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Effect of genotypes in different tea-growing locations in Kenya on some micronutrients content of Black Tea

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ABSTRACT: Human beings and plants require essential elements for normal physiological functions and survival. Tea beverages are the most widely consumed fluids after water. If the beverages have appreciable levels of micronutrients, they could contribute to the alleviation of micronutrients deficiencies in human beings. Different tea cultivars/clones have been developed and distributed to farmers in various locations for production of the tea beverages. However it remains obscure the levels of the essential elements in their resultant black teas. This study evaluated the levels of some micronutrients different tea clones planted in a single site and determined if the levels of the micronutrients varied in the same pattern when same clones were planted in different regions in Kenya under similar agronomic inputs. The clones varied significantly ($P \leq 0.05$) in their micronutrient levels even when planted in a single location showing that clones different abilities to absorb the micronutrients from the soil. The patterns of variations were different when the clones were planted in different locations. It is therefore clear that environmental factors of growth have an influence in the ability of the clones to absorb the micronutrients. Thus, it is necessary to identify region specific clones that can absorb the micronutrients in the region to optimize the micronutrient content of resultant black teas.

KEYWORDS: Camellia sinensis; *Clones; Micronutrients; Location of production*

Introduction

Beverages from tea (*Camellia sinensis*) are the most widely consumed fluids after water,[1,2] partly due its beneficial medical properties mainly attributed to polyphenols.[2–4] Micronutrients deficiencies are problematic worldwide, and the wide consumption of tea could contribute significantly to the management of micronutrients "hidden hunger" if tea contains high enough micronutrients and factors governing the micronutrient levels in tea are properly understood. In Kenya, tea is grown in the highlands East and West of the Great Rift Valley in Kenya, and Kenya is now the 4th leading tea producer and the highest exporter of black tea.[5] Kenya is, therefore, a significant contributor to world tea supply and could contribute largely to hidden hunger management if factors influencing micronutrients levels in tea are understood and controlled. With the expansion of the tea industry, cultivation of tea has been extended to agro-ecological zones initially thought to be marginal for tea. Similarly, genotypes bred and selected in Kenya are now cultivated in the other Africa countries.[6]

The main sources of micronutrients in plants are their growth media, agro inputs and soil.[7] Plants take up the elements from the soil and under certain conditions, high levels can be accumulated in the leaves.[8] The mineral constituents of tea leaves normally differ according to the geological source.[9] For tea grown in different parts of the world, varying levels of minerals have been recorded.[10–12] Elements such as Ca, Na, K, Mg and Mn have been shown to be present in mg/g quantities while elements such as Cr, Fe, Co, Ni, Cu, Zn, Se and Cd are in μg/g level.[10,12] However factors which control the levels of these micronutrients in the tea plant have not been determined. Consequently, it is not known if the tea cultivars developed in Kenya, widely grown in Africa and consumed in many parts of the world can be a significant source of the micronutrients.

Several studies have demonstrated wide responses in yields,[13,14] yield partitioning,[13] growth,[15,16] shoot population density[17] and dry matter partitioning[16] of tea genotypes to different growing environments. Such variations occur even within 10-km radius.[18,19] The black tea quality, the black tea aroma,[20,21] volatile flavour compounds composition[22,23] and black tea plain quality parameters[24] also varied widely with geographical area of production. It has been assumed that large differences in climatic conditions are necessary for significant differences to be observed. Consequently, many tea-growing countries have centralized their clonal selection/ breeding programmes in single locations believing that superior genotype selected in one location maintains desirable attri-

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butes wherever it is grown. This is despite the fact that tea plants selected in one location and planted in other locations do not usually match the performance at the site of selection.[25] These observations indicate the micronutrients content of black teas may vary in same clones grown in different environments. Indeed, micronutrients levels in teas changed with locations,[10,26] but genotypes also varied in these studies. It was therefore not possible to isolate the effects of locations from those of genotypes.

Under similar agronomic practices, different clones have varying abilities to absorb micronutrients from the soil.[27] The study was conducted to establish that if same cultivars planted in a single location in Kenya have different abilities to extract the essential micronutrients and whether the variation follow the same pattern in the clones when planted in different locations.

