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VARIATIONS IN THE PRECURSORS OF PLAIN BLACK TEA QUALITY PARAMETERS DUE TO LOCATION OF PRODUCTION AND NITROGEN FERTILIZER RATES IN EASTERN AFRICAN CLONAL TEA LEAVES

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SUMMARY

Theaflavins contribute to astringency and brightness while thearubigins contribute to colour and mouth feel of black tea. Green leaf flavan-3-ols influence levels and distribution of theaflavins and thearubigins in black tea and are black tea quality precursor compounds. Caffeine also contributes to tea quality. Although location of production and nitrogenous fertilizer rates influence black tea quality, it is not known if the variations arise from the levels and distribution of the precursor compounds in green leaf or other factors. The variations and distribution of the flavan-3-ols and caffeine in young green leaves of clone TRFK 6/8 due to nitrogen fertiliser rates in seven locations within Eastern Africa were evaluated. Green leaf comprising two leaves and a bud were harvested from each plot, and subjected to HPLC analysis for caffeine, total polyphenol, dihydroxyflavan-3-ols, trihydroxyflavan-3-ols, ratios of trihydroxyflavan-3-ols to dihydroxyflavan-3-ols and total catechins levels. Results were subjected to statistical analysis using split plot design, with locations as main treatments and nitrogen rates as the sub-treatment. Caffeine and flavan-3-ols levels changed ($p \leq 0.05$) with location of production, demonstrating that even with use of same cultivar and similar agronomic management quality of tea from one location cannot be replicated in another location. Caffeine levels increased ($p \leq 0.05$) with rise in nitrogen fertilizer rate in all locations, but the extent depended on location. Total polyphenols and individual flavan-3-ols showed an inverse quadratic response, except EGCG that linearly decreased ($p \leq 0.05$) in some locations, due to increasing rates of nitrogen fertilizer. Similar responses in the black tea quality parameters had been observed in previous studies. The black tea quality results were therefore directly influenced by the green leaf precursor compound patterns. Region specific nitrogenous fertilizer rates need development to ensure high tea quality.

INTRODUCTION

In tea trade, African black teas are classified as plain to medium flavoury. Such teas are valued for taste and colour characteristics; factors attributed to the non-volatile components of black tea. The black tea theaflavins contribute to the astringency (briskness) and brightness while thearubigins contribute to the colour and thickness (mouth-feel) of plain black tea (Biswas *et al.*, 1971; 1973). The theaflavins and

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Table 1. Formation of theaflavins from flavan-3-ols.

Epicatechin (EC) + Epigallocatechin(EGC)	→	Simple theaflavin (TF).
EC + Epigallocatechin gallate (EGCG)	→	Theaflavin-3-gallate (TF-3-g)
Epicatechin gallate (ECG) + EGC	→	Theaflavin-$3'$-gallate (TF-$3'$-g)
ECG + EGCG	→	Theaflavin-3, $3'$- digallate (TF dg)

thearubigins are products of polyphenols oxidation during black tea processing (Davis *et al.*, 1997; Jhoo, 2007). The polyphenols, (flavanols, flavonol glycosides, polyphenolic acids and depsides) make up to between 30% and 40% of the dry weight in tea shoots (Harbowy and Balentine, 1997). Successful relationships have been demonstrated between total theaflavins levels of Central and Southern African plain black teas and sensory evaluations or prices (Ellis and Cloughley, 1981; Hilton *et al.*, 1973; Wright *et al.*, 2002). Consequently, total theaflavins levels were suggested as objective quality parameter for plain black teas (Ellis and Cloughley, 1981). Such relationships were positive but insignificant for Kenya (Owuor *et al.*, 1986) and Sri Lanka (Roberts and Fernando, 1981). When the contributions of the individual theaflavins were normalised to account for their differences in astringencies, the normalised factor, theaflavin digallate equivalent, showed good relationship with sensory evaluation for both Kenyan and Central/Southern African black teas (Owuor *et al.*, 2006). This confirmed that theaflavins are indeed black tea quality parameter. Although high polyphenols levels in green tea leaf were assumed to lead to high black tea levels of theaflavins and thearubigins (Erturk *et al.*, 2010; Hilton *et al.*, 1973; Yao *et al.*, 2005), the composition of the polyphenols has been shown to be more critical to black tea quality than total polyphenols *per se*. The flavan-3-ols levels and composition successfully predicted black tea quality (Owuor and Obanda, 2007; Wright *et al.*, 2000).

