Journal of Tea Science Research
2014, Vol.4, No.2, 17-26 http://jtsr.sophiapublisher.com

Effect of Genotypes in Different Environments on Micronutrient Content of Black Tea

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Journal of Tea Science Research, 2014, Vol.4, No.2 DOI: 10.5376/jtsr.2014.04.0002

Abstract Essential elements are needed in the day to day living of both human beings and the plants including tea (*Camellia sinensis*). Different clones of tea have been developed and distributed to farmers in various locations however it remains obscure the levels of the essential elements in their resultant black teas. This study aimed at establishing the micronutrient levels of different clones planted in a single site and also establish whether the levels of the micronutrients varied in the same pattern when planted in different regions. It was found that the different clones varied significantly ($p \leq 0.05$) in their micronutrient levels when planted in a single location under similar agronomic practices and this did not follow a similar pattern when the clones were planted in different locations. Thus there is need to identify region specific clones in order to optimize the micronutrient content of resultant black teas.

Keywords *Camellia sinensis*; Clones; Micronutrients; Agronomic practices

Introduction

Tea (*Camellia sinensis*) is widely grown in the highlands East and West of the Great Rift Valley in Kenya. With the expansion of the industry, cultivation of this perennial tree crop 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 areas of Eastern Africa. These genotypes had not been previously tested in these areas which differ widely in environmental conditions such as total rainfall and its distribution, temperature and edaphic factors. Successful tea genotypes must be adapted to a wide range of climatic and edaphic conditions. Tolerances to drought, cold and frost, high solar radiation, and high soil pH, etc. are among the major environmental factors that affect the adaptation and performance of tea in different sites (Wachira et al., 2002).

The main sources of micronutrients in plants are their growth media, agro inputs and soil (Subbiah et al., 2007). Plants take up the elements from the soil and under certain conditions, high levels can be accumulated in the leaves (Lasheen et al., 2008). The mineral constituents of tea leaves normally differ according to the geological source (Marcus et al., 1996). A number of papers regarding the determination of the mineral contents of tea have been published (Mokgalaka et al., 2004; Mondal et al., 2004; Cao et al., 1998). Several elements such as Ca, Na, K, Mg and Mn are present in mg/g quantities while elements such as Cr, Fe, Co, Ni, Cu, Zn, Se and Cd are present in µg/g level (Cao et al., 1998; Mokgalaka et al., 2004). However such data have not been generated for the clones that are widely grown in East Africa. Tea is largely out crossing and inherently self infertile. Every plant produced is therefore unique. Clonal tea plants are derived from one bush (a mother bush) through vegetative propagation. Cultivars {culti (vated)+var (iety)} are plants that have been purposely selected and maintained through cultivation. Cultivars are normally registered and protected under law (Kamau, 2008). Several clones have been developed and released to farmers (Anon, 2002: Othieno, 1988). Several studies have demonstrated wide response in yield (Ng'etich et al., 2001; Wachira et al., 1990; Wackremaratne, 1981), yield partitioning (Ng'etich et al., 2001), growth (Ng'etich and Stephens, 2001a,

Preferred citation for this article:
Omwoyo et al., 2014, Effect of Genotypes in Different Environments on Micronutrient Content of Black Tea, Journal of Tea Science Research, Vol. 4, No. 2 17-26 (doi: 10.5376/jtsr.2014.04.0002)
Received: 19 Jan., 2014 **Accepted:** 24 Jan., 2013 **Published:** 08 Feb., 2014