Materials and Methods

Sites for Sample Collection and Clones

This experiment was superimposed on an ongoing GxE experiment, in which 20 widely cultivated (commercial) genotypes of tea clones: TRFK 7/9, TRFK 303/259, TRFK 303/1199, TRFK 54/40, TRFK 31/8, BBK 35, TRFK 6/8, TRFK 31/27, TRFK 12/12, TRFK 303/909, APH S15/10, TRFK 57/15, TRFK 56/89, TRFK 12/19, TRFK 11/26, STC 5/3, TRFK 7/3, TRFK 303/577, EPK TN 14-3 and TRFK 2x1/4 planted in Kangaita Tea Farm East of the Great Rift Valley, Timbilil Estate in Kericho and Kipkebe Estate in Sotik. The sites' altitudes, latitudes, longitudes and year of plantation are given in Table 1. At each site, plots were arranged in a randomized complete block design with three replicates.[25] The tea was planted in a 122-cm × 61-cm rectangular spacing.[28] Nitrogen, as NPKS 25:5:5:5 compound fertilizer at a single dose of 120 kg N/ha was applied during the year of plantation and 200 kg N/ha/year in subsequent years. Plucking was done at 10–14 days intervals depending on leaf availability. The plants were under uniform management and agronomic practices. One kilogram of leaf was plucked from each plot and miniature black tea processed.[21] The unsorted black tea samples from these clones were also analysed for the micronutrients.

Analysis of Black Tea for the Micronutrients

In 2000, a modified standard procedure described in AOAC was followed for the preparation of samples for analysis of essential minerals.[29] Accurately weighed 1.0000 g black tea was used for Mn, Fe, Zn and Cu analyses while 2.0000 g black tea was used for Se analysis. The weighed samples were transferred into ashing tubes and kept in a muffle furnace at 460°C for 12 hr. The cooled ashed samples were digested using double acid (concentrated hydrochloric and nitric acids in a 1:1 ratio) and hydrogen peroxide in the ratio of 2:3. The crucible containing acid solution was kept on a hot plate and evaporated to dryness, the final residue was dissolved in 5 ml of 0.05 M HCl acid solution. The final volume was made up to 25 ml for Mn, Fe, Zn and Cu analysis and to 10 ml for Se analysis. Standard solutions were prepared by diluting the stock solution (Sigma Aldrich) with 0.05 M HCl acid. The micronutrients were analysed using atomic absorption spectrophotometer (Shimadzu AA-6200 Model, Japan).

Statistical Analysis

The data was analysed using a randomized complete block design in a 2-factorial arrangement, with sites as the main treatments and clones as sub-treatments. MSTAT-C statistical package (Michigan State University, MI) was used for ANOVA.

Results

The clones used here were commercial clones, most of which were initially selected at Timbilil site. Their selections were based on yield, not micronutrient content. The effect of location of production and cultivars on the micronutrients contents of black teas are presented in Tables 2–6. The levels of the micronutrients different clones significantly ($P \leq 0.05$) varied from clone to clone and did not follow any specific pattern when the clones are planted in different geographic locations. Indeed, the mean levels of the micronutrients also varied significantly ($P \leq 0.05$). For Mn (Table 2), clone TRFK 11/26 recorded the highest mean Mn levels while clone TRFK 303/1199 recorded the lowest levels. The highest levels of Mn in Timbilil, Kipkebe and Kangaita were recorded in clones TRFK 7/9, TRFK 31/27, EPK TN14-3, respectively. But clones TRFK 7/3, STC 5/3 and BBK 35 had the lowest concentrations of Mn in Timbilil, Kipkebe and Kangaita, respectively. There were significant ($P \leq 0.05$) differences in mean levels of Mn in the three sites. The highest mean Mn levels were recorded in Kipkebe while the lowest levels were recorded in Timbilil. There was a significant ($P \leq 0.05$) interaction between the clones and locations.