The flavan-3-ols, comprising of (+)-catechin (C), epicatechin (EC), epicatechin gallate (ECG), gallic acid (GA), epigallocatechin (EGC) and epigallocatechin gallate (EGCG) dominate green leaf polyphenols (Hilton *et al.*, 1973). The simple catechins (dihydroxyflavan-3-ols) undergo oxidative dimerization with the gallocatechins (trihydroxyflavan-3-ols) to produce theaflavins (Table 1). In Central and Southern African black teas, high levels of EC and EGC (Hilton *et al.*, 1973) and EC and ECG levels (Wright *et al.*, 2000) were associated with high quality black teas. But in Kenya, high EGCG and low EC levels were indicators of high black tea quality potential of clonal tea bushes (Owuor and Obanda, 1997; 2007; Owuor *et al.*, 2006). The green leaf EGCG levels correlated significantly with black tea total theaflavin, liquor brightness and sensory evaluation, while EC correlated positively with thearubigins and negatively with theaflavin digallate equivalent and sensory evaluation (Owuor and Obanda, 2007). The sum of gallated flavan-3-ols (flavan-3-gallates), trihydroxyflavan-3-ols (gallocatechins) and ratios of trihydroxyflavan-3-ols:dihydroxyflavan-3-ols in green tea leaf predicted plain black tea quality potential (Owuor and Obanda, 2007). Indeed, flavan-3-ols composition was a better quality indicator than total polyphenols or total flavan-3-ols (Owuor and Obanda, 1997; 2007). These results demonstrated the importance of the flavan-3-ols composition and levels as indicators of black tea quality potential of green tea leaf. Large clonal differences were observed in the distribution of the flavan-3-ols in green leaf and individual theaflavins in Kenyan (Owuor *et al.*, 2006) and Southern and Central African plain black teas (Wright *et al.*, 2002). Earlier, it was suggested that sources of plain teas could be predicted from the distribution pattern of the individual theaflavins (McDowell *et al.*, 1991). The composition and/or levels of the flavan-3-ols from which the individual theaflavins arise (Table 1) could therefore be dependent on location of production. Results from Kenya showed that the composition of the individual theaflavins were dependent on cultivars (Owuor and Obanda, 1997). For cultivars grown in one location, the flavan-3-ol composition pattern was clonal specific at a set plucking standard (Magoma *et al.*, 2000) suggesting their composition could be genetically controlled. The pattern further remained constant although the levels varied in the same cultivars subjected to uniform agronomic inputs in different locations (Cherotich *et al.*, 2013; Kwach *et al.*, 2013). However, it was not known if varying agronomic inputs on same cultivar could change the levels and composition of the flavan-3-ols.

Nitrogenous fertilizer application is the most costly agronomic input in tea production, after harvesting (Bonheure and Willson, 1992). The expense is justified as nitrogen application increases yields (Msomba *et al.*, 2014) though high rates reduces black tea quality (Hilton *et al.*, 1973; Owuor *et al.*, 1987b) even in the same cultivar under same management practices at various locations (Owuor *et al.*, 2013). But it is not known if the variations were due to changes in polyphenols levels or composition of the flavan-3-ols in green tea leaf. Caffeine is an important black tea quality parameter (Ashihihara and Crozier, 2001; Spiller, 1998). At a single site, caffeine levels increased with nitrogenous fertilizer (Owuor *et al.*, 1987b), varied with year of prune (Owuor and Lang'at, 1988), season (Yao *et al.*, 2005) and location of production (Akhlas *et al.*, 2003). However, it is not known if the extent in caffeine increase with nitrogenous fertilizer rates varies with location of production. The objective of this research was to determine whether the composition of flavan-3-ols varies with location, and whether there is an interaction between nitrogen fertilizer and location on levels of caffeine and polyphenols in the Eastern Africa tea growing regions.

MATERIALS AND METHODS

Experimental set up

Clone TRFK 6/8 is the most widely grown tea variety in Eastern Africa, constituting 80% of Rwanda tea, 60% of Kenya clonal tea and 35–40% of Tanzania tea (Kwach *et al.*, 2014; Msomba *et al.*, 2014). A fertilizer trial on clone TRFK 6/8 was set in seven tea estates in the tea growing locations of Eastern Africa in 2008 (Table 2). At each site, nitrogenous fertilizer rates (0, 75, 150, 225 and 300 kg N ha^{-1} $year^{-1}$) as NPKS 25:5:5:5 trial was laid out in a Randomized Complete Block Design replicated 3 times (Kwach *et al.*, 2014; Msomba *et al.*, 2014). Each plot comprised 30 tea plants of clone TRFK 6/8. The plants at each site were pruned between April and May 2008 before the first fertilizer treatment applications so that all the plants were in same pruning cycle. The first fertilizer treatments were applied in September/October

Table 2. The study sites coordinates and altitude in metres above mean sea level (m amsl).

