 204 studied clones. CV% = 5.4, LSD (P < 0.05) = 2.73.

Clones used in this study can therefore be exploited to generate diverse high value tea products, especially as raw materials for formulation in cosmetic products and development of polyphenol rich tablets used as pharmacologically active products. This area has not been exploited but has potential to generate significant revenue to the industry. The polyphenolic contents of the studied clones were observed to be slightly different compared to those documented in the clonal catalogue at the TRI[33]. This variation could be due to gradual climatic changes and changes in the durations of rains, cold and dry seasons[34, 35, 36] which might eventually influence synthesis and accumulation of the polyphenol compounds. Sampling times could also be responsible for the observed differences since the leaf samples for this study were collected during the dry season which contributes to increased synthesis of polyphenols[37].

From the correlation analysis, the total polyphenolic contents were influenced by the total catechin content (r= 0.521, P 0.001) as well as the individual catechins fractions with ECG and EGCG fractions showing the most contribution (r= 0.358, P 0.001 and r= 0.375, P 0.001 respectively). The observed differences in the total phenolic content between the evaluated cultivars is an important trait for tea breeders and tea manufacturers as well since it allows for selection of potential clones with desired quality potential[4, 24] either for propagation or manufacture of polyphenolic rich teas and extracts.

Total Catechins

The Total Catechin (TC) levels varied significantly (P≤0.05) among the clones with mean value of 19.64%. Clone AHP F7/346 had the highest catechins content of 25.42% followed by clones TRFK 301/3 (24.82%), TRFK 11/52 (24.45%), TRFK 31/8 (24.38%), TRFK 381/5 (24.28%), TRFK 100/5 (23.96%), BBK 7 (23.80%) and TRFK 382/2 (23.79%) in that order, while clone TRFK 657/1 had the lowest TC at 9.46% **(Figures 3 and 4)**. The observed wide variations in total catechins are indicative of the inherent genetic variations among the clones and show the presence of suitable clones with diverse potential in manufacture of high quality tea products. Most of the clones in the high catechins cluster were of the Assamica origin and are suitable for manufacture of oolong and orthodox or CTC black teas. The clones in the low and medium catechin clusters are majorly of Chinary origin and would be suitable in manufacture of high quality green teas.

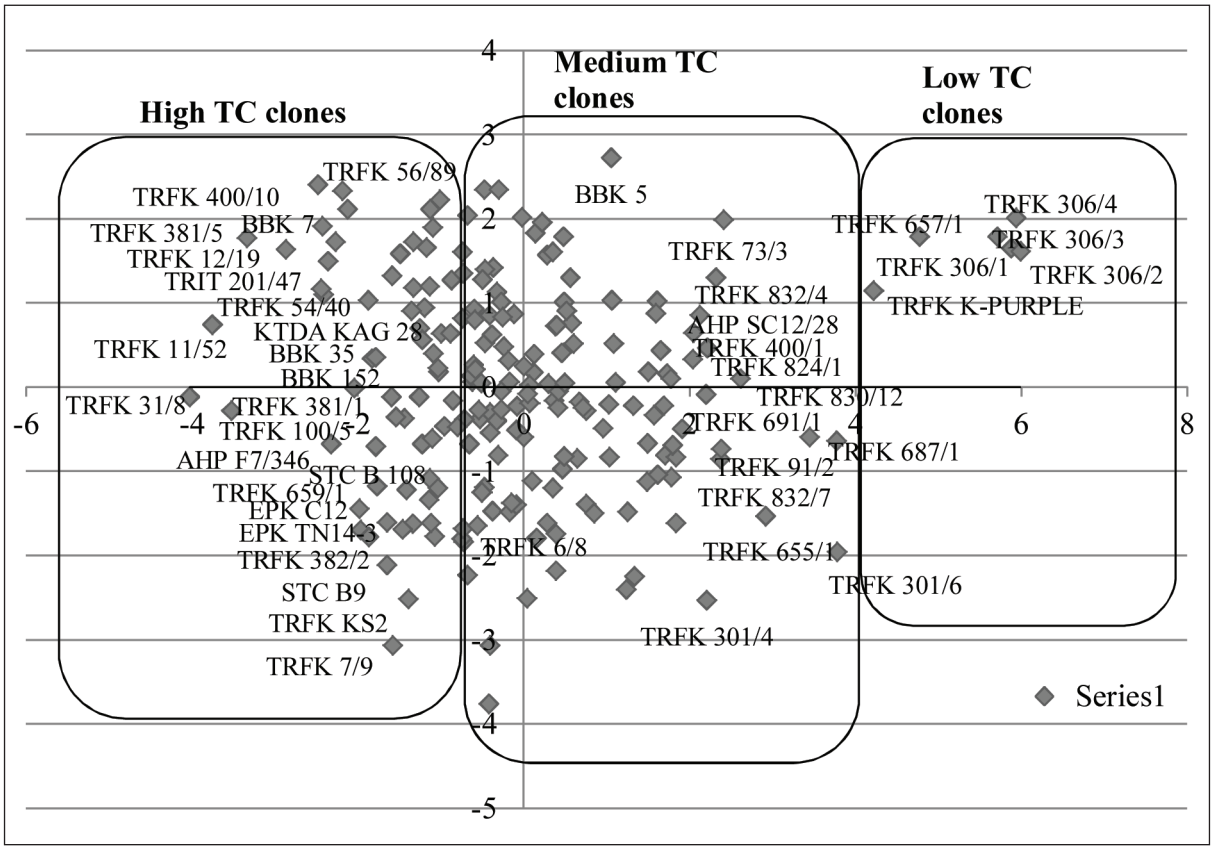

Figure 3: Principle component analysis on variation in Total Catechins among the 204 clones.

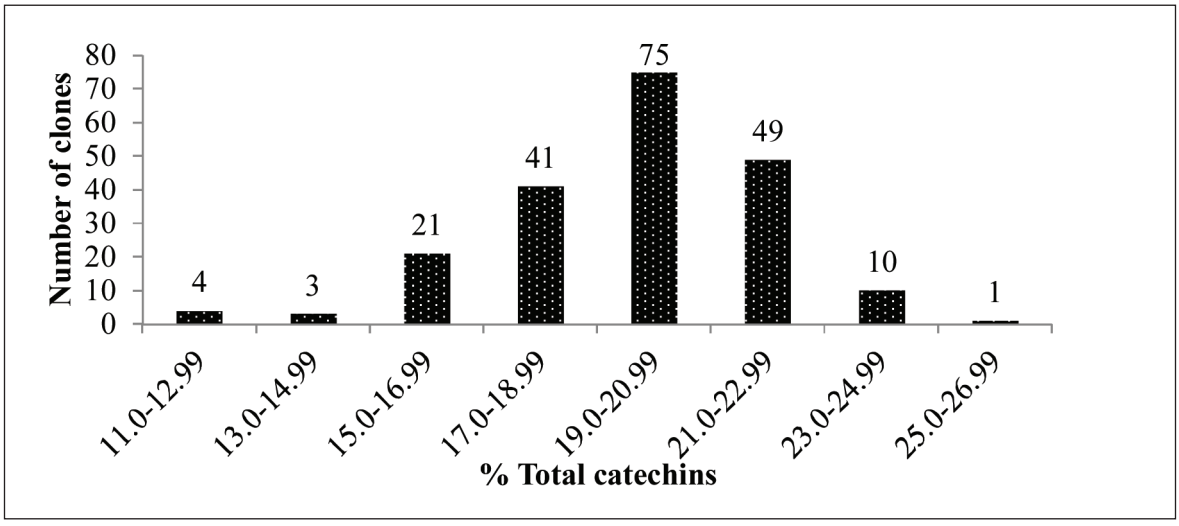

Figure 4: Variation in total catechins among the 204 studied clones. CV% =9.5, LSD (5%) =3.67.