Clone TRFK 6/8 had significantly ($P \leq 0.05$) higher mean levels of Fe than other clones while clone BBK 35 had the lowest levels (Table 3). In Kipkebe, clones TRFK

Table 1: Site Locality and Hisotry of the Different Clones

Site Locality/history	Kipkebe	TRFK Timbilil	Kangaita
Altitude (m)	1872	2178	2100
Latitude Longitude	00 45'S 3505'E	00 22'S 350 21'E	0030'S 37016'E
Year planted	1997	1986	1991
Plantation age*	14	25	20

*As at year 2011.

7/9, TRFK 303/1199, TRFK 54/40 recorded the highest Fe levels while clone TRFK 31/27 had the highest Fe levels in Kangaita. Clone BB35, TRFK 57/15 and TRFK 7/9 had the lowest Fe concentrations in Timbilil, Kipkebe and Kangaita, respectively. Clone TRFK 303/259 had the highest mean levels of Zn while clone STC 5/3 recorded the least Zn level (Table 4). Higher levels of Zn can be achieved by planting clones TRFK 57/15, TRFK 54/40 and TRFK 303/259 in Timbilil, Kipkebe and Kangaita, respectively, since there black teas had significantly ($P \leq 0.05$) higher levels of Zn. Clones STC 5/3 and TRFK 2x1/4 recorded significantly ($P \leq 0.05$) lower levels of Zn in Timbilil. In Kipkebe, lower levels of Zn were from clones TRFK 7/9, TRFK 56/89, APH S15/10, TRFK 303/999 and STC 5/3.

Clone TRFK 56/89 recorded the highest mean Cu levels among the studied clones while clone EPK TN14-3 recorded the lowest levels (Table 5). The mean Cu levels significantly ($P \leq 0.05$) differed in the three locations with Timbilil recording significantly ($P \leq 0.05$) higher levels of Cu while Kangaita tea farm recorded the lowest levels. In Timbilil, clones TRFK 6/8 and TRFK 303/259 had higher Cu levels while EPK TN14-3 had the lowest levels. Clones TRFK 56/89 and EPK TN14-3 had the highest and lowest Cu levels, respectively, in Kipkebe. In Kangaita, clones TRFK 303/1199, TRFK 31/8 and TRFK 12/99 had significantly ($P \leq 0.05$) higher levels of Cu while lower levels were recorded from clones TRFK 6/8 and TRFK 303/999. There were significant ($P \leq 0.05$) interactions in Cu between location of production and clones. For Se (Table 6), clone TRFK 303/999 had significantly ($P \leq 0.05$) higher mean levels than the other clones while clone TRFK 12/19 recorded the lowest levels. Clones TRFK 2x1/4, APH S15/10 and TRFK 54/40 had significantly ($P \leq 0.05$) higher concentration levels of Se in Timbilil, Kipkebe and Kangaita, respectively. Lower levels were recorded from clones TRFK 12/19, TRFK 2x1/4 and TRFK 7/9 in Timbilil, Kipkebe and Kangaita, respectively. There was also significant ($P \leq 0.05$) interaction effect between the cultivars and location of production in Se contents.

Discussion

For tea grown in different parts of the world, varying levels of minerals had been recorded.[10–12] However, in these studies, the genetic make-up of the plants was not explained. The results presented herein demonstrate that different clones have varied abilities to absorb micronutrients in single locations. For enhancement of micronutrients availability in individual locations, clones with ability to extract high amounts of the individual micronutrient should be identified. However, same clone had different ability to absorb the micronutrients in different locations. Similar variations in composition of some chemical constituents of tea from the same country had been observed in other studies. In China, the F, Al (Ref. 30) and Cu (Ref. 31) contents of tea from different farms within Sichuan Province varied. In Turkey, Fe and Mn levels of tea from different regions were different.[32] The genetic make-ups of the teas used in these studies were not indicated, and the noted differences could be in part due to genotypes thus to effectively maximize the micronutrient content in the resultant black teas, then region-specific clones should be adopted that can optimally absorb the micronutrients. These results were similar to results from Wushwush farms in Ethiopia on the levels of essential and non-essential elements, in which there were significant variations in the minerals of five different clones planted in four unit farms under similar agronomic practices.[27] Earlier, large variations had been shown in mature leaf nutrients contents grown at the same location and that the nutrients contents were not related to the yields.[33] Recently clone BBK 35 was shown to have different mature leaf nutrient levels when grown in different regions in Kenya under same agronomic inputs.[34] Similar variations were recently observed in the plain tea quality parameters.[35,36] Successful cultivars of most crop species and tea, in particular, should be adapted to a wide range of climatic and edaphic conditions. Indeed even the yields significantly differed in the different environments.[25] Results presented here reaffirm that different clones have varying abilities to absorb nutrients from the soil similar to other studies,[27,37] leading to clonal varia-