Country	Site	Latitude	Longitude	Altitude
Kenya	Timbilil Tea Estate (Tea Research Foundation of Kenya)	0° 22′S	35° 21′E	2180
	Changoi Tea Estate	0° 30′S	35° 13′E	1860
	Sotik Tea Estate	0° 36′S	35° 04′E	1800
Rwanda	Kitabi Tea Estate	2° 32′S	29° 26′E	2231
	Mulindi Tea Estate	1° 27′S	30° 01′E	1800
Tanzania	Maruku Tea Estate	1° 23′S	31° 45′E	1488
	Katoke Tea Estate	1° 36′S	31° 41′E	1217

2008, depending on the onset of rain at the individual site. Subsequently, fertilizer was applied annually as single dose in September. The plots were plucked on a 14 days harvesting intervals.

Leaf sampling, extraction and HPLC analysis of flavan-3-ols

In May 2012, leaf (100 g) samples were collected by random hand plucking of two leaves and a bud for the determination of caffeine and polyphenols contents. The leaf was steamed for 1 min, and then dried in an oven at 80 °C to a constant weight. The dried leaf was cooled and crushed to a powder (Owuor and Obanda, 2007). About 125 mg of the powder from each sample was extracted in 25 mL acetonitrile water (1:1 v/v) mixture at room temperature for 30 min with constant shaking. Total polyphenols was determined as outlined in the International Organization for Standardization method (ISO-14502-1, 2005). Flavan-3-ols and caffeine levels were determined using a solvent gradient Shimadzu HPLC with UV detector as described in the International Organization for Standardization (ISO-14502-2, 2005) method. The statistical analyses were done using MSTAC as a split plot design, with locations as main treatment split for rates of nitrogen.

RESULTS

There was good baseline HPLC chromatogram resolution for caffeine and the individual flavan-3-ols (Figure 1). Caffeine levels varied significantly ($p \leq 0.05$) with locations (Table 3), with Rwanda sites recording the highest levels, while sites in Tanzania had the lowest levels. At all locations, caffeine increased significantly ($p \leq 0.05$) with rise in nitrogen rates. There were no significant interactions effects between location of production and nitrogen rates on caffeine levels in tea.

Significant ($p \leq 0.05$) changes in total polyphenols (Table 4), simple catechins (dihydroxyflavan-3-ols) (C, EC, and ECG (epicatechin gallate) (Table 5), GA and EGC (Table 6), trihydroxy:dihydroxyflavan-3-ols ratio, gallated:non gallated flavan-3-ols (flavan-3-gallates: flavan-3-ols) ratio (Table 7), total catechins, total flavan-3-ol gallic acid esters and simple (non-ester) flavan-3-ols (Table 8) in green leaf of clone TRFK 6/8 were recorded due to location of production and nitrogen fertilizer rates. The Kenya sites recorded higher ($p \leq 0.05$) values of total polyphenols, EGCG,

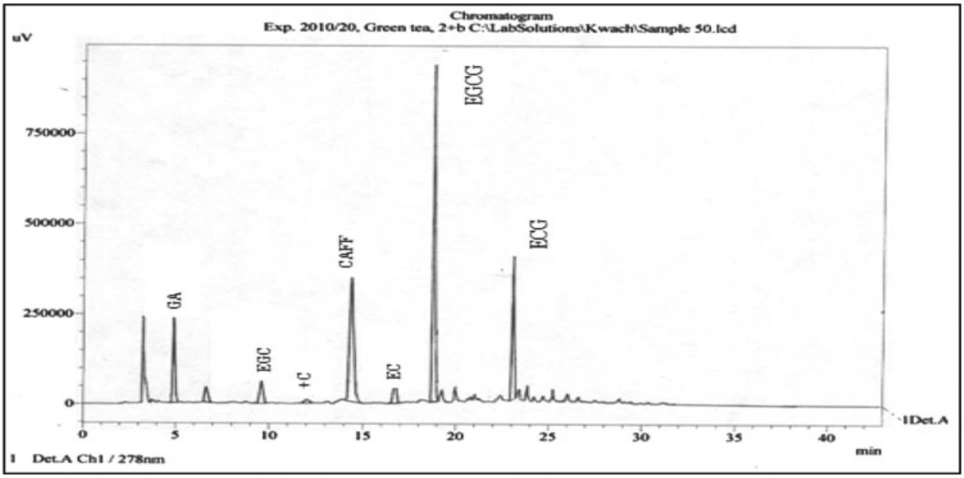

Figure 1. HPLC profile of green tea leaf caffeine and favan-3-ols at 278 nm.

