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Variations of Fatty Acids Levels in Young Shoots of Clonal Tea with Location of Production and Nitrogenous Fertilizer Rates in the Kenya Highlands

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ABSTRACT

Tea leaves contain unsaturated fatty acids, key precursors of volatile compounds contributing to tea aroma quality. Tea is cultivated in areas with diverse environmental conditions. Nitrogenous fertilizers influence tea yields and quality. A previous single site study demonstrated that increasing nitrogenous fertilizer rates raised fatty acid levels. However it is not known if the magnitudes and patterns of the responses are replicated in different locations due to variations in growth factors. Nonetheless, there is a single fertilizer rate recommendation in all tea growing locations in Kenya. This study assessed possible variations in patterns and magnitudes of fatty acids in a single cultivar grown under similar nitrogenous fertilizer rates in different locations. Trials were conducted in five locations using clone BBK 35, receiving varying nitrogenous fertilizer rates. Fatty acids in two leaves and a bud were quantified as methyl esters. The levels varied ($P \leq 0.05$) with locations and increased ($P \leq 0.05$) with nitrogenous fertilizer rates. The rates of increase differed with locations leading to significant ($P \leq 0.05$) interaction effects. Thus, similar fertilizer rates in different locations result in different fatty acid levels, explaining differences in tea aroma quality from different locations even with the same agronomic inputs. The results demonstrate the need to develop region-specific agronomic inputs for the production of same tea quality.

Keywords*: Camellia sinensis*, Fatty acids, Location of production, Nitrogenous fertilizer application rates, Tea.

INTRODUCTION

Tea, *camellia sinensis* (L.) O. Kuntze is cultivated under diverse environments ranging from 49°N, outer Carpathians to 30°S, Natal South Africa (Shoubo, 1989) and altitudes varying from sea level in Japan to above 2,700 m above mean sea level (amsl) in Olenguruone in Kenya and Gisovu, Rwanda (Owuor *et al.*, 2008). In Kenya it is grown on the foothills of the Aberdares and Mount Kenya in the East of the Great Rift Valley and Mau ranges, Nandi, Kisii and Kakamega Hills in the West of the Great Rift Valley. The plant is adaptable to geographical areas with large differences in climate and physical features which affect rates of growth, leading to differences in yields and quality. Due to the high demand for tea beverages, many regions not traditionally known for tea production are opening up resulting in an increased area of land under tea cultivation (Anonymous, 2009). Several studies have demonstrated variations in tea yields due to production locations (Ng'etich and Stephens, 2001a; 2001b; Wachira, *et al.*, 2002; Owuor *et al.*, 2009). Black tea quality also varies due to environmental factors (Gulati and Ravichranath, 1996) and geographical area of production (Borse *et al.*, 2002; Fernandez

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et al., 2002; Li *et al.*, 2007; Maredo-Pineiro *et al.*, 2003; Owuor *et al*., 2008). In these previous studies, it has been difficult to isolate the variations due to area of production as different genotypes were used. More recently, the yields (Owuor *et al*., 2009) and plain quality parameters (Owuor *et al*., 2008; 2010a, 2010b) were demonstrated to vary with regions for the same cultivar under similar agronomic practices. However, no study has investigated the variation of aroma quality parameters or the precursors of the aroma compounds with location in one cultivar under similar agronomic inputs.

