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RELATIONSHIP BETWEEN TEA (*CAMELLIA SINENSIS)* LEAF UPTAKE OF MAJOR NUTRIENTS, NITROGEN, PHOSPHOROUS AND POTASSIUM (NPK) AND LEAF ANATOMY OF DIFFERENT VARIETIES GROWN IN THE KENYAN HIGHLANDS

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ABSTRACT

Uptake of major nutrients (NPK) through the leaf studies were carried out on three varieties of tea grown in the Kenyan Highlands. A foliar fertilizers trial was setup in three sites comprising of 36 plots per site in the major tea growing regions in Kenya. The uptake of NPK through leaf tissue and the role of leaf anatomy were investigated. Two foliar fertilizers tested were Foliar Fertilizer 1 (FF1) and Foliar Fertilizer 2 (FF2), a positive control of Soil Fertilizer (SF) and a blank were used to standardize the method. Leaf anatomical studies were done by determining the stomata count of the third leaf of sample plants from each plot in all the trial sites. Results showed significant correlation between stomata count and first mature leaf nutrients; N ($r=0.387$, $p\leq0.05$), P ($r=0.32$, $p\leq0.01$) and K% ($r=-0.014$, $p\leq0.01$). Tea yields were found to correlate significantly with NPK nutrient uptake; N% $r=0.453$ ($p\leq0.01$), P% $r=-0.332$, $p\leq0.01$ and K% $r=-0.373$, $p\leq0.05$. Stomatal patterns and density responded to the environment, where Kericho site (west of Rift Valley) had significantly higher stomata count than Kirinyaga and Meru sites both in east of Rift valley at HSD= 6.5, 5.6 at $p\leq0.05$ respectively. Overall the leaf anatomy, i.e. epidermal layers (<50µm thick), palisade layers (50µm thick) and mesophyll layers (150-200µm) were not affected by fertilizer application.

KEYWORDS: Tea, Leaf Uptake, Leaf Anatomy, Foliar Fertilizer

INTRODUCTION

Tea is a perennial mono-crop and is highly demanding in terms of inputs i.e. fertilizer, since its production is occasioned by high nutrient removal on harvesting [12]. Globally, fertilizers have been experiencing instability in prices due to imbalances between supply and the rapidly expanding demand from various sectors [10]. Kenya solely relies on imported standard soil applied NPK 25:5:5 fertilizer, for tea production and has recently been experiencing challenges arising from the high fertilizer prices. Foliar fertilizer application has been reported to increase yield from 12 to 25 percent when compared to soil fertilizer application [11, 13].There are advantages in that the application rates are much lower than for soil applications, and uniform application is easily achieved [16]. Foliar fertilizer applications have the greatest impact on new flush as the young leaves readily absorb nutrients [7]. Old leaves are green factories where photosynthesis takes place to produce the essential metabolites utilized in plant growth and development. By applying foliar fertilizers the leaf's activity is expected to increase resulting to high chlorophyll level and thus photosynthesis [17]. Therefore, nutrients are absorbed right at the site where they are needed, whereas much of soil fertilizers may never go to plants [11], as nutrients may be lost through the processes of leaching, runoff, volatilization and fixation thus rendering them unavailable to plants or causing environmental pollution [6]. Foliar applied fertilizers have only been successfully used to correct copper and zinc nutrient deficiencies in Malawi and Kenya respectively [2] and magnesium in Nigeria [17]. They successfully reduced nutritional stress in tea plants in relatively short periods.

In Kenya, soil fertilizer application rate is based on the number of bushes (50Kgs NPK for 700 bushes), this not withstanding that different tea clones have different genetic potential to yields and response to nutrient supply [3]. Stomata are small adjustable pores found on surface of most aerial plants [15]. The two specialized cells in the epidermis, guard cells, are morphologically distinct from general epidermal cells, and are responsible for controlling stomata aperture [9]. Open stomata keep water and nutrients moving through the plant and allow the exchange of carbon dioxide and oxygen between the air inside and outside the leaf, a process that is critical for photosynthesis. Stomatal aperture has the greatest control over stomata conductance, with stomata density being secondary [15]. Stomatal patterns alter with response to the environment [4]. Studies have shown that changes in CO_2 and light levels elicit changes in stomata numbers. According to Oosterhuis, 2009 [18], stomata penetration can only occur in the brief period after application while spray deposit remain liquid [18]. The mechanisms governing uptake of foliar applied nutrients involve entry of the nutrients into the leaf tissue and subsequent translocation to other parts of the plant [16]. Foliar absorption involves both active and passive processes [8], entry of nutrients through aqueous pores found on the cuticle of the leaves, then the cell walls of the epidermal cells and finally through the plasma membrane by active transport [1, 5, 20]. The ability of the leaf to absorb nutrients from the surface depends on the degree of permeability of the leaf epidermis and the presence and density of stomata. Free-hand sections of living plant tissues provide adequate information for rapid and inexpensive microscopic observation of their internal structure [19]. This research embarked on establishing the uptake of nutrients through the tea leaf tissue and evaluates the role leaf anatomy plays in the absorption of the nutrients.