Individual catechins

The levels of the individual catechin fractions varied significantly (P0.05) among the studied clones except for the simple catechins (+C). The observed variations in individual catechins among the clones show differences in quality potential of the assayed clones that could be utilized in the diversification endeavor. The individual catechins fractions are the precursor compounds in the formation of black tea theaflavins and thearubigins emphasizing their importance as tea quality markers[4,24]. Epigallocatechin gallate (EGCG) fraction was significantly higher (P0.05) at 7.86% followed by Epigallocatechin (EGC) at 6.46% and Epicatechin gallate (ECG) at 2.93%. Epicatechin (EC) and Catechin (+C) were present in significantly lower amounts with mean values of 1.87% and 0.53% respectively. This order of abundance is however bound to differ depending on the clone as also shown

in a previous study[23]. The relatively low levels of ECG and EC imply that the two molecules are of major importance in formation of black tea theaflavins as they are the most limiting[38]. Further, the high levels of EGCG serve as a useful clonal selection criterion for potential plain black tea quality parameters as shown in a previous study [23].

Epigallocatechin gallate (EGCG)

Results for the clonal variations in the EGCG fraction are shown in **Figures 5** and **6**. Clones TRFK 306/2 and TRFK 381/5 had the lowest and highest EGCG levels at 3.63% and 11.68%, respectively. Majority of the assayed clones had more than the average EGCG content (7.86%), suggesting that most are of high quality. In addition to clone TRFK 381/5, other high EGCG rich clones include TRFK 31/8(11.09%), TRFK 12/19(11.09%), TRFK 11/4(10.69%), TRFK 100/5(10.62%), TRFK 31/11(10.82%), TRFK 56/89(10.86%), BBK 5(10.67%), BBK 7(11.05%), TRFK 400/10 (11.25%), TRFK 11/52 (11.41%) all of which are known for high quality black tea[33]. Clones TRFK 400/10, TRFK 381/5 and TRFK 11/52 have not been released to farmers for commercial cultivation due to either their below average yield stability or poor tolerance to abiotic stresses[39].Their release for cultivation in relatively favorable environments may however provide an excellent source of EGCG in green tea. Further, their high level in the green leaf has been shown to correlate positively with black tea sensory evaluation[4, 23,24].

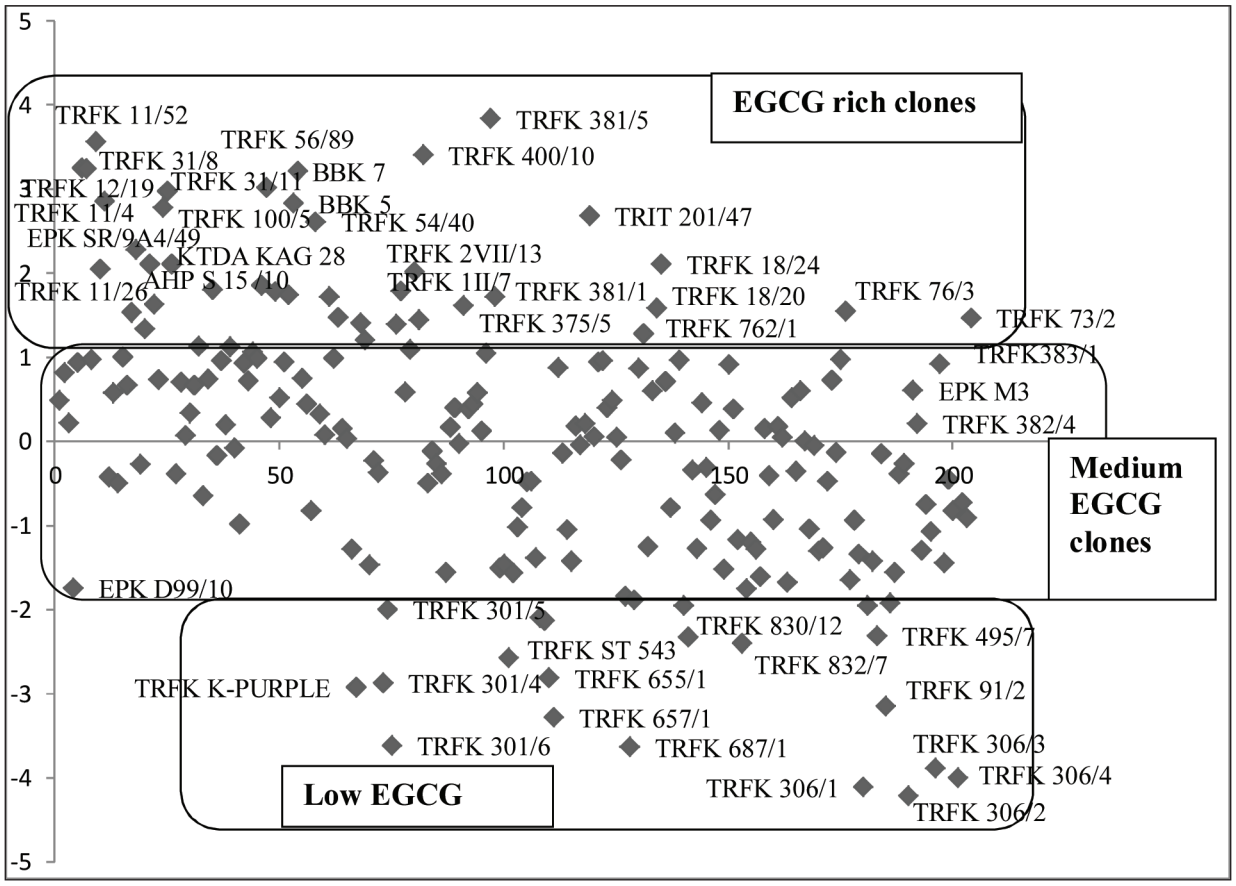

Figure 5: Principle component analysis on variation in EGCG among the 204 clones

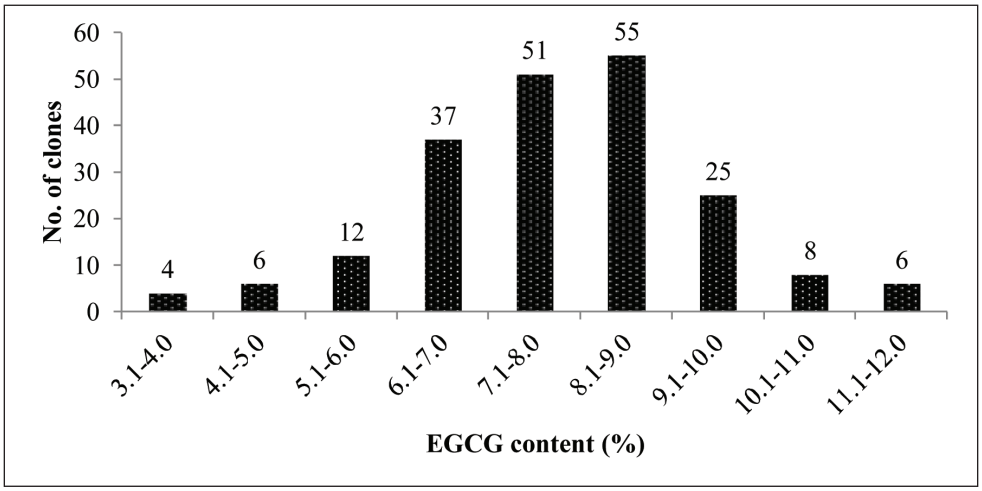

Figure 6: Variation in EGCG among the 204 studied clones. CV% =12.4, LSD (5%) =1.92.