Aromatic teas are preferred due to their unique flavors, making aroma an important quality attribute of tea. The flavor of tea is due to a unique mixture of volatile flavor compounds (VFC) (Yamanishi *et al.*, 1966; 1968; Mick *et al.*, 1984), dominated by compounds formed from unsaturated fatty acids plus other lipids (Sanderson and Graham, 1973; Yamanishi, 1981). Although the lipids contents in tea leaves makes up only around 4-9% dry weight of fresh tea leaf (Wright and Fishwick, 1979; Mahanta *et al.*,1985), lipid metabolism plays an important role in the biogenesis of black tea flavor (Bondarovitch *et al.*, 1967; Mahanta *et al.*, 1993). During black tea production, unsaturated fatty acids, mainly linolenic and linoleic acids undergo enzyme catalysed degradation to form volatile flavor compounds (VFC) (Robinson and Owuor, 1992, Hatanaka *et al.*, 1982; Sekiya *et al.*, 1984; Selvendran *et al.*, 1978; Saijo and Takeo, 1972; Kajiwara, Sekiya *et al.*, 1982), normally the C_6 aldehydes, alcohols and acids. Heptanal, heptanol, nonanol, nonanal plus E-2-noneneal 2,2, Z-4- nonadienal and E-2, E-4-nonadienal reported in tea (Robinson and Owuor, 1992) are also products of unsaturated fatty acids breakdown. In some tea, the C_6 alcohols and aldehydes, especially E-2-hexenal, E-2-hexenol and Z-3-hexenol from linolenic acid constitute over 60% of the aroma compounds (Owuor *et al.,* 2008; 1988) although over 600 VFCs have been reported in tea (Robinson and Owuor, 1992). Heptanal and heptanol are products of palmitoleic degradation while nonanol and nonanal are from oleic acid. Several volatile esters are also formed from the alcohols (Robinson and Owuor, 1992). The mechanism of the degradation process has been widely elucidated (Hatanaka *et al*., 1982; Sekiya *et al*., 1984; Kajiwara *et al*., 1982). *Cis*-4,5-epoxy-E-2-decenal (Gardener and Salke, 1984; Gassenmeier and Schieberle, 1994) and *trans*-4,5-epoxy-E-2-decenal (Kumazawa *et al.*, 2006) are products of linoleic acid degradation. These compounds reduce black tea quality (Mahanta *et al.,* 1988; Robinson and Owuor, 1992) but enhance green tea quality (Wang *et al.*, 1988). The levels of these VFCs increase with increase in amounts of unsaturated fatty acids in green tea leaves (Owuor *et al.*,1990) leading to poor aroma quality of black tea (Owuor *et al*., 1988). However, in green tea, high levels of these compounds are desired (Wang *et al.,* 1988) as they improve the green aroma in the tea. Fatty acids are therefore important quality precursors of black tea. High rates of nitrogenous fertilizer lowered the aroma quality of black tea (Owuor *et al.*, 1987) partly due to the increase of fatty acids contents with increase in nitrogenous fertilizer rates (Owuor, *et al*., 1990). However, these studies were performed at single sites and it is not known if the patterns and magnitudes of the changes in a single cultivar is the same when grown in varying locations.

Variations in tea leaves fatty acids are due to locations and nitrogenous fertilizer rates. Although fertilizer application is the second most expensive agronomic input in tea production after harvesting (Ellis and Grice, 1981), it is indispensible in tea production. Studies have shown tea yield benefits due to fertilizer application (Owuor *et al*., 2010a; Bonheure and Willson, 1992) thus fertilizer application is a prerequisite in tea production. Nitrogen is the most important nutrient in tea production. In Japan, 1,200 kg N ha^{-1} $year^{-1}$ is recommended and up to

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3,000 kg N ha^{-1} $year^{-1}$ has been applied to green tea (Watanabe, 1995) to enhance green tea quality. Studies relating the fatty acids responses to nitrogen rates were conducted at a single site (Owuor *et al*., 1990). However, uniform rates of nitrogenous fertilizer are recommended within Kenya (Othieno, 1988) and the actual rate is dependent on the level of production. It is not known if a similar nitrogenous fertilizer application rate results in the similar amounts of aroma precursor compounds (especially fatty acids) in different locations. This study evaluated the variations of fatty acids levels due to nitrogenous fertilizer rates changes in different locations using one tea genotype.