Table 3. Variations in caffeine levels (mg g^{-1} DM) in 2 + a bud of clone TRFK 6/8 with location of production and nitrogen fertilizer rates.

Kg N ha^{-1} $year^{-1}$	Timbilil	Changoi	Sotik	Mulindi	Kitabi	Maruku	Katoke	Mean *N*-rate
0	30.2	28.1	27.6	41.8	34.5	15.5	16.7	27.8
75	32.4	32.3	29.5	46.0	36.4	16.3	16.8	30.0
150	33.0	36.3	30.0	46.2	37.6	17.7	17.5	31.2
225	34.4	36.6	33.1	47.9	40.6	19.1	17.5	32.8
300	35.1	36.6	33.4	49.4	40.9	19.6	18.3	33.3
Mean	33.0	34.0	30.7	46.3	38.0	17.6	17.4	
C.V. (%)				6.3				
LSD, ($p \leq 0.05$)				1.8				1.7

Table 4. Variations in total polyphenols levels with location of production and nitrogenous fertilizers rates (% gallic acid equivalent (GAE)).

Kg N ha^{-1} $year^{-1}$	Timbilil	Changoi	Sotik	Mulindi	Kitabi	Maruku	Katoke	Mean *N*-rate
0	29.45	30.14	27.75	19.47	24.34	22.29	20.92	24.91
75	29.01	29.75	27.26	19.24	24.20	22.40	21.02	24.70
150	28.31	29.58	27.14	18.28	23.71	22.24	20.11	24.20
225	29.18	27.78	27.92	19.57	24.44	23.12	21.45	24.78
300	29.24	28.08	28.07	19.64	25.70	23.76	21.43	25.13
Mean	29.04	29.07	27.63	19.24	24.48	22.76	20.99	
C.V. (%)				1.96				
LSD, ($p \leq 0.05$)				0.43				0.42
Interactions				0.82				

total gallocatechins, gallated:non gallated catechins, total catechins and total gallated catechins than in Tanzania and Rwanda sites which were close.

Generally, the total polyphenol (Table 4), dihydroxyflavan-3-ols (Table 5), trihydroxyflavan-3-ols (Table 6), and total flavan-3-ols and flavan-3-gallate (Table 8)

Table 5. Variations in clone TRFK 6/8 dihydroxyflavan-3-ols levels (mg g^{-1} DM) with location of production and rates of nitrogenous fertilizer.

Catechin	*N*-rate kg N ha^{-1} year^{-1}	Timbilil	Changoi	Sotik	Mulindi	Kitabi	Maruku	Katoke	Mean *N*-rate
(+) C	0	4.5	3.3	2.1	2.7	6.6	3.0	3.4	3.7
	75	4.5	3.2	1.7	2.5	6.6	2.8	3.0	3.5
	150	4.5	2.7	1.3	2.1	6.4	2.5	2.5	3.2
	225	5.1	3.3	2.5	2.6	6.8	2.9	2.9	3.7
	300	5.6	3.6	2.6	2.7	7.0	2.9	3.6	4.0
	Mean	4.8	3.2	2.1	2.5	6.7	2.8	3.1	
	C.V. (%)				8.2				
	LSD, ($p \leq 0.05$)				0.3				0.3
EC	0	24.1	17.4	14.7	18.2	17.2	13.5	13.3	16.9
	75	22.1	14.6	14.0	14.2	16.5	12.8	12.6	15.3
	150	21.3	14.3	13.4	10.9	14.0	12.3	9.3	13.6
	225	23.5	14.6	14.6	17.1	16.9	13.2	15.5	16.5
	300	25.4	15.3	15.1	17.3	18.6	14.1	15.8	17.4
	Mean	23.3	15.2	14.4	15.5	16.6	13.2	13.3	
	C.V. (%)				9.6				
	LSD, ($p \leq 0.05$)				1.4				1.3
	Interaction				2.6				
ECG	0	26.4	28.8	27.1	8.4	27.4	17.0	16.9	21.7
	75	24.9	28.6	25.6	7.6	21.9	16.9	16.2	20.3
	150	23.4	27.2	25.4	7.6	21.7	16.9	13.7	19.4
	225	25.8	28.9	27.5	7.8	24.4	16.7	16.7	21.1
	300	26.5	29.2	28.0	8.0	27.6	17.5	18.5	21.2
	Mean	25.4	28.5	26.7	7.9	24.6	17.0	16.4	
	C.V. (%)				5.2				
	LSD, ($p \leq 0.05$)				1.0				0.9
	Interaction				1.8				
Total simple catechins (dihydroxyflavan-3-ols)	0	55.0	49.6	43.9	29.3	51.2	33.5	33.6	42.3
	75	51.5	46.3	41.3	24.3	45.0	32.5	31.9	39.0
	150	49.3	44.2	40.1	20.6	42.1	31.8	25.5	36.2
	225	54.3	46.7	44.7	27.5	48.1	32.8	35.1	41.3
	300	57.5	48.1	45.6	28.0	53.2	34.5	37.9	43.6
	Mean	53.5	47.0	43.1	26.0	47.9	33.0	32.8	
	C.V. (%)				4.3				
	LSD, ($p \leq 0.05$)				1.5				1.5
	Interaction				2.9				