Epicatechin gallate (ECG)

Results for the ECG fraction are presented in **Figures 7 and 8.** Clone STC M3 had the lowest ECG content of 1.03% while TRFK 301/6 had the highest with 6.04% followed by clones TRFK 832/8 (5.64%), TRFK 655/1 (5.23%), TRFK 301/4 (5.16%), TRFK 301/3 (4.98%), TRFK 301/1 (4.84%), TRFK 824/1 (4.60%), TRFK 598/1 (4.53%) and TRFK 831/1 (4.50%). The high ECG levels observed in this study are highly desirable as high quality black tea predictors because during black tea manufacture, they polymerize with EGCG to form theaflavin digallate, which is the most potent theaflavin. Their levels in the green leaf have been shown in previous studies to correlate positively with black tea quality[23]. Among the ECG-rich clones, only TRFK 301/4 is grown commercially. This implies that most of the unreleased clones provide suitable raw materials in the manufacture of high quality green and black teas.

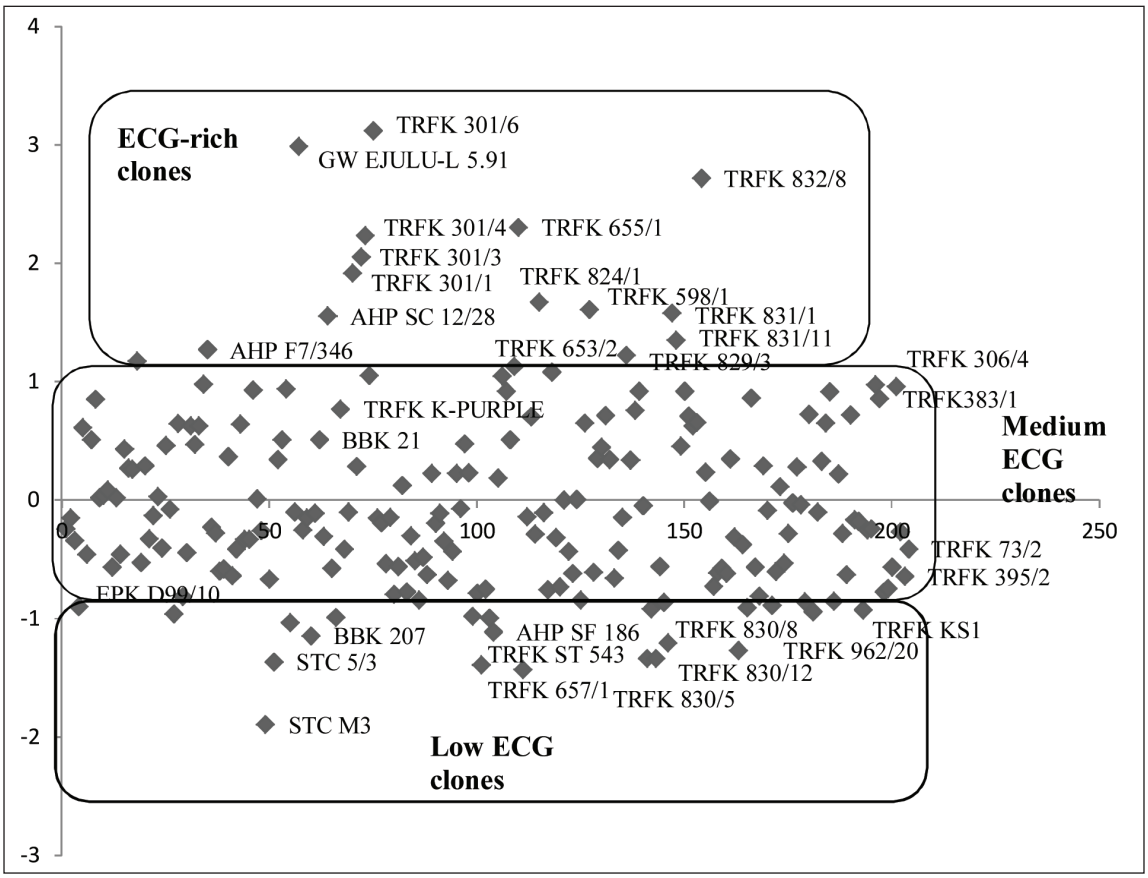

Figure 7: Principle component analysis on variation in ECG among the 204 clones

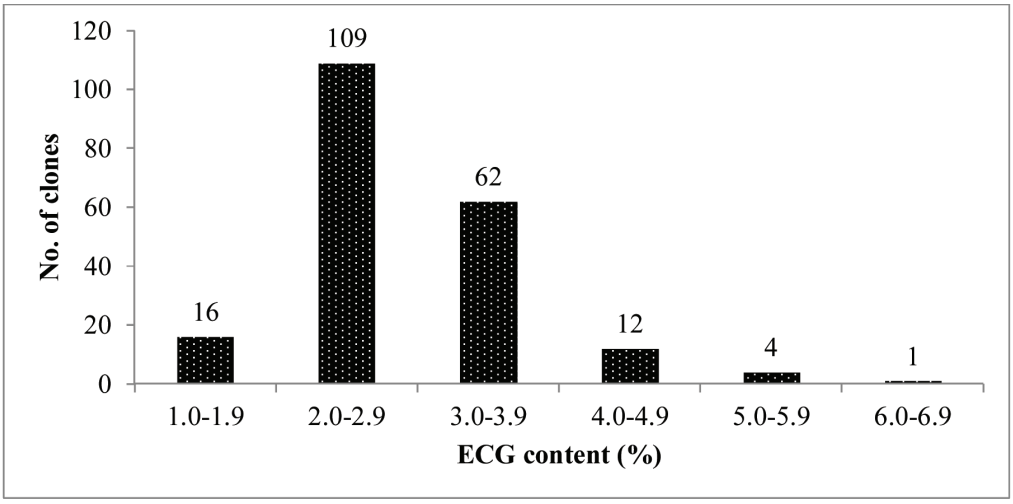

Figure 8: Variation in ECG among the studied 204 clones. CV% = 21.9, LSD (5%) =1.26.