MATERIALS AND METHODS

The trials were laid out in five main tea growing regions of Kenya at Karirana (altitude 2260 m above mean sea level (amsl), latitude 1° 6'S, latitude 36° 39'E), Timbilil (altitude 2,180 m amsl, latitude 0° 22'S, longitude 35° 21'E), Changoi (altitude 1,860 m amsl, latitude 0° 29'S, Longitude 35° 14'E), Sotik Highlands (altitude 1,800 m amsl, latitude 0° 35'S, longitude 35° 5'E) and Kipkebe (altitude 1,800 m amsl, latitude 0° 41'S, longitude 35° 5'E). The mean annual rainfalls (1997-2010) for the sites were 1,730, 2,170, 1,800, 1,600 and 1,490 mm, respectively. Clone BBK 35 plantations were planted in 1991, 1986, 1989, 1974 and 1978, respectively and at the time of sampling the tea plants were 17, 22, 19 and 30 years old, respectively. Although tea yields vary with age (Kamau *et al*, 2008), the differences in the age of the plantations used in this study was not large enough to cause significant yield difference. Indeed, in a well-managed mature tea plantation of up to 80 years, aging does not lower the yielding ability within the same genotype (Kamau *et al*., 2008). However, apart from very young or moribund tea plantations, black tea quality differences have not been reported to vary with the age of mature tea bushes. The plantations had been uniformly managed and samples with known past cultivation history, were selected from each site. The plants were regularly pruned after every four years and the prunings were left *in situ.* As a result, the soils organic carbon was not a limiting production factor. The trial were laid out in a randomized complete block design with five nitrogenous fertilizer rates (0, 75, 150, 225 and 300 kg ha^{-1} $year^{-1}$) replicated three times. The fertilizer was applied in the form of NPKS 25:5:5:5 in a single dose broadcast in November every year from 1997. Each effective plot comprised 60 plants surrounded by a line of tea bushes that served as guard rows. The plots were uniformly managed. Prior to the experiments, all the plots were receiving 150 kg N ha^{-1} $year^{-1}$ as NPKS 25:5:5:5. The standard plucking of two leaves and a bud was done in all the plots at 7 day plucking intervals.

Samples were collected by plucking 100 g of two leaves and a bud from each plot in May 2008. Within one hour of sampling, the leaves were steamed for 1 minute to deactivate the enzymes responsible for oxidative degradation reactions in tea leaves. The leaves were oven dried at 96°C until a constant weight was reached. The dried leaves were then ground to a fine powder using a coffee grinder. From each dried sample, 10 g was weighed together with 0.015 of heptadecanoic acid (as an internal standard). Each sample was then extracted twice with 2:1 (v/v) chloroform and methanol mixture for three hours with continuous stirring at room temperature. The mixture was shaken together with 20 ml dilute potassium chloride solution. The organic layer was separated and solvent removed under reduced pressure. The lipids were transesterified and esterified to their corresponding methyl esters (Owuor *et al*., 1990). To the lipids mixture, in a round bottomed flask fitted with a condenser, 10 ml 0.5N methanolic sodium hydroxide solution was added. A small volume of hydrofuran was added to improve the solubility of the lipids. The mixture was

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refluxed for ten minutes. About 10 ml of boron trifluoride-methanol complex (about 14% BF_3) was added and the mixture was refluxed for a further two minutes. The solution was cooled to room temperature and 5 ml hexane was added and the mixture was refluxed again for two minutes. A saturated sodium chloride solution was added and the hexane layer was separated and dried with anhydrous sodium sulphate. The extraction was done twice and activated silica gel was added to the hexane layer with continuous stirring until all the chlorophyll was removed from the solution. The silica gel was filtered off and hexane removed under reduced pressure using a rotary evaporator. The fatty acid methyl esters (FAMES) in a small amount of hexane were stored in a sample bottle before injecting into the GC.

The GC analysis was carried out as described by Munavu (1983). The GC was fitted with fused 50 m silica gel capillary columns coated carbowax 20 m, (a film thickness of 0.20 µm, 0.25 mm inner diameter) and flame ionization detector (FID). The temperature program was isothermal at 160°C for 4 minutes then 160-170°C at 0.6 °C min^{-1} for 9 minutes and then 170-200°C at 3.5 °C min^{-1} for 4 minutes. The flow rate for the carrier gas was 3.0 mL min^{-1} N_2/air, for makeup gases 30.0 ml min^{-1} N_2/air and for detector gases 40.0 mL min^{-1} H_2 and 400.0 mL min^{-1} air, respectively. The detector temperature was 230°C. Volumes of 0.1 µL were injected. The samples were identified by corroborative retention times of authentic fatty acid standards and the quantification was done by use of the internal standard.