METHODOLOGY

Experimental Sites

The trial was set up in September 2010, and comprised of three experimental sites which represent the geographically different major tea growing regions in Kenya (East and West of the Great Rift Valley) -Timbilil estate, TRFK, Kericho, clone TRFK 31/8; KTDA-Kangaita farm, Kirinyaga, clone TRFK 6/8; Michimikuru Ltd Co. farm, Meru, clone EPK D99/10. Each site comprised of 36plots laid in a randomized complete block design with three replications of three fertilizer types; two NPK foliar fertilizers (FF1 and FF2) and the convectional NPK soil applied fertilizer (SF).

Table 1: Location, Elevation and Climatic Characteristics of Experimental Sites

Site	Clone	Latitude	Longitude	Elevation (M)	Mean Annual Temp (^{O}C)	Mean Annual Rainfall (Mm)
Timbilil	TRFK 31/8	0^0 22' S	35^0 32'E	2180	16.6	2175
Kangaita	TRFK 6/8	0^0 26' S	37^0 15'E	2020	15.5	2040
Michimikuru	EPK DPP/10	0^0 11'N	37^0 51'E	1950	17.3	2379

*** Source:** Tea Research Foundation of Kenya Weather Reports

Fertilizers and Application Rates

Two foliar fertilizers and one soil applied fertilizer were used in the fertilizer trial; *Maj Tea foliar* fertilizer, a water soluble formulation with the elemental composition; NPK 24:24:18 + Trace elements 0.9 MgO, 0.1625 Fe(EDTA), 0.16 Cu, 0.08 Zn, 0.0325 B, 0.0012 Mo, and 0.08 Mn (EDTA). The pH of a 10% solution was 3-4, with a density of 1.40; *T-foliar* SPS fertilizer and plant booster containing NPK 20:5:5 + S+ MgO + Trace Elements; and the soil chemically compounded fertilizer containing NPK 25:5:5. The fertilizers were coded as Foliar Fertilizer 1 (FF1) for Maj Tea foliar, Foliar Fertilizer 2 (FF2) for T-foliar, and Soil Fertilizer (SF) for NPK 25:5:5. FF1 was applied every 2 months, FF2 every 3 months and SF applied once per year.

Application rates for the foliar fertilizers were Nil, Half rate, Full rate and Double rates. The specific fertilizers were coded as, $FF1_0$, $FF1_{½}$, $FF1_1$, $FF1_2$, and $FF2_0$, $FF2_{½}$, $FF2_1$, $FF2_2$, for Maj and T foliar fertilizer respectively, and 0, 75, 150, and 225 Kg N/ha/year for SF which was treated as the positive control. The amount of fertilizers applied for both foliar and soil fertilizers were calculated based on the number of bushes per plot and the spacing of the tea bushes. The average amounts applied per plot for each fertilizer type are shown in Table 2**.**

Table 2: Amounts of Fertilizers Applied

Sites	Treatments	Rates	Amounts of Fertilizer Applied (G)	Amounts of N,P,K In Each Rate (G)		
				N (G)	P (G)	K (G)
KERICHO	FF1	Half	10.5	2.5	2.5	1.9
		Full	21	5.1	5.1	3.8
		Double	42	10.0	10.0	7.6
	FF2	Half	23	4.64	1.16	1.16
		Full	46	9.25	2.31	2.31
		Double	92	18.5	4.63	4.63
	SF	Half	390	97.5	19.5	19.5
		Full	780	194.9	39.0	39.0
		Double	1169	292.3	58.5	58.5
KIRINYAGA	FF1	Half	10.6	2.6	2.6	1.9
		Full	22	5.2	5.2	3.9
		Double	43	10.4	10.4	7.9
	FF2	Half	23	4.64	1.16	1.16
		Full	46	9.28	2.32	2.32
		Double	92	18.6	4.6	4.6
	SF	Half	418	104.5	20.9	20.9
		Full	836	209.0	41.8	41.8
		Double	1254	313.6	62.7	62.7
MERU	FF1	Half	13	3.1	3.1	2.3
		Full	26	6.1	6.1	4.6
		Double	52.5	12.5	12.5	9.4
	FF2	Half	27	5.6	1.4	1.4
		Full	55	11.1	2.8	2.8
		Double	111	22.3	5.6	5.6
	SF	Half	502	125.4	25.1	25.1
		Full	1003	250.7	50.1	50.1
		Double	1505	376.4	75.3	75.3