Epigallocatechin (EGC)

Results of the EGC fraction are exhibited in **Figures 9** and **10**. Clone TRFK 306/4 had the lowest contents of EGC at 1.74%, while clone TRFK 7/9 had the highest contents at 9.94% followed by EPK D99/10 (9.80%), STC B9 (9.11%), TRFK KS2 (9.33%), TRFK KS1 (9.10%), TRFK 412/2 (8.50%), TRFK 382/2 (8.84%), TRFK 301/3 (8.67%), AHP M2/10/51 (8.63%), TRFK 100/5 (8.60%), KTDA B1 (8.57%), TRFK 31/8 (8.77%), EPK C12 (8.77%), EPK C182/40 (8.54%), EPK H81/22 (8.83%) and EPK TN 14-3 (8.70%). Clones TRFK KS2, TRFK KS1, TRFK 412/2, TRFK 382/2 and TRFK 301/3 are yet to be released for commercial cultivation. These EGC rich clones would be highly suitable in high quality black tea manufacture. EGC combines with EC and ECG to form simple theaflavin and theaflavin monogallate respectively. In their study, Owuor *et al*[24] found that EGC had significant contribution to black tea quality parameters owing to the fact that, EGC being a trihydroxyflavan-3-ol, has lower redox potential and therefore is oxidized faster implying that they could be the limiting factor during theaflavin formation.

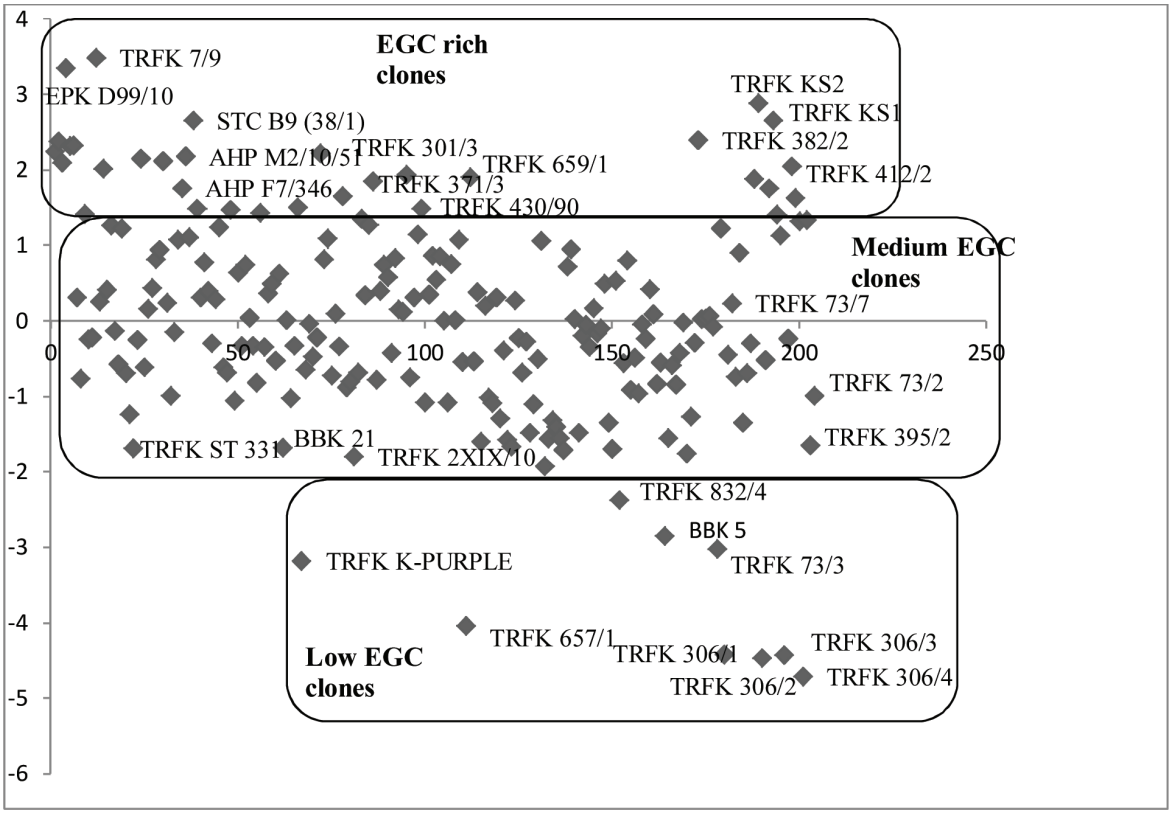

Figure 9: Principle component analysis on clonal variation in EGC among the 204 clones

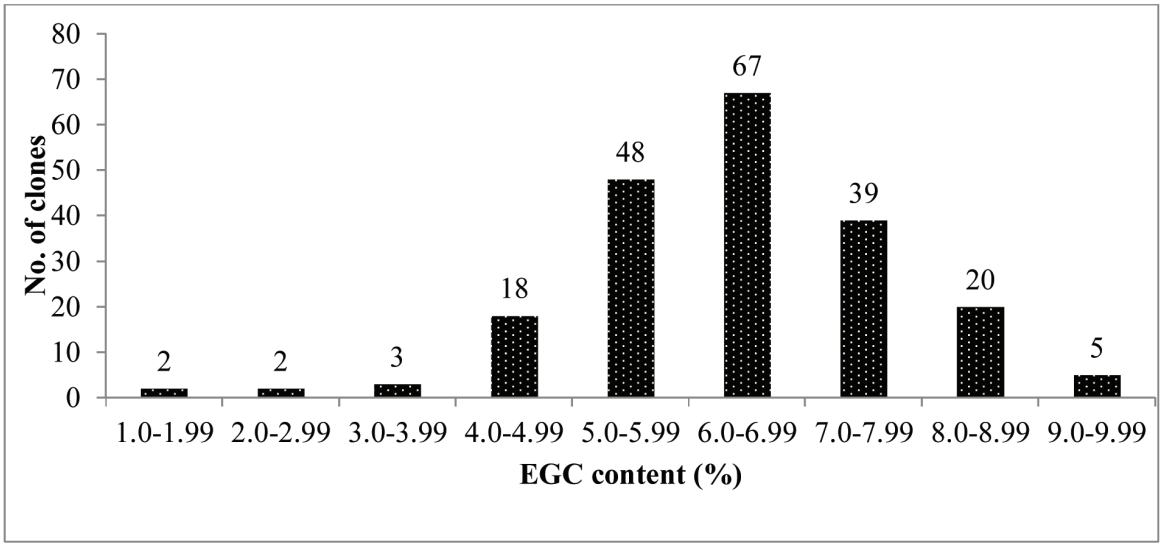

Figure 10: Variation in EGC among the studied 204 clones. CV% = 15.6, LSD (5%) = 1.98.

Epicatechin (EC)

Results of the EC contents among the 204 clones are shown in **Figure 11**. TRFK 301/6 had the highest EC level of 5.41%, while TRFK 420/13contained the lowest with 0.77%. Apart from TRFK 301/6, other EC rich clones include TRFK 655/1 (4.60%), TRFK 301/5 (4.24%), TRFK 301/1 (4.15%), TRFK 301/4 (3.81%), TRFK 91/1 (3.44%), TRFK 653/2 (3.14%) and TRFK 301/3 (3.12%). Among the more than average EC content clones, only TRFK 301/5 and TRFK 301/4 have been released for commercial utilization. Such EC-rich clones could be utilized in manufacture of less astringent high quality green and black teas. Several previous studies have demonstrated inconsistent results on the contribution of EC to overall black tea quality parameters with some showing positive correlation to black tea sensory evaluation[38]. A study by Obanda *et al.*, [23] showed that EC correlated negatively, though insignificantly, to black teas sensory evaluation suggesting that it contributed minimally to black tea quality.