levels decreased with rates of nitrogen up to 150 kg nitrogen ha^{-1} year^{-1}. Above this fertilizer rate, there were increases in these parameters with nitrogenous fertilizer rates. For total polyphenol, dihydroxyflavan-3-ols and trihydroxyflvan-3-ols, this pattern was clearer in Timbilil, Mulindi, Kitabi and Katoke. But in Changoi, there was decline in levels of total polyphenols up to beyond 225 kg nitrogen ha^{-1} year^{-1}, while in Maruku the levels increased with increase in rates of nitrogen fertilizer. The gallated:non gallated flavan-3-ols and trihydroxy/dihydroxy falvan-3-ol ratios (Table 7) however, showed an inverse response curve. There were positive quadratic responses peaking up at about 150 kg nitrogen ha^{-1} year^{-1} and thereafter declining. In Maruku, there were linear increases in the two ratios with rise in nitrogen fertilizer rates. EGCG, however, showed different patterns (Table 6). In Kenya, the levels decreased with increase

Table 6. Variations in trihydroxyflavan-3-ols levels (mg g^{-1}) DM with location of production and nitrogen rates.

Catechin	*N*-rate kg N ha^{-1} $year^{-1}$	Timbilil	Changoi	Sotik	Mulindi	Kitabi	Maruku	Katoke	Mean *N*-rate
GA	0	7.8	7.4	6.9	22.8	6.5	3.3	3.5	8.3
	75	7.6	7.2	6.4	22.6	6.5	2.9	3.0	8.0
	150	7.4	7.1	6.5	22.2	6.3	2.7	2.8	7.9
	225	7.9	7.9	6.5	24.1	6.9	3.1	3.2	8.5
	300	8.0	8.0	6.8	24.5	7.4	3.4	3.6	8.8
	Mean	7.8	7.5	6.6	23.2	6.7	3.1	3.2	
	C.V. (%)				11.2				
	LSD, ($p \leq 0.05$)				0.8				0.8
EGC	0	47.4	48.6	44.4	17.5	36.3	42.8	41.4	39.8
	75	46.2	47.3	42.6	17.0	35.9	42.5	41.0	38.9
	150	44.1	47.1	42.3	14.7	32.0	42.3	40.0	37.5
	225	46.4	47.9	46.2	16.9	36.6	43.5	42.7	40.0
	300	47.6	48.7	46.3	17.1	39.5	44.7	43.2	41.0
	Mean	46.4	47.9	44.4	16.6	36.1	43.2	41.7	
	C.V. (%)				6.1				
	LSD, ($p \leq 0.05$)				2.2				2.1
EGCG	0	84.2	95.8	82.3	25.1	49.3	43.4	30.6	58.7
	75	84.7	96.7	82.3	28.5	54.6	46.1	34.4	61.0
	150	82.3	97.4	82.4	25.4	56.6	45.7	32.8	60.4
	225	83.1	75.3	81.9	27.2	52.7	51.9	33.5	58.0
	300	79.3	75.9	82.0	26.8	56.9	55.0	29.7	57.9
	Mean	82.7	88.2	82.2	26.6	54.0	48.4	32.2	
	C.V. (%)				6.4				
	LSD, ($p \leq 0.05$)				3.4				3.3
	Interaction				06.4				
Total gallo catechin	0	139.5	151.8	133.6	65.4	92.2	89.4	75.5	106.8
	75	138.6	151.2	131.3	68.1	97.0	91.5	78.3	108.0
	150	133.8	151.6	131.2	62.2	95.0	90.7	75.6	105.7
	225	137.5	131.1	134.6	68.2	96.3	98.4	79.4	106.5
	300	135.0	132.7	135.1	68.4	103.8	103.1	76.5	107.8
	Mean	136.9	143.7	133.2	66.5	96.8	94.6	77.1	
	C.V. (%)				4.3				
	LSD, ($p \leq 0.05$)				4.1				NS
	Interaction				7.8				

of nitrogen rates while in Rwanda and Tanzania sites the patterns were not clear. Although all parameters changed significantly ($p \leq 0.05$), due to nitrogenous fertiliser rates, the changes in total trihydroxyflavan-3-ols (Table 6) were not significant. The extent of the variations in these parameters due to nitrogenous fertilizer rates changed with location of production, causing significant ($p \leq 0.05$) interactions effects between nitrogen rates and location of production.