Table 2: Clonal Black Tea Mn Levels (μg/g) and Relative Ranking based on Mn Levels to Growing Environments

Clones	Mn concentrations (μg/g)				Ranking			
	Site			Mean clones	Site			Mean clones
	Timbilil	Kipkebe	Kangaita		Timbilil	Kipkebe	Kangaita	
TRFK 7/9	1466.67	1777.33	720.67	1321.55	1	3	9	2
TRFK 303/259	832.00	1264.00	618.33	904.78	16	17	15	19
TRFK 303/1199	550.67	1291.67	731.33	857.89	20	15	7	20
TRFK 54/40	916.67	1438.00	636.00	996.89	13	13	14	15
TRFK 31/8	705.33	1534.33	636.67	958.78	18	12	13	18
BBK 35	938.00	1575.67	483.33	999.00	12	10	20	14
TRFK 6/8	912.00	1428.33	660.00	1000.11	14	14	10	13
TRFK 31/27	760.67	2073.33	530.33	1121.44	17	2	18	7
TRFK 12/12	1123.00	1261.33	659.33	1014.56	9	18	11	12
TRFK 303/999	1172.00	1565.33	729.33	1155.56	6	11	8	6
APH S15/10	847.33	1764.00	745.00	1118.78	15	4	5	9
TRFK 57/15	1464.00	1696.00	653.67	1271.22	2	8	12	3
TRFK 56/89	1164.67	1757.33	568.67	1163.56	7	6	16	5
TRFK 12/19	1107.33	1722.67	528.67	1119.56	10	7	19	8
TRFK 11/26	1446.00	2356.00	823.00	1541.67	3	1	1	1
STC 5/3	1296.67	925.00	734.67	985.44	4	20	6	17
TRFK 7/3	673.33	1760. 00	544.67	992.78	19	5	17	16
TRFK 303/577	1241.67	1632.67	761.00	1211.78	3	9	4	4
EPK TN14-3	1156.00	1244.00	764.67	1054.89	8	19	3	10
TRFK 2x1/4	1093.67	1269.00	796.00	1052.89	11	16	2	11
Mean site	1043.38	1566.82	666.27					
C.V (%)		4.36						
LSD ($P \leq 0.05$)		37.44		47.02				
Interactions		79.46						

tion in mature leaf nutrient levels.[33]

There were significant interactions effects between location of production and the cultivars for all the micronutrients studied demonstrating that the clones absorbed the micronutrients differently in the single location and did not follow the same pattern when the clones planted in other locations. The results demonstrate that there is need to evaluate clones in intended extension sites to ensure ability to absorb nutrients if that tea cultivar is intended to contribute to management of "hidden hunger". There is need to generate additional data on widely grown clones in different locations to help in managing the micronutrient levels in black tea. Also, clonal and location-specific agronomic recommendations need to be developed to strike a balance in all the micronutrients from black tea in order to effectively optimize them in black tea.