2001b), shoot population density (Balasuriya, 1999) and dry matter partitioning (Ng'etich and Stephens, 2001b) of tea genotypes to different environments (Carr and Stephens, 1992; Wachira et al., 1990; Wachira et al., 2002) including water stress (Carr, 1997), temperature (Tanton, 1982a; 1982b) and altitude (Obaga et al., 1989; Squire et al., 1993). Such variations occur even within 10-km radius (Ng'etich and Stephens, 2001a; 2001b; Ng'etich et al., 2001; Obaga et al., 1989; Squire et al., 1993). In terms of the black tea quality, the black tea aroma (Aisaka et al., 1978; Owuor and Obanda, 1996), volatile flavor compounds composition (Horita and Owuor, 1987; Yamanishi et al., 1968) and black tea plain quality parameters (Owuor et al., 1986a; 1986b) varied widely with geographical area of production. Previous studies assumed that large differences in climate are necessary for significant quality differences to be observed. As a result many tea-growing countries have centralized their clonal selection/breeding programmmes in single locations. It has been thought that a superior genotype selected in one location maintains its desirable attributes within the country. However tea plants selected in one location and planted in other locations have usually not matched the performance at the site of selection (Wachira et al., 1990, 2002). One such reason for such difference has been altitude, which affects rates of growth, even when other agronomic/cultural practices are similar. All these observations are indicators that there might be variations in the micronutrient content of the resultant black teas due to clones in different environments. Again different clones are known to have different abilities to absorb micronutrients from the soil when they are under similar agronomic practices (Yemane et al., 2008). The effect of region on growth is a known factor in determining the micronutrient content in tea (Mokgalaka et al., 2004). The level of micronutrient in teas has been demonstrated to change with locations in India (Kumar et al., 2005). In recent studies, tea quality parameters (Jondiko, 2010; Owuor et al., 2008; 2009; 2010a; 2010b) and quality precursors (Okal, 2011) were demonstrated to change with geographical area of production. Indeed even the yields (Owuor et al., 2009; 2010a; Wachira et al., 2002) and quality (Owuor et al., 2010a) vary with geographical area of production, the variation occurring in unpredictable patterns. Region specific cultivars that have the ability to extract optimal amounts of the essential micronutrients have not been identified it is thus necessary to determine whether the levels of micronutrients in black tea from several clones planted in a single location vary and whether the variation follow the same pattern in the clones when planted in different locations.

Results and Discussion

Effect of genotypes in different environments on micronutrient content of black tea

The clones used here were commercial clones, most of which were initially selected at the Timbilil site. Their selections were based on yield, not micronutrient content. The effect of location of production and cultivars on Mn content of black teas is presented in Table 1. The concentration levels of the 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. This indicates that different clones have different abilities to absorb Mn from the soils. Clone TRFK 11/26 recorded 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 indicating that the clones have different abilities to absorb micronutrients in different locations. The highest mean Mn levels were recorded in Kipkebe while the lowest levels were recorded in Timbilil. There was a significant interaction between the clones and different locations meaning that the responses did not occur in the same pattern.

Significant ($p \leq 0.05$) differences were observed in Fe levels due to location of production and clones (Table 2). 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. The mean levels of Fe varied significantly ($p \leq 0.05$) from clone to clone meaning that the clones have varied abilities to absorb Fe from the soil. There were significant ($p \leq 0.05$) differences in the mean Fe levels in the three locations indicating that the clones have varied abilities to absorb Fe when

Table 1 Clonal black tea Mn levels (μg/g) and relative ranking based on Mn levels to growing environments

Clone	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≤0.05)	37.44			47.02				
Interactions	79.46							

planted in different locations. In Kipkebe clones TRFK 7/9, TRFK 303/1199, TRFK 54/40 recorded the highest Fe levels while clone TRFK 31/27 had 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. The results indicate that clones in Kipkebe tea farm had the highest mean Fe levels while Timbilil recorded the lowest levels. There were significant ($p \le 0.05$) interaction effects between the clones and geographical area of production meaning the responses occurred in different patterns. Thus the use of cultivars that extract high levels of Fe will assist in increasing the Fe content of the resultant black teas.

The concentration levels of Zn significantly ($p \le 0.05$) varied due to different clones planted in different locations (Table 3). This also implies that it is not possible to produce black teas with similar Zn content from different cultivars planted in a single location or even in different locations. The mean Zn levels significantly ($p \le 0.05$) varied among the clones indicating that the different clones absorb Zn differently. Clone TRFK 303/259 had the highest mean levels of Zn while clone STC 5/3 recorded the least Zn level. There were significant ($p \le 0.05$) differences in mean levels of Zn in the different sites indicating that the clones have varied abilities to absorb Zn when subjected to different locations.

Table 2 Clonal black tea Fe levels (µg/g) and relative ranking based on Fe levels to growing environments.

Clone	Fe concentrations (µg/g)				Ranking			
	Site			Mean clone	Site			Mean clone
	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	7	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≤0.05)			4.27	5.37				
Interactions		9.07						

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≤0.05) higher levels of Zn. Clones STC 5/3 and TRFK 2x1/4 recorded significantly (P≤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. There were significant (p≤0.05) interactions between location of production and the cultivars meaning the clones absorb Zn differently in the single location and do not follow the same pattern when planted in other locations.