DISCUSSION

Caffeine in tea is a stimulant (Bokuchava and Skobeleva, 1969) and plays an important role in cream formation in black tea (Roberts, 1962). The levels of green leaf caffeine at different locations were in similar range to those in clones grown in different locations in Kenya (Kwach *et al.*, 2013) and Northeast India black teas (Dev Choudhury *et al.*,

Table 7. Variations in flavan-3-ols ratios with location of production and N rates.

Catechin	*N*-rate kg N ha^{-1} year^{-1}	Timbilil	Changoi	Sotik	Mulindi	Kitabi	Maruku	Katoke	Mean *N*-rate
Gallated/Non gallated ratio	0	1.45	1.80	1.79	0.88	1.28	1.02	0.82	1.29
	75	1.51	1.93	1.85	1.09	1.30	1.08	0.89	1.39
	150	1.51	1.95	1.89	1.19	1.50	1.10	0.90	1.43
	225	1.46	1.59	1.74	0.96	1.28	1.15	0.83	1.29
	300	1.35	1.55	1.72	0.94	1.30	1.18	0.77	1.26
	Mean	1.46	1.76	1.80	1.01	1.33	1.11	0.84	
	C.V. (%)				7.27				
	LSD, ($p \leq 0.05$)				0.09				0.08
	Interaction				0.16				
Trihydroxy/ dihydroxy (Gallo catechin /simple catechin) ratio	0	2.54	3.07	3.04	2.23	1.80	2.67	2.25	2.51
	75	2.69	3.26	3.18	2.83	2.15	2.82	2.45	2.77
	150	2.72	3.43	3.27	3.06	2.26	2.85	2.97	2.94
	225	2.54	2.80	3.01	2.49	2.00	3.02	2.26	2.59
	300	2.35	2.75	2.97	2.44	1.95	2.99	2.04	2.50
	Mean	2.57	3.06	3.09	2.61	2.03	2.87	2.39	
	C.V. (%)				7.23				
	LSD, ($p \leq 0.05$)				0.17				0.16
	Interaction				0.32				

Table 8. Variations in sum of flavan-3-ols (mg g^{-1} DM) with location of production and nitrogen rates.

Catechin	*N*-rate kg N ha^{-1} year^{-1}	Timbilil	Changoi	Sotik	Mulindi	Kitabi	Maruku	Katoke	Mean *N*-rate
Total Catechins (flavan-3-0ls)	0	186.6	194.0	170.6	71.9	136.9	119.6	105.7	140.8
	75	182.4	190.3	166.2	69.8	135.5	121.1	107.2	138.9
	150	175.7	188.7	164.9	60.7	130.7	119.7	98.3	134.1
	225	183.9	169.8	172.8	71.5	137.5	128.1	111.3	139.3
	300	184.4	172.8	173.9	71.9	149.6	134.2	110.7	142.5
	Mean	182.6	183.1	169.7	69.2	138.0	124.6	106.7	
	C.V. (%)				3.5				
	LSD, ($p \leq 0.05$)				4.4				4.2
	Interaction				8.3				
Total gallated catechins (flavan-3-gallates)	0	110.6	124.6	109.4	33.6	76.7	60.4	47.5	80.4
	75	109.6	125.3	107.9	36.1	76.5	63.0	50.6	81.3
	150	105.7	124.6	107.9	33.0	78.3	62.6	46.5	79.8
	225	108.9	104.1	109.4	35.0	77.1	68.6	50.3	79.1
	300	105.8	105.1	109.9	34.8	84.6	72.5	48.2	80.1
	Mean	108.1	116.8	108.9	34.5	78.7	65.4	48.6	
	C.V. (%)				5.0				
	LSD, ($p \leq 0.05$)				3.5				NS
	Interaction				6.7				
Total non gallated catechins	0	76.0	69.4	61.2	38.4	60.1	59.2	58.2	60.4
	75	72.8	65.0	58.3	33.7	59.0	58.1	56.6	57.7
	150	70.0	64.1	57.0	27.7	52.4	57.1	51.8	54.3
	225	75.0	65.7	63.3	36.6	60.3	59.6	61.0	60.1
	300	78.6	67.6	63.9	37.1	65.1	61.7	62.6	62.4
	Mean	74.5	66.4	60.8	34.7	59.4	59.1	58.1	
	C.V. (%)				5.0				
	LSD, ($p \leq 0.05$)				2.5				2.4

1991). The variation in black tea quality as measured by caffeine is therefore partly dependent on location of production in Eastern Africa.