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Table 3: Clonal Black Tea Fe Levels (µg/g) and Relative Ranking based on Fe Levels to Growing Environments

Clones	Fe concentrations (µg/g)				Ranking			
	Site			Mean clones	Site			Mean clones
	Timbilil	Kipkebe	Kangaita		Timbilil	Kipkebe	Kangaita	
TRFK 7/9	53.67	77.33	44.67	58.56	8	2	20	13
TRFK 303/259	54.67	69.67	51.67	58.67	5	10	17	11
TRFK 303/1199	53.67	74.33	85.00	71.00	8	4	1	3
TRFK 54/40	57.33	74.00	64.00	65.11	5	5	10	4
TRFK 31/8	44.00	72.00	55.33	57.11	19	6	15	18
BBK 35	37.00	63.00	67.33	55.78	20	18	3	20
TRFK 6/8	96.67	71.00	64.33	77.33	1	1	7	1
TRFK 31/27	73.33	70.00	76.33	73.22	2	9	2	2
TRFK 12/12	54.67	68.00	65.00	62.56	5	14	5	5
TRFK 303/999	51.33	68.67	53.67	57.89	11	12	16	16
APH S15/10	57.67	70.67	45.67	58.00	4	8	18	14
TRFK 57/15	46.33	60.00	66.67	57.67	17	20	4	17
TRFK 56/89	52.33	77.00	56.00	61.78	10	3	14	6
TRFK 12/19	54.33	69.33	59.00	60.89	7	11	13	8
TRFK 11/26	48.00	87.00	45.33	60.11	15	1	19	9
STC 5/3	49.67	68.33	65.00	61.00	12	13	15	7
TRFK 7/3	49.67	64.67	64.33	59.56	12	15	7	10
TRFK 303/577	45.67	61.00	64.33	57.00	18	19	7	16
EPK TN14-3	47.33	63.33	63.33	58.00	16	17	11	14
TRFK 2x1/4	48.67	64.00	63.33	58.67	14	16	11	11
Mean site	53.80	69.67	61.02					
C.V (%)		8.85						
LSD ($P\leq0.05$)		4.27		5.37				
Interactions		9.07						

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Table 4: Clonal Black Tea Zn Levels (µg/g) and Relative Ranking based on Zn Levels to Growing Environments

Clones	Zn concentrations (µg/g)				Ranking			
	Site			Mean clones	Site			Mean clones
	Timbilil	Kipkebe	Kangaita		Timbilil	Kipkebe	Kangaita	
TRFK 7/9	27.67	23.67	18.33	23.22	18	19	14	18
TRFK 303/259	59.00	43.67	28.67	43.78	2	2	2	1
TRFK 303/1199	49.00	35.33	30.33	38.22	6	4	1	3
TRFK 54/40	49.00	47.67	24.00	40.22	6	1	4	2
TRFK 31/8	48.00	25.00	23.67	32.22	8	14	9	11
BBK 35	43.67	35.67	24.33	34.56	12	3	5	7
TRFK 6/8	32.00	24.00	21.33	25.78	17	17	11	16
TRFK 31/27	52.67	34.67	24.33	37.22	4	5	5	5
TRFK 12/12	33.67	33.00	23.33	30.00	16	7	10	13
TRFK 303/999	42.00	24.33	21.33	29.22	13	16	11	14
APH S15/10	57.00	24.67	24.00	35.22	3	15	7	6
TRFK 57/15	63.33	32.00	18.00	37.78	1	8	15	4
TRFK 56/89	37.00	23.67	16.33	25.67	15	19	16	17
TRFK 12/19	46.33	25.33	25.33	32.33	10	13	3	10
TRFK 11/26	39.67	25.67	15.67	27.00	14	11	18	15
STC 5/3	26.33	24.00	13.67	21.33	20	17	20	20
TRFK 7/3	46.67	33.33	14.67	31.56	9	6	19	12
TRFK 303/577	44.33	31.33	24.00	33.22	11	9	7	8
EPK TN14-3	52.67	25.67	21.33	33.22	4	11	11	8
TRFK 2x1/4	26.67	26.33	16.00	23.00	19	10	17	19
Mean site	43.83	29.95	21.43					
C.V. (%)		10.61						
LSD ($P \leq 0.05$)		2.65		3.32				
Interactions		5.62						

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Table 5: Clonal Black Tea Cu Levels (µg/g) and Relative Ranking based on Cu Levels to Growing Environments