Leaf Anatomical Studies

Stomata Count

The samples of third leaf were collected in the mid morning between 9-10am from the three sites. A coat of commercially available clear nail varnish was applied on the upper (abaxial) and lower (adaxial) epidermis of the samples collected and allowed to dry. The coat was then peeled off and mounted on a slide using a few drops of water. Physical counting of the stomata was done for the leaf under the light microscope at 25X magnification.

Measurements of Anatomical Features in Tealeaf

Small pieces of second, third and fourth freshly plucked leaf samples were cut using free hand and processed for permanent slide preparation (with embedding) by fixing the with Formalin Acetic Alcohol (FAA) for 24 hours, dehydrating in ascending series of alcohol: 30% alcohol, 70% alcohol, 90% alcohol and 100% alcohol twice, clearing with xylene: alcohol ratios of 1:3, 1:1 and 3:1 for day 1, 2, 3 days respectively and day 4 and 5, clearing was done using pure xylene. Impregnation of the leaf tissue in wax was done in an oven at 60^oC using xylene: wax ratios of 3:1, 1:1, and 1:3

respectively for the first 3 days and then changed into pure wax for about five times. The plant material was embedded in pure wax followed by sectioning using a Leitz Wetzlar, 1400 microtome. The sections were mounted on slides by smearing haupts adhesive solution and the dried slides stained using Safranin "O". Scoring was done using a light microscope. The parameters scored in micrometers were; thickness of upper epidermis, thickness of palisade layer, thickness of spongy mesophyll layer and thickness of the lower epidermis.

Statistical Analysis

All the determinations were carried out in triplicate and the data were subjected to one-way analysis of variance (ANOVA) whereby analysis for each variable was separately done i.e. by fertilizer type (Blank, FF1, FF2, SF) and by rates of application (zero, half, full and double). This was followed by the Tukey-Kramer range test to establish the honest significant difference (HSD) in means between the various group means at $p<0.05$ confidence level. HSD is minimum distance between two group means that must exist before the difference between the two groups is considered statistically significant.

RESULTS

Stomata Count

In all the three sites, there were no significant differences in stomata count by either the fertilizer type or by rates (Table 3). However, pair wise comparison between sites (Table 4) showed significant differences with tea at the Kirinyaga site (clone TRFK 6/8) having the lowest stomata count and Kericho (clone TRFK 31/8) having significantly ($p<0.05$) higher count than the other two sites. This may be attributed to clonal differences in each site and environmental factors.

Table 3: Stomata Count Analysis by Fertilizer Types and by Rates at the 3 Sites

	Group (X_1) vs. Group(X_2)	Group Means X_1	X_2	Mean Dif(X_2- X_1)	HSD
KERICHO	ZERO vs. $FF1_{1/2}$	210	233	22	2
	ZERO vs. $FF1_1$	210	233	23	2
	ZERO vs. $FF1_2$	210	202	-8	1
	$FF1_{1/2}$ vs. $FF1_1$	233	233	1	0
	$FF1_{1/2}$ vs. $FF1_2$	233	202	-30	2
	$FF1_1$ vs. $FF1_2$	233	202	-31	2
	ZERO vs. $FF2_{1/2}$	250	320	70	3
	Group (X_1) vs. Group(X_2)	**Group Means X_1**	**X_2**	**Mean Dif(X_2- X_1)**	**HSD**
KIRINYAGA	ZERO vs. $FF2_1$	250	226	-23	1
	ZERO vs. $FF2_2$	250	219	-31	1
	$FF2_{1/2}$vs. $FF2_1$	320	226	-93	3
	$FF2_{1/2}$vs. $FF2_2$	320	219	-101	4
	$FF2_1$ vs. $FF2_2$	226	219	-8	0
	ZERO vs. $SF_{1/2}$	249	297	48	2
	ZERO vs. SF_1	249	259	10	0
	ZERO vs. SF_2	249	217	-32	1
	$SF_{1/2}$ vs. SF_1	297	259	-38	1
	$SF_{1/2}$ vs. SF_2	297	217	-80	3
	SF_1 vs. SF_2	259	217	-42	1
	ZERO vs. $FF1_{1/2}$	219	205	-15	2
MERU	ZERO vs. $FF1_1$	219	194	-25	3
	ZERO vs. $FF1_2$	219	197	-22	2
	$FF1_{1/2}$ vs. $FF1_1$	205	194	-10	1
	$FF1_{1/2}$ vs. $FF1_2$	205	197	-7	1
	$FF1_1$ vs. $FF1_2$	194	197	3	0
	ZERO vs. $FF2_{1/2}$	218	195	-23	2

