VARIATIONS IN *Camellia sinensis* (L.) LEAF NUTRIENTS AND POLYPHENOLS LEVELS WITH GENOTYPES, NITROGENOUS FERTILIZER RATES, SEASONS AND PLUCKING INTERVALS IN EASTERN AFRICA TEA GROWING REGIONS

BY

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DECLARATION BY THE CANDIDATE

I certify that this thesis has not been previously presented for a degree in Maseno University or in any other university. The work reported herein is my original work and all sources of information have been supported by relevant references.

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DEDICATION

This work is dedicated to my beloved sons Bowa Kwach Bowa and Fortune Omondi Kwach, and daughter Bertha Anyango Kwach

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ABSTRACT

In Eastern Africa, teas are grown in high rainfall areas causing fast nutrients depletion. Foliar nutrients diagnostic limits developed for seedling tea have been in use since 1970s and they have been adopted for clonal tea in Eastern Africa without validation. It is not known if the limits are suitable for clonal teas. Tea farmers usually import high quality clones across different regions; however their quality has not been replicated in new regions. Factors causing the quality variations and how the quality precursor compounds vary with agronomic inputs, season and locations are unknown. Nitrogen fertilizer rates and harvesting intervals influence tea yields and quality. However, it is not known how nitrogen rates influence the leaf nutrients and black tea quality precursors in Eastern Africa. This study assessed suitability of seedling tea diagnostic limits on clonal tea in different locations, the effect of genotypes, seasons and location of production on leaf caffeine and flavan-3-ols in Kenya and the influence of nitrogenous fertilizer rates and plucking interval on mature leaf nutrients and nitrogen rates on flavan-3-ols in different locations in Eastern Africa. The research was superimposed on two ongoing trials: a clonal trial involving twenty clones in Kenya and a fertilizer trial on clone TRFK 6/8 in Eastern Africa. Leaf nutrients, flavan-3-ols and caffeine levels were determined. The data was analyzed using appropriate factorial design for each trial. Leaf nutrients varied ($P \leq 0.05$) with clones and locations with interactions ($P \leq 0.05$) between clones and locations. The level of nutrients in clonal leaf did not concur with set limits in seedling tea. Increasing nitrogen rates decreased P, K, Ca and Mg levels but increased levels of N, Mn, Zn, and Fe in mature leaf. Plucking frequency did not affect leaf nutrients. Caffeine and flavan-3-ols varied ($P \leq 0.05$) with location, genotype and fertilizer rate but not with season. Flavan-3-ols and their ratios all varied ($P \leq 0.05$) with clones and nitrogen rates but not with site and season. These results demonstrate that diagnostic nutrients limits set for seedling tea may not be suitable for clonal tea. Variations in caffeine and the flavan-3-ols explain the previously observed plain black tea quality changes with clones, locations and nitrogen rates. It is recommended that region and clonal specific foliar diagnostic guidelines be developed, clones be tested and recommended for areas of their optimal quality potential and region specific nitrogen fertilizer rates for clones be developed in the growing regions.

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LIST OF ABBREVIATIONS/ACRONYMS

AAS	Atomic absorption spectrophotometer
AOAC	Association of Official Analytical Chemists
B	Boron
Ca	Calcium
Cu	Copper
CV	Coeffient of variation
EC	Epicatechin - [2-(3,4-Dihydroxyphenyl)-3,4-dihydro-2H-1-benzo-pyran-3,5,7-triol]
ECG	Epicatechin gallate – [(*2R,3R*)-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3,4-dihydro-2H-chromen-3-yl]3,4,5-trihydroxybenzoate.
EDTA	Ethylenediaminetetraacetic acid
EGC	Epigallocatechin - (*2R,3R*)-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-1-benzopyran-3,5,7-triol
EGCG	Epigallocatechin gallate [(*2R,3R*)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl) chroman-3-yl]3,4,5-trihydroxybenzoate
FAO	Food and Agricultural Organization
Fe	Iron
GA	Gallic acid – 3,4,5-trihydroxybenzoic acid
GAE	Gallic acid equivalent
Gall	Gallated
Gallo	Trihydroxy
GC	Gas chromatograph

HPLC	High pressure liquid chromatograph
ISO	International Organization for Standardization
K	Potassium
LSD	Least significant difference
Mg	Magnesium
Mn	Manganese
N	Nitrogen
nm	Nanometre
NS	Not significant
P	Phosphorus
ppm	Parts per million
RNA	Ribonucleic acid
rpm	Revolutions per minute
S	Sulphur
TC	Total catechins
TGC	Total gallocatechins
TnGC	Total non gallated catechins
TRFCA	Tea Research Foundation of Central Africa
TRFK	Tea Research Foundation of Kenya
TRIEA	Tea Research Institute of East Africa
TRIT	Tea Research Institute of Tanzania
UPASI	United Planters' Association of Southern India
UV-Vis	Ultra Violet-Visible

DEFINITIONS OF TERMS

Clones/Cultivars/ Genotype: Plants/varieties that are derived from one mother bush by a method of vegetative propagation and maintained through cultivation. They have the same genetic constitution.

Seedling tea: Tea plants that are derived from seeds, consisting of a broad spectrum of genotypes.

Polyphenols: Flavanols, flavonols, flavonol glycosides, polyphenolic acids and depsides put together.

Plain black tea: Black teas valued for their taste and colour characteristics.

CHAPTER ONE

1 INTRODUCTION

1.1 Background to the study

Tea (*Camellia sinensis, (L) O. Kuntze*) is an evergreen plant classified in the *Theaceae* family (Bokuchava and Skobeleva, 1969; Hara *et al.*, 1995). Commercially, there are three varieties of *Camellia sinensis*:- the China type (*Camellia sinensis* variety *sinensis*), the Assam type (*Camellia sinensis* variety *assamica*) and the hybrid type (*Camellia sinensis* variety *assamica* ssp *lasiocalyx* (Barnejee, 1992). Recently, two new varieties *Camellia sinensis* variety *pumbilimba* and *Camellia sinensis* variety *kucha* have been identified in China for commercial exploitation (Yao *et al.*, 2008). Tea plants are commercially grown in a wide range of latitudes, ranging from 45^{o}N (Russia) to 30^{o}S (South Africa), and longitudes from 150^{o}E (New Guinea) to 60^{o}W (Argentina) (Shoubo, 1989) and altitudes ranging from sea level in Japan to 2,700 m above mean sea level (amsl) in Olenguruone, Kenya and Gisovu, Rwanda (Owuor *et al.*, 2008a), demonstrating its adaptability to various geographical and environmental factors. Such variations affect growth factors, soil quality and nutrients supply (Anon, 2002). Tea plant can tolerate quite large deviations from normal nutrients levels before the first visible signs of deficiency begin to appear in the foliage. This necessitates regular foliar analysis to cushion the tea plants from "hidden hunger" that would reduce yields (Anon, 2002; Owuor *et al*., 2008b; Msomba *et al*., 2011) and influence plain black tea quality (Owuor *et al*., 1987b, 1997, 2010a).

Plants absorb nutrients mainly from the soil. Due to continuous tea cropping (Dang, 2002), high nutrient leaching (Owuor *et al*., 1997) and surface run-off (Othieno, 1988) in the high rainfall areas where tea is grown, soil nutrients diminish, making it necessary to replenish nutrients in form of inorganic fertilizers for high yields to be sustained. But excessive use of fertilizers may lead to low quality teas (Owuor *et al*., 1987b, 1991, 1997, 2010a) and also reduce

yields (Owuor *et al.*, 1997, 2008b) while at the same time degrade soil quality (Othieno *et al*., 2000; Kamau *et al*., 2008). It is however not known if, and how, the uptake of nutrients by tea clones varies with location of production in Kenya.

In Kenya, smallholder tea sector now owns about 81% of land under tea (Ogola and Kibiku, 2004). Their productivity is, however, about 50% of that realized by the estate sector despite the fact that they plant high yielding clonal teas, mainly TRFK 6/8 (Wachira, 2002). For clonal tea grown in one field under same agronomic input supply, different clones have varying nutritional requirements (Wanyoko and Njuguna, 1983). This difference may be made worse by genotype x environment (GxE) interactions (Wachira *et al.*, 2002). Indeed, it was recently shown that even the same clone grown in different regions (Owuor *et al*., 2008a) and receiving same agronomic inputs, produce varying yields (Owuor *et al.*, 2009; 2010a) and quality (Owuor *et al.*, 2009; 2010a; 2010b) and has different mature leaf nutrients levels (Kamau *et al*., 2005). The low productivity in smallholder tea sector could, in part, be due to inefficient and inappropriate fertilizer use arising from seedling tea based diagnostic system that may not be relevant to clonal tea. It is therefore necessary to evaluate the relevance of current nutrients diagnostic system on clonal tea.

Plant tissue analysis is considered a reliable technique to establish plant nutrients status (Tolhurst, 1976; TRFCA, 1990; Bonheure and Willson, 1992; Othieno, 1988; Kamau *et al*., 2005). Different countries and regions use different types of leaves to predict the tea bush nutritional requirements (Bonheure and Willson, 1992). Thus, while the mature leaf has been observed to be sensitive and a good predictor for most macronutrients deficiencies in East Africa (Tolhurst, 1976; Othieno, 1988) and Central Africa (TRFCA, 1990), mature leaf was only sensitive predictor for phosphorus deficiency in Sri Lanka (Sivapalan *et al.*, 1986) and potassium deficiency in South India (UPASI, 1987). The younger second, third or fourth leaves have been

used in foliar analysis for nutrients demand diagnosis in India, Indonesia, Taiwan and USSR (Ranganathan, 1998). For tea in Kenya, the use of mature leaf, defined as leaf from which new shoot has grown after last harvest, has been adopted by the advisory system to assess the problems relating to nutrients management (Tolhurst, 1976; Owuor and Wanyoko, 1983; Othieno, 1988) and the critical nutrients levels have been set (Owuor and Wanyoko, 1983; Bonheure and Willson, 1992). But the set limits were based on mature seedling tea. With the expansion of tea, the amount of clonal tea in Kenya is now over 60% (Wachira, 2002). When grown in the same field under same nutrients regimes, the different clones have different abilities to absorb nutrients and/or partition the nutrients, thus differ in their leaf nutrients levels (Wanyoko and Njuguna, 1983). Therefore, the set nutrients norms for seedling tea may not be optimal for clonal tea. It is necessary to establish if the set nutrients limits are relevant for use in clonal tea, especially when grown in different regions. It is also not known if the third leaf being used in other countries (Ranganathan, 1998) may be more accurate for clonal tea in Kenya and if there is a relationship between nutrients levels in mature and third leaf.

In Eastern Africa, tea growing areas fall in several agro-ecological regions, differing widely in elevation and climatic factors but with favourable soil and light conditions. Despite the differences, many agronomic inputs are uniform throughout the region (Owuor *et al*., 2011a). Nitrogen fertilizer and harvesting are the most expensive agronomic inputs in tea production (Bonheure and Willson, 1992). Clone TRFK 6/8 is widely grown in the region and constitutes 80% of Rwanda tea, 60% of Kenya clonal tea and 35-40% of Tanzania tea (Msomba *et al*., 2011; Owuor *et al*., 2011a). Nutrients diagnosis in this region is based on mature leaf nutrients, plucked any time diagnosis is required, irrespective of the plucking frequency of the two leaves and a bud for processing, and the recommended nitrogenous fertilizer rates set, based on mature leaf, diagnosis vary between 100 and 250 kg N ha^{-1} $year^{-1}$ (Anon, 2002). However, it is not

established if plucking interval of the two leaves and a bud affects nutrients amounts in mature leaf. The agronomic recommendations in use in the Eastern Africa tea growing regions have been adopted from the recommendations made by Tea Research Institute of East Africa without re-testing in new growing areas. The uniform agronomic recommendations currently used in tea production in the Lake Victoria basin may be inappropriate in some regions. There is therefore need to evaluate if clone TRFK 6/8 nutrients uptake is influenced by area of production in Eastern Africa and if mature leaf nutrients levels of this clone are stable to plucking interval of two leaves and a bud.

Beverages from tea plant are popular in the world but in recent times more interest has been focused on tea due to its relatively higher flavonoid content which has elicited many pharmacological activities (Ho *et al.*, 1994; Hollman and Arts, 2000). Green tea is made without enzymatic oxidation of polyphenols, as polyphenol oxidase is inactivated by heat during the early stages of processing (Hara *et al.*, 1995). Thus, the polyphenols present in green tea are the same as those in fresh tea leaves. Most Eastern Africa black tea are classified as plain teas valued for their taste and colour characteristics. Plain black tea quality is influenced by non volatile precursor quality chemical components in green leaf (Wright *et al*., 2000; Owuor *et al.*, 2006; Owuor and Obanda, 2007) which usually consist of flavan-3-ols (catechins) (Bailey *et al.*, 1990; Ding *et al.*, 1992), flavonols (Bailey *et al.*, 1990; McDowell *et al.*, 1991), oxidation products of green tea polyphenols, mainly theaflavins and thearubigins (Takino *et al.*, 1964; Brown *et al.*, 1966, 1969; Deb and Ullah, 1968) and caffeine (Bhatia, 1964; Deb and Ullah, 1968; Millin *et al*., 1969). Green tea leaves contain high levels of polyphenols mainly flavan-3-ols that are responsible for the formation of theaflavins and thearubigins in plain black tea (Deb and Ullah, 1968). Theaflavins have astringent tastes, and contribute to the briskness of plain black tea (Deb and Ullah, 1968). Whereas plain black tea quality has been shown to vary with geographical area

of production (Owuor *et al*., 2008a, 2009), nitrogenous fertilizer rates (Owuor *et al*., 2010a) and seasonal fluctuations in variables such as rainfall, temperature and humidity (Owuor *et al*., 1991b; Owuor, 1992; 1994), variation in plain black tea quality green leaf chemical precursors such as caffeine, total polyphenols and individual flavan-3-ols with geographical area of production, seasons and nitrogen rates has not been quantified in the Eastern African tea growing region. Indeed tea clones with high plain black tea quality potentials that have been planted in new growing areas in the region have not replicated plain black tea quality in the new areas (Owuor *et al*., 2011b).

1.2 Statement of the problem

In Kenya, smallholder farmers own about 81% of land under tea, planted with high yielding clonal tea. However, their productivity, after using nutrients management norms developed for seedling tea, is only about 50% of the estate sector. Further, Eastern Africa tea growing region also widely adopted the fertilizer regimes recommended for seedling tea based on mature leaf nutrients contents for the widely grown clone TRFK 6/8. The low productivity in smallholder tea sector could, in part, be due to inefficient and inappropriate fertilizer use arising from a diagnostic system (mature leaf nutrients contents) not relevant for clonal tea grown in different regions. It is also not known if plucking frequency of the tender shoots (two leaves and a bud), for processing, causes variations in levels of clonal tea mature leaf nutrients, or if the third leaf being used in other countries may be more accurate for clonal tea in Kenya and if there is a relationship between nutrients levels in mature and third leaf.

Tea farmers in Eastern Africa tea growing region usually import genetic materials across the borders with the assumption that the genotype with good quality attributes in one location maintains the status irrespective of where the plant is grown. However, tea planters have not

managed to replicate plain black tea quality in new production regions. This may be attributed to variations in green leaf caffeine, total polyphenols and individual levels. It is, however, not known how these green leaf chemical quality precursors vary with genotype, seasons and geographical location of production in the region.

1.3 Broad objective

The broad objective of this research was to assess the suitability of seedling tea leaf diagnostic limits on clonal tea, the influence of location of production, nitrogenous fertilizer rates and plucking intervals on clonal leaf nutrients levels, variations in plain black tea quality precursor levels with genotype, season, location of production and nitrogenous fertilizer rates in Eastern Africa.

1.4 Specific objectives

1. To assess if the nutrients critical limits set for seedling tea is relevant for different clones grown in Kenya, and compare suitability of leaf of different ages as guide in diagnostic chemical nutrients limits.
2. To assess if the use of mature leaf for nutrients deficiency diagnosis based on seedling tea is relevant for clone TRFK 6/8 in different growing regions of Eastern Africa and if mature leaf nutrients levels vary with nitrogenous fertilizer rates and plucking intervals.
3. To assess the variations in levels of caffeine and flavan-3-ols of tender clonal tea shoots (2 leaves and a bud) with geographical area of production and season in Kenya.
4. To assess the variations in caffeine and flavan-3-ols in clone TRFK 6/8 with nitrogenous fertilizer rates in Kenya, Tanzania and Rwanda.

1.5 Null hypotheses

i. The set leaf nutrients diagnostic limits for seedling tea are not suitable for clones in different locations in Kenya, and leaf age does not influence chemical nutrients levels.

ii. Seedling tea based mature leaf diagnostic limits are not suitable for clone TRFK 6/8 in the Eastern African tea growing regions. Nitrogenous fertilizer rates and plucking intervals have no effect on mature leaf nutrients contents of the clone.

iii. The levels of caffeine and flavan-3-ols in clones receiving uniform agronomic practices in Kenya do not vary with geographical area of production and season.

iv. The levels of caffeine and flavan-3-ols in clone TRFK 6/8 in Eastern Africa tea growing regions do not vary with nitrogenous fertilizer rates.

If the null hypotheses are not significant, the alternative hypotheses shall be adopted.

1.6 Justification of the study

Assessment of the variations of levels of leaf nutrients in the genotypes can help in the development of relevant leaf age and leaf nutrients limits for nutrients diagnosis that would aid in setting fertilizer use recommendations for the clones in different locations. This would lead to fertilizer use efficiency that lowers the high production costs while increasing yields and improving plain black tea quality thus reducing poverty in tea growing areas. It would also conserve the environment by eliminating inappropriate application of fertilizers that cause soil quality degradation (Othieno *et al*., 2000; Kamau *et al.*, 2008). Variations of levels of mature leaf nutrients with tender shoot (2 leaves and a bud) plucking interval will help to establish if mature leaf nutrients are stable to plucking interval and therefore foliar nutrients diagnosis using this leaf can be done at any plucking interval. Otherwise, there would be need to determine appropriate plucking interval for mature leaf for nutrients management in tea.