RESULTS AND DISCUSSION

A typical gas chromatogram of fatty acids methyl esters is presented in Figure 1. The individual fatty acids were identified using authentic samples. There was a good baseline separation for all fatty acids methyl esters, thus facilitating accurate quantification of the individual fatty acids. The variations in the levels of saturated fatty acids, unsaturated fatty acids and total fatty acids are presented in Tables 1, 2, and 3 respectively. The biochemical transformations of saturated fatty acids in green tea leaves during black tea processing have not been quantified. Indeed, their contribution to tea quality in is not known. However, they are present in appreciable amounts in tea leaves. Although, in earlier studies, lauric acid (C12:0) and myristic acid (C14:0) were not detected in some cultivars

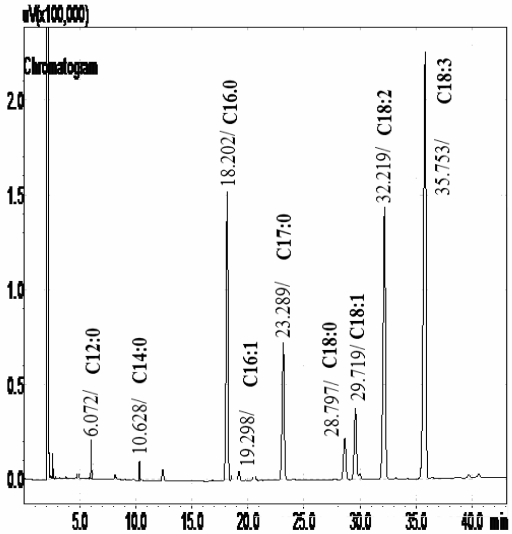

Figure 1. Gas chromatogram of green tea leaves fatty acids methyl esters.

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Table 1. Variations of saturated fatty acids (mg g^{-1} dry leaf) due to location and Nitrogen rates.

		Nitrogen rate (kg N ha^{-1} $year^{-1}$)					Mean
FA	Location	0	75	150	225	300	location
C12:0	Karirana	0.0114	0.0249	0.0573	0.1470	0.4556	0.1392
	Timbilil	0.0151	0.0298	0.0464	0.0619	0.0758	0.0458
	Changoi	0.0186	0.0916	0.1667	0.2840	0.4018	0.1926
	Sotik Highlands	0.0147	0.418	0.0921	0.1916	0.3178	0.1316
	Kipkebe	0.0025	0.0065	0.0144	0.2386	0.6918	0.1908
	Mean rate	0.0125	0.0389	0.0754	0.1846	0.3886	
	CV (%)			4.15			
	LSD, $P \leq 0.05$			0.0056			0.0056
	Interaction, $P \leq 0.05$			0.0095			
C14:0	Karirana	0.0065	0.0843	0.1451	0.2504	0.4791	0.1931
	Timbilil	0.0177	0.0461	0.0619	0.0984	0.1300	0.0708
	Changoi	0.0069	0.0551	0.1299	0.2983	0.7149	0.24104
	Sotik Highlands	0.0123	0.0744	0.1105	0.2025	0.3504	0.1500
	Kipkebe	0.0119	0.0898	0.2554	0.4809	0.7222	0.3120
	Mean rate	0.0111	0.0669	01406	0.2661	0.4793	
	CV (%)			34.98			
	LSD, $P \leq 0.05$			0.0714			0.0714
	Interaction, $P \leq 0.05$			0.1219			
C16:0	Karirana	0.7227	1.2267	1.4140	1.8545	2.4740	1.5384
	Timbilil	0.3695	1.3875	1.6173	2.2977	2.5549	1.6454
	Changoi	0.5274	0.7593	1.2686	1.9999	3.4361	1.5983
	Sotik Highlands	0.1778	0.9023	1.1670	1.5154	1.8682	1.1261
	Kipkebe	1.1708	1.3404	1.5372	1.6371	1.8979	1.5167
	Mean rate	0.5936	1.1232	1.4008	1.8609	2.4462	
	CV (%)			2.41			
	LSD, $P \leq 0.05$			0.0363			0.0363
	Interaction, $P \leq 0.05$			0.06192			
C18:0	Karirana	0.2911	0.4356	0.6222	1.4852	1.9238	0.9516
	Timbilil	0.1892	0.3956	0.4879	0.6784	1.1624	0.5827
	Changoi	0.2253	0.3959	0.8724	1.1099	1.9560	0.9119
	Sotik Highlands	0.1131	0.2456	0.4224	0.5446	1.1190	0.4889
	Kipkebe	0.2255	0.4031	0.4470	0.5405	0.7780	0.4788
	Mean rate	0.2089	0.3752	0.5704	0.8717	1.3878	
	CV (%)			10.54			
	LSD, $P \leq 0.05$			0.0730			0.0730
	Interaction, $P \leq 0.05$			0.1246			