Clones	Cu concentrations (µg/g)				Ranking			
	Site			Mean clones	Site			Mean clones
	Timbilil	Kipkebe	Kangaita		Timbilil	Kipkebe	Kangaita	
TRFK 7/9	11.67	11.00	11.00	11.22	14	19	4	12
TRFK 303/259	14.67	15.67	11.00	13.78	5	2	4	3
TRFK 303/1199	13.00	15.00	12.33	13.44	9	6	2	5
TRFK 54/40	13.67	15.33	11.00	13.33	7	3	4	6
TRFK 31/8	12.67	14.67	12.67	13.33	12	7	1	6
BBK 35	15.33	13.67	11.00	13.33	3	10	4	6
TRFK 6/8	14.67	11.33	7.33	11.11	5	18	18	15
TRFK 31/27	13.67	13.67	9.00	12.11	7	10	10	9
TRFK 12/12	9.67	11.67	8.00	9.78	19	16	15	18
TRFK 303/999	13.00	13.00	7.67	11.22	9	13	17	12
APH S15/10	15.00	15.33	11.00	13.78	4	3	4	3
TRFK 57/15	11.00	14.00	66.67	11.22	18	9	13	12
TRFK 56/89	17.67	16.00	9.00	14.22	1	1	10	1
TRFK 12/19	16.33	13.33	12.33	14.00	2	12	2	2
TRFK 11/26	12.00	15.33	8.00	11.78	13	3	15	10
STC 5/3	11.33	13.00	10.00	11.44	17	13	9	11
TRFK 7/3	12.00	14.33	7.00	11.11	13	8	19	15
TRFK 303/577	11.67	12.00	8.67	10.78	14	15	13	17
EPK TN14-3	7.67	10.67	9.00	9.11	20	20	10	20
TRFK 2x1/4	11.67	11.67	6.00	9.78	14	16	20	18
Mean site	12.92	13.53	9.53					
C.V (%)		12.54						
LSD ($P \leq 0.05$)		1.18		1.48				
Interactions		2.51						

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Table 6: Clonal Black Tea Se Levels (µg/g) and Relative Ranking based on Se Levels to Growing Environments

Clones	Se concentrations (µg/g)				Ranking			
	Site			Mean clones	Site			Mean clones
	Timbilil	Kipkebe	Kangaita		Timbilil	Kipkebe	Kangaita	
TRFK 7/9	1.60	2.37	1.30	1.76	13	7	19	17
TRFK 303/259	2.37	2.37	1.60	2.11	5	7	17	12
TRFK 303/1199	2.33	2.03	3.67	2.68	6	11	2	2
TRFK 54/40	1.40	1.57	4.43	2.47	18	15	1	6
TRFK 31/8	1.47	1.43	3.13	2.01	15	17	4	13
BBK 35	3.30	1.47	2.97	2.58	2	16	5	3
TRFK 6/8	1.46	1.70	1.43	1.53	17	13	18	19
TRFK 31/27	2.63	1.36	1.70	1.90	4	19	14	15
TRFK 12/12	1.47	2.13	1.17	1.59	15	10	20	18
TRFK 303/999	2.33	2.57	3.33	2.74	6	5	3	1
APH S15/10	2.26	3.53	1.73	2.51	10	1	12	4
TRFK 57/15	1.60	3.43	2.47	2.50	13	2	6	5
TRFK 56/89	1.30	2.60	2.10	2.00	19	4	11	14
TRFK 12/19	1.27	1.43	1.70	1.47	20	17	14	20
TRFK 11/26	1.73	2.36	2.40	2.17	11	9	7	9
STC 5/3	2.27	2.40	1.73	2.13	8	6	12	11
TRFK 7/3	1.63	1.73	2.30	1.89	12	12	8	16
TRFK 303/577	2.27	2.93	2.13	2.44	8	3	10	7
EPK TN14-3	3.17	1.67	1.63	2.16	3	14	16	10
TRFK 2x1/4	3.33	1.33	2.17	2.28	1	20	9	8
Mean site	2.06	2.12	2.26					
C.V. (%)		9.27						
LSD ($P \leq 0.05$)		0.16		0.20				
Interactions		0.33						

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