Investigation of the variations of plain black tea quality precursors in different genotypes and locations will provide a practical method of selecting suitable cultivars for specific areas to ensure the quality potentials for clones is not compromised, while the effect of nitrogenous fertilizer rates on clone TRFK 6/8 quality precursors will help in setting region specific nitrogenous fertilizer rates that optimize plain black tea quality in the clone. This will make Eastern African plain black tea more competitive in the global market and increase foreign exchange earnings for the countries.

1.7 Study limitations

- Sampling was delayed due to hailstorm in some sites and prolonged drought in others.
- Sporadic rainfall incidences were realized in seasons generally considered dry.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Botany of tea

Tea (*Camellia sinensis*, (L) O. Kuntze) is an evergreen plant in the *Theaceae* family (Bokuchava and Skobeleva, 1969; Hara *et al.*, 1995). Three commercial varieties of *Camellia sinensis* are:- the China type (*Camellia sinensis* variety *sinensis*), the Assam type (*Camellia sinensis* variety *assamica*) and the hybrid (*Camellia sinensis* variety *assamica ssp lasiocalyx* (Barnejee, 1992). However, two new varieties *Camellia sinensis* variety *pumbilimba* and *Camellia sinensis* variety *kucha* have been identified in China for commercial exploitation (Yao *et al.*, 2008). Tea plants grow in latitudes ranging from 45^{o}N (Russia) to 30^{o}S (South Africa), and longitudes ranging from 150^{o}E (New Guinea) to 60^{o}W (Argentina) (Shoubo, 1989) and altitudes ranging from sea level in Japan to 2,700 m above mean sea level (amsl) in Olenguruone, Kenya and Gisovu, Rwanda (Owuor *et al.*, 2008a). The wide range in longitudes latitudes and altitudes indicate that the plant is highly adaptable to various environments.

Tea trees can attain a height of up to 20-30 m and can have a very long lifespan. Some trees more than 1500 years old are still thriving in their original forests of Yunnan Province in the south-western China (Hara *et al.*, 1995). The plant is maintained as an evergreen shrub by regular pruning (Hara *et al.*, 1995). Closer to the equator, tea leaves are harvested all year round while further away from the equator, harvesting is seasonal (Hara *et al.*, 1995). The variations in the environment and growing conditions cause large differences in growth that are reflected in yields (Uddin *et al.*, 2005), plain black tea quality (Owuor, 1992; Owuor and Kwach, 2012) and fatty acids (Okal *et al*., 2012a, 2012b). It is however not known how variations in growing conditions influence the leaf nutrients levels and plain black tea quality precursors.

In Kenya, tea is grown on the foothills of Aberdare ranges and Mount Kenya in the East of the Great Rift Valley and the Mau Ranges, Nandi, Kisii and Kakamega Hills in the West of the Great Rift Valley at altitudes ranging from 1300 m to 2700 m amsl (Anon, 2005). Smallholder tea sector in Kenya has grown over the years and now accounts for over 61% of the total production (Figure 1) and own about 81% of land under tea (Figure 2), planted with high yielding clonal tea (Wachira, 2002) but leaf nutrients diagnostic norms being used on these clonal tea were set for seedling tea. The productivity of these smallholder farmers is, however, only about 50% that realised by the estate sector (Figure 3) (Ogola and Kibiku, 2004), probably due to inefficient management (Othieno, 1994) and inefficient fertilizer use (Anon, 2002; Othieno, 1988). It is therefore necessary to establish clonal specific nutrients deficiency diagnostic norms. This would guide fertilizer use efficiency in order to boost production and quality while lowering production costs, since fertilizer application is the second most expensive agronomic input after harvesting (Bonheure and Willson, 1992).

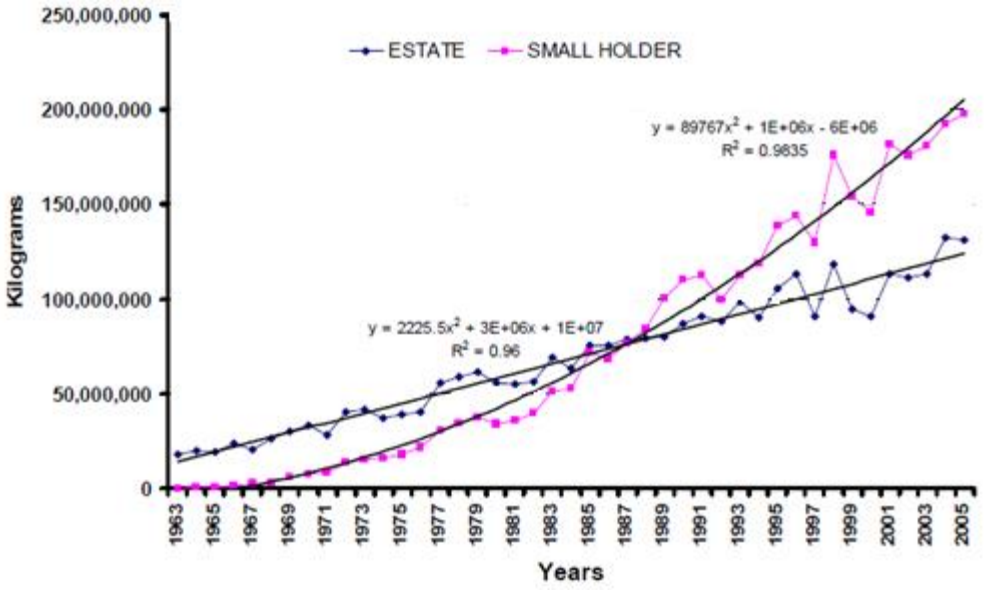


Figure 1: Tea production in the smallholder and the larger estate sub-sectors in Kenya 1963 - 2005.
Source: Tea Board of Kenya Records

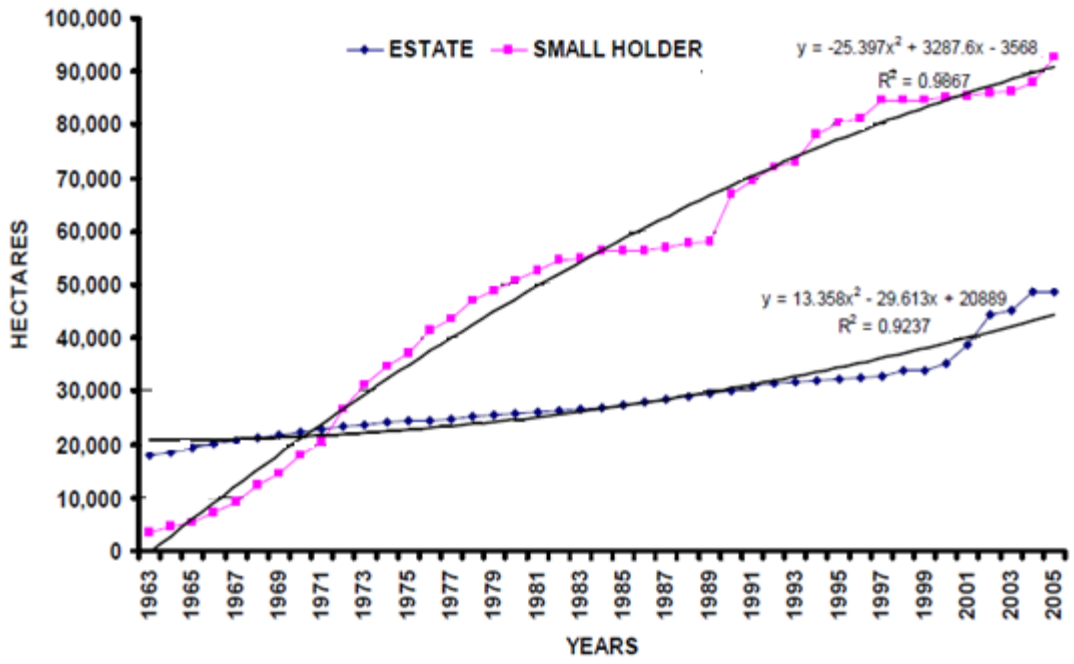


Figure 2: Area under tea production in the smallholder and the large estate sub-sectors in Kenya 1963 - 2005.
Source: Tea Board of Kenya Records

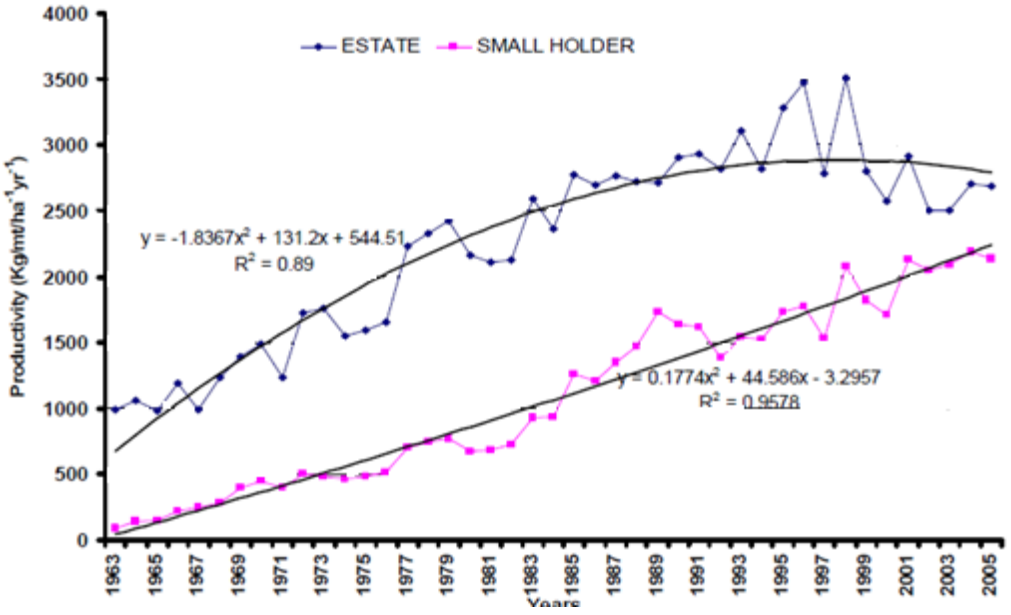


Figure 3: Tea productivity performance in the smallholder and the large estate sub-sectors in Kenya 1963 - 2005.
Source: Tea Board of Kenya Records

In Eastern Africa, tea growing areas fall in several agro-ecological regions, differing widely in elevation and climatic factors but with favourable soil conditions. For example, in Tanzania tea is grown in the districts of Mufindi, Rungwe, Njombe, Muheza, Korogwe, Lushoto, Bukoba, Muleba, and has lately been introduced in Tarime at altitudes ranging between 1200 m to 1900 m amsl (TRIT, 2006). In Kenya, it is grown on the foothills of Aberdare ranges and Mount Kenya in the East of the Great Rift Valley and the Mau ranges, Nandi, Kisii and Kakamega Hills in the West of the Great Rift Valley at altitudes ranging from 1300 m to 2700 m amsl, while in Rwanda, tea is grown at altitudes between 1800 m to 2700 m amsl (Owuor *et al*., 2008a). Clone TRFK 6/8 is widely grown in the region. It constitutes about 80% of Rwanda tea, 60% of Kenya clonal tea and 35-40% of Tanzania tea (Owuor *et al*., 2011a). Despite the differences in elevation and climatic factors in the tea growing areas, agronomic inputs are mainly uniform throughout the region (Owuor *et al*., 2011a). For instance, nutrients diagnosis is based on mature leaf with recommended nitrogenous fertilizer rates varying between 100 and 250 kg N ha^{-1} $year^{-1}$ (Anon, 2002). Most of these agronomic recommendations were seedling tea based and adopted from TRIEA withuto re-testing for appropriateness in clones in other tea growing areas. The uniform agronomic recommendations currently used in tea production in the Lake Victoria basin may be inappropriate in some regions. There is need to evaluate if clone TRFK 6/8 nutrients uptake is influenced by area of production in Eastern Africa and if mature leaf is stable for nutrients diagnosis in the whole region.

2.2 Tea nutrition

Green tea leaf minerals include both micro and macro elements that accumulate in the plant. The minerals are responsible for changes in the state of colloids in plant cell and directly affect cell metabolism in green tea leaf (Bokuchava and Skobeleva, 1969). In many cases

minerals function as catalysts of biochemical reactions. They are involved in changes in protoplasm turgor and permeability, are often the centres of electrical phenomena in tea. Mineral elements make up 4-5% of dry matter of the fresh tea leaf (Bokuchava and Skobeleva, 1969).

The tea nutrients are mainly supplied from the soil via organic or inorganic inputs (Willson, 1969; Bonheure and Willson, 1992; Kamau *et al.*, 2005). The nutrients in tea soils diminish with continuous cropping and harvesting, as harvesting tea removes high quantities of nutrients in the crop leaf (Owuor *et al*., 1997). The macro nutrients removed from tea plantations via harvesting are nitrogen (N), phosphorus (P), and potassium (K). The other macro nutrients removed from the tea plantations are sulphur (S), magnesium (Mg), and calcium (Ca), and the micro-nutrients iron (Fe), manganese (Mn), boron (B), copper (Cu) and zinc (Zn) (Bonheure and Willson, 1992; Kamau *et al.*, 2005). In addition, high nutrient leaching (Owuor *et al*., 1997) and surface run-off (Othieno, 1988) in the high rainfall areas where tea is grown also reduce nutrients availability in tea soils. The nutrients have a variety of functions in the tea plant that affect either yield or quality. To ensure and sustain high yields and quality, these nutrients should be monitored regularly and if necessary appropriate and timely replenishment done through fertilizer application. While it is known that excessive use of fertilizers may lead to low quality plain black teas (Owuor *et al*., 1987b, 1991, 1997, 2010a), reduce yields (Owuor *et al.*, 1997, 2008b), increase unsaturated fatty acid levels (Okal *et al*., 2012a, 2012b) and degrade soil quality (Othieno *et al*., 2000; Kamau *et al*., 2008), it is not known how the uptake of nutrients by clones and levels of plain black tea quality precursors vary in tea shoots with geographical location of production. Similarly, how leaf nutrients and quality precursors of a single clone in different geographical locations of Eastern Africa would respond to different nitrogenous fertilizer rates has not been determined.

Previous studies have demonstrated that plucking intervals affect tea yield and black tea quality. Short plucking intervals increased both yields (Odhiambo, 1989; Owuor *et al*., 2009, 2013a; Owuor and Kwach, 2012) and black tea quality (Barua *et al*., 1986; Owuor *et al*., 1990, 1997, 2000, 2009; Owuor and Odhiambo, 1993, 1994). Short plucking intervals increased fatty acid levels (Okal *et al*., 2012a, Owuor *et al*., 2013b), however, it had no effect on soil chemical properties (Kamau *et al*., 2008). No study has been done to establish if plucking interval of tender shoots (2 leaves and a bud), for plain black tea manufacture, causes variations in nutrients levels of clonal tea mature leaf (used for nutrients management) in Eastern Africa and if the variation is specific to geographical area of production.

2.3 Benefits of nutrients to tea

Nitrogen, phosphorus and potassium are the most critical nutrients in the fertilization programme of tea (Cloughley, 1983; Wanyoko, 1983, 1988; Bonheure and Willson, 1992; Kamau *et al*., 2008; Owuor *et al*., 2008b). Nitrogen constitutes 2–4 percent dry matter of plants (Roy *et al*., 2006) and the highest content is in young harvestable tea shoots (Dang, 2005). Plants absorb nitrogen either as the nitrate ion (NO_3^-) or the ammonium ion (NH_4^+) (Roy *et al*., 2006). Nitrogen is part of chlorophyll (the green pigment in leaves) and is an essential constituent of all proteins, nucleotide, hormones, protoplasm, vitamins etc (Wickremasinghe and Krishnapillai, 1986; Roy *et al*., 2006). The commercial portion of tea crop is the leaf. Tea production is highly responsive to application of nitrogen fertilizer as it induces more vegetative growth thus increasing leaf yield (Wickremasinghe and Krishnapillai, 1986). Nitrogen nutrition in tea affect the levels of amino acids (Liang *et al.*, 1990), fatty acids (Owuor *et al*., 1990c, 2013b; Okal *et al*., 2012b), plant pigments (Wicknemasinhe and Perera, 1966), plain black tea quality parameters (Iwasa, 1977; Owuor *et al.*, 1991b, 1997, 2000, 2009) and volatile flavour

compounds composition (Owuor *et al.*, 1987c, 2000). Nitrogen deficiency in plants results in a marked reduction in growth rate reducing crop yield (Obaga and Othieno, 1987; Roy *et al*., 2006). Substantial quantity of nitrogen is continuously lost due to harvesting, removal of twigs, leaching, surface run-off and volatilization under conditions of high rainfall and temperature where tea is grown (Chaudhry, 1985). Response of tea crop to nitrogen is dependent on adequate availability of other nutrients, and therefore may vary from area to area and from country to country. However, very high levels of nitrogen fertilizer application are uneconomical and may become negative (Wanyoko, 1983; Bonheure and Willson, 1992). The nitrogenous fertilizer use on tea varies from country to country (Bonheure and Willson, 1992; Owuor and Wanyoko, 1996). The lowest fertilizer use per hectare per year is in Vietnam at 36 to 40 kg N, while highest is in Japan at 800 kg N. Despite these variations, tea yields were generally below 2000 kg made tea (mt) per hectare per year in most of the tea producing countries in the 1980s. In Kenya, tea responded economically to increasing nitrogen fertilizer rates up to about 500 kg N/ha/yr but became less profitable at rates above 150-200 kg N/ha/yr (Owuor, 1985).