in Kenya (Owuor *et al*., 1990) and palmitic acid (C16:0) in Assam, India tea (Bhuyan and Mahanta, 1989), (Ramaswamy and Ramaswamy, 2000), in this study all the acids were present in detectable amounts (Figure 1, Table 1). The order of occurrence of the saturated fatty acids was n-hexadecanoic (palmitic) acid (C16:0) > n-octadecanoic (stearic) acid (C18:0) > n-tetradecanoic (myristic) acid (C14:0) > n-dodecanoic (lauric) acid (C12:0) in all locations and nitrogenous fertilizer rates. Similar patterns of occurrence of the fatty acids had been reported in India

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Table 2. Variation of unsaturated fatty acids (mg g^{-1} dry leaf) due to location and Nitrogen rates.

FA	Location	Nitrogen rate (kg N ha^{-1} $year^{-1}$)					Mean location
		0	75	150	225	300	
C16:1	Karirana	0.01270	0.0292	0.0419	0.0759	1.0264	0.0572
	Timbilil	0.0072	0.0166	0.0290	0.0371	0.0547	0.0289
	Changoi	0.0049	0.0204	0.0384	0.0463	0.0699	0.0360
	Sotik Highlands	0.0049	0.0265	0.0563	0.0704	0.0863	0.0489
	Kipkebe	0.0060	0.0131	0.0263	0.0387	0.0860	0.0340
	Mean rate	0.0071	0.0212	0.0384	0.0537	0.0847	
	CV (%)			6.21			
	LSD, $P \leq 0.05$			0.0033			0.0033
	Interaction, $P \leq 0.05$			0.0055			
C18:1	Karirana	0.0749	0.1948	0.3609	0.6191	0.7404	0.3980
	Timbilil	0.1541	0.2515	0.3750	0.4851	0.7022	0.3936
	Changoi	0.0377	0.2736	0.4939	0.5739	1.0503	0.4859
	Sotik Highlands	0.0357	0.1994	0.3011	0.3747	0.5456	0.2913
	Kipkebe	0.2111	0.4086	0.4847	0.5520	0.6325	0.4578
	Mean rate	0.1027	0.2656	0.4031	0.5210	0.7342	
	CV (%)			1.887			
	LSD, $P \leq 0.05$			0.0079			0.0079
	Interaction, $P \leq 0.05$			0.0134			
C18:2	Karirana	0.7462	1.5016	1.6172	2.7396	3.3419	1.9893
	Timbilil	0.7462	1.3983	1.7613	2.2168	2.8309	1.6802
	Changoi	1.1939	0.9919	1.2261	2.2293	3.2580	1.6859
	Sotik Highlands	0.7242	1.2711	1.5650	1.7985	2.1486	1.4991
	Kipkebe	0.7123	1.1469	1.4316	1.6395	1.9430	1.3881
	Mean rate	0.8313	1.2619	1.5202	2.1247	2.7045	
	CV (%)			1.60			
	LSD, $P \leq 0.05$			0.0274			0.0274
	Interaction, $P \leq 0.05$			0.0468			
C18:3	Karirana	1.2814	1.5978	2.8590	3.9285	4.8547	2.9043
	Timbilil	1.0683	1.7046	1.9899	2.4080	3.1688	2.0679
	Changoi	0.7460	1.1824	1.6589	2.5174	4.0298	2.0269
	Sotik Highlands	1.1679	1.5460	1.9428	2.2054	2.9049	1.9534
	Kipkebe	0.6293	1.3399	1.6175	2.2553	3.6323	1.8949
	Mean rate	0.9786	1.4741	2.0136	2.6629	3.7181	
	CV (%)			2.02			
	LSD, $P \leq 0.05$			0.0444			0.0444
	Interaction, $P \leq 0.05$			0.0758			
Total Unsaturated fatty acids	Karirana	2.1152	3.3233	4.8789	7.3631	9.0635	5.3488
	Timbilil	2.4235	3.3710	4.1551	5.1437	6.7565	4.3700
	Changoi	1.5127	2.4683	3.4176	5.3670	8.4081	4.2347
	Sotik Highlands	1.9208	3.0430	3.8652	4.4479	5.6854	3.7925
	Kipkebe	1.6261	2.9084	3.5601	4.4854	6.2938	3.7748
	Mean rate	1.9197	3.0228	3.9754	5.3614	7.2415	
	CV (%)			1.25			
	LSD, $P \leq 0.05$			0.0545			0.0545
	Interactions, $P \leq 0.05$			0.0931			

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Table 3. Variations of total fatty acids (mg g^{-1} of dry leaf) due to location and Nitrogen rates.