Table 3: Contd.,

ZERO vs. $FF2_1$	218	205	-13	1
ZERO vs. $FF2_2$	218	207	-11	1
$FF2_{1/2}$ vs. $FF2_1$	195	205	11	1
$FF2_{1/2}$ vs. $FF2_2$	195	207	12	1
$FF2_1$ vs. $FF2_2$	205	207	2	0
ZERO vs. $SF_{1/2}$	207	215	8	1
ZERO vs. SF_1	207	226	19	2
ZERO vs. SF_2	207	212	5	1
$SF_{1/2}$ vs. SF_1	215	226	11	1
$SF_{1/2}$ vs. SF_2	215	212	-4	0
SF_1 vs. SF_2	226	212	-15	2
ZERO vs. $FF1_{1/2}$	214	214	0	0
ZERO vs. $FF1_1$	214	213	-1	0
ZERO vs. $FF1_2$	214	211	-3	1
$FF1_{1/2}$ vs. $FF1_1$	214	213	-1	0
$FF1_{1/2}$ vs. $FF1_2$	214	211	-3	1
$FF1_1$ vs. $FF1_2$	213	211	-2	0
ZERO vs. $FF2_{1/2}$	215	224	9	1
ZERO vs. $FF2_1$	215	201	-14	1
ZERO vs. $FF2_2$	215	205	-10	1

Group (X_1) vs. Group(X_2)	**Group Means X_1 X_2**		**Mean Dif(X_2- X_1)**	**HSD**
$FF2_{1/2}$ vs. $FF2_1$	224	201	-23	1
$FF2_{1/2}$ vs. $FF2_2$	224	205	-20	1
$FF2_1$ vs. $FF2_2$	201	205	3	0
ZERO vs. $SF_{1/2}$	218	183	-35	3
ZERO vs. SF_1	218	232	14	1
ZERO vs. SF_2	218	226	9	1
$SF_{1/2}$ vs. SF_1	183	232	49	4.8210*
$SF_{1/2}$ vs. SF_2	183	226	43	4
SF_1 vs. SF_2	232	226	-6	1

Starred values (*) represent significant differences at p =0.05

Table 4: Stomata Count Comparison in the Three Sites

Site (X_1) Vs. Site(X_2)	**Group Means X_1 X_2**		**Mean Dif (X_2- X_1)**	**HSD**
Kericho vs. Kirinyaga	242.97	208.42	34.56	6.4796*
Kericho vs. Meru	242.97	213.08	29.89	5.6046*
Kirinyaga vs. Meru	208.42	213.08	4.67	0.88

Correlations between stomata count, 1st mature leaf nutrients and yields were done (Table 5). The 1st mature leaf is used as a diagnostic tool as to the nutrient content in the tea plant [14] In Kirinyaga site, stomata correlates positively with N% at r=0.387, $p \leq 0.05$, which is indicative of uptake of nutrients. In Kericho, yields correlated significantly with N% r=0.453 ($p \leq 0.01$), with P% r=-0.332, $p \leq 0.01$ and with K% r=-0.373, $p \leq 0.05$ showing that N, P & K were absorbed and hence influenced yields. In Meru, there was a significant correlation between yields and stomata count at r=-0.335, $p \leq 0.05$ again showing influence of uptake in yields.

Table 5: Correlation Matrix (Pearson) between Stomata Count, 1st Mature Leaf Nutrients and Yields for the Three Sites

Kericho	**N%**	**P%**	**K%**	**Ca%**	**Mg%**	**Mn%**	**Stomata**	**Yield**
N%	**1**							
P%	-0.189	**1**						
K%	-0.053	-0.089	**1**					
Ca%	0.068	**0.345***	**-0.381***	**1**				
Mg%	0.012	0.059	0.22	0.075	**1**			
Mn%	-0.106	**0.453****	**-0.354***	**0.730*****	**-0.422***	**1**		