Increasing nitrogen fertilizer application rate impairs black tea quality (Owuor *et al*., 1987d, 1997, 2010; 2013a; Owuor and Odhiambo, 1994; Okal *et al*., 2012b; Owuor, 2001). The plain quality of black tea is largely determined by the concentration of theaflavins (TFs) and thearubigins (TRs) that are oxidation products of catechins (Biswas *et al*., 1973; Hazarika *et al*., 1984; McDowell *et al*., 1995). High rates of nitrogen have been shown to lower tha black tea quality (Venkatesan and Ganapathy, 2004) by lowering theaflavins levels (Cloughley, 1983; Cloughley *et al*., 1983; Owuor *et al*., 1997, 2000). Indeed flavanol composition in tea leaves influence theaflavins formation (Hilton *et al*., 1973). However, previous results regarding the effect of nitrogen fertilization on the concentrations of catechins and their oxidation derivatives (TFs and TRs) are elusive. Hilton *et al* (1973) showed that nitrogen fertilization diminished (-)-

EGC, (-)-EC in young shoots, whereas it either increased or decreased (-)-EGCG concentration. In contrast, another study showed that total polyphenol concentration is increased by nitrogen fertilization (Venkatesan and Ganapathy, 2004). In other experiments, it decreased (Hilton *et al*., 1973; Cloughley, 1983; Owuor and Odhiambo, 1994), did not change (Owuor *et al*., 1991b; 2000) or even increased (Owuor *et al*., 1987d) theaflavins and thearubigins concentrations. In South Africa, total chlorophyll content increased with increasing nitrogenous fertilizer rate up to 200 kg N/ha, theaflavin content decreased with increasing nitrogenous fertilizer rate with the largest drop being at 200 kg N/ha, while the total colour of the infusion followed an almost identical pattern to theaflavin content. Black tea quality consequently deteriorated with increasing nitrogenous fertilizer rate and was described as 'grassy' for the highest nitrogen rates (Lelyveld *et al*., 1990). In Central Africa, increasing nitrogen application rates and harvesting older shoots (shoots older than two leaves and a bud) adversely reduced black tea liquor quality as assessed by theaflavin analysis, and the value of the product as assessed by tea tasters' sensory examination (Cloughley, 1983). The information concerning the effect of nitrogen fertilization on aroma property of tea is scanty. Experiments showed that concentrations of aldehyde, geraniol, cis-furanoid oxide of linalool, methyl ester of salicylic acid and dihydroactinidiolide were increased, while those of linalool, pentanol α-terpineol, beta-nerolidol and some unidentified compounds were decreased by nitrogen fertilization (Tsanava *et al*., 1991). Application of nitrogen fertilizers was found to increase fatty acid contents, leading to high level of undesirable aroma in black tea (Owuor *et al*., 1987d, 2013b; Owuor and Odhiambo, 1994; Okal *et al*., 2012b). It is not known how the plain black tea precursors, especially flavan-3-ols and caffeine vary with geographical area of production particularly at varying nitrogenous fertilizer rates.

Previously, variations in tea yields (Han *et al*., 2008) and plain black tea quality (Venkatesan *et al*., 2003; Venkatesan and Ganapathy, 2004) among tea cultivars have been shown with increase in nitrogenous fertilizer. Increasing nitrogen fertilizer rate in combination with rise in tea base temperature increased yield by increasing shoot population (Obaga and Ng'etich 1989). However, excessive use of nitrogenous fertilizer reduced the quality of plain black tea (Cloughley, 1983; Owuor and Othieno, 1996; Owuor and Wanyoko, 1996). There was a decline in palin black tea total colour with increase in nitrogen rate (Owuor *et al*., 1997, 2000). Also there was increase of caffeine and decrease of flavour index with higher rates of nitrogenous fertilizers (Owuor *et al*., 1997, 2000). The extent of these variations were however dependent on the genotypes (Owuor and Othieno 1996; Owuor and Wanyoko 1996) and the environment (Owuor *et al*., 2010b). But, high rates of nitrogen did not increase tea yields economically when applied beyond 300 kg N ha^{-1} $year^{-1}$ and reduced plain black tea quality (Owuor *et al*., 1997, 2000; Venkatesan *et al*., 2004; Venkatesan and Ganapathy 2004). The yields and quality of plain black tea showed dissimilar response patterns to increased nitrogen fertilizer rate, suggesting the importance of optimizing the two parameters (Venkatesan *et al*., 2004) for economic production. Excessive use of nitrogenous fertilizers degrade soil quality by increasing soil acidity and leaching of base nutrients, especially extractable calcium and magnesium (Kamau *et al*., 2008). Recommendations on fertilizer composition and rates are usually based on the ratio and amounts of nutrients removed (Dang, 2005).

Tea soil nitrogen replenishment is usually through application of nitrogenous fertilizers. The recommended rates of nitrogen fertilizer application for mature tea vary from country to country (Bonheure and Willson, 1992). The general fertilizer recommendation for tea soils of Indonesia having yield less than 4000 kg/ ha made tea is 360 kg N/ha/yr (Wibowo, 1994), in Sri Lanka, the recommended nitrogen rate is 160 kg N/ha/yr (Kemmler, 1986), while in Central

Africa, it is 165 kg N/ha/yr for high yielding areas (Kemmler, 1986). In Kenya the rate varies between 100 and 250 kg N/ha/year depending on yield performance (Othieno, 1988; Ruto *et al*., 1994). However, some farmers believe that the yield response is limitless and thus apply more than the recommended rates. Although it was thought that a high yielding clone like HP S15/10, which has yielded up to10995 kg made tea (mt)/ha/year under commercial estate practice in Kenya (Oyamo, 1992) might require more than the recommended rates of nitrogen (Othieno, 1988), recently it was demonstrated using this clone that there was no significant yield response beyond 200 kg N/ha/year which was also the most profitable rate of nitrogen fertilizer application (Owuor *et al*., 1997, 2008b). However, rates upto 300 kg N/ha/year are considered a normal practice for high yielding clones (Owuor *et al*., 2008b). Though different clones with varying yield potentials are grown across different locations in Kenya, the recommended nitrogen fertilizer rate of 150 kg N/ha/year (Othieno, 1988; Anon, 2002) is in use for all the the tea plantations in most locations, especially in smallholder tea sector with the assumption that wherever the tea plants grow, the rate would be ideal for optimal yields and plain black tea quality. However, when planted in one field, different tea genotypes had varying ability to extract nutrients from the soil resulting in variations in leaf nutrients content of different clones grown at the same site (Wanyoko and Njuguna 1983; Nyirenda 1991). The levels of nutrients in different parts of tea bushes also varied (Ruan *et al*., 2003; Dang 2005; Kamau 2008). Even a single clone grown in different regions but receiving same agronomic inputs had varying leaf nutrients contents (Kamau *et al*., 2005). This suggests that nutrients removal from the soil by tea plant is dependent on both genotypes and location of production which could lead to variations in amounts of fertilizer needed to optimise yields and quality. Indeed, yield (Owuor *et al*., 2009, 2010a, 2011a, 2011b; Msomba *et al*., 2011) and plain black tea quality (Owuor *et al*., 2009, 2010a, 2011b) variations have been reported for a single clone grown in different regions but

receiving same agronomic inputs. For both yield and quality, responses to nitrogen varied with geographical area of production and areas with good response to nitrogen suffered more in quality decline due to high rates of nitrogen (Owuor *et al*., 2010a). In one genotype, yield and quality responses patterns to nitrogenous fertilizers varied from one location to the other (Owuor *et al*., 2010a) and the variations in yields and black tea quality are likely to be larger further from the equator since the environmental conditions controlling growth are more variable (Owuor *et al*., 2010a). The variations in black tea quality between same clone grown in Kenya and in Malawi were attributed to the growing conditions (Owuor *et al*., 2006) which are largely uncontrollable in tea production, tea being a rain-fed crop. Whereas cross border collaboration may be useful, the Eastern African countries of Uganda, Tanzania, Rwanda and Burundi have largely used agronomic production technologies developed in Kenya, without further testing for appropriateness and these technologies may not be relevant for the new production areas. It is also not known how quality precursor compounds variy with genotypes and nitrogenous fertilizer application rates in different locations of production within Eastern Africa.

Phosphorus is much less abundant in plants compared to nitrogen and potassium, having a concentration of about one-fifth to one-tenth that of nitrogen in plant dry matter (Roy *et al*., 2006). But phosphorus plays a major role in the growth of tea plants, especially in the form of new shoots and roots. It is necessary for the transformation of energy, takes part in the metabolism of fats and is involved in the utilization of nitrogen (Bonheure and Willson, 1992). In the tea leaf, phosphorus is found in both organic and inorganic forms, with main organic compounds being phytin, hexosomonophosphate, and hexosodiphosphate, while inorganic phosphorus is mainly orthophosphoric acid derivatives (Bokuchava and Skobeleva, 1969). Phosphorus is very mobile within the plants and migrates to young leaves where photosynthetic activity is highest (Wickremasinghe and Krishnapillai, 1986). The availability of phosphorus in

the soil is at highest when the soil has a pH between 5.5 and 7.0 and declines rapidly as the soil pH falls below 5.5 or rises above 7.0 (Bhattacharya and Dey, 1983). In a very acidic soil, phosphorus is combined with hydroxides of iron and aluminium to form complexes which are insoluble in water making phosphorus unavailable to the plant (Bhattacharya and Dey, 1983). In Sri-Lanka, phosphorus uptake by tea plants was found to be influenced by genotype (Zoysa *et al*., 1999). Application of high rates of nitrogenous fertilizer reduced mature leaf phosphorus (Owuor *et al*., 1990f; 2011) for clonal tea in Kenya. The use of the NH_4^+ form of fertiliser increased acidification in tea rhizosphere compared with bulk soil and this enhanced the effectiveness of phosphate rock fertiliser utilisation by tea plants (Zoysa *et al*., 1998a, 1998b). Increase in phosphate fertilizer application rate increased tea yield and improved black tea quality in Japan (Salukvadze, 1980). Highest plain black tea quality with respect to total colour and percent brightness were obtained with annual phosphorus rate of 60 kg P_20_5 /ha (Sharma *et al*., 2005) in India. In South Africa, regardless of season, application of phosphorus fertilizers increased quadratically the total polyphenols in bush tea, with most of the increase occurring between 300 $kgha^{-1}yr^{-1}$. Linear relationships between leaf phosphorus with total polyphenols in tea were also observed (Mogotlane, 2007; Mudau, 2007) implying that phosphorus influences plain black tea quality. In Eastern Africa most experiments done showed no response by mature tea leaf to phosphate application. However, linear response to phosphate application has also been reported in the region (Anon, 1965), particularly on the typical acid tea soils when there is an undisturbed mulch layer on the soil surface (Willson, 1975d). But in Kenya, there was no plain black tea quality response to phosphatic fertilizer application (Owuor *et al*., 1998). The remedial phosphorus application to tea soils in Eastern Africa is based on recommendations drawn from seedling tea. It is not known if these recommendations are relevant in the whole

region since most Kenyan tea (Wachira, 2002) and tea in Eastern Africa (Msomba *et al*., 2011; Owuor *et al*., 2011a) are now mainly clonal.

The major constituent in the ash of young tea leaves is potassium, which reaches 50% of the total ash constituent. Potassium plays a major role in triggering the growth of young tissues and for maintenance of an optimum turgor needed for cell elongation and cell division (Ranganathan and Natesan, 1985). Potassium regulates water usage of the plant particularly in the process of absorption and transpiration (Lacaille, 1966). The status of potassium in tea plants can change rapidly because of the high rate of removal from the crop through harvesting, (Willson, 1975c). When NPK fertilizer is used, potassium leaching is triggered by excess ammonium ions in the fertilizer (Owuor *et al.,* 1987a). The level of availability of potassium in most tea soils is low because of their acidity (Willson, 1975c). In Eastern Africa responses to mature leaf potassium increased with potash application only when the pH was below 5.2 (Willson, 1975c). When applied as NPK formulation, there was decline in leaf potash with rise in rates of the fertilizer (Owuor *et al*., 2011a). Continuous tea cropping without potash fertilization leads to low yields and ultimately death of the plants (Willson, 1975c). Tea yield responses to potassium application were reported from various countries (Godziashvili and Peterburgsky 1985; Rahman and Jain, 1985; Krishnapillai and Ediriweera, 1986; Malenga and Grice, 1991; Sharma and Sharma, 1995). Yield increases by application of potassium-fertilizers were reported in various areas of China producing different types of teas (Wu and Ruan, 1994). Responses of yield to potassium fertilization however relate to availability of potassium in soils, which explains examples where no yield response was obtained (Owuor et al., 1988; Kamau *et al*. 1999) in Kenya, possibly because of high potassium supplying capacity in soil. In addition to yield response, the quality of tea was improved due to increase of total free amino acid in green tea and theaflavins and thearubigins in black tea (Ruan *et al*., 1998; 1999; Venkatesan and

Ganapathy, 2004). However, in Kenya, there was no quality response to potassium fertilization (Owuor *et al*., 1998). Potash tea soil remediation in Eastern Africa is based on seedling tea without reevaluation in clonal tea and the new regions on the effects on quality precursors or if location of production has influence on leaf potassium levels.

The other essential macronutrients for tea are calcium (Willson, 1975a) and magnesium (Willson, 1975b). Calcium ranks with magnesium, phosphorus and sulphur in the group of least abundant macronutrients in plants. Calcium influences the water economy, synthesis of proteins, and neutralization of acids, root development and absorption of nitrogen (Willson, 1975a). It is also required for the growth of apical meristems. Calcium forms part of the structure of cell walls (calcium pectate) and cannot be replaced by other bases. In Eastern Africa, calcium applied to the soil was deleterious to the yield of mature tea or growth of the young plant (Willson, 1975a). However, calcium uptake by tea plant is third to nitrogen and potassium (Othieno, 1992). A considerable amount of calcium is therefore required by tea plant. On the average, between 10 to 20 kg calcium is removed annually through harvesting fields yielding 2000 kg made tea ha^{-1} $year^{-1}$ (Othieno, 1992). Calcium lowers quality of plain black tea by lowering solubility of polyphenols and increasing cream formation (Jobstl *et al.*, 2005). Calcium deficiency is characterized by brittle old leaves covered with discoloured areas at the edge of the lamina, which then become dark brown in colour (Willson, 1975a; Roy *et al*., 2006). No work has been done to establish the effects of location of production on leaf calcium of clonal teas in Eastern Africa.

Magnesium is third, after nitrogen and potassium, in terms of importance for economic growth of the tea plant (Willson, 1975c). Magnesium occupies the centre-spot in the chlorophyll molecule and, thus, is vital for photosynthesis. It is the only mineral constituent of the chlorophyll molecule that regulates photosynthesis (Willson, 1975c). It also acts as an activator

of many enzyme systems involved in carbohydrate metabolism and synthesis of nucleic acids and in translocation of sugars (Bonheure and Willson, 1992). There is synergism, in availability to plants, between magnesium and phosphorus and antagonism between magnesium and potassium (Wickremasinghe and Krishnapillai, 1986) and high level of application of potassium along with nitrogen fertilizer reinforce this antagonism leading to magnesium deficiency as reported in many parts of South Indian tea fields (Jayaganesh *et al*., 2011). The Magnesium deficiency is characterized by the yellowing of old leaves and an inverted **"V"** shaped yellow tint between the veins and premature leaf fall from the affected bushes (Othieno, 1992). Application of magnesium sulphate is recommended (Vankatesan, 2006) for high yielding tea fields in South India as the magnesium content of most of the tea soils are generally low to medium and not adequate to maintain high productivity of tea in the area. Application of magnesium sulphate along with nitrogen and potassium application increased yield and quality constituents of made tea in South India (Jayaganesh *et al*., 2011). In Eastern African tea growing regions, the effect of locations of production on mature leaf magnesium has not been established.

The essential micronutrients for tea include manganese, iron, copper and zinc (Bonheure and Willson, 1992). Manganese activates several enzymes and functions as an auto-catalyst. It is essential for splitting the water molecule during photosynthesis (Roy *et al*., 2006). High manganese inhibit tea polyphenol levels but increase total amino acids while low manganese levels increase yield of tea leaves (Gohain *et al*., 2001), implying that low manganese levels may help to improve yield and quality of plain black tea. It is not known how mature leaf manganese varies with location of production in the Eastern Africa tea growing regions. The content of iron in the tea plant is limited though it plays a significant role, being part of the enzyme peroxidase which is involved in oxidoreductive processes (Bokuchava and Skobeleva, 1969). It plays a role in the synthesis of chlorophyll, carbohydrate production, cell respiration, and in nitrogen

assimilation (Roy *et al*., 2006), hence would influence yields and quality of plain black tea. Polyphenols content of tea leaves increased with addition of smaller amounts of iron but a sharp decline was noted with higher iron dosage (Kuzhandaivel and Venkatesan, 2011). No data has been generated in Eastern Africa to show how clonal leaf iron changes with location of production. Copper is involved in chlorophyll formation and is a part of several enzymes (Roy *et al*., 2006). Polyphenol oxidase, responsible for black tea fermentation, belongs to a family of copper-containing oxidoreductases that catalyse the oxidation of mono- and *o*-diphenols to *o*-diquinones (Takino *et al.*, 1964; Bonheure and Willson 1992). Teas grown on copper deficient soils do not ferment (Harler, 1971; Clowes and Mitini-Nkhoma, 1987). Application of copper and zinc are necessary to improve fermentation process in black tea (Sedaghathoor *et al*., 2009). Application of copper sulphate at summer especially on drought years can improve tea quality (Sedaghathoor and Bagheri, 2009). It has not been established how leaf copper levels vary with location of production within the Eastern African tea growing region. Zinc is required directly or indirectly by several enzymes systems, auxins and affects formation of flavan-3-ols (Iwasa, 1977) which are plain black tea quality precursor compounds. Zinc deficiency has been found to reduce the growth of tea and was first recognized in Sri Lanka but has now been confirmed in other parts of the world (Tolhurst, 1973). Its deficiency is corrected by foliar application of zinc oxide (Othieno, 1992). Zinc has been shown to increase the yield of mature and semi-mature tea (Barua and Dutta, 1972; Dootson, 1974; Malenga *et al*., 1982). Wang *et al*., (1993) reported improvements in both yield and quality with zinc application on young tea. In Kenya, annual tea yields increased with frequency of foliar application of zinc oxide (Wanyoko *et al*., 1992). No work has been done in Eastern Africa to establish variations of clonal tea leaf zinc with location of production.