		Nitrogen rate (kg N ha^{-1} $year^{-1}$)					Mean
FA	Location	0	75	150	225	300	location
Total fatty acids	Karirana	3.1470	5.0950	7.1175	10.9002	14.3960	8.1311
	Timbilil	2.0149	5.2299	6.3686	8.2800	10.6795	6.7146
	Changoi	2.2910	3.7701	4.8551	9.0591	14.9169	6.9784
	Sotik Highlands	2.2372	4.3070	5.6572	6.9019	9.3408	5.6888
	Kipkebe	3.0368	4.7481	5.8141	7.3825	10.3837	6.2730
	Mean rate	2.7454	4.6300	5.9625	8.5048	11.9434	
	CV (%)			5.11			
	LSD, $P \leq 0.05$			0.3503			0.3503
	Interaction, $P \leq 0.05$			0.5980			

(Ramaswamy and Ramaswamy 2000) and Japan (Anan and Nakagawa, 1977). Again, similar to observations at single sites (Owuor *et al.,* 1990); there were significant increases ($P \leq 0.05$) in the levels of saturated fatty acids with nitrogenous fertilizer rates indicating that the nitrogenous fertilizer contributes to the formation of fatty acids (Table 1). However, the magnitude and rates of the changes varied with increasing fertilizer rates at different locations leading to significant ($P \leq 0.05$) interactions effects. These results demonstrate that the formations of the saturated fatty acids are influenced by environmental, climatic and cultural factors that varied with location of production when fertilizer rates were held constant.

The changes in the levels of unsaturated fatty acids with location and increasing rates of nitrogenous fertilizer are presented in Table 2. The unsaturated fatty acids which break down into group I VFC varied significantly ($P \leq 0.05$) with location. High amounts of group I VFC impair black tea quality (Mahanta *et al.,* 1988; Robinson and Owuor, 1992). The proportions of the unsaturated fatty acids was in the order of linolenic (octadecatrienoic) acid (C18:3) □ linoleic (octadecadienoic) acid (18:2) □ oleic (octadecaenoic) acid (18:1) □ palmitoleic (hexadecaenoic) acid (16:1) at all locations. Similar patterns of occurrence of the fatty acids but at slightly different ratios had been noted in different varieties in Kenya (Owuor *et al*., 1990) and in India (Bhuyan *et al.*, 1991). The results explain the observation of the levels and order of occurrence of the respective VFC arising from the unsaturated fatty acids in black tea aroma (Owuor *et al.,* 1988; Mahanta *et al.,* 1988). The order of dominance of the unsaturated fatty acids changed with locations. Linolenic acid was in the order of Karirana> Changoi> Timbilil> Sotik Highlands> Kipkebe, linoleic acid varied in the order of Karirana> Timbilil> Changoi> Sotik Highlands> Kipkebe, oleic acid was in the order of Changoi> Timbilil> Kipkebe> Karirana> Sotik Highlands and palmitoleic acid was in the order of Karirana> Sotik Highlands> Changoi> Kipkebe> Timbilil. The amounts of individual VFCs arising from the respective unsaturated fatty acid is therefore expected to change in the same pattern. Although there had been a study of variations in the VFCs composition in some Kenyan tea varieties with location of production (Owuor *et al.,* 1988), there was no attempt to relate the VFCs to their precursor compounds. But in a previous study (Ramaswamy and Ramaswamy, 2000), the lipid composition was demonstrated to be dependent on tea species or genotype. The results presented here further demonstrate that even in a single genotype, the lipid composition can be very variable and influenced by agronomic inputs such as nitrogenous fertilizer rates. The results further show that the area of

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production influences the lipid composition of tea leaves even when agronomic inputs are held constant. This demonstrates that biosynthetic factors controlling the formation of the individual fatty acids maybe varying with location. The variations in the levels of unsaturated fatty acids with regions explain the previously observed variation of aroma quality with location (Owuor *et al*., 1988, 2008). Indeed, the results presented herein show that it may not be possible to produce black teas of the same aroma quality from different tea growing regions.