Similar increases in caffeine levels with increase in nitrogenous fertilizer rates had been recorded in black teas at single locations (Owuor *et al.*, 1987b) and at different agro ecological zones in Pakistan (Akhlas *et al.*, 2003). Thus, irrespective of region of production, increase in nitrogen fertilizer rates enhances caffeine levels in tea leaves. Cultural practices such as year of prune (Owuor and Lang'at, 1988), plucking standards (Owuor *et al.*, 1987a), plucking intervals (Owuor and Odhiambo, 1994) and season of production (Yao *et al.*, 2005) previously influenced caffeine levels in tea produced at single sites. Agronomic inputs are therefore critical in the determination of levels of caffeine in tea. The lack of significant interactions effects between nitrogen fertilizer rates and locations of production demonstrated that the patterns of changes in caffeine levels due to nitrogen fertilizer rates were not influenced by environmental factors at location of production, or nitrogen fertilization.

The levels of all polyphenols were within the limits reported in clonal tea grown in Kenya (Cherotich *et al.*, 2013; Kwach *et al.*, 2013). The green tea leaf total polyphenols (Table 4) and flavan-3-ols levels (Tables 5, 6 and 8) varied ($p \leq 0.05$) due to location of production and nitrogenous fertilizer rates. These variations may in part explain the previously observed black tea quality changes with location of production (Owuor *et al.*, 2010a) and nitrogen fertilizer rates in Kenya (Owuor *et al.*, 2010b; 2013) and Pakistan (Akhlas *et al.*, 2003). The quality variations were therefore in part arising from the composition of the precursor compounds as demonstrated herein. These variations ($p \leq 0.05$) in total polyphenols and flavan-3-ols with location of production show that the potential of clone TRFK 6/8 to make black tea of high quality varies with locations even when agronomic inputs are identical. Black tea quality variations with location of production had been attributed to several factors including soil types, soil fertility (Bonheure and Willson, 1992), temperatures (Tanton, 1982), rainfall and rainfall distribution (Othieno *et al.*, 1992) and altitudes (Mahanta *et al.*, 1988). These factors could be partly responsible for the variations in the polyphenols observed in this study.

The levels and composition of the dihydroxy- and trihydroxyflavan-3-ols control the composition of the individual theaflavins (Owuor and Obanda, 2007; Owuor *et al.*, 2006). The relative astringencies of the four predominant theaflavins in black tea i.e. theaflavin digallate, theaflavin-3-gallate, theaflavin-3′-gallate and theaflavin, are 6.4:2.2:2.2:1, respectively (Sanderson *et al.*, 1976). High ECG and EGCG content in fresh leaf lead to the formation of high amounts of gallated theaflavins levels in black tea (Madanhire, 1995), a parameter associated with higher black tea quality (Owuor and Obanda, 1997; 2007). The total theaflavins *per se* may not be critical to plain black tea quality estimation as the ratios and sum of the individual theaflavins (Owuor *et al.*, 2006; Wright *et al.*, 2002). Total trihydroxyflavan-3-ols and EGCG levels in green tea leaf were indicators of Kenyan black tea quality (Owuor and Obanda, 2007; Owuor *et al.*, 2006) while total theaflavins in black tea and EGC in green leaf were Central Africa black tea quality indicators (Hilton *et al.*, 1973; Wright *et al.*, 2000). Tea leaf combining high flvan-3-ol levels with high trihydroxy:dihydroxy flavan-3-ols ratio have potential to produce high quality black tea quality through formation of high amounts of theaflavins (Owuor and Obanda, 2007; Owuor *et al.*, 2006). Since trihydoxyflavan-3-ols have lower redox potentials than dihydroxyflavan-3-ols (Bajaj *et al.*, 1987), low levels of trihydroxyflavan-3-ols may limit formation of theaflavins.