2.4 Tea leaf nutrients as a diagnostic tool

Plant tissue analysis is considered a reliable technique to establish nutrients deficiencies and/or oversupply (Tolhurst, 1976; Othieno, 1988; TRFCA, 1990; Bonheure and Willson, 1992). Tea nutrition advisory service was initiated in East Africa tea growing region in 1968 (Willson and Hainsworth, 1968). This system based its recommendation on the results of soil analysis (Willson, 1969) and leaf analysis (Anon, 1971; Tolhurst, 1969, 1970, 1971a). Leaf analysis was found to be more precise in predicting the nutritional requirement of the tea bush (Tolhurst, 1971b) and the results could be used to recommend remedial fertilizer application (Tolhurst, 1971c), however, it was noted that the results could be affected by season and irrigation (Tolhurst, 1971d). Leaf sampling instructions were provided to ensure that uniform materials were analysed (Anon, 1972). The leaf analysis system was appraised in 1972 and subsequently recommended as a guide to fertilizer programmes (Owuor and Wanyoko, 1983; Tolhurst, 1976).

Different countries and regions use different types of leaves to predict the tea bush nutritional requirements (Bonheure and Willson, 1992). Thus while the mature leaf has been observed to be sensitive and a good predictor for most macronutrients deficiencies in Eastern Africa (Tolhurst, 1976; Othieno, 1988) and Central Africa (TRFCA, 1990), mature leaf was only sensitive predictor for phosphorus deficiency in Sri Lanka (Sivapalan *et al.,* 1986) and potassium deficiency in South India (UPASI, 1987). The younger second, third or fourth leaves (Figure 4) have been used in foliar analysis nutrients demand diagnosis in India, Indonesia, Taiwan and USSR (Ranganathan, 1998). For tea in Kenya, the use of mature leaf has been adopted by the advisory system to assess the problems in nutrients management (Tolhurst, 1976; Owuor and Wanyoko, 1983; Othieno, 1988) and the critical nutrients levels have been set (Table 1) (Owuor and Wanyoko, 1983). The limits (Table 1) were based on mature leaf of seedling tea.

For third leaf, limits outlined in Table 2 have been suggested to be ideal (Bonheure and Willson, 1992).

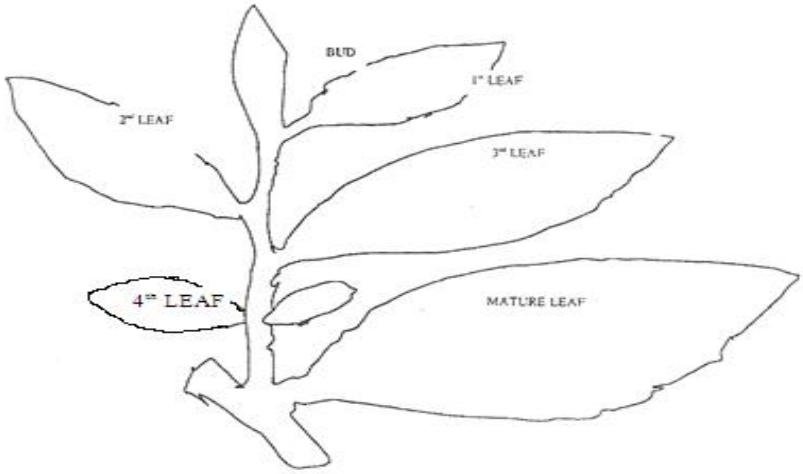


Figure 4: A sketch of the young (two leaves and a bud), third, fourth and mature leaves.

Table 1: Critical levels of nutrients in the mature tea leaf in East Africa

Nutrient	Deficient	Borderline	Adequate
Nitrogen	Below 3.00%	3.00 to 3.50%	Above 3.50%
Phosphorous	Below 0.15%	0.15 to 0.17%	Above 0.17%
Potassium	Below 1.20%	1.20 to 1.50%	Above 1.50%
Magnesium	Below 0.10%	0.10 to 0.13%	Above 0.13%
Zinc	Below 10 ppm		

Source: Owuor and Wanyoko, 1983

Table 2: Critical levels of nutrients in the third leaf of tea shoot

	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Mn (ppm)	Fe (ppm)	Zn (ppm)	Cu (ppm)
Deficient	3.00	0.35	1.60	0.05	0.05	50	60	20	20
Subnormal	4.00	0.40	2.00	0.10	0.10	100	100	25	15
Normal	5.00	0.50	3.00	0.35	0.30	5000	500	50	30

Source: Bonheure and Willson, 1992

In Eastern Africa, clone TRFK 6/8 constitutes 80% of Rwanda tea, 60% of Kenya clonal tea and 35-40% of Tanzania tea (Owuor *et al.*, 2011a). When grown in the same field under same nutrients regimes, the different clones varied in their abilities to extract nutrients from the soils and thus had different leaf nutrients levels (Wanyoko and Njuguna, 1983). Despite the differences in elevation, climatic and edaphic factors in the Eastern African tea growing regions, most of the agronomic recommendations being used in most of these regions have been adopted from TRIEA without re-testing for appropriateness in the new areas. Mature leaf nutrients norms (Othieno, 1988; Tolhurst, 1976) are one such recommendation. It is necessary to establish if the seedling tea mature leaf nutrients currently used in clonal tea are relevant for the cultivars, especially when grown in different tea growing regions. Use of third leaf for nutrients norms diagnosis has been successful in some countries especially India (Ranganathan, 1998). Indeed Bonheure and Willson (1992) suggested the use of third leaf and provided norms for the diagnosis in all teas (Table 2). However, it is not known if the third leaf being used in other countries may be more accurate for clonal tea in Kenya and if there is a relationship between nutrients levels in mature and third leaf. There is also need to evaluate if clone TRFK 6/8 nutrients uptake is influenced by area of production in Eastern Africa and if mature leaf is stable for nutrients diagnosis in the whole region.

2.5 Polyphenols in green tea leaf

Tea shoots contain a full complement of enzymes, biochemicals mainly secondary metabolites including polyphenols, carbohydrates, proteins and lipids. The production of polyphenolic constituents and other secondary metabolites in the tea shoots is assumed to be a means of chemical defense against insects, birds, and animals, which would consume the plant as food (Beart *et al.*, 1985). Tea shoot is distinguished by its remarkably high content of

polyphenols and methyl xanthines (caffeine and other purines, such as theobromine and theophylline) (Harbowy and Balentine, 1997).

Flavanols (catechins), flavonol glycosides, polyphenolic acids and depsides put together are referred to as total polyphenols and make up about 30% of the dry weight in a tea shoot (Harbowy and Balentine, 1997). Biosynthetic pathway of catechins in tea shoots is presented in Figure 5. The C_6 (A) catechin ring is produced by the acetic-malonic acid pathway and C_3-C_6 (B) ring is produced by the shikimic-cinnamic acid pathway starting from the glucose pool (Iwasa, 1977). Factors that affect production of glucose such as the amounts of available nutrients may influence the amounts and composition of green tea leaf polyphenolic compounds. (Iwasa, 1977).

Catechins in tea shoots include epicatechin (EC) **(1)**, catechin (C) **(2)**, epigallocatechin (EGC) **(3)**, gallocatechin (GC) **(4)**, epicatechin gallate (ECG) **(5)**, catechin gallate (CG) **(6)**, epigallocatechin gallate (EGCG) **(7)**, and gallocatechin gallate (GCG) **(8)** (Hilton *et al.*, 1975; Forrest and Bendall, 1969; Hara *et al.*, 1995). Two minor catechin digallates, epicatechin digallate (ECDG) **(9)** and epigallocatechin digallate (EGCDG) **(10)** (Coxon *et al.*, 1972; Nonaka *et al.*, 1983; Hashimoto *et al.*, 1987) have also been detected in tea. The major catechins are EGCG **(7)**, EGC **(3)**, ECG **(5)**, and EC **(1)** (Caffin *et al.*, 2004). Other catechins such as (+) C **(2)** and GC **(4)** are present in smaller quantities in tea, whereas the gallocatechin gallates GCG **(8)** and CG **(6)** found in tea may be products of racemisation and not "native" to the tea plant (Roberts, 1962). The methyl esters of ECG **(5)** and EGCG **(7)** have also been identified in tea (Zeeb *et al.*, 2000).

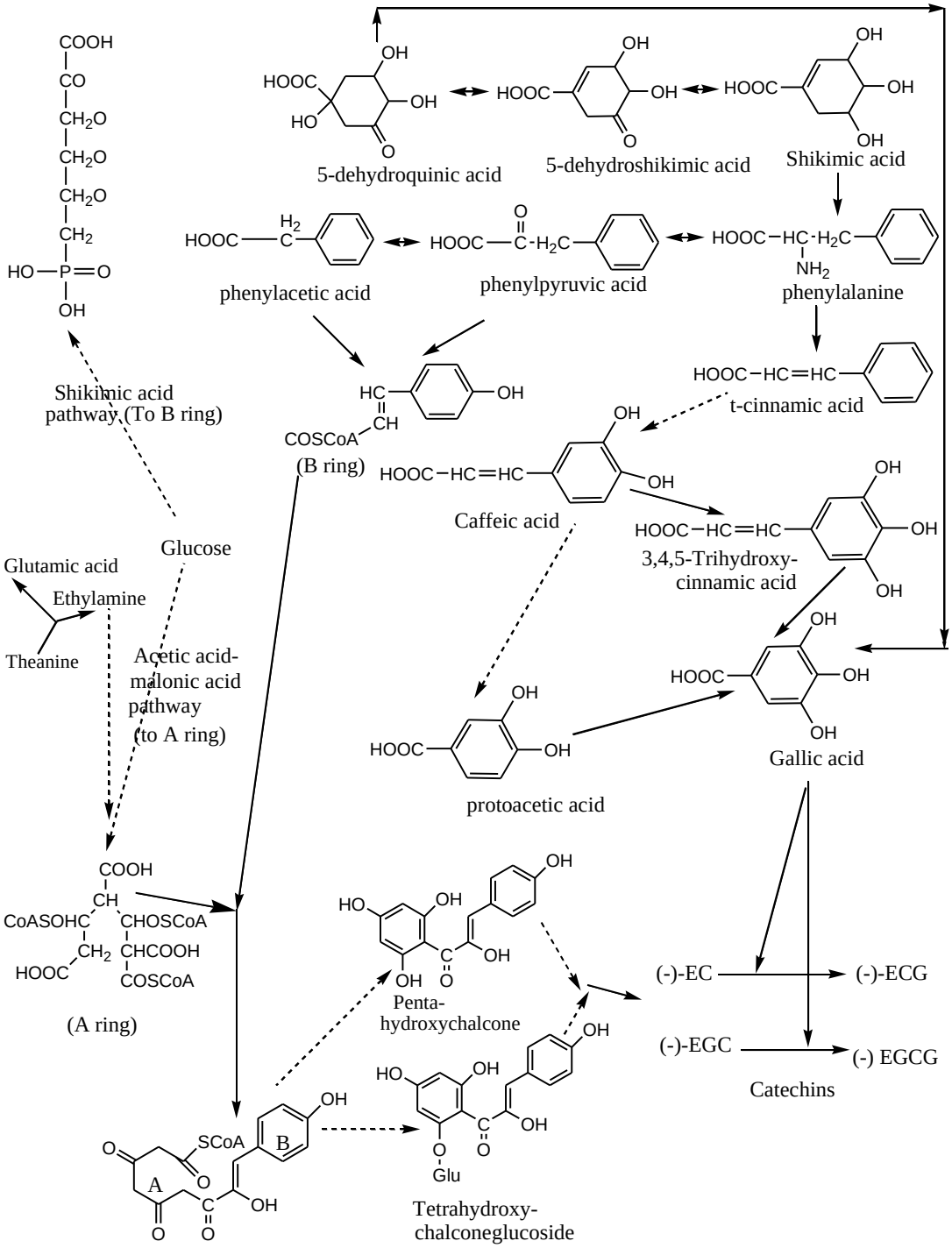
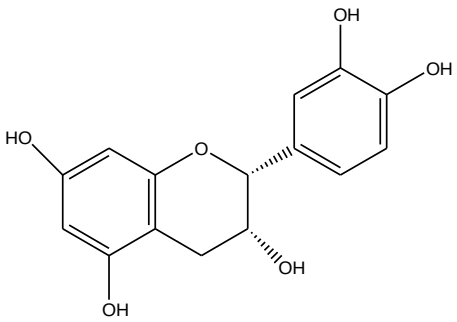


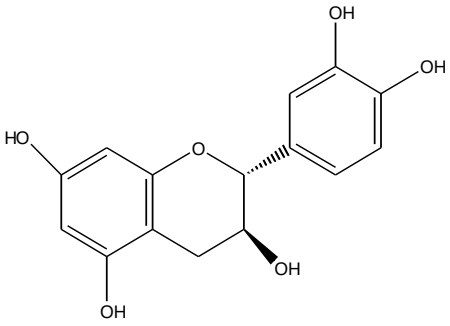
Figure 5: Biosynthetic pathway of catechins in tea leaves (Iwasa, 1977)

Major chemical reactions associated with the making of plain black tea, defined as tea whose quality is determined by colour and taste characteristics, occur during fermentation stage. In this stage, the leaves are macerated to break down subcellular compartments to allow cytoplasmic polyphenol oxidase (PPO)(EC 1.10.3.1) to oxidize the flavan-3-ols in the vacuoles (Wright *et al*., 2002). The main consequence of this enzymatic oxidation process (fermentation) is dimerization of the flavan-3-ol monomers to form theaflavins (TFs) and thearubigins (TRs) which determine plain black tea quality.

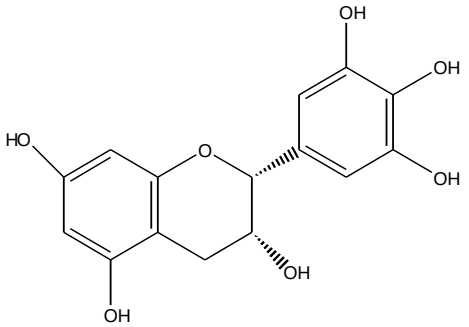
The levels of catechins depend on tea bush variety and rate of growth (Robertson, 1983b). The rate of growth is affected by parameters such as nitrogen fertilizer application, pruning and environmental factors. Large variations in quality of tea have been recorded due to changes in climate (Howard, 1978), environmental conditions (Gulati and Ravichranath, 1996) and geographical areas of production (Marcos *et al*., 1998; Fernandez *et al*., 2002; Moredo-Pineiro *et al*., 2003). Although it had been thought that large variations are necessary in these factors for quantifiable quality differences (Owuor *et al*., 1998), quality can vary even within Kenya, where tea grows almost uniformly throughout the year due to proximity to the equator.



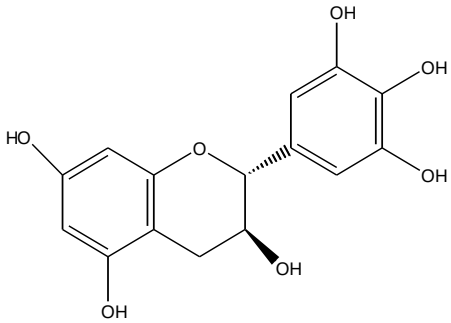
1; (-)-Epicatechin



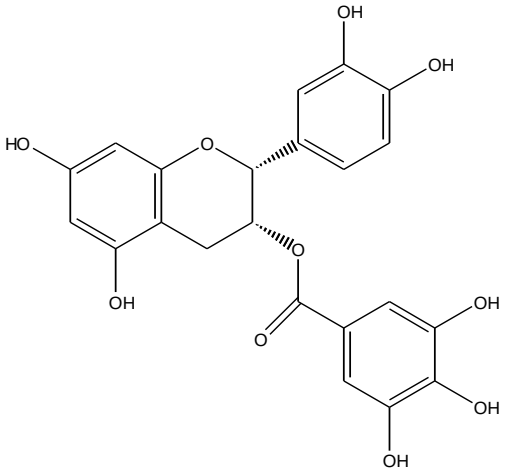
2; (+)-Catechin



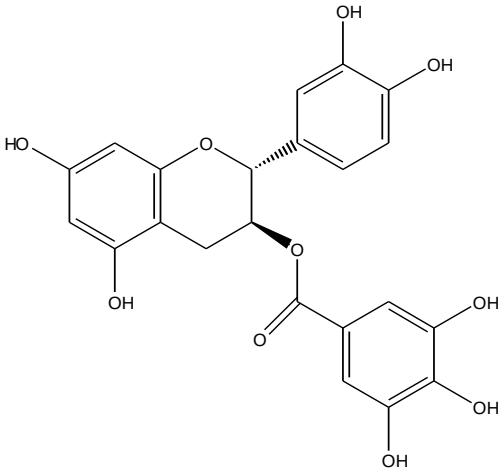
3; (-)-Epigallocatechin



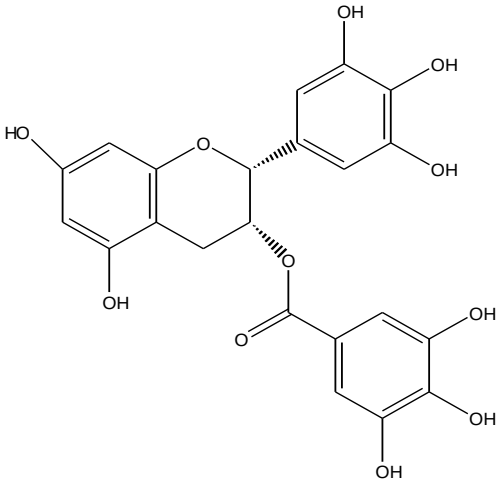
4; (-)-Gallocatechin



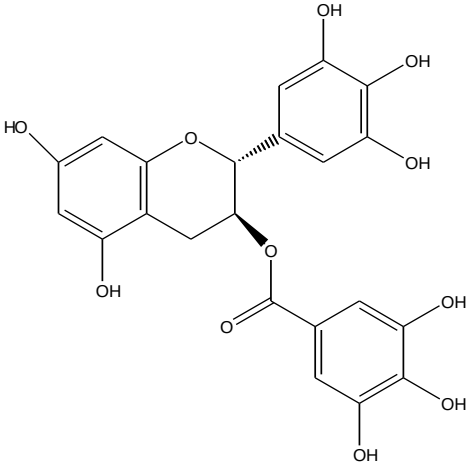
5; (-)-Epicatechin gallate



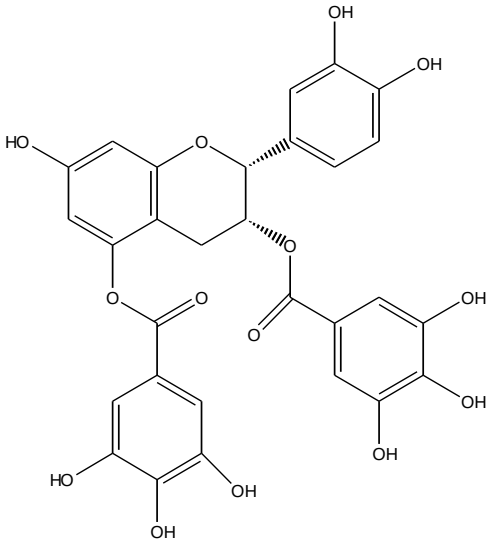
6; (-)-Catechin gallate



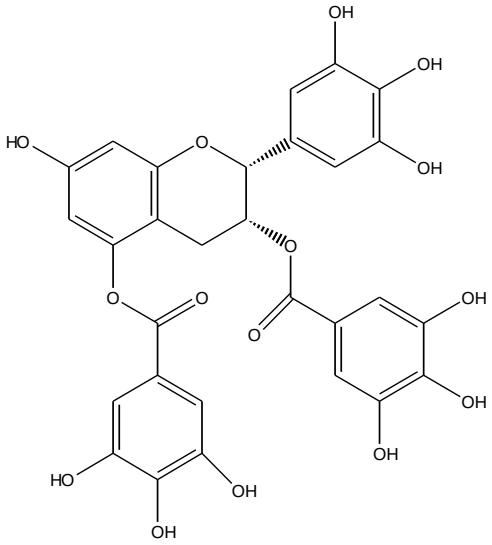
7; (-)-Epigallocatechin gallate



8; (-)-Gallocatechin gallate



9; (-)-Epicatechin digallate



10; (-)-Epigallocatechin digallate

Enzymes activity in tea leaf is influenced by fertilization management programme (Kanazawa *et al*., 2005). At high nitrogen rates of 450 kg N/ha/yr, both polyphenols and amino acid contents of green tea leaf increased with increase in potassium dose in South India (Vankatesan *et al*., 2004). Plucking standard has also been found to affect plain black tea quality. Coarse plucking standard (plucking leaves older than 2 leaves and a bud) reduces plain black tea quality (Owuor *et al*., 1987) by lowering catechins levels (Forrest and Bendall, 1969) and changing polyphenol oxidase, isoenzyme composition and activity (Takeo and Baker, 1973; Thanaraj and Seshadri, 1990; Obanda and Owuor, 1992).