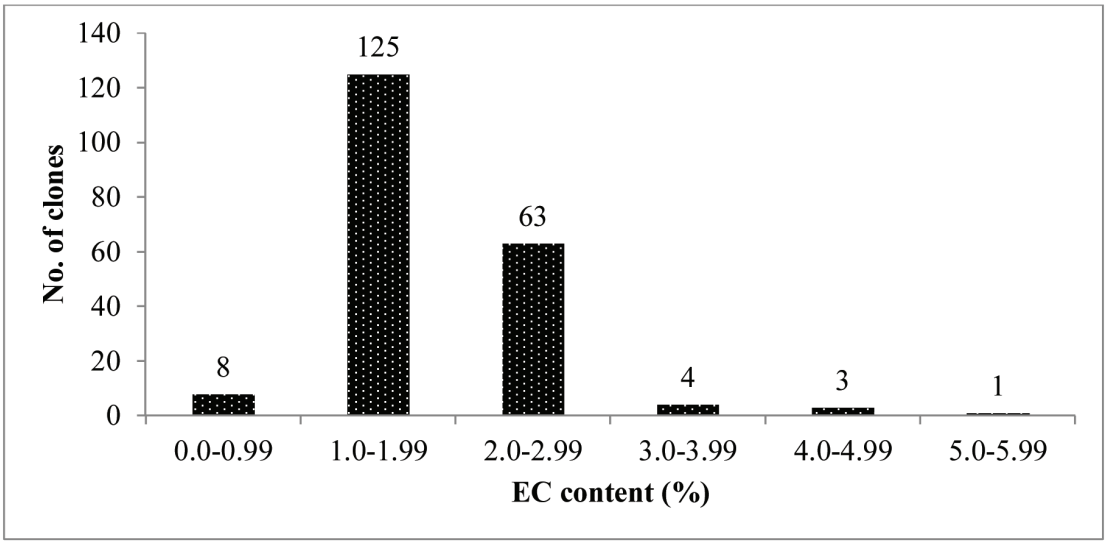

Figure 11: Variation in EC among the studied 204 clones. CV% = 33.5, LSD (5%) =1.24.

Catechin (+C)

Results for the levels of simple catechins (+C) fraction are shown in **Figure 12.** Though no significant differences were observed amongst the clones in the contents of simple catechin (+C), clones TRFK ST 536 and TRFK 306/1 had the lowest and highest contents of 0.21% and 1.52%, respectively. Other high +C clones include TRFK 306/2(1.44%), TRFK 306/3(1.48%), EPK SR/18V/49 (0.84%), BBK 152(0.80%), TRFK 371/3(0.80%), TRFK 371/2(0.76%) and TRFK 73/5 (0.76%).

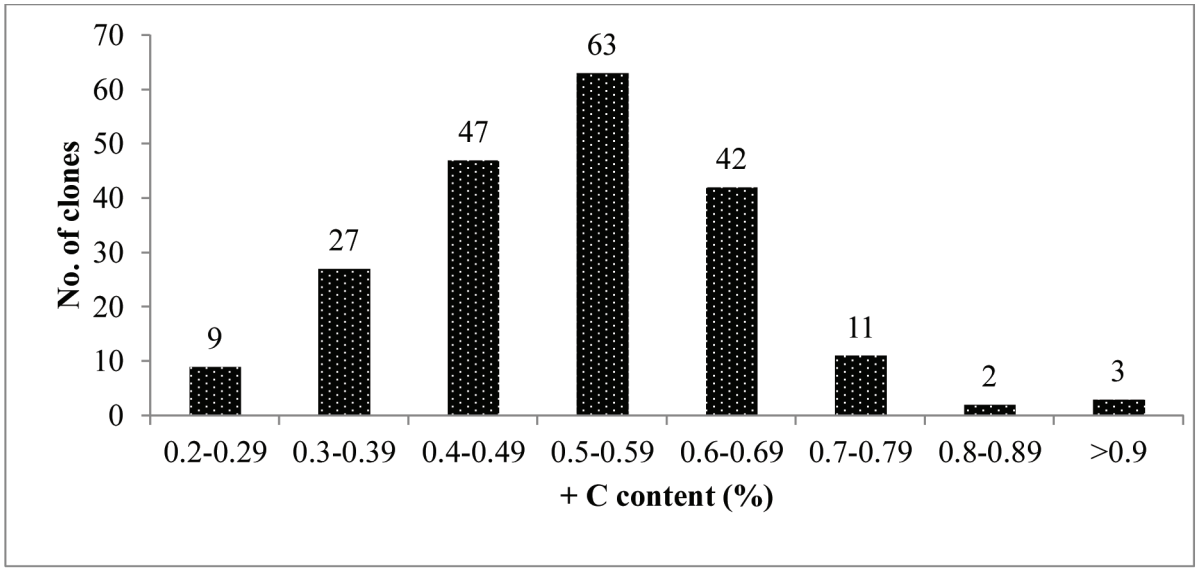

Figure 12: Variation in +C among the studied 204 clones

Catechins are important compounds in tea quality[4, 24, 38, 40, 41] and accounts for two thirds of the total polyphenolic content in tea plants. The major catechins in tea include catechins (+C), epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG). In this study, the sampled cultivars contained EGCG fraction in largest amounts followed by EGC and ECG, while +C and EC were present in significantly lower amounts. Similar results were obtained in studies done by Karori *et al*[42,43] and Ender *et al*[44]. Correlation analysis showed a significant (contribution of the individual catechin fractions to the TC content with EGC contributing the most (r=0.600***) followed by EGCG (r=0.593***), ECG (r=0.425***) and EC (r= 0.365***). Usually, tea clones contain EGC and EGCG in high levels with the EGCG fraction accounting for up to 50% of the total catechins content. A change in the levels of these two catechin fractions is expected to greatly affect the total catechin composition of the tea leaves. The purple leaf colored clones (TRFK 306/1, TRFK 306/2, TRFK 306/3 and TRFK 306/4) had significantly lower catechin levels compared to the green leaf cultivars. The purple teas contain high levels of anthocyanins. Synthesis of both compounds (anthocyanins and catechins) shares the same substrate, anthocyanidins[31]. The purple teas are believed to over-express the enzyme anthocyanin synthase, responsible for synthesis of anthocyanins rather than leucoanthocyanin reductase in the catechins biosynthetic pathway. This observation is believed to be in response to some environmental stimuli such as high temperatures since both compounds are secondary metabolites derived from the general phenyl propanoid pathway[45]. Ninety (90) clones recorded a TC level (21.69%) greater than the standard reference clone, TRFK 6/8 (20.28%). In green tea manufacture, a prescreening procedure to ascertain suitability of the clones in terms of high catechins has not been standardized in Kenya. Instead, all the available clones are combined indiscriminately with the only measure taken being the steaming process that deactivates polyphenols oxidase. Since clones with high TC content (> 20%) are normally very astringent and therefore disliked by tasters of green teas, clones producing low TC content would be preferable for green tea processing[4,24,38,40,41]. On the contrary, clones with high TC contents are normally suitable for black CTC or orthodox teas. Since catechins are the major constituents and quality markers of tea, the total catechin-rich clones such as AHP F7/346 (25.42%), TRFK 301/3 (24.82%), TRFK 11/52 (24.45%), TRFK 31/8 (24.38%), TRFK 381/5 (24.28%), TRFK 100/5 (23.96%), BBK 7 (23.80%) and TRFK 382/2 (23.79%) can be commercially exploited in manufacture of catechins extracts for use either in pharmacological or cosmetic industries.