Similar to observations at a single site (Owuor *et al.,* 1990), there was a significant ($P \leq 0.05$) increase in fatty acids levels with increase in the nitrogenous fertilizer rates (Tables 1; 2, and 3). The rate of increase in the fatty acids due to increasing nitrogenous fertilizer as exemplified by the total fatty acids, changed with location of production (Figure 2, Table 3). This study for the first time has therefore established that the same cultivar of tea under similar fertilizer rate may not produce the same levels of fatty acids leading to different aroma qualities when growing in different locations. The magnitude and rates of increase of the total fatty acids (Figure 2), total unsaturated fatty acids and individual fatty acids with changes in nitrogenous fertilizer amounts varied at different locations. This caused significant ($P \leq 0.05$) interactions effects between nitrogen rates and locations of production for the fatty acids (Tables 1; 2, and 3). The results demonstrate that the biosynthetic patterns of the fatty acids are influenced by the variations in environmental factors, even under nearly similar climatic conditions.

High quality tea cultivars have been assumed to produce similar quality attributes in all areas of growth under similar fertilizer regimes if planted within one country or region where climatic variations are thought to be minimal. In Kenya, tea is grown in altitudes varying from 1,300 m to 2,700 m amsl almost along the equator (Othieno, 1988). Within such range of latitudes and altitudes, the climatic variations are

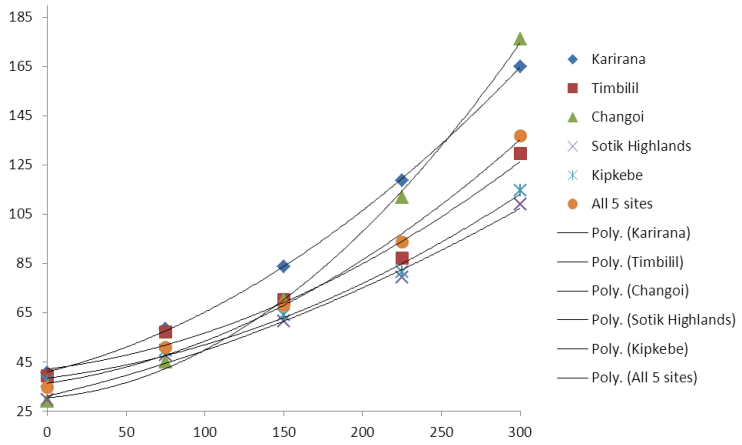

Figure 2: Changes in total fatty acids (mg10g^{-1} dry leaf) due to rates of Nitrogen (kg N ha^{-1}year^{-1}) at different locations.

$Y = 0.0008x^2 + 0.1583x + 40.969$, $R^2 = 0.9999$ (Karirana)
$Y = 0.0007x^2 + 0.08x + 42.059$, $R^2 = 0.9799$ (Timbilil)
$Y = 0.0014x^2 + 0.0513x + 30.424$, $R^2 = 0.9986$ Changoi)
$Y = 0.0003x^2 + 0.1511x + 31.107$, $R^2 = 0.9932$ (Sotik Highlands)
$Y = 0.0006x^2 + 0.0787x + 38.403$, $R^2 = 0.9941$ (Kipkebe)
$Y = 0.0008x^2 + 0.0923x + 36.306$, $R^2 = 0.996$ (Mean of all)

Where Y = Total fatty acids, and X = rate of nitrogenous fertilizer.