In Eastern Africa, tea growing areas fall in several agro-ecological regions, differing widely in elevation and climatic factors but with favourable soil and light conditions. Despite the differences in both edaphic and non edaphic factors in the region, tea farmers import genotypes with good quality attributes across geographical areas of production with the assumption that the genotypes will retain their quality potentials in the new areas, however this has not been the case

(Owuor *et al*., 2011b). This could be attributed to variations in the green leaf plain black tea quality precursor compounds. The variations in levels of plain black tea quality precursor compounds with geographical location of production (environmental factors), season of production and genotype has not been determined for the popular clones grown in Kenya. Similarly, the variations in plain black tea quality precursors with location of production and nitrogenous fertilizer rates has not been done for the popular clone TRFK 6/8 in Eastern Africa tea growing region.

2.6 Catechins and plain black tea quality

In trade, African black teas are classified as plain to medium flavours. Such teas are valued for their taste and colour characteristics; factors which are attributed to the non-volatile components of tea. Theaflavins contribute to the astringency (briskness) and brightness while thearubigins contribute to the colour and thickness (mouth-feel) of plain black tea. Successful relationships have been demonstrated between the total theaflavins levels of central African plain black teas and sensory evaluations or prices (Hilton and Ellis, 1972; Hilton and Palmer-Jones, 1975: Cloughley, 1981, 1983; Ellis and Cloughley, 1981). Such relationships were positive but less successful for Kenya plain black teas (Owuor *et al.,* 1986). It has been suggested that total theaflavins level is an objective quality parameter for plain black teas (Ellis and Cloughley, 1981; Davis, 1983), however theaflavins levels have showed little relationship with sensory evaluations for some plain black teas (Othieno and Owuor, 1984) such as Kenyan plain black teas that showed better relationships between aroma tea quality and sensory valuation (Owuor *et al.,* 1988a; Owuor, 1992).

High levels of EC and EGC in green leaf (Hilton *et al*., 1973; Robertson, 1983b) have been associated with plain black tea quality. EC and ECG were found to correlate well with quality of

Southern and Central African plain black teas (Wright *et al*., 2000). In Kenya, high levels of green leaf ECG (Obanda and Owuor, 1997), EGCG (Owuor and Obanda, 2007) and low levels of EC (Owuor and Obanda, 2007) were found to be good indicators of plain black tea quality potential of clonal tea bush. In recent studies on Kenyan clonal teas, theaflavin digallate equivalent (TDE), which is a normalizing factor for the various theaflavins with different astringency, and brightness were demonstrated to be good plain black tea quality indicators (Owuor and Obanda, 1997; Owuor *et al*., 2006). Green leaf EGCG levels correlated significantly with plain black tea total theaflavin, liquor brightness and sensory evaluation, while EC correlated positively with thearubigins and negatively with theaflavin digallate equivalent and sensory evaluation (Owuor and Obanda, 2007). In the same study, high levels of sum of gallated catechins, trihydroxyflavan-3-ols and ratios of trihydroxyflavan-3-ols to dihydroxyflavan-3-ols were reported as parameters in green tea leaf that may be used in predicting plain black tea quality potential of Kenyan tea clones (Owuor and Obanda, 2007). The sum of gallated flavan-3-ols correlated significantly and positively with theaflavin digallate equivalent levels but negatively with thearubigins. Sum of trihydroxyflavan-3-ols (gallocatechins) positively correlated with brightness and sensory evaluation but negatively with thearubigins. The sum of simple catechins (dihydroxyflavan-3-ols) correlated positively with thearubigins and negatively with total theaflavins, theaflavin digallate equivalent, brightness and sensory evaluation. The ratios of trihydroxyflavan-3-ols to dihydroxyflavan-3-ols correlated positively with brightness and sensory evaluation but negatively with thearubigins (Owuor and Obanda, 2007).

Theaflavin (Figure 6), which contributes to the astringency (briskness) and brightness of plain black tea, is formed from anaerobic oxidation of green leaf flavan-3-ols during manufacture, catalysed by the enzyme polyphenol oxidase (Figure 7). Large differences were observed in the distribution of the individual theaflavins in Kenyan plain black teas and those

from Central Africa (Wright *et al*., 2002; Owuor *et al*., 2006). These could be due to the differences in geographical area of production (McDowell *et al*., 1991) or the genetic differences (Magoma *et al*., 2000) in the cultivars used in the studies.

The formation of a single theaflavin molecule requires a dihydroxy and a trihydroxy flavan-3-ol (Robertson, 1983b) (Table 3). However, the trihydroxy flavan-3-ols generally have lower redox potentials than dihydroxy flavan-3-ols (Bajaj *et al*., 1987). Their levels therefore could diminish faster (Owuor *et al*., 1994) during black tea fermentation. The thearubigins constitute between 10 and 20% of the dry weight of plain black tea, some thearubigins are formed from oxidative breakdown of the theaflavins and polymerization of polyphenols (Roberts, 1962). Molecular oxygen is involved in theaflavin breakdown during fermentation (Robertson, 1983a). Higher levels of theaflavins and thearubigins and higher ratios of theaflavins to thearubigins lead to more astringent teas (Obanda *et al*., 2001). The ratios of theaflavins to thearubigins are influenced by initial simple and gallocatechin composition of the green leaf (Robertson, 1983a). However there is lack of knowledge on how catechin levels, distribution and ratios vary with locations, clones and nitrogenous fertilizer rates in the Eastern Africa tea growing region.

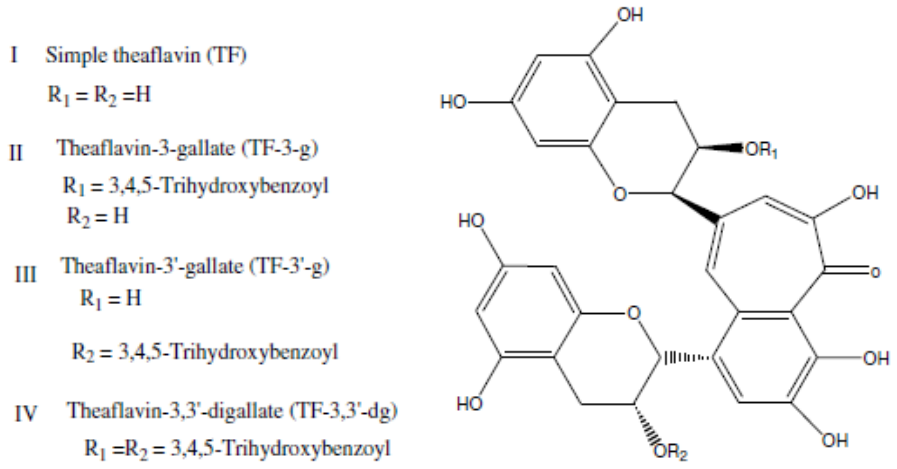


Figure 6: The major individual theaflavins in plain black teas

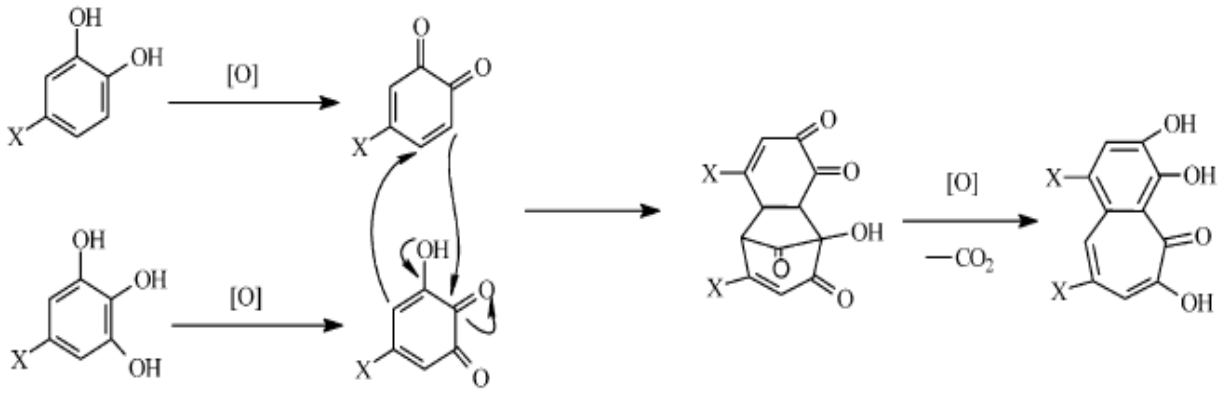


Figure 7: Mechanism for theaflavin formation

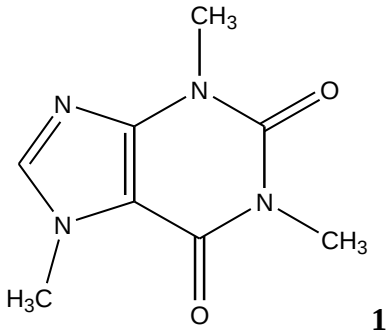
Table 3: Formation of theaflavins from flavan-3-ol

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

It is therefore necessary to quantify the effect of nitrogenous fertilizer rates and geographical locations on the amounts of initial green leaf catechins as this can present a practical way of selecting suitable genotypes for specific locations.

2.7 Caffeine

Caffeine (1,3,5-trimethylxanthine) **(11)**, is a naturally occurring substance in the leaves, seeds or fruits of some plant species worldwide. The most common sources of caffeine are coffee, cocoa beans, cola nuts and tea leaves (Barone and Roberts, 1996). Caffeine is a pharmacologically active substance and depending on the dose, can be a mild central nervous system stimulant (Barone and Roberts, 1996). It does not accumulate in the body over the course of time and is normally excreted within several hours of consumption (Barone and Roberts, 1996). In its pure state, it is an intensely bitter white powder (Arnaud, 1987).



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Caffeine is regarded as an important constituent of tea, bestowing mood and cognitive-enhancing properties (Chow and Kramer, 1990; Bokuchava and Skobeleva, 1969). The important role that caffeine plays in plain black tea quality characteristics has been acknowledged. Bhatia (1964), Deb and Ullah (1968), Millin *et al*. (1969) observed that caffeine contributes towards the briskness of plain black tea. It complexes with the polyphenols in tea, mainly theaflavins (Roberts, 1962; Collier *et al*., 1972). This complex modifies the taste characteristics of both caffeine and theaflavins (Millin *et al*., 1969) making the tea taste brisker (Sanderson *et al*., 1976). A quality characteristic which contributes positively to plain black tea evaluation is its ability to form a coloured precipitate or "cream" when its infusion is cooled (Roberts 1962; Smith 1968). The extent of "cream" is a complex of flavan-3-ols, their oxidation products, caffeine and certain nutrients. Its formation is dependent on the amount of caffeine present in the tea. Seasonal, genetic, agronomic and cultural factors, as well as processing practices might influence the caffeine content of made teas to some extent (Owuor, 1987).

In Argentina, caffeine content in tea leaf decreased gradually during most of the season after an early rapid increase (Malec and Vigo, 1988). In central Africa, the highest level of caffeine was found during the peak harvesting season when shoot growth rate was most rapid (Cloughley, 1982). In addition, studies on Assam tea showed that caffeine content decreased progressively through the season (Wood *et al*., 1964). Shoot maturity, variety, season, fertilizers, pruning, processing, grading and location have effects on the caffeine content of tea (Owuor and

Chavanji, 1986, 1988; Dev Choudhury *et al*., 1991). At a single location in Kenya, caffeine levels increased with nitrogen fertilizer rates (Owuor *et al*, 1987d), clones (Owuor and Chavanji, 1986, 1988), plucking standards (Owuor *et al*., 1987b), plucking frequency and height of mechanical harvesting (Owuor *et al*., 1991a) and season of production (Owuor and Chavanji, 1986, 1988). In a single clone, caffeine levels changed with location of production (Owuor *et al*., 1987e). In Eastern Africa tea growing region, high quality tea clones have been imported across boundaries and planted in new growing regions, however the clones have not replicated quality in the new areas. It is not known if variations in caffeine levels are specific to locations, genotypes, nitrogenous fertilizer rates and season in the Eastern Africa tea growing regions.

CHAPTER THREE

3 METHODOLOGY

3.1 Experimental sites and Materials

3.1.1 Experimental sites

This research was superimposed on two on-going trials/experiments. The first one was a clonal trial involving twenty clones namely: TRFK 11/26, TRFK 6/8, TRFK 7/9, TRFK 54/40, TRFK 56/89, TRFK 12/12, TRFK 12/19, TRFK 31/27, TRFK 31/8, TRFK 7/3, EPK TN 14-3, AHP S15/10, BBK35, 2X1/4, TRFK 303/1199, TRFK 303/259, TRFK 303/577, TRFK 303/999, TRFK 57/15 and STC 5/3. The clones were of the same age after pruning (except in Mulindi) and were subjected to same agronomic input supply. This experiment was established in Timbilil (1992), Kipkebe (1996) and Kangaita (2000) tea growing regions in Kenya by the Botany Division of Tea Research Foundation of Kenya. From this experiment, two leaves and a bud, third leaf and mature leaf samples were taken during cold and wet season for analysis of variation in nutrients levels with location of production and genotypes. Two leaves and a bud samples were also taken from this trial in cold and wet, and in warm and dry seasons for analysis of variations in caffeine and flavan-3-ols with location of production and season of production.

The second trial was a fertilizer trial on clone TRFK 6/8 set by the Chemistry Divisions of Tea Reseasrch Foundation of Kenya (TRFK), Tea Research Institute of Tanzania (TRIT) and Office Des Cultures Industrielles du Rwanda The' (OCIR The') in 2002 . The clone was subjected to five different nitrogenous fertilizer rates in eight different locations within the Eastern Africa tea growing growing regions. The locations were: Changoi, Sotik and Timbilil in Kenya, Ngwazi, Maruku and Katoke in Tanzania, and Kitabi and Mulindi in Rwanda. From this experiment, mature leaf samples were taken for determination of variations in mature leaf nutrients levels with nitrogenous fertilizer rates, location of production and plucking interval of

the tender leaves (two leaves and a bud). Further, two leaves and a bud samples from this experiment was used to assess variations in levels of caffeine and flavan-3-ols with nitrogenous fertilizer rates and location of production. The study sites coordinates are shown in Table 4.

Table 4: The study sites coordinates and altitude in metres above mean sea level (m amsl)

Country	Site	Latitude	Longitude	Altitude
Kenya	Timbilil (Tea Research Foundation of Kenya)	0^o 22'S	35^o 21'E	2180
	Changoi	0^o 30'S	35^o 13'E	1860
	Kipkebe	0^o 39'S	35^o 02'E	1800
	Sotik Tea	0^o 36'S	35^o 04'E	1800
	Kangaita	0^o30'S	37^o16'E	2100
Rwanda	Kitabi	2^o 32'S	29^o26'E	2231
	Mulindi	1^o 27'S	30^o 01'E	1800
Tanzania	Maruku Tea Estate	1º23' S	31º 45'E	1488
	Katoke Tea Estate	1º 36'S	31º 41'E	1217
	Ngwazi Tea Research Station (NTRS)	8 º32'S	35 º10'E	1840

3.1.2 Materials

3.1.2.1 Chemicals

The solvents; Methanol (99.8%) and acetonitrile (HPLC grade) were analytical grades purchased from Sigma-Aldrich. The other reagents: NaOH, HCl (37%), HNO_3 (66%), H_2SO_4 (97%), 20 volume hydrogen peroxide, EDTA, ascorbic acid and Folin Ciocalteau phenol reagent used were also analytical grades from Sigma-Aldrich. Caffeine standard, mixed catechins standard and AAS standards were also purchased from Sigma-Aldrich.