Table 5: Contd.,

Stomata	0.152	-0.23	-0.113	-0.089	0.015	-0.22	**1**	
Yield	**0.453****	**-0.332***	**-0.373***	-0.123	0.149	**-0.372***	0.266	**1**
Kirinyaga	N%	P%	K%	Ca%	Mg%	Mn%	Stomata	Yield
N%	**1**							
P%	**-0.345***	**1**						
K%	-0.118	**0.664*****	**1**					
Ca%	-0.076	-0.05	-0.051	**1**				
Mg%	-0.138	0.058	0.05	**0.874*****	**1**			
Mn%	-0.078	-0.174	-0.224	**0.885*****	**0.747*****	**1**		
Stomata	**0.387***	-0.164	-0.006	-0.247	-0.319	-0.208	**1**	
Yield	0.236	-0.187	0.077	-0.137	0.019	-0.239	0.11	**1**
Meru	N%	P%	K%	Ca%	Mg%	Mn%	Stomata	Yield
N%	**1**							
P%	**0.790*****	**1**						
K%	**0.713*****	**0.663*****	**1**					
Ca%	-0.114	-0.057	-0.049	**1**				
Mg%	-0.111	-0.013	-0.045	**0.906*****	**1**			
Mn%	0.219	0.127	-0.053	**0.461****	0.186	**1**		
Stomata	-0.012	**0.320****	0.014	-0.017	0.031	0.048	**1**	
Yield	0.15	0.054	-0.137	-0.053	-0.13	-0.025	**0.335***	**1**

*Values in **bold*** are significantly different from 0 at p ≤0.05*
*Values in **bold**** are significantly different from 0 at p ≤0.01*
*Values in **bold***** are significantly different from 0 at p≤0.001*

Tea Leaf Anatomical Features

The lower and the upper epidermis layers were <50µm thick, the palisade thickness was about 50µm while the spongy mesophyll layer was between 150-200 µm thick for all the fertilizer treatments. There was no significance difference in thickness of the tealeaf layers with the different types of fertilizers used.

DISCUSSIONS

The study confirmed that tea has stomata only on the abaxial surface of the leaf which is collaborated by other studies [16]. Several factors influence the absorption of leaf-applied nutrients. High air humidity stimulates the absorption due reduction in drying of droplets and causes swelling of cuticular membrane which increases the absorption of hydrophilic compounds. This emphasized the need to strictly apply the foliar fertilizer very early in the morning or late in the evening when the ambient temperatures are low. In this study relatively low amounts of foliar solution were used for the test fertilizers (Table 2). This affects the rate of absorption of the foliar applied nutrients. It has been reported that the passive transport of nutrients into the epidermal cells positively correlates to concentration of nutrients albeit to a certain concentration beyond which they cause burning and scorching of the leaf [16, 20]. Stomatal patterns and density respond to the environment [4], and this explains why Kericho site had significantly higher stomata count than Kirinyaga HSD= 6.5 and Meru HSD= 5.6 sites at p≤0.05. Kericho herein represents the West of Rift Valley while Meru and Kirinyaga represent the East of the Rift Valley and the two regions are geographically different. While studies have shown the influence of applied nutrients to the physiology of tea, e.g. photosynthesis of tea being strongly related to leaf N, increased levels of chlorophyll after increased applications of N and K, there does not seem to have any influence of the nutrients on the tea leaf anatomy since the epidermal layers, the palisade layers and the mesophyll layers did not differ significantly with the different fertilizer types.

CONCLUSIONS

Relationship between leaf anatomy and uptake was established from the positive correlation N% ($r=0.387$, $p\leq0.05$), P% ($r=0.32$, $p\leq0.01$) and K% ($r=-0.014$, $p\leq0.01$) between NPK of first mature leaf and stomata count. This is indicative of influence of the stomata on the uptake of the nutrient. Moreover, yield was found to correlate significantly with NPK nutrient levels which also shows influence of uptake of nutrients on the yields; N% $r=0.453$ ($p\leq0.01$), P% $r=-0.332$, $p\leq0.01$ and K% $r=-0.373$, $p\leq0.05$.

Leaf anatomy was found to be independent of fertilizer types, Zero, FF1, FF2 and SF and their rates of applications since no significant changes in stomata count. The epidermal layers (<50µm thick), the palisade layers (50µm thick) and the mesophyll layers (150-200µm) did not differ significantly ($p\leq0.05$) with the different fertilizer types. Stomatal density was found to alter with response to environment with Kericho site (clone TRFK 31/8) recording significantly higher counts than Kirinyaga site (clone TRFK 6/8) HSD= 6.5, $p\leq0.05$ and Meru site (clone EPK D99/10) HSD= 5.6, $p\leq0.05$

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