The chemical composition of the fresh tea leaves especially the individual catechins contributes to the quality of the resulting black tea[4, 24, 38, 41]. During the aeration, the individual catechins fractions react together to form respective theaflavin products in this manner EC + EGC =TF, EC + EGCG= TF-3-g, ECG + EGC= TF-3'-g, ECG + EGCG= TF-3, 3'-dg. It is therefore possible to optimize the production of

tea products that are rich in specific biomolecules by utilizing clones with the desired combination of catechins fractions[4, 24, 38, 40, 41]. In this study, clones such as GW EJULU, TRFK 301/6, TRFK 832/8, TRFK 655/1, TRFK 301/3, TRFK 824/1, TRFK 831/1, AHP SC 31/37, AHP SC 12/28, TRFK K-purple, TRFK 6/8, AHP F7/346, TRFK 301/2, TRFK 301/1 and TRFK 301/4 can be used for developing high quality black tea product rich in theaflavin-3, 3'-digallate (T-3, 3'-DG). During the selection, a lot of emphasis was based on high ECG levels rather than EGCG since it was the most limiting catechins during the reaction. The ratio between the two fractions ECG:EGCG was maintained at a minimum level with the levels of the aforementioned clones as follows; 5.91:7.02, 6.04:4.23, 5.64:6.09, 5.23:5.03,4.98:7.47,4.60:6.42,4.50:7.21, 3.21:7.79,4.48:8.00, 3.69:4.92, 2.46:7.35, 4.19:9.64, 3.21:7.61, 4.84:6.37 and 5.16:4.97, respectively. This is the reason why some clones e.g. TRFK 381/5 with high EGCG amounts were not selected due to a wide EGCG:ECG ratio. Clones TRFK 301/6, TRFK 655/1 and TRFK 301/1 were found to have high EC and EGC levels and are suitable in manufacture of black teas with high simple theaflavin fraction. The TRFK 301 clonal series especially TRFK 301/1, TRFK 301/3, TRFK 301/5 and TRFK 301/6, all Cambods, had higher levels of EC and ECG, the most limiting compounds during black tea formation making them perfect for manufacture of black teas with the various theaflavin fractions. The diversification process requires several approaches to ensure that resulting products meet the different consumer tastes. Kenya, being a major producer of black tea will benefit from utilization of these clones as the resulting products will be sold as high quality and branded black teas, a strategy expected to boost demand for our products consequently increasing returns to producers.

Clones TRFK 301/5 and TRFK 301/4 with high EGC/EC fractions of 7.54:4.24 and 7.26:3.81, respectively, but relatively low EGCG/ECG fractions were also selected for manufacture of less astringent and bitter green teas. EGCG and ECG contribute to most of green tea's bitter and astringent taste which some consumers find pleasant. In Kenya where the main product is black tea, green tea production is yet to grow despite the potential health benefits associated with the product. The awareness on its benefits offers a potential market which is poorly exploited in Kenya. Production of high quality green tea products which could be marketed predominantly as a health drink will open up a new line of premium brands envisaged to place the country in the forefront of the niche tea market. Since the EGCG fraction possesses much of green tea's potency, several clones not yet released for commercial cultivation such as TRFK 11/52, EPK SR/9A4/49, BBK 5, BBK 7, TRFK 400/10, TRFK 381/5 and TRIT 201/47 should be utilized. The clones can also be exploited as raw materials for extraction and sold as nutritional and pharmaceutical supplements inform of tablets, capsules or health drinks. Green tea extracts can also be used in cosmetic products such as moisturizers, hair care products and sunscreens where they act as preservatives in the products and also help prevent skin damage by the sun due to their antioxidative activities. However, due to the high content of catechins in the Kenyan clonal tea leaves, levels of residual catechins in Kenyan black teas are comparable with those of green teas from other countries[46]. This implies that Kenyan black tea is as good as green tea from China or any other green tea manufacturing country.

Caffeine

Results of Caffeine levels among the evaluated clones are presented in Figures 13 and 14 below. Caffeine levels varied significantly between 1.96% for clone TRFK 687/1 and 4.37% for TRFK 371/5with an average value of 3.23%. Other high caffeine containing clones included TRFK 12/12 (4.26%), TRFK 381/5 (4.23%), TRFK 400/7 (4.14%), TRFK 371/3 (4.11%), TRFK 830/6 (4.11%), TRFK 430/52 (4.10%), TRFK 11/52 (4.09%), STC B9 (4.04%), TRFK 829/3 (4.04%) and TRFK 381/1(3.95%). Only TRFK 12/12, STC B9 and TRFK 371/3 have been released for commercial utilization. On the other hand, low caffeine clones other than TRFK 687/1, that would be suitable for manufacture of low caffeine beverages include; TRFK 73/7(2.04), TRFK 830/12 (2.14%), TRFK 306/2 (2.18%), TRFK 306/4 (2.18%), TRFK 306/1 (2.21%), AHP PC 81 (2.25%), TRFK 306/3 (2.31%) and TRFK 830/5 (2.38%).

Caffeine belongs to a group of purine-based compounds collectively known as methyl xanthenes and contributes to the tea's bitter taste/astringency. Synthesis and accumulation of caffeine in the tea plant is genotype-dependent[23,47,48] and affects the caffeine levels. Selection of low caffeine tea clones such as TRFK 306 clonal series (i.e. purple tea cultivars),

TRFK 687/1, TRFK 73/7 and TRFK 830/12 offers a better alternative to caffeine sensitive consumers since the decaffeination process could result in loss of attributes such as aroma. However, clones endowed with high caffeine levels such as TRFK 12/12, TRFK 11/52, STC B9, TRFK 430/52, TRFK 400/7, TRFK 371/3, TRFK 371/5, TRFK 381/5, TRFK 381/1, TRFK 829/3 and TRFK 830/6 should be utilized in manufacture of caffeine-rich teas. Owing to its pharmacological properties, caffeine is often been included in some over the counter analgesics and also added as an ingredient in most commercial soft drinks in the market such as cola, chocolates and energy drinks. Therefore extraction of caffeine from the caffeine-rich clones for use as health supplement or to fortify foodstuff provides a viable market for diversified tea products. This line of value addition ought to be embraced since production and marketing of food stuffs with important dietary components are being determined solely by differing consumer tastes, a strategy that will serve to increase demand for Kenyan tea products, hence better returns.