3.1.2.2 Instruments

The instruments used were: Memmert Oven (D-91126), Kjeldahl digester (Gerhardt 2200), Flame Photometer (Corning 400), Atomic Absorption Spectrophotometer (SpectrAA-30), UV-visible Spectrophotometer (Cecil-393) and solvent gradient HPLC (Shimadzu LC-20A) with a C6 capillary column.

3.2 Methodology and research design

The twenty clones in the clonal trial were planted in a Randomized Complete Block (RCBD) comprising twenty plots of different clones with each plot containing twenty plants of same clone. The complete blocks were replicated three times in Timbilil, Kipkebe and Kangaita (Wachira *et al*., 2002) tea growing regions of Kenya. Although the plants were at different ages, all the sites had mature clonal tea bushes in the same pruning cycle life. The clones were subjected to the same agronomic inputs including NPKS 25:5:5:5 at 150 kg N $ha^{-1}year^{-1}$. Leaf samples for nutrients analysis were obtained during "cold and wet" (March/April, 2010) season, while 'two leaves and a bud' for quality precursors were obtained during 'cold and wet' (March/April, 2010), and 'warm and dry' (November, January, 2010/2011) seasons. The data from the trial were analysed as factorial two in Randomized Complete Block Design.

The second trial was a fertilizer trial on clone TRFK 6/8. This experiment was conducted in eight sites: Timbilil, Changoi and Kipkebe in Kenya, Ngwazi, Maruku and Katoke in Tanzania, and Mulindi and Kitabi in Rwanda. At each site, a 5 by 3 factorial experiment was laid out in Randomized Complete Block Design and replicated 3 times (Appendix 1). The main treatments are the 8 sites with nitrogen rates (0, 75, 150, 225 and 300 kg N ha^{-1} $year^{-1}$ as NPKS (25:5:5:5) as a sub treatment and plucking frequency (7, 14 and 21 days intervals) the sub-sub treatment. A sub-sub plot comprised 30 bushes of clone TRFK 6/8 (Msomba *et al*., 2011; Owuor *et al*., 2011a). Tea at each site was pruned between April and August 2008 so that all plants were in same pruning cycle life. The trial was analysed as 5 by 3 factorial split design for the 8 locations for mature leaf nutrients.

3.3 Sampling and sample preparation

3.3.1 Leaf sampling for analysis of nutrients

3.3.1.1 Sampling from first experiment (Clonal trial)

Two leaves and a bud, third leaf and mature leaf samples (ca. 100 g each), were hand plucked randomly from plots in all the sites. Sampling was done a month after fertilizer application during 'cold and wet' season (March/April, 2010) when water was not expected to limit nutrients uptake and the plants had recovered from effects of drought. The samples were put in labelled paper envelopes and oven dried at 105^{o}C for 4.5 hours before milling to powder form using coffee mill. The powdered samples were kept in labelled paper envelopes for laboratory nutrients analysis.

3.3.1.2 Sampling from second experiment (Fertilizer trial)

About 100 g mature leaves were sampled by hand plucking, on a 7, 14 and 21 day plucking rounds in January/February, 2010. The samples were then put in labelled paper envelopes and oven dried at 105^{o}C for 4.5 hours before milling to powder form. The powdered samples were kept in labelled paper envelopes for laboratory analysis of nutrients.

3.3.2 Leaf sampling for evaluation of quality precursors

Leaf sampling was done according to the method of Owuor and Obanda (2007). Leaf samples were collected by random hand plucking of two leaves and a bud.

3.3.2.1 Sampling from first experiments (Clonal trial)

About 200 g freshly harvested two and a bud samples were hand plucked in two seasons: 'cold and wet' (March/April, 2010) and 'warm and dry' (November/January, 2010/2011). The

freshly harvested leaf samples were then immediately steamed for 1 minute then dried in an oven at 80^{o}C to ensure constant weight. The dried leaf was then ground to powder form and stored in a freezer (-20^{o}C) prior to extraction.

3.3.2.2 Sampling from second experiment (Fertilizer trial)

Fresh two and a bud leaf samples (ca 200 g) were hand plucked once on a 14 day plucking round in January/February, 2010. Freshly harvested leaf samples were immediately steamed for 1 minute. The steamed leaf was then dried in an oven at 80^{o}C to ensure constant weight. The dried leaf was then ground to powder form. The powdery samples were stored in a freezer (-20 ^{o}C) prior to extraction.

3.4 Analytical methods

3.4.1 Leaf nutrients extraction and determination

Leaf nutrients extraction and determination was done according to modified AOAC Official Methods, 2000. For nitrogen, 1.00 g of the dried ground tea leaf samples was micro-Kjeldahl digested using 1 mL analytical grade sulphuric acid, in presence of copper-selenium catalyst for 4 hours at 350^{o}C. The digest was allowed to cool to room temperature and 10 mL of distilled water added. This was then distilled with 40% NaOH and 2% boric acid using Conway mixed indicator (methyl red and bromocresol green) in a Markham still distiller. The distilled sample was titrated against standardized HCl. Percent nitrogen was then calculated using the expression:

$$\%N\ in\ leaf\ material = \frac{V \times N \times 14}{10 \times Wt}$$

Where V = titre value, N is normality of HCl and Wt is weight of leaf (1.00 g).

AOAC (2000) method was used for potassium, magnesium, manganese and calcium determinations. Dried and ground mature tea leaf (0.25 g) was put in specimen tubes and ashed in a muffle furnace for 4.5 hours at a temperature of 450^{o}C until grayish white ash was obtained. This was then cooled and digested with 0.5mL of 2:3 double acid-peroxide mixture. The double acid was prepared by mixing equal volumes of 1:1 HCl (acid to water) and 1:1 HNO_3 (acid to water), with 20 volume hydrogen peroxide. This was evaporated to dryness on a hot plate under low heat and ventilation. The sample was then extracted with 25 mL of 0.05 N HCl for 12 hours. This extract was diluted by pippeting 0.2 mL into 25 mL volumetric flask, adding 5 mL strontium chloride and making to the mark with distilled water. This diluted extract was analyzed for potassium using Corning 400 Flame Photometer and for magnesium, manganese and calcium using SpetrAAS-30 atomic absorption spectrophotometer.

For phosphorus analysis, 5 mL of composite/colouring solution (mixture of ammonium vanadate and ammonium molybdate) was added to a pippeted 0.2 mL extract and allowed to stand for 30 minutes to form colour in the dark before being run in a UV-vis (Cecil-393) at a wavelength of 250 nm (AOAC, 2000).

3.4.2 Extraction of polyphenols from leaf samples

The method described by the International Organization for Standardization (ISO) was used (ISO 14502-2, 2005). Each sample (0.200 g) was weighed in triplicate into an extraction glass tube. The tubes were placed in a water bath at 70°C and 5 mL of 70% methanol at 70°C added. The tubes were loosely stoppered and the contents gently mixed on a Votex mixer. The tubes were continually heated in water bath for ten minutes, with mixing after every 5 minutes. The tubes were then removed from water bath, allowed to cool for a few minutes and then centrifuged at 3000 rpm for 10 minutes. The supernatant was carefully decanted into clean

graduated glass and the extraction procedure repeated. The extracts were combined and made up to 10 mL with cold 70% methanol. This extract was stored at 4°C awaiting determination of total polyphenols, caffeine and catechins.

3.4.2.1 Analysis of total polyphenols

Total polyphenols analysis was done according to ISO 14502-1 (2005). One mL of the extract was diluted to 100 mL with distilled water and 5.0 mL of Folin-Ciocalteau phenol reagent was added and mixed. Within 5 min after the addition of Folin-Ciocateau phenol reagent, 4.0 mL of sodium carbonate solution was added and allowed to stand for 60 min at room temperature. Optical density was then measured in a 10 mm cell on a UV-Vis spectrophotometer (Cecil-393) set at 765 nm. Further, gallic acid standard solutions (1.0 mL) corresponding to 10, 20, 30, 40 and 50 ppm of anhydrous gallic acid was made in different graduated tubes and the volume made 100 mL with distilled water. These were then treated the same way with Folin-Ciocalteau phenol reagent and sodium carbonate solution, allowed to stand for 60 min at room temperature before taking their optical densities in a spectrophotometer at 765 nm. The polyphenol amounts were calculated from a standard curve generated from gallic acid standards and expressed as percent gallic acid equivalent (% GAE) on dry matter basis.

3.4.2.2 HPLC determination of catechins and caffeine

Tha determination was done according to ISO-14502-02 (2005). For analysis, 1 mL of the methanol leaf extract was diluted to 5 mL with a stabilizing solution (10% acetonitrile, with 500 µg of EDTA and ascorbic acid). Catechins and caffeine were determined using the gradient HPLC (Shimadzu LC-20A with a C6 capillary column). The diluted sample extract (1.0 mL) was injected splittless into the HPLC under the following conditions: Flow rate of the mobile phase;

1.0 mL/min, binary gradient conditions; 100% mobile phase A (9% acetonitrile water v:v, 2% acetic acid and 20 µg/mL EDTA) for 10 minutes, then over 15 minutes, a linear gradient to 68% mobile phase A, 32% mobile phase B (80% acetonitrile water + 2% acetic acid + 20 µg/mL EDTA), holding at this composition for 10 minutes. This was then reset to 100% mobile phase A and allowed to equilibrate for 10 minutes before next injection. Column temperature; 35°C ± 0.5°C. The UV detector; 278 nm. The identification of individual catechins and caffeine was done by comparing retention times from sample chromatograms with those obtained from a mixed standard solution [gallic acid (5 µg/mL) + (50 µg/mL caffeine) + (50 µg/mL catechin) + (50 µg/mL epicatechin) + (100 µg/mL epigallocatechin) + (100 µg/mL epigallocatechin gallate) + (50 µg/mL epicatechin gallate)] under the same chromatographic conditions. Quantification was done by external standardization using caffeine.

3.5 Statistical analysis of results

The data was subjected to analysis of variance (ANOVA) using MSTAT-C (1993) statistical package. Factorial two design was used for analysis of nutrients in experiment 1 (clonal trial), with genotype and location of production as the factors, and for caffeine and flavan-3-ols analysis in experiment two (fertilizer trial on clone TRFK 6/8), with nitrogenoud fertilizer rate and location of production as the factors. Factorial three design was used for analysis of nutrients in experiment two (fertilizer trial on clone TRFK 6/8), where location of production, plucking interval and nitrogenous fertilizer rate were the factors, and for analysis of caffeine and flavan-3-ols in exxxperiment one (clonal trial), with location of production, season of production and genotype as the factors. The means were separated using least significant difference (LSD) and regressed to establish inter-related factors.

CHAPTER FOUR

4 RESULTS AND DISCUSSION

4.1 Nutrients variations in mature leaf, third leaf and young tea leaf with location of production and genotypes

Plant tissue analysis has been claimed to be a more direct and unique method of assessing soil fertility by use of growing plants (Jackson, 1960; Chapman and Pratt, 1961), especially for tea plants (Akmelov and Bairamov, 1968; Willson, 1969; Tolhurst, 1972; Lin, 1969). For use as a diagnostic method, the age of the leaf must be clearly defined (Bould, 1968). Consequently, norms to guide use of mature (Tolhurst, 1976; TRFCA, 1990) and third (Bonheure and Willson, 1992) leaf were set as shown in Tables 1 and 2. Clonal tea plants used in these trials received the recommended fertilizer rate of 150 kg N/ha/year (Othieno, 1988) and did not show any visual signs of deficiency (Bonheure and Willson, 1992; Anon, 2002). Consequently, any nutrients levels observed below the set limits (Tolhurst, 1976; Owuor and Wanyoko, 1983; Othieno, 1988; Bonheure and Willson, 1992) could only demonstrate the poor suitability of the seedling tea-based tissue analysis method to guide fertilizer use in clonal tea. The mature leaf was thought to be more stable (Tolhurst, 1976; TRFCA, 1990) and was adopted for setting nutrients norms in Kenya. However, the recommended plucking standard is two leaves and a bud (Othieno, 1988). The nutrients in two leaves and a bud therefore are fair estimates of the amounts of nutrients removed with the crop.

The variations in the macro nutrients: nitrogen, phosphorus and potassium are presented in Tables 5, 6 and 7, respectively. Nutrients levels observed were below the set limits (Tolhurst, 1976; Owuor and Wanyoko, 1983; Othieno, 1988; Bonheure and Willson, 1992) and this therefore could only demonstrate the poor suitability of the seedling tea-based tissue analysis diagnosis method to guide fertilizer use in the clonal tea. For mature leaf, third leaf, and two

leaves and a bud, there were significant ($P \leq 0.05$) variations in all the three nutrients with clones and locations despite the fact that all the tea plants received same amount of fertilizer as recommended (; Othieno, 1988; Bonheure and Willson, 1992; Anon, 2002).

Table 5: Variations in mean nitrogen levels (%) with location of production and cultivars

	Mature leaf				3rd Leaf				2 leaves and a bud			
Clone	Timb	Kipk	Kang	Mean clone	Timb	Kipk	Kang	Mean clone	Timb	Kipk	Kang	Mean clone
TRFK7/9	3.62	3.45	3.25	3.44	3.66	3.68	3.33	3.56	4.76	4.70	5.51	4.66
TRFK303/259	3.62	3.67	3.55	3.61	3.68	3.64	3.30	3.54	5.17	4.63	4.68	4.83
TRFK303/1199	3.18	3.28	2.67	3.04	3.29	3.35	3.10	3.25	4.45	4.46	4.39	4.44
TRFK54/40	3.42	3.51	2.97	3.30	3.25	3.50	3.08	3.28	4.52	4.61	4.34	4.49
TRFK31/8	3.11	3.62	2.76	3.16	3.52	3.85	2.92	3.43	4.51	4.68	4.48	4.56
BB35	3.10	3.31	2.91	3.11	3.61	3.63	3.09	3.44	4.97	5.06	4.40	4.81
TRFK6/8	3.55	3.64	3.15	3.44	3.38	3.36	2.97	3.24	3.83	4.38	4.11	4.11
TRFK31/27	3.54	3.40	3.30	3.41	3.58	3.50	3.36	3.48	5.62	5.15	5.15	5.31
TRFK12/12	3.44	3.46	3.39	3.43	3.62	3.77	3.37	3.59	4.71	4.43	5.23	4.79
TRFK303/999	3.26	3.32	3.19	3.26	3.26	3.57	3.00	3.28	4.35	4.28	4.21	4.28
AHPS15/10	3.30	3.66	2.73	2.89	3.57	3.39	2.72	3.23	4.38	4.33	4.06	4.26
TRFK57/15	3.74	3.65	3.28	3.56	3.83	3.60	3.42	3.62	4.73	4.84	4.54	4.70
TRFK56/89	3.25	3.32	3.30	3.29	3.58	3.50	2.89	3.32	4.85	5.14	4.42	4.80
TRFK12/19	3.39	3.34	3.21	3.32	3.36	3.61	3.30	3.42	454	4.51	4.78	4.61
TRFK11/26	3.57	3.39	3.40	3.46	3.30	3.45	3.29	3.35	4.39	4.39	4.49	4.42
STC5/3	3.68	3.58	2.89	3.38	3.21	3.42	2.97	3.20	4.61	4.35	4.21	4.39
TRFK7/3	3.16	3.25	3.25	3.22	3.60	3.40	3.42	3.47	4.77	4.80	4.54	4.70
TRFK303/577	3.41	3.49	3.04	3.32	3.35	3.18	2.91	3.15	3.69	3.75	4.09	3.85
EPKTN14-3	3.64	3.10	3.17	3.30	3.60	3.82	3.21	354	4.64	4.75	4.45	4.55
TRFK2xI/4	3.43	2.80	3.26	3.17	3.45	3.45	3.40	344	4.31	4.40	4.47	4.39
Mean site	3.41	3.36	3.13		3.48	3.53	3.15		4.59	4.57	4.48	
C.V. (%)		6.28				6.22				4.66		
LSD, $P \leq 0.05$												
				0.21				0.21				0.21
		0.17				0.17				0.17		
Interactions		0.35				NS				0.35		

Key: Timb = Timbilil, Kipk = Kipkebe, Kang = Kangaita

The mean site values for mature leaf nitrogen at all the sites (Table 5) were at what was considered borderline in the foliar analysis advisory service (Tolhurst, 1976; Othieno, 1988). The mean clonal mature leaf levels were only within the adequate levels for clones TRFK 303/259 and TRFK 57/15, while clone AHP S15/10 was within the deficient level and seventeen other

clones were within the borderline deficiency limit. Levels of mature leaf nitrogen were generally borderline to adequate supply limits at Timbilil and Kipkebe, but at Kangaita, except for clone TRFK 303/259 that had adequate leaf nitrogen, six clones fell within the deficient limits while thirteen clones showed borderline deficiency. The significant ($P \leq 0.05$) interactions between leaf nitrogen in clones and location indicated that the pattern of change in clonal leaf nitrogen was not uniform. These results demonstrated that the nitrogen limits guidelines being used (Tolhurst, 1976; Owuor and Wanyoko, 1983; Othieno, 1988) may not be suitable on clonal tea plantations in all the three locations.

The levels of nitrogen (Table 5) in the 3rd leaf did not reach the 5% content suggested as adequate supply (Table 2) (Bonheure and Willson, 1992), for all clones and in all locations. Although no suggested limit has been published for the nutrients in two leaves and a bud, this is the recommended leaf for harvesting (Othieno, 1988). At all sites and in all clones higher levels of nitrogen were recorded in younger leaves than older leaves, showing that tea partitions more nitrogen to the young leaves. This suggests that for tea with high yields as had been demonstrated for these clones (Wachira *et al.*, 2002), substantial amounts of nitrogen nutrient is harvested with crop. The noted variations in mature leaf nitrogen were replicated in the 3rd leaf and 2 leaves and a bud, indicating the unique ability of individual clones to absorb nitrogen from the soil. Irrespective of the leaf that is used in foliar analysis, there were significant ($P \leq 0.05$) variations in leaf nitrogen with clone/cultivar and location of production, suggesting that limits to guide foliar analysis advisory system should be different for individual clones and also depend on location of production.