The effect of region of production and cultivars on Cu content of black teas is presented in Table 4. There were significant (p≤0.05) variations in the Cu levels among the clones. This indicates that the clones have varied abilities to absorb Cu from the tea soils. Clone TRFK 56/89 recorded the highest mean Cu levels among the studied clones while clone EPK TN14-3 recorded the lowest levels. The mean Cu levels significantly (p≤0.05) differed in the three locations with Timbilil recording significantly (p≤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

Table 3 Clonal black tea Zn levels (µg/g) and relative ranking based on Zn levels to growing environments

Clone	Zn concentrations (µg/g)				Ranking			
	Site			Mean clone	Site			Mean clone
	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≤0.05)			2.65	3.32				
Interactions		5.62						

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 between location of production and clones indicating that the clones behaved differently in the locations of production and that the response patterns were not similar. Thus it is necessary to determine the source and genotype of the teas that could increase the Cu content in the resultant black teas.

The changes in black tea Se levels due to location of production and clones are presented in Table 5. The clones showed significant ($p \leq 0.05$) variations in black tea Se levels. This implies that the clones have varied abilities to absorb Se. This was confirmed by the fact that clone TRFK 303/999 had significantly ($p \leq 0.05$) higher mean levels of Se than the other clones while clone TRFK 12/19 recorded the lowest levels. Such variations significantly ($p \leq 0.05$) differed at different locations indicating that the clones have varied abilities to absorb Se when planted in different locations. Clones TRFK 2x1/4, APH S15/10 and TRFK 54/40 had significantly ($p \leq 0.05$) higher concentration

Table 4 Clonal black tea Cu levels (μg/g) and relative ranking based on Cu levels to growing environments.

Clone	Cu concentrations (μg/g)				Ranking			
	Site			Mean clone	Site			Mean clone
	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	8.67	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≤0.05)			1.18	1.48				
Interactions		2.51						

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. These differences are expected since Se is known to be naturally available in the soils for absorption by the tea plants (Ip, 1998) and the different cultivars have varied abilities to absorb this nutrient even when under similar agronomic practices in different locations. There was also significant ($p \leq 0.05$) interaction effect between the cultivars and location of production indicating that the response pattern of the clones was different in the different locations. Thus for increasing the Se content in black teas, cultivars with a high ability to extract Se should be adopted.

The results presented herein demonstrate that different clones have varied abilities to absorb micronutrients in single locations and same clone has different ability to absorb the micronutrients in different locations. The genetic makeup of the teas used in these study was 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. 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 (Shu et

Table 5 Clonal black tea Se levels (μg/g) and relative ranking based on Se levels to growing environments

Clone	Se concentrations (μg/g)				Ranking			
	Site			Mean clone	Site			Mean clone
	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≤0.05)		0.16		0. 20				
Interactions		0.33						

al., 2003) and Cu (Jin et al., 2008) contents of tea from different farms within Sichuan Province varied. In Turkey, Fe and Mn levels of tea from different regions were different (Sahin et al., 1991).

These results were similar to recent research done in the Wushwush farms in Ethiopia on the levels of essential and non-essential elements where there were significant variations in the elements of five different clones planted in four unit farms under similar agronomic practices (Yemane et al., 2008). 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 (Wanyoko and Njuguna, 1983). Recently clone BBK 35 was shown to have different mature leaf nutrient levels when grown in different regions in Kenya under same agronomic inputs (Kamau et al., 2005). Similar variations were recently observed in the plain tea quality parameters (Owuor et al., 2010a, 2010b). Successful cultivars of most crop species, successful tea genotypes should be adapted to a wide range of climatic and edaphic conditions. Tolerance to drought, cold, frost high solar radiation and high pH are among the major environmental factors that affect adaptation and performance of tea in different sites (Wachira et al., 2002). The clones that are grown in a single site (Owuor et al., 1988, 1987a, 1987b) and even in different environments (Owuor et al., 2010a) exhibited variations in their black tea chemical composition.

Indeed even the yields significantly differed in the different environments (Wachira et al., 2002). Previous research demonstrated wide response ranges among tea genotypes to different environments (Carr, 1997; Obaga et al., 1988; Tanton, 1982a; Wickremaratne, 1981; Carr and Stephens, 1992). Indeed, dry matter partitioning (Ng’etich and Stephenes, 2001a; Magambo and Canell, 1981; Magambo and Waithaka, 1983) and quality (Owuor et al., 2010b) of tea vary with clones and location of production. However, different clones have varying abilities to absorb nutrients from the soil (Yemane et al., 2008; Wanyoko, 1981) leading to clonal variation in mature leaf nutrient levels (Wanyoko and Njuguna, 1983).