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considered minimal, although the soil physiochemical parameters were diverse at the five sites (Table 4) (Kamau *et al.*, 2008b). The soil chemical parameters varied from site to site. All the soils were acidic in reaction, as typical of tea soils (Othieno, 1988). Despite these, uniform agronomic inputs are recommended for tea in Kenya (Othieno, 1988). However, the results presented herein from fields situated within latitude 1^{o} 6' S and 0^{o} 41' s at altitudes varying from 1,800 to 2,260 m amsl exhibited large ($P \leq 0.05$) variations in cultivar BBK35 fatty acids with location of production (Tables 1 and 2). Similar variations were also documented in plain black tea quality parameters of one cultivar (Owuor *et al.*, 2009; 2010a, 2010b), suggesting that plain black tea quality precursors may be varying in a similar manner. It may not therefore be possible to produce black tea of the same quality even from a single cultivar when it is planted in different locations/geographical regions even where climatic variations are not large. In other studies, yields (Wachira *et al.,* 2002) and plain black tea quality (Owuor *et al.,* 2010b) of the same clones differed with geographical locations in Kenya. The patterns and magnitudes of the variations changed from clone to clone. These results demonstrate the need to develop region and clonal specific agronomic inputs to optimize the production of precursor chemicals in green tea leaf for the production of high quality teas.

Generally, the amounts, intensity and distribution of rainfall, cloud cover, hail damage, frost, humidity, temperature, sunshine hours and intensity, soil type and fertility gradient, etc, can vary widely even within a short radius. These factors are known to influence biosynthetic patterns. Even though these factors were not investigated in this study, it is apparent that they were not constant in all areas of study. These factors expose the plants to different stress levels in different locations leading to different activities of enzymes involved in the fatty acid synthesis in the plants. The observed variations are therefore not unique. But the variations underscore the fact that it may not be possible to produce black teas with the same aroma compounds levels with the use of the same agronomic inputs such as nitrogenous fertilizers. The current blanket agronomic inputs recommendations for tea in all regions of Kenya (Othieno, 1988) may not therefore be realistic. In conclusion, the results presented here demonstrate that although the fatty acids increased with increasing rates of nitrogen fertilizers at all locations, the magnitude and patterns of changes in the individual fatty acids varied in an unpredictable manner. Validation of the recommended agronomic inputs level at different locations may be a viable way of ensuring production of high quality tea beverages. Indeed, for the production of high quality tea beverages, development of region specific recommendations is necessary.

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تغییرات سطوح اسیدهای چرب در شاخه‌های جوان چای کلونال با محل تولید و میزان کود نیتروژنی در ارتفاعات کنیا

ا. و. اکال، پ. اوکیندا اوور، د. م. کامو، و ل. و. ا. مانگورو

چکیده

برگ چای حاوی اسیدهای چرب اشباع نشده، پیش سازهای اصلی ترکیبات فرارعامل کیفیت چای از نظر عطر می‌باشد. چای در مناطق با شرایط متنوع زیست محیطی کشت می‌شود. کودهای نیتروژنی بر عملکرد و کیفیت چای موثرند. یک مطالعه قبلی در تنها یک رویشگاه نشان داد که افزایش میزان کود نیتروژنی سطح اسیدهای چرب را افزایش داد. با این حال مشخص نیست که میزان و الگوهای پاسخ در نقاط مختلف با توجه به تنوع در عوامل رشد قابل تکرار باشند. با این حال، در تمام نقاط کشت چای در کنیا یک میزان کود توصیه می‌شود. این مطالعه به بررسی تغییرات در الگوها و مقدار اسیدهای چرب در یک رقم چای تحت تیمار کود نیتروژنی مشابه در نقاط مختلف رویش می‌پردازد. مطالعه در پنج منطقه با استفاده از کلون BBK 35 با میزان دریافت کود نیتروژنی مختلف انجام شد. اسیدهای چرب در دو برگ و یک جوانه بر حسب متیل استر اندازه گیری شدند. مقدار اسیدهای چرب با منطقه رویش ($P \leq 0.05$) تغییر کرد و با میزان کود نیتروژنی افزایش یافت ($P \leq 0.05$). نرخ افزایش در مناطق مختلف رویش اختلاف داشت که منجر به اثرات متقابل معنی دار($P \leq 0.05$) گردید. بنابراین، میزان کود مشابه در مناطق مختلف، سطوح مختلف اسید چرب را سبب می شود که توضیح دهنده تفاوت در کیفیت عطر چای در مناطق مختلف حتی با ورودی‌های زراعی یکسان است. نتایج نیاز به ایجاد ورودی-های زراعی خاص هر منطقه برای تولید چای با همان کیفیت را نشان می دهند.

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