The changes in the levels of leaf phosphorus with locations and clones are presented in Table 6. There were significant ($P \leq 0.05$) variations in the leaf phosphorus levels with location of production and clones. Indeed the mean data for clones showed that only clone TRFK 7/9 had

borderline mature leaf phosphorus level. At all locations and in every clone the data revealed that phosphorus supply was adequate. However, a closer look at the data showed that one clone grown in the three different locations could exhibit very large leaf phosphorus variations.

Table 6: Variations in leaf phosphorus levels (%) with location of production and cultivars

	Mature leaf				3rd Leaf				2 leaves and a bud			
Clone	Timb	Kipk	Kang	Mean clone	Timb	Kipk	Kanga	Mean clone	Timb	Kipk	Kang	Mean clone
TRFK7/9	0.17	0.18	0.17	0.17	0.20	0.26	0.20	0.22	0.23	0.28	0.19	0.24
TRFK303/259	0.19	0.23	0.24	0.22	0.22	0.24	0.21	0.22	0.32	0.25	0.20	0.26
TRFK303/1199	0.19	027	0.26	0.24	0.20	0.26	0.20	0.22	0.31	0.28	0.22	0.27
TRFK54/40	0.18	0.28	0.28	0.25	0.22	0.29	0.19	0.23	0.28	0.25	0.19	0.24
TRFK31/8	0.17	0.22	0.25	0.21	0.20	0.26	0.23	0.23	0.22	0.29	0.20	0.24
BBK35	0.15	0.23	0.24	0.21	0.21	0.29	0.19	0.23	0.25	0.22	0.24	0.23
TRFK6/8	0.19	0.21	0.22	0.21	0.20	0.20	0.18	0.19	0.23	0.21	0.24	0.23
TRFK31/27	0.17	0.21	0.21	0.20	0.21	0.24	0.20	0.22	0.37	0.26	0.21	0.28
TRFK12/12	0.19	0.29	0.28	0.25	0.22	0.27	0.22	0.24	0.23	0.34	0.22	0.26
TRFK303/999	0.14	0.24	0.23	0.21	0.20	0.28	0.20	0.23	0.28	0.22	0.21	0.23
AHPS15/10	0.17	0.27	0.26	0.23	0.23	0.29	0.22	0.25	0.29	0.31	0.21	0.27
TRFK57/15	0.20	0.15	0.25	0.20	0.22	0.29	0.25	0.25	0.39	0.23	0.30	0.30
TRFK56/89	0.15	0.20	0.21	0.19	0.22	0.25	0.24	0.24	0.26	0.24	0.22	0.24
TRFK12/19	0.18	0.20	0.20	0.19	0.23	0.28	0.20	0.24	0.31	0.26	0.19	0.25
TRFK11/26	0.15	0.22	0.20	0.19	0.20	0.23	0.19	0.21	0.28	0.26	0.19	0.24
STC5/3	0.19	0.21	0.32	0.24	0.21	0.22	0.18	0.20	0.24	0.20	0.17	0.20
TRFK7/3	0.16	0.19	0.21	0.19	0.21	0.28	0.21	0.23	0.28	0.27	0.16	0.24
TRFK303/577	0.19	0.18	0.20	0.19	0.22	0.24	0.21	0.22	0.23	0.26	0.21	0.23
EPKTN14-3	0.19	0.22	0.24	0.22	0.21	0.25	0.25	0.24	0.34	0.24	0.18	0.26
TRFK2xI/4	0.19	0.20	0.23	021	0.21	0.26	0.24	0.23	0.31	0.28	0.23	0.27
Mean site	0.18	0.22	0.23		0.21	0.26	0.21		0.28	0.26	0.21	
C.V. (%)		9.64				8.62				8.17		
LSD, P ≤ 0.05												
				0.02				0.02				0.02
		0.02				0.02				0.02		
Interactions		0.03				0.03				0.03		

Key: Timb = Timbilil, Kipk = Kipkebe, Kang = Kangaita

A total of eight clones in Timbilil and one clone in Kipkebe had borderline mature leaf phosphorus content. The results demonstrated that use of mature leaf to evaluate deficiency of the nutrient in clonal tea may work well in Kipkebe and Kangaita, but performs very poorly in Timbilil. However, for the 3rd leaf, no clone had phosphorus level that could be considered

adequate. The diagnostic level set (Bonheure and Willson, 1992), is therefore too high for clonal tea in Kenya. Like nitrogen, there was general rise in leaf phosphorus in younger tea leaves.

The average mature leaf potassium was borderline for clonal tea in Kangaita, while adequate for both Timbilil and Kipkebe clonal tea (Table 7). There were two clones with mean values below 1.20% considered deficient, while seven clones had borderline deficiency and eleven clones had adequate levels (Tolhurst, 1976; Owuor and Wanyoko, 1983; Othieno, 1988).

Table 7: Variations in leaf potassium levels (%) with location of production and cultivars

	Mature leaf				3rd Leaf				2 leaves and a bud			
Clone	Timb	Kipk	Kang	Mean clone	Timb	Kipk	Kang	Mean clone	Timb	Kipk	Kang	Mean clone
TRFK7/9	1.43	1.26	1.31	1.33	1.58	2.26	1.30	1.72	1.89	2.41	1.80	2.03
TRFK303/259	1.80	1.66	1.61	1.69	1.74	2.79	1.67	2.07	2.07	2.60	1.74	2.13
TRFK303/1199	1.66	1.59	1.32	1.52	1.79	2.50	1.46	1.92	1.98	2.65	1.84	2.16
TRFK54/40	1.19	1.94	1.18	1.44	1.51	2.32	1.33	1.72	1.90	2.48	1.75	2.05
TRFK31/8	1.46	2.14	1.27	1.62	1.80	2.31	158	1.90	2.05	2.40	1.73	2.06
BBK35	1.09	1.46	1.02	1.19	1.73	2.19	1.38	1.77	1.93	2.43	1.62	1.99
TRFK6/8	1.64	1.62	1.31	1.52	1.82	2.34	1.32	1.83	2.03	2.56	1.77	2.12
TRFK31/27	1.65	2.18	1.30	1.71	1.79	2.41	1.62	1.94	2.15	2.46	1.88	2.17
TRFK12/12	1.52	2.11	1.43	1.69	1.78	2.49	1.73	2.00	1.94	2.38	1.83	2.05
TRFK303/999	1.63	2.10	1.36	1.70	2.11	2.24	1.55	1.97	2.27	2.68	1.72	2.22
AHPS15/10	1.74	2.21	1.46	1.80	2.02	2.69	1.58	2.10	2.04	2.90	1.70	2.21
TRFK57/15	1.55	1.46	1.34	1.45	1.95	2.59	1.70	2.08	2.15	2.63	2.02	2.27
TRFK56/89	1.12	1.57	1.25	1.31	1.66	2.24	1.69	1.86	2.00	2.68	2.04	2.24
TRFK12/19	1.37	1.59	1.37	1.45	1.67	2.47	1.53	1.89	2.01	2.66	1.68	2.12
TRFK11/26	1.39	1.35	1.29	1.34	1.86	2.26	1.59	1.90	2.02	2.55	1.78	2.12
STC5/3	1.14	1.26	1.18	1.19	1.35	2.04	1.40	1.60	1.90	2.41	1.67	1.99
TRFK7/3	1.42	1.57	1.40	1.46	1.76	2.68	1.58	2.01	2.11	2.69	1.78	2.19
TRFK303/577	1.91	1.50	1.25	1.55	2.12	2.43	1.79	2.11	2.02	2.60	1.94	2.19
EPKTN14-3	1.58	1.80	1.56	1.64	1.60	2.06	1.51	1.72	1.91	2.31	1.68	1.97
TRFK2xI/4	2.00	1.81	1.34	1.72	1.82	2.48	1.90	2.07	2.14	267	2.02	2.28
Mean site	1.52	1.71	1.33		1.77	2.39	1.56		2.02	2.56	1.80	
C.V. (%)		11.00				7.48				7.06		
LSD, P ≤ 0.05												
				0.16				0.14				0.15
		0.13				0.11				0.12		
Interactions		0.28				0.24				NS		

Key: Timb = Timbilil, Kipk = Kipkebe, Kang = Kangaita

At Kangaita, except for clones TRFK 303/259 and EPK TN 14-3, other clones had either borderline potassium (fifteen clones) or deficiency of potassium (three clones), while in Kipkebe, potassium in mature leaf was adequate except in four clones where there was mild deficiency, and in Timbilil, four clones showed potassium deficiency, five clones were borderline and eleven clones had adequate levels. There were large variations ($P \leq 0.05$) in mature leaf potassium with locations and clones. As a result, the guidelines based on mature leaf potassium (Table 1) may not be appropriate for clonal tea and at different locations. For the 3rd leaf, no clone at any location had adequate level of potassium. The suggested guidelines (Bonheure and Willson, 1992) may therefore not be suitable for clonal tea. There was rise in potassium levels as the leaves became younger implying that high amount of potassium is harvested with two leaves and a bud.

The other macronutrients essential for tea are calcium (Willson, 1975b) and magnesium (Willson, 1975a). There are no recommended diagnostic levels set for calcium for mature leaf, but magnesium is considered deficient when mature leaf levels are below 0.10% (Table 1) (Owuor and Wanyoko, 1983; Othieno, 1988). The variations in leaf calcium with age of leaf, location and clones are presented in Table 8. The clones exhibited significantly ($P \leq 0.05$) different abilities to absorb calcium from the soil and this also varied ($P \leq 0.05$) with locations. The large variations demonstrated possible difficulties in setting single diagnostic levels that can be used in all clones at different locations. In the 3rd leaf, all samples had calcium levels higher than 0.35% suggested as optimal level (Bonheure and Willson, 1992). These results suggest that the calcium levels in Kenyan tea soils are either adequate, or the clones used in this study were efficient in calcium extraction from the soil. The observation supports why calcium fertilizers are not normally applied to tea in Kenya. Unlike nitrogen, phosphorus and potassium (Tables 5, 6 and 7), there was increase in

calcium levels as the leaf mature, demonstrating that the nutrient is stored more in the older tea leaves.

Table 8: Variations in leaf calcium levels (%) with location of production and cultivars

	Mature leaf				3rd Leaf				2 leaves and a bud			
Clone	Timb	Kipk	Kang	Mean clone	Timb	Kipk	Kang	Mean clone	Timb	Kipk	Kang	Mean clone
TRFK7/9	0.95	0.98	0.89	0.94	0.46	0.57	0.53	0.52	0.39	0.47	0.35	0.40
TRFK303/259	0.88	1.26	0.93	1.02	0.49	0.54	0.39	0.47	0.38	0.36	0.30	0.34
TRFK303/1199	1.11	1.12	1.07	1.10	0.51	0.68	0.39	0.53	0.34	0.55	0.35	0.41
TRFK54/40	0.89	0.68	0.85	0.81	0.52	0.58	0.45	0.52	0.37	0.50	0.35	0.40
TRFK31/8	1.45	0.75	1.54	1.25	0.48	0.58	0.70	0.58	0.28	0.41	0.29	0.33
BBK35	0.87	0.62	0.99	0.83	0.36	0.53	0.38	0.42	0.23	0.25	0.24	0.24
TRFK6/8	0.70	0.93	1.07	0.90	0.38	0.44	0.54	0.46	0.29	0.40	0.32	0.34
TRFK31/27	1.12	1.06	1.16	1.11	0.60	0.66	0.55	0.60	0.40	0.46	0.31	0.39
TRFK12/12	1.04	1.24	1.02	1.10	0.47	0.58	0.50	0.51	0.30	0.39	0.29	0.33
TRFK303/999	1.04	0.98	1.11	1.04	0.57	0.59	0.54	0.57	0.34	0.40	0.35	0.36
AHPS15/10	1.06	1.18	1.34	1.19	0.46	0.57	0.58	0.54	0.31	0.41	0.45	0.39
TRFK57/15	1.02	1.33	1.10	1.15	0.44	0.51	0.54	0.50	0.21	0.43	0.30	0.31
TRFK56/89	1.42	1.13	1.16	1.24	0.44	0.49	0.53	0.49	0.34	0.40	0.39	0.38
TRFK12/19	1.19	1.25	1.01	1.14	0.55	0.58	0.53	0.55	0.31	0.43	0.29	0.34
TRFK11/26	0.94	1.08	1.04	1.02	0.51	0.60	0.50	0.54	0.37	0.46	0.35	0.39
STC5/3	0.94	1.12	1.10	1.06	0.49	0.59	0.40	0.50	0.27	0.42	0.35	0.35
TRFK7/3	1.14	1.34	0.90	1.13	0.50	0.46	0.56	0.51	0.34	0.41	0.36	0.37
TRFK303/577	0.79	0.86	1.19	0.95	0.60	0.42	0.46	0.49	0.31	0.34	0.36	0.34
EPKTN14-3	0.86	0.95	0.83	0.88	0.40	0.43	0.54	0.46	0.26	0.36	0.28	0.30
TRFK2xI/4	1.14	1.19	1.47	1.27	0.63	0.75	0.81	0.73	0.36	0.42	0.44	0.41
Mean site	1.03	1.05	1.09		0.49	0.56	0.56		0.32	0.41	0.34	
C.V. (%)			12.44			3.36				9.02		
LSD, P ≤ 0.05												
				0.13				0.04				0.03
		0.01				0.03				0.02		
Interactions		0.22				0.06				0.05		

Key: Timb = Timbilil, Kipk = Kipkebe, Kang = Kangaita

Magnesium levels also varied significantly ($P \leq 0.05$) in tea leaves type due to clones and location of production (Table 9). Using mature leaf as a guide, on the average, all clones at all locations extracted adequate amounts of magnesium from the soil. However, significant ($P \leq 0.05$) variations in magnesium levels with location and clones were observed (Table 9), suggesting differences in the ability of the plants to extract the nutrient from the soil. Bonheure

and Willson (1992) had suggested 0.30% as level of magnesium in the third leaf considered adequate (Table 2). There was no clone at any site with this level of magnesium in the third leaf (Table 9), although the plants did not have any visual signs of magnesium deficiency (Bonheure and Willson, 1992; Anon, 2002). Kamau (2002) reported similar observations and findings on magnesium levels in clonal tea. The norms set for third leaf magnesium levels are therefore unrealistic for Kenyan clonal tea plantations. The levels of magnesium decreased with increase in leaf age.

Table 9: Variations in leaf magnesium levels (%) with location of production and cultivars

	Mature leaf				3rd Leaf				2 leaves and a bud			
Clone	Timb	Kipk	Kang	Mean clone	Timb	Kipk	Kang	Mean clone	Timb	Kipk	Kang	Mean clone
TRFK7/9	0.09	0.13	0.08	0.10	0.12	0.18	0.08	0.13	0.20	0.21	0.15	0.19
TRFK303/259	0.11	0.16	0.10	0.12	0.18	0.17	0.12	0.15	0.25	0.22	0.18	022
TRFK303/1199	0.07	0.18	0.10	0.12	0.18	0.18	0.12	0.16	0.23	0.22	0.17	0.21
TRFK54/40	0.06	0.13	0.10	0.10	0.10	0.15	0.10	0.11	0.15	0.19	0.17	0.17
TRFK31/8	0.13	0.18	0.14	0.15	0.14	0.21	0.12	0.16	0.19	0.23	016	0.19
BBK35	0.14	0.16	0.12	0.14	0.12	0.18	0.11	0.14	0.20	0.21	0.15	0.19
TRFK6/8	0.11	0.15	0.10	0.12	0.17	0.16	0.11	0.15	0.20	0.21	0.16	0.19
TRFK31/27	0.07	0.17	0.12	0.12	0.15	0.19	0.12	0.15	0.21	0.23	0.18	0.21
TRFK12/12	0.12	0.25	0.13	0.17	0.17	0.20	0.12	0.16	0.19	0.19	0.16	0.18
TRFK303/999	0.12	0.23	0.13	0.16	0.15	0.18	0.13	0.15	0.22	0.22	0.17	0.20
AHPS15/10	0.14	0.21	0.16	0.17	0.19	0.21	0.16	019	0.23	0.30	0.19	0.24
TRFK57/15	0.15	0.27	0.13	0.18	0.18	0.20	0.17	0.18	0.23	0.23	0.19	0.22
TRFK56/89	0.12	0.17	0.10	0.13	0.12	0.17	0.11	0.13	0.19	0.22	016	0.19
TRFK12/19	0.17	0.19	0.12	0.16	015	0.22	0.13	017	0.21	0.26	0.17	0.21
TRFK11/26	0.07	0.14	0.13	0.11	011	0.17	0.13	0.14	0.18	0.21	0.17	0.19
STC5/3	0.11	0.15	0.11	0.13	0.15	0.18	0.13	0.16	0.22	0.22	0.16	0.20
TRFK7/3	0.11	0.17	0.13	0.14	0.16	0.19	014	0.17	0.27	0.24	0.17	0.23
TRFK303/577	0.20	0.17	0.14	0.17	0.22	0.20	0.15	0.19	0.25	0.23	0.19	0.23
EPKTN14-3	0.13	0.14	0.13	0.13	0.15	0.17	0.13	0.15	0.18	0.20	0.17	0.18
TRFK2xI/4	0.18	0.19	0.16	0.18	0.16	0.21	0.17	0.18	0.21	0.22	0.19	0.21
Mean site	0.12	0.18	0.12		0.15	0.19	0.13		0.21	0.22	0.17	
C.V. (%)		12.31				10.75				11.65		
LSD, P $\leq$ 0.05												
				0.02				0.02				0.02
		0.01				0.01				0.02		
Interactions		0.03				0.03				NS		

Key: Timb = Timbilil, Kipk = Kipkebe, Kang = Kangaita

The essential micronutrients for tea include manganese, zinc, copper and iron (Bonheure and Willson, 1992). The changes in leaf manganese levels due to locations and clones are presented in Table 10. The clones showed significant ($P \leq 0.05$) variations in mature leaf manganese. Such variations differed ($P \leq 0.05$) with locations. The levels of manganese in the 3rd leaf of all clones, and at all sites were above 0.005% (50 ppm) considered optimal (Bonheure and Willson, 1992). With continuous application of nitrogenous fertilizers, there are normally increase in soil pH which in turn increases soil available manganese (Kamau *et al.*, 1998). Since nitrogen fertilization forms the basis of tea nutrition, it is unlikely manganese deficiency will be prevalent in Kenya tea soils. There was decline in manganese levels in younger leaves compared to older leaves. However, the change from mature uppermost leaf to the third leaf was more drastic than the change from third leaf to two leaves and a bud.