The results together with data presented herein demonstrate clear evidence that there is need to generate additional data on widely grown clones in different locations to help in managing the micronutrient levels in black tea. It is necessary that clonal and location specific agronomic recommend-dations are developed that will strike a balance in all the micronutrients from black tea in order to effectively optimize them in black tea.

Conclusion

The different clones showed varied ($p \leq 0.05$) micronutrient content when planted in a single location under similar agronomic practices and did not follow a similar pattern when the clones were planted in different locations.

Materials and Methods

Sites for sample collection and clones

This experiment was superimposed on an ongoing GxE experiment. Twenty widely cultivated (comercial) 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, whose altitude, latitude, longitude and year of plantation are given in Table 6, were used in this trial. At each site, plots were arranged in a randomized complete block design with three replicates (Wachira et al., 2002). The tea was planted in a 122cm by 61 cm rectangular spacing as detailed in Tea Growers Handbook (Anon, 2002). Nitrogen inform of NPKS 25:5:5:5 compound fertilizer at a single dose of 120 kg/ha was applied during the year of plantation and 200 kg/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 (Owuor and Reeves, 1986). The unsorted black tea samples from these clones were also analyzed for the micronutrients.

Table 6 Site locality and history for the different clones

Site (Locality/ history)	Kipkebe	TRFK/Timbilil	Kangaita
Altitude (m)	1872	2178	2100
Latitude	0^{0} 45’S	0^{0} 22’S 35^{0}	0^{0}30’S
longitude	35^{0}5’E	21’E	37^{0}16’E
Year planted	1997	1986	1991
Plantation age*	14	25	20

Note: * As at year 2011; Source: Individual/ estate records

Analysis of black tea for the micronutrients

A modified standard procedure described in AOAC (2000) was followed for the preparation of samples for analysis of essential minerals. The made tea samples were weighed on a digital analytical balance (Mettler Toledo, Switzerland) with $\pm$0.0001 g precision. Accurately weighed 1.0000g black tea for analyzing Mn, Fe, Zn and Cu while 2.0000 g black tea for Se analysis were transferred into ashing tubes and kept in a muffle furnace for ashing at 460 °C for 12 hours. The ashed samples were digested using double acid (concentrated hydrochloric and nitric acids in a1:1 ratio) and hydrogen peroxide in the ratio of 2:3. Care was taken to ensure that all ash came into contact with the acid. All the chemicals used were of analytical grade obtained from Sigma Aldrich. Further the crucible containing acid solution was kept on a hot plate and evaporated to dryness. The final residue was dissolved in 0.05 M hydrochloric acid solution for extraction and made up to 25 mL for Mn, Fe, Zn and Cu analysis and to 10 mL for Se analysis. Working standard solutions were prepared by diluting the stock solution with 0.05M hydrochloric acid. The Mn, Fe, Zn, Cu and Se in made tea samples was analyzed using atomic absorption spectrophotometer (Shimadzu AA-6200 Model, Japan) under standard instrumental conditions (Table 7).

Statistical analysis

The data was analyzed 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.

Table 7 Atomic absorption flame emission spectrophotometer (Shimadzu AA−6200) experimental parameters

Element	Mn	Se	Cu	Zn	Fe
Lamp current (mA)	10	23	10	6	8
Wavelength (nm)	279.5	196.0	324.8	213.9	243.3
Slit width (nm)	0.2	0.7	0.7	0.7	0.7
Mode	BGC-D_2	HVG-1	BGC-D_2	BGC-D_2	BGC-D_2
Flame Type	Air-C_2H_2	Air-C_2H_2	Air-C_2H_2	Air-C_2H_2	Air-C_2H_2
Fuel flow (L/min)	2.0	1.8	2.0	2.0	1.8
Prespraytime (sec)	3	3	3	3	3
Intergration time (sec)	5	5	5	5	5
Callibrations (ppm)	0.5~2.0	0.2~3.2	0.8~3.2	0.2~1.2	1.0~8.0
MDL (ppm)	0.06	0.20	0.04	0.011	0.08

Note: MDL: Machine detection limit; BGC-D_2: Deterium background correction (compensates for matrix interferences)

Acknowledgment

We are grateful to the Tea Research Foundation of Kenya for facilitating field trials of the experiments and we are also thankful to the Inter-University Council for East Africa, Lake Victoria Research Initiative (VicRes) who funded the research.

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