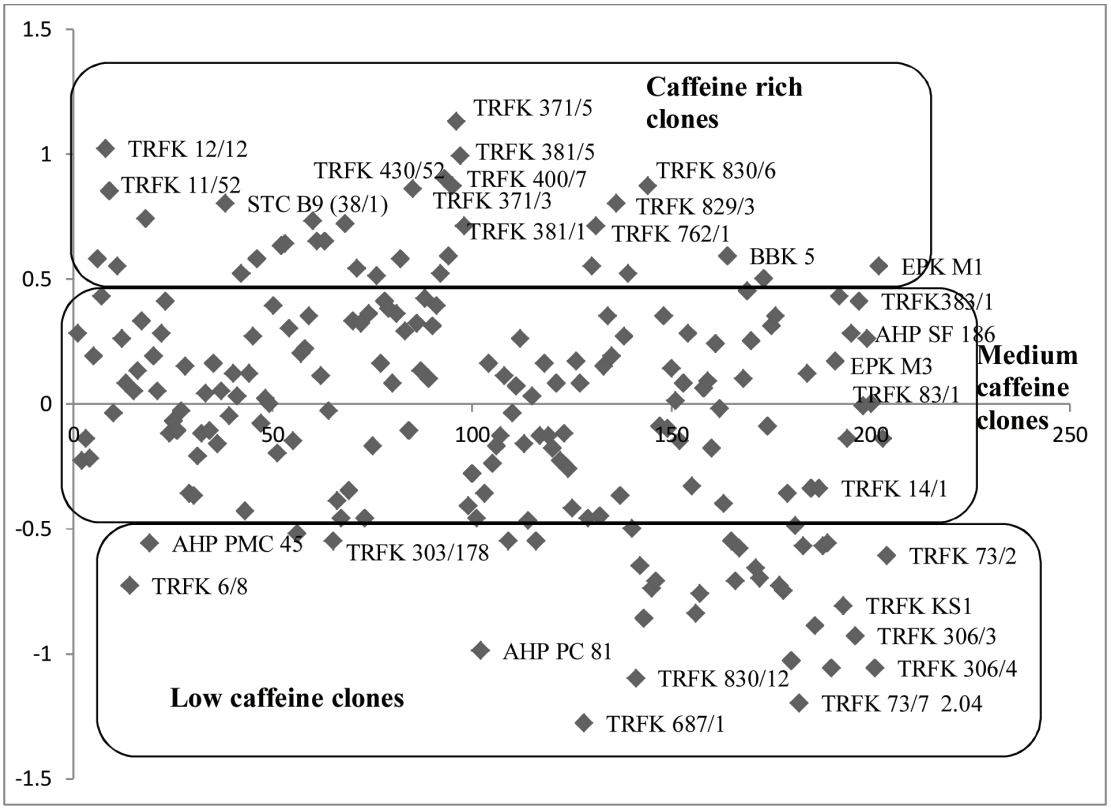

Figure 13: Principle component analysis on variation in caffeine among the 204 clones

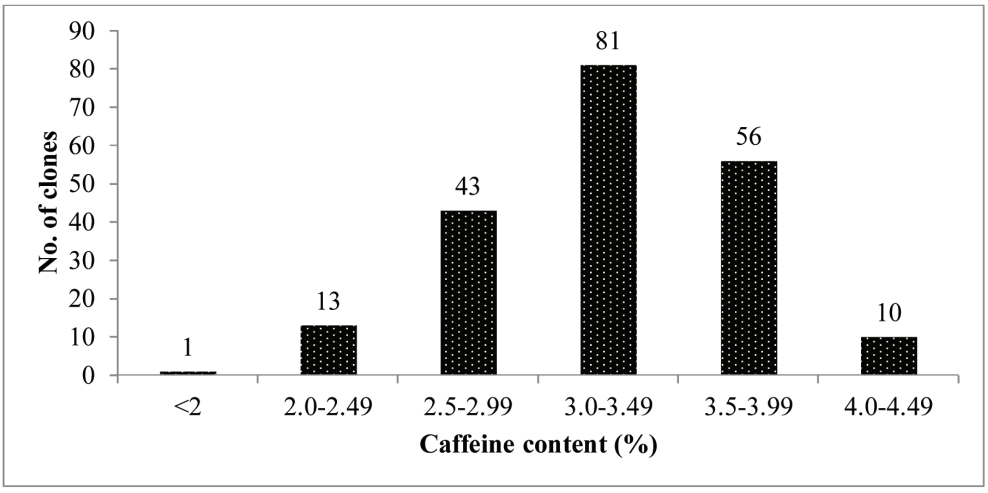

Figure 14: Variation in Caffeine contents among the studied 204 clones. CV% = 13, LSD (5%) = 0.83.

Anthocyanins

Results for the levels of total anthocyanins and anthocyanins profiles among the clones are presented in **Table 1**. The total anthocyanins levels varied though not significantly ($p<0.05$) among the four clones of purple tea studied. Clone TRFK 306/1 had the highest content of 1319 mg/l followed by TRFK 306/2 with 1260 mg/l, TRFK 306/3 with TRFK 1168 mg/l, while TRFK 306/4 had the least amounts of anthocyanins with a value of 986 mg/l. The levels of the anthocyanin fractions were not significantly different ($p<0.05$) among the clones except for the Malvidin fraction. The anthocyanidin pigment, Malvidin was present in highest amounts in the purple tea clones followed by peonidin, kuromanin, pelargonidin, cyanidin, iadein and lastly delphinidin as also observed in a study by Kerio *et al*[49].

Table 1: Contents of total anthocyanins (mg/l) and anthocyanins fractions in the four purple colored clones. CV% = 6.3, LSD (5%) = 237.1.

Clone	Iadein	kuromanin	delphinidin	Cyanidin	pelargonidin	peonidin	melvidin	T.ANTHC
306/1	36.6	81.5	17.9	62.1	64.3	464.0	593.0	1319.4
306/2	33.2	81.2	16.5	58.3	66.8	420.0	583.5	1259.6
306/3	33.6	70.3	16.4	60.5	59.1	398.6	529.4	1168.0
306/4	36.4	67.5	15.4	58.2	58.5	318.7	431.0	985.6

Anthocyanins are a group of flavonoid compounds that contribute to the attractive colours of fruits, vegetables and flowers imparting red, orange, purple, violet and blue colours[49,50]. They have been used in the food industries as natural food colorants, preservatives and in the manufacture of cosmetics such as soaps and shampoos but recently many studies are focusing on their nutritional value. Recent studies have supported their health-enhancing roles such as antioxidant[51], anti-inflammatory[52,53], antimicrobial[54,55], antiartherosclerotic[56] and anticarcinogenic activities[57] further increasing their use as nutritional and pharmacological supplements. Their water soluble nature can be used to diversify their use in food industries mainly to prevent lipid peroxidation which contributes to deterioration in food quality and development of unpleasant odors.

Chlorogenic acid

Significant differences ($P\leq 0.05$) in the contents of chlorogenic acid among the 15 clones were observed (**Figure 15**). Clones AHP SC 31/37, TRFK 831/11 and TRFK 6/8 had the highest content of 0.131%, 0.067% and 0.066%, respectively, while clone TRFK 301/6 had the lowest amounts of 0.05%.