It had been anticipated that the ratio gallated:non gallated flaval-3-ols would be constant as formation of the flavan-3-ols were claimed to be genetically controlled (Magoma *et al.*, 2000). However, the recent literature (Cherotich *et al.*, 2013; Kwach *et al.*, 2013) and these results demonstrate that the flavan-3-ols levels vary widely with location of production in a single cultivar. Environmental conditions of production is therefore a major factor controlling flavanols formations and hence the quality of black tea. The variations in the polyphenols, especially flavan-3-ols levels and flvan-3-ols ratios with location of production observed show that even with a single cultivar, it may not be possible to produce same quality black tea in different locations, further supporting previous observations and conclusion on clonal black teas (Owuor *et al.*, 2008; 2009; 2010a; 2010b; Wright *et al.*, 2000).

Variations in black tea quality due to nitrogen fertilizer rates at single sites have been widely documented (Hilton *et al.*, 1973; Owuor *et al.*, 2013; Venkatesan and Ganapathy, 2004; Venkatesan *et al.*, 2004). Similar variations were observed in the polyphenols (Tables 4–8) at different locations. Increasing nitrogen fertilizer rates reduced black tea quality both at single locations (Hilton *et al.*, 1973; Owuor *et al.*, 1987b) and in various locations (Akhlas *et al.*, 2003; Owuor *et al.*, 2010a; 2013). Total theaflavins and thearubigins levels declined with increase in nitrogen fertilizer rates at a single site in clone TRFK 6/8 (Owuor and Odhiambo, 1994) and in clone BBK 35 at different locations (Owuor *et al.*, 2013) in Kenya. In clone AHP S15/10 in Kenya, such decline was only up to 159 kg nitrogen ha^{-1} year^{-1} but thereafter increased with rise in the fertilizer rates (Owuor *et al.*, 1987b). Similar decline in flavan-3-ols (Tables 4–6, 8) with increasing nitrogenous fertilizer rates up to 150 kg nitrogen ha^{-1} year^{-1} were observed. Although these results contradict observations from India where theaflavins, thearubigins (Venkatesan and Ganapathy, 2004), total polyphenols and flavan-3-ols (Venkatesan *et al.*, 2004) increased with rise in nitrogen fertilizer rates, the results demonstrate that pattern of change in flavan-3-ols production with rates of nitrogen direct the formation of theaflavins and thearubigins.

Application of above 180 nitrogen ha^{-1} year^{-1} reduced dihydroxyflavan-3-ols in Pakistan (Akhlas *et al.*, 2003) and (–)-EGC and (–)-EGCG levels in Malawi (Hilton *et al.*, 1973). Results presented herein contradict these observations as levels of EGC and other flavan-3-ols declined up to 150 kg N ha^{-1} year^{-1}, except EGCG, that increased linearly with increasing rates of nitrogen. Indeed total flavan-3-ols increased at higher rates of nitrogen fertiliser, while total flavan-3-ol gallates declined. The contradicting results may be attributed to differences in growing conditions and/or cultivars used in the studies. However, the pattern of the variations in the trihydroxy:dihydroxyflavan-3-ols and gallated:non gallated flavan-3-ols ratios due to nitrogenous fertilizer rate (Table 7) explain the earlier reports (Hilton *et al.*, 1973; Owuor *et al.*, 1987b; Venkatesan and Ganapathy, 2004) in which high rates of nitrogenous fertilizer reduced black tea quality. The reduction in plain black tea quality at higher nitrogen rates may be as a result of depression of gallated flavan-3-ols and enhanced levels of non gallated flavan-3-ols that would lead to lower levels of theaflavin digallate equivalent, a reliable black tea quality parameter (Owuor and Obanda, 1997; 2007; Owuor *et al.*, 2006).

The extents of variations in the polyphenols due to nitrogen fertilizer rates changed with locations causing significant ($p \leq 0.05$) interactions effects. Thus, even in the same clone, nitrogenous fertilizer rate that ensures optimum black tea quality may be region specific within the tea growing regions of Eastern Africa. In a recent study (Msomba *et al.*, 2014) optimal nitrogen fertilizer rate for realization of optimal yield varied with location of production. The optimum nitrogen fertiliser rate for production of high quality black tea and yields may therefore be region specific. The response in polyphenols production in Maruku Estate did not follow the same pattern as responses at the other sites. The differences were noted even between Maruku and Katoke that were within 30 km away from each other. Further investigations are necessary to understand the causes of this change in pattern of response.

In conclusion, caffeine and flavan-3-ols levels changed significantly ($p \leq 0.05$) with location of production and nitrogen fertiliser rates. The extent of the responses to nitrogen rates varied with locations. It is recommended that region specific agronomic inputs, especially nitrogen rates need to be developed to ensure production of tea leaves with optimal green leaf precursor levels that culminate to production of high black tea quality.

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