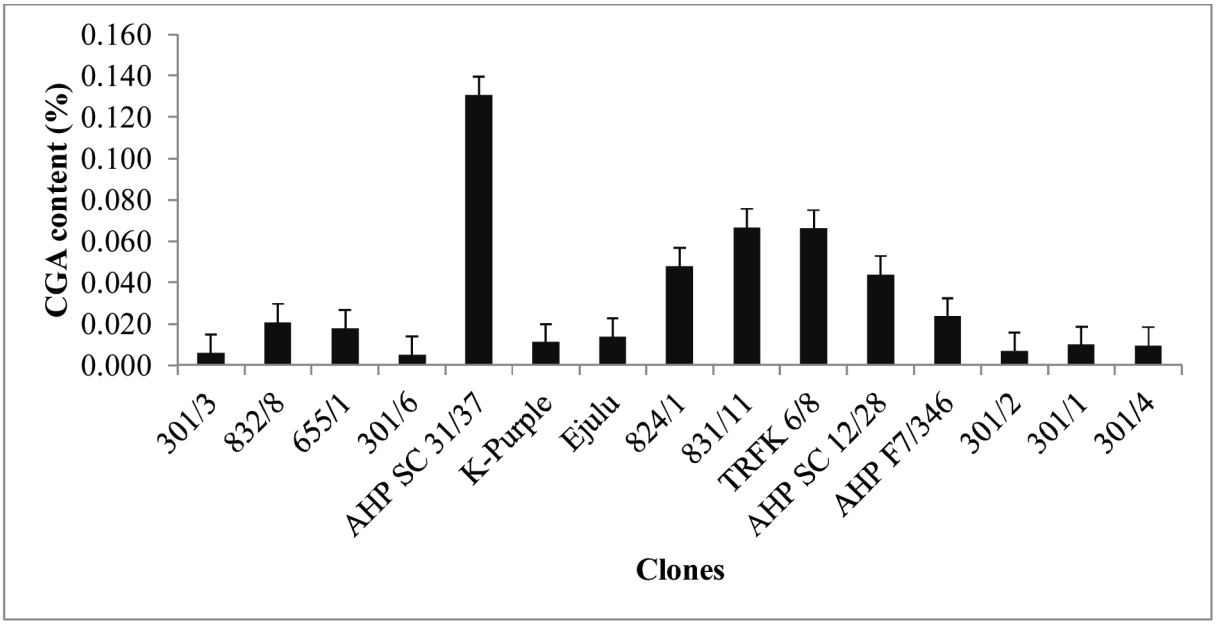

Figure 15: Variation in chlorogenic acid among the 15 clones assayed.CV%= 0.7, LSD (5%) =0.0098.

Chlorogenic acid is a phenolic acid found mainly in green coffee beans but also in other plants such as tea, sunflower seeds, potatoes, fruits such as apples, pears, eggplant and blueberries although in smaller quantities[58]. Research work previously carried out on chlorogenic acid has been primarily on sources such as coffee and thus there is scarce information available on tea[59]. Results obtained in this study revealed that clones AHP SC 31/37, TRFK 831/8 and TRFK 6/8 had the highest quantities (0.13%, 0.07% and 0.06% respectively) and are thus suitable in manufacture of high chlorogenic acid teas. Further, the chlorogenic acid can be extracted and the resulting products sold as health enhancing nutraceuticals. Previous studies on chlorogenic acid in coffee have shown that it possesses health benefits such as reduction of the relative risk of cardiovascular disease[60], diabetes type 2[61], antibacterial[62], anti-inflammatory activities[63], antihypertensive[64], anti-obesity[65] and modulation of glucose metabolism in humans[66]. Given its potential health benefits, inclusion of chlorogenic acid as a novel quality marker in tea will help in branding Kenyan tea products, further increasing the demand of her teas in the world market.

Theanine

Significant differences (P≤ 0.05) in theanine composition among the 15 clones assayed were observed **(Figure 16)**. Clones TRFK 6/8, TRFK 831/11, TRFK 655/1, TRFK 301/1 and 301/3 had the highest theanine content of 1.7%, 1.565%, 1.505, 1.46% and 1.255% respectively while clone AHP SC 12/28 had the lowest with 0.32%.

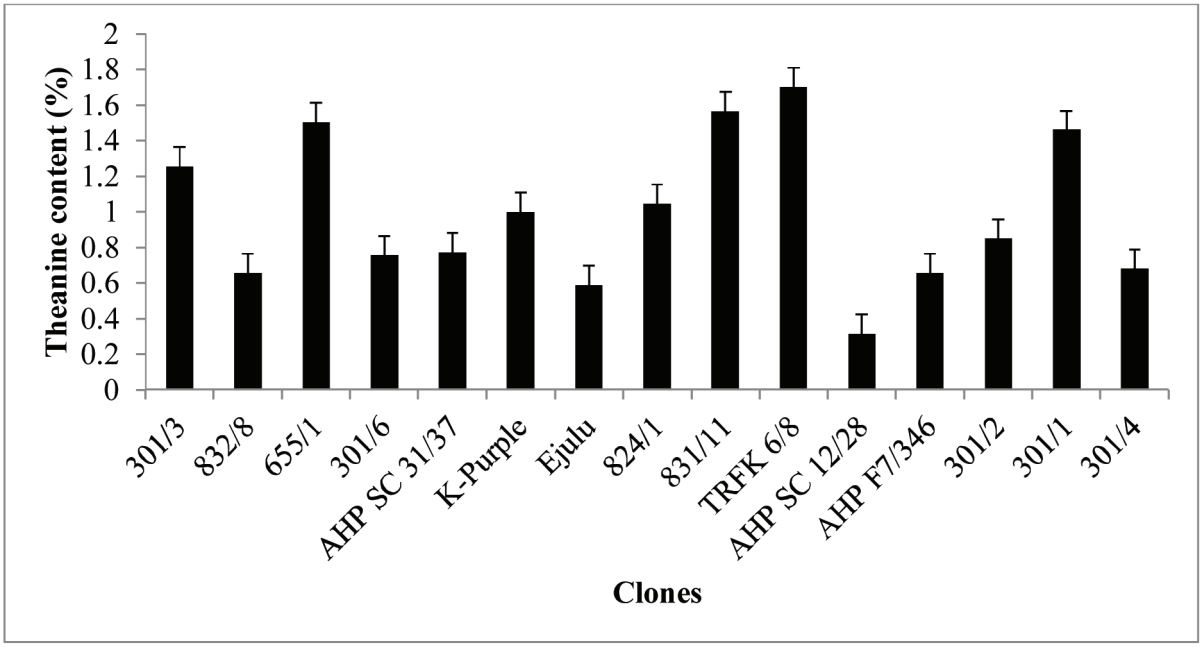

Figure 16: Variation in theanine composition among the 15 assayed clones. CV%= 14.7, LSD (5%) =0.31.

Theanine is the most physiologically important and abundant amino acid component in tea leaves responsible for giving tea infusions the sweet umami taste[67]. Clones with high theanine levels can be utilized as raw materials for production of theanine rich green teas. Owing to its significant contribution to taste and reported health-enhancing benefits particularly on the central nervous system (CNS) by inducing a state of mental alertness, relaxation and anti-hypertensive abilities, its content in tea leaves is considered a very important aspect of tea quality. Additionally, green teas with high theanine levels have been shown to command high market prices[68], further emphasizing the importance of identification of theanine rich tea clones for utilization of novel tea products. Interestingly, the tea plant also stands out as the main available source of theanine in the human diet, the second source being the mushroom *Xerocomus badius*[69]. Previous studies have shown that accumulation of theanine in tea leaves is influenced by sunlight whereby shaded tea plants accumulate more theanine compared to those exposed to sunlight[70]. Exposure to sunlight converts theanine

into its constituent compounds, glutamic acid and ethylamine which is further utilized by the tea plant in synthesis of catechins. As Kenyan tea is largely cultivated without shade, consideration of integrating suitable agro forestry/N-fixing trees in theanine-rich clones cultivated areas may further enhance these unique phytochemical in the final premium product.

Conclusion

The assayed parameters revealed the diversity in the chemical composition of Kenyan tea clones. These variations clearly indicate the inherent qualities in Kenyan tea that are largely untapped in development of diverse tea products. The observed chemical and therefore quality differences based on clones and regions show that Kenyan teas have a remarkable diversity in biochemical attributes and thus suitable for development of diversified tea products with remarkable geographical indications. Utilization of the selected clones for the development of high value tea products is expected to increase demand both locally and internationally, increasing returns to producers.

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