Zinc is an important micronutrient in tea, and where deficiency is detected, this is corrected through foliar application of zinc oxide (Othieno, 1988; Anon, 2002). In mature leaf, level of zinc varied ($P \leq 0.05$) with clones and location (Table 11). Although the zinc levels appeared within the accepted range (Owuor and Wanyoko, 1983; Othieno, 1988) for all clones in Timbilil, except in STC 5/3, and in Kipkebe, except in clones TRFK 56/89, TRFK 11/16 and STC 5/3, only clone TRFK 303/577 showed adequate zinc levels in Kangaita (Table 11). The levels recorded in the 3rd leaf were much lower than the 50 ppm considered adequate (Bonheure and Willson, 1992) for optimal growth. High amounts of zinc were harvested with crop in two leaves and a bud. Copper is also an important micronutrient in tea, and low levels have detrimental effects on plain black tea quality. Polyphenol oxidase responsible for fermentation process is a copper-protein compound, and teas grown on copper deficient soils do not ferment (Harler, 1971; Clowes and Mitini-Nkhoma, 1987). There were significant ($P \leq 0.05$) variations in leaf copper levels in different clones and at different locations (Table 12).

Table 10: Variations in leaf manganese levels (%) with location of production and cultivars

	Mature leaf				3rd Leaf				2 leaves and a bud			
Clone	Timb	Kipk	Kang	Mean clone	Timb	Kipk	Kang	Mean clone	Timb	Kipk	Kang	Mean clone
TRFK7/9	0.47	0.48	0.21	0.39	0.19	0.28	0.12	0.20	0.19	0.21	0.07	0.17
TRFK303/259	0.61	0.51	0.14	0.42	0.22	0.22	0.07	017	0.13	0.17	0.06	0.12
TRFK303/1199	0.49	0.45	0.22	0.39	0.21	0.30	0.08	0.20	0.10	0.17	0.07	0.12
TRFK54/40	0.38	0.40	0.16	0.35	0.22	0.26	0.04	0.17	0.14	0.19	0.06	0.13
TRFK31/8	0.57	0.36	0.34	0.42	0.18	0.26	0.12	0.19	0.12	0.20	0.06	0.13
BBK35	0.50	0.53	0.17	0.40	0.19	0.22	0.05	0.15	0.14	0.20	0.04	0.13
TRFK6/8	0.30	0.43	0.20	0.32	0.22	0.25	0.07	0.18	0.15	0.19	0.06	0.14
TRFK31/27	0.46	0.31	0.22	0.33	0.22	0.30	0.10	0.20	0.13	0.25	0.05	0.14
TRFK12/12	0.52	0.54	0.23	0.43	0.15	0.29	0.09	0.18	0.16	0.17	0.06	0.13
TRFK303/999	0.43	0.42	0.20	0.35	0.18	0.26	0.14	0.19	0.16	0.21	0.07	0.14
AHPS15/10	0.52	0.53	0.23	0.43	0.17	0.28	0.10	0.19	0.13	0.23	0.07	0.14
TRFK57/15	0.44	0.61	0.22	0.42	0.15	0.31	0.10	0.18	0.19	0.22	0.06	0.16
TRFK56/89	0.60	0.40	0.12	0.37	0.17	0.20	0.06	0.15	0.15	0.22	0.05	0.14
TRFK12/19	0.41	0.66	0.11	0.39	0.14	0.31	0.09	0.18	0.15	0.22	0.05	0.14
TRFK11/26	0.43	0.46	0.23	0.37	0.22	0.34	0.12	0.22	0.19	0.28	0.08	0.18
STC5/3	0.51	0.48	0.33	0.44	0.21	0.22	0.08	0.17	0.17	014	0.07	0.13
TRFK7/3	0.42	0.61	0.24	0.41	0.16	0.28	0.10	0.18	0.09	0.22	0.05	0.12
TRFK303/577	0.39	0.55	0.29	0.41	0.19	0.27	0.10	0.18	0.17	0.21	0.07	0.15
EPKTN14-3	0.45	0.45	0.27	0.39	0.13	0.25	0.12	0.16	0.16	0.16	0.07	0.13
TRFK2xI/4	0.48	0.51	0.23	0.40	0.15	0.24	0.12	0.17	0.15	0.17	0.07	0.13
Mean site	0.47	0.48	0.22		0.18	0.27	0.09		0.15	0.20	0.06	
C.V. (%)		7.24				8.49				7.48		
LSD, P $\leq$ 0.05												
				0.03				0.02				0.01
		0.02				0.01				0.01		
Interactions		0.05				0.03				0.02		

Key: Timb = Timbilil, Kipk = Kipkebe, Kang = Kangaita

The levels of copper in mature leaf to guide foliar analysis advisory system have not been set. However, for clones grown in the same location, large variations were observed suggesting that setting a single level for all clones may not be accurate. For 3rd leaf, most clones had lower than the 15 ppm content considered borderline deficiency and none reached the 30 ppm considered adequate (Bonheure and Willson, 1992). But these clones did not have any copper deficiency problem and fermented very well (Owuor *et al.,* 2010b). This may indicate that the levels set for seedling tea nutrients diagnosis may not be relevant for clonal tea. Copper Levels generally increased with increase in leaf age.

Table 11: Variations in leaf zinc levels (ppm) with location of production and cultivars

	Mature leaf				3rd Leaf				2 leaves and a bud			
Clone	Timb	Kipk	Kang	Mean clone	Timb	Kipk	Kang	Mean clone	Timb	Kipk	Kang	Mean clone
TRFK7/9	16	10	9	12	18	10	9	12	24	21	15	20
TRFK303/259	17	10	8	12	18	13	12	14	57	41	29	42
TRFK303/1199	13	13	9	12	28	16	12	19	48	32	28	36
TRFK54/40	13	15	9	12	20	24	8	17	46	44	22	37
TRFK31/8	11	10	7	9	22	13	12	16	43	21	22	29
BBK35	11	10	7	9	18	13	13	15	44	33	24	34
TRFK6/8	11	10	7	9	13	12	12	12	28	24	20	24
TRFK31/27	11	13	7	10	17	16	14	16	53	35	26	38
TRFK12/12	15	15	9	13	22	15	12	16	28	33	23	28
TRFK303/999	14	12	8	11	17	14	10	14	43	27	19	30
AHPS15/10	11	12	6	10	25	14	11	17	51	27	25	34
TRFK57/15	15	13	9	12	21	24	14	20	61	31	18	37
TRFK56/89	11	9	7	9	15	11	8	11	30	23	18	24
TRFK12/19	11	10	7	9	16	16	10	14	45	27	25	32
TRFK11/26	10	3	6	8	17	11	10	13	35	27	17	26
STC5/3	9	9	6	8	11	9	10	10	23	23	12	20
TRFK7/3	11	10	7	9	17	12	11	14	45	30	18	31
TRFK303/577	16	14	10	13	27	17	13	19	49	30	28	34
EPKTN14-3	10	10	9	9	16	13	10	13	44	27	20	30
TRFK2xI/4	11	11	11	11	17	13	10	13	26	28	18	23
Mean site	12	11	8		18	14	11		41	29	21	
C.V. (%)		12.89				11.11				7.35		
LSD, P ≤ 0.05												
				1				2				2
		1				1				2		
Interactions		2				3				4		

Key: Timb = Timbilil, Kipk = Kipkebe, Kang = Kangaita

Significant ($P \leq 0.05$) differences were observed in iron levels due to location of production and clones (Table 13). Although there are no set norms to guide diagnostic mature leaf iron levels, the large variations demonstrate that setting such level to serve many clones in different locations can be problematic.

In the third leaf, there were either deficient (less than 60 ppm) or borderline (less than 100 ppm) levels and no clone reached the adequate (500 ppm) level (Bonheure and Willson, 1992). Thus although there were no visible signs of iron deficiency, the third leaf set limits for seedling

tea could not be diagnostic to delineate demand for corrective measure in clonal tea at all locations.

Except for third leaf nitrogen, two leaves and a bud phosphorus, magnesium and copper levels, there were significant ($P \leq 0.05$) interactions effects between the leaf nutrient levels of clones and locations. This demonstrates that the patterns of nutrients uptake and partitioning varied with clones and in individual clones, the extent or rate of variations were not uniform.

Table 12: Variations in leaf copper levels (ppm) with location of production and cultivars

	Mature leaf				3rd Leaf				2 leaves and a bud			
Clone	Timb	Kipk	Kang	Mean clone	Timb	Kipk	Kang	Mean clone	Timb	Kipk	Kang	Mean clone
TRFK7/9	8	10	8	9	9	10	7	9	10	12	10	11
TRFK303/259	8	20	7	12	10	11	5	9	14	14	10	13
TRFK303/1199	8	9	9	9	10	11	8	10	13	16	11	13
TRFK	9	10	8	9	8	11	5	8	12	15	10	12
TRFK31/8	6	10	16	11	8	12	7	9	12	13	11	12
BBK35	7	9	6	7	9	11	4	8	12	14	10	12
TRFK6/8	6	6	8	7	6	9	5	7	8	11	8	9
TRFK31/27	8	10	7	8	8	11	5	8	12	15	9	12
TRFK12/12	6	11	6	8	8	10	7	8	10	12	9	10
TRFK303/999	6	11	6	8	8	11	9	9	13	13	8	11
AHPS15/10	7	10	6	7	8	10	10	9	14	16	10	13
TRFK57/15	6	11	7	8	6	9	9	8	11	14	9	11
TRFK56/89	6	8	5	6	9	9	9	9	15	15	10	13
TRFK12/19	8	13	7	10	9	16	9	11	15	15	11	14
TRFK11/26	7	12	6	8	11	12	9	10	13	15	9	12
STC5/3	7	6	8	7	7	9	8	8	11	14	9	11
TRFK7/3	7	6	6	6	8	10	7	9	10	13	7	10
TRFK303/577	9	7	6	8	12	10	9	10	10	13	9	11
EPKTN14-3	7	8	6	7	7	10	8	8	9	11	8	9
TRFK2xI/4	9	7	6	7	10	10	8	9	10	12	7	10
Mean site	7	10	7		9	11	7		12	14	9	
C.V. (%)		15.8				12.4				12.9		
LSD, $P \leq 0.05$												
				1				1				1
		1				1				1		
Interactions		2				2				NS		

Key: Timb = Timbilil, Kipk = Kipkebe, Kang = Kangaita

Table 13: Variations in leaf iron levels (ppm) with location of production and cultivars

	Mature leaf				3rd Leaf				2 leaves and a bud			
Clone	Timb	Kipk	Kang	Mean clone	Timb	Kipk	Kang	Mean clone	Timb	Kipk	Kang	Mean clone
TRFK7/9	67	102	76	81	36	70	56	54	50	70	42	54
TRFK303/259	95	141	74	103	40	68	59	56	46	70	51	56
TRFK303/1199	73	78	71	74	44	69	57	56	58	70	84	71
TRFK54/40	80	101	67	83	41	65	56	54	55	72	64	64
TRFK31/8	90	80	103	91	38	66	61	55	49	70	57	59
BBK35	95	100	89	94	37	67	46	50	37	61	68	55
TRFK6/8	57	109	90	85	36	69	49	51	42	71	63	59
TRFK31/27	66	72	90	76	41	72	68	60	72	69	74	72
TRFK12/12	75	89	95	86	34	61	50	48	40	67	65	57
TRFK303/999	108	96	101	102	50	76	58	61	47	65	58	57
AHPS15/10	78	68	96	81	41	76	52	57	52	68	48	56
TRFK57/15	77	103	65	82	48	71	57	58	44	60	63	56
TRFK56/89	94	92	73	86	41	57	48	49	48	73	53	58
TRFK12/19	96	103	73	91	47	75	54	59	46	66	59	57
TRFK11/26	83	99	75	86	60	74	58	64	44	82	46	57
STC5/3	79	107	66	84	41	53	45	46	51	65	66	60
TRFK7/3	98	130	84	104	39	62	53	51	43	66	61	56
TRFK303/577	79	103	98	93	57	62	57	58	44	63	62	56
EPKTN14-3	94	109	88	97	45	68	61	58	43	65	64	58
TRFK2xI/4	74	104	85	88	52	65	52	56	42	62	64	56
Mean site	83	99	83		43	67	55		48	68	60	
C.V. (%)		5.03				4.84				4.61		
LSD, P ≤ 0.05												
				4				3				3
		3				2				3		
Interactions		7				4				4		

Key: Timb = Timbilil, Kipk = Kipkebe, Kang = Kangaita

Indeed the regression coefficients (r^2) between the levels of same nutrient in different locations were low and not significant (Table 14) further demonstrating that there were variations in the patterns of nutrients uptake and partitioning with location of production. The regression coefficients (r^2) between same nutrient contents in leaf of different age in same clones at individual locations were not significant (Table 15), implying the clones partitioned nutrients differently even in same location. Similarly, the regression coefficients (r^2) between different nutrients in leaves of same age in same location were also not significant (Table 16), a further

indication that clones partition different nutrients differently in their leaves even in the same location. Although several studies have reported dry matter partitioning in different clones (Magambo and Waithaka, 1983; Ng'etich and Stephens, 1995), no study has reported nutrient partitioning in clonal teas. Results presented here demonstrate that such variations can be large in clones or even in same clone grown in different locations. Similar variations were recently observed in the plain tea quality parameters (Owuor *et al.,* 2010b).

The results further demonstrate the difficulties encountered in the use of tissues analysis to guide fertilizer use recommendations. Indeed, despite the wide use of the tissue analysis, there are no uniform recommendations across many major tea growing countries including Sri Lanka (Wicremasighe and Krisnapillai, 1986), South India (Venkatamarani, 1987) and Papua New Guinea (Southern, 1969).

For more definitive and accurate tissue analysis diagnostic system, collection of more data from clonal tea that are widely grown is necessary. The recommendations based on seedling tea (Tolhurst, 1976; Owuor and Wanyoko, 1983; Othieno, 1988) are unreliable and inaccurate to guide foliar analysis advisory system for clonal tea.

Table 14: Regression coefficients (r^2) of linear regression analyses between same nutrients in different leaves from different regions.

		Timb	Kipk	Kang		Timb	Kipk	Kang		Timb	Kipk	Kang
						Mature leaf						
	Timb	1.000				1.000				1.000		
N	Kipk	0.046	1.000		P	0.133	1.000		Cu	0.004	1.000	
	Kang	0.240	0.019	1.000		0.004	0.027	1.000		0.016	0.000	1.000
	Timb	1.000				1.000				1.000		
K	Kipk	0.106	1.000		Ca	0.059	1.000		Zn	0.233	1.000	
	Kang	0.320	0.091	1.000		0.274	0.000	1.000		0.304	0.296	1.000
	Timb	1.000			Mn	1.000			Fe	1.000		
Mg	Kipk	0.155	1.000			0.018	1.000			0.097	1.000	
	Kang	0.342	0.227	1.000		0.000	0.027	1.000		0.010	0.100	1.000
						Third leaf						
	Timb	1.000				1.000				1.000		
N	Kipk	0.222	1.000		P	0.158	1.000		Cu	0.098	1.000	
	Kang	0.149	0.084	1.000		0.080	0.086	1.000		0.028	0.001	1.000
	Timb	1.000				1.000				1.000		
K	Kipk	0.185	1.000		Ca	0.288	1.000		Zn	0.209	1.000	
	Kang	0.253	0.195	1.000		0.096	0.078	1.000		0.133	0.065	1.000
	Timb	1.000				1.000				1.000		
Mg	Kipk	0.203	1.000		Mn	0.003	1.000		Fe	0.066	1.000	
	Kang	0.356	0.345	1.000		0.134	0.167	1.000		0.074	0.249	1.000
						Two leaves and a bud						
	Timb	1.000				1.000				1.000		
N	Kipk	0.661	1.000		P	0.012	1.000		Cu	0.665	1.000	
	Kang	0.356	0.165	1.000		0.086	0.012	1.000		0.394	0.348	1.000
	Timb	1.000				1.000				1.000		
K	Kipk	0.284	1.000		Ca	0.248	1.000		Zn	0.305	1.000	
	Kang	0.111	0.073	1.000		0.179	0.125	1.000		0.412	0.310	1.000
	Timb	1.000				1.000				1.000		
Mg	Kipk	0.260	1.000		Mn	0.017	1.000		Fe	0.071	1.000	
	Kang	0.240	0.221	1.000		0.171	0.008	1.000		0.081	0.141	1.000

Table 15: Regression coefficients (r^2) of linear regression analyses between nutrients in different leaves of clonal tea

		Timbilil			Kipkebe			Kangaita		
		Mature	3rd leaf	2+bud	Mature	3rd leaf	2+bud	Mature	3rd leaf	2+bud
	mature	1.000			1.000			1.000		
N	3rd leaf	0.020	1.000		0.000	1.000		0.441	1.000	
	2+bud	0.006	0.277	1.000	0.012	0.209	1.000	0.243	0.461	1.000
	mature	1.000			1.000			1.000		
P	3rd leaf	0.068	1.000		0.043	1.000		0.001	1.000	
	2+bud	0.053	0.062	1.000	0.159	0.100	1.000	0.001	0.102	1.000
	mature	1.000			1.000			1.000		
K	3rd leaf	0.396	1.000		0.051	1.000		0.010	1.000	
	2+bud	0.254	0.470	1.000	0.010	0.381	1.000	0.064	0.417	1.000
	mature	1.000			1.000			1.000		
Ca	3rd leaf	0.047	1.000		0.020	1.000		0.430	1.000	
	2+bud	0.012	0.047	1.000	0.060	0.266	1.000	0.171	0.155	1.000
	mature	1.000			1.000			1.000		
Mg	3rd leaf	0.268	1.000		0.343	1.000		0.670	1.000	
	2+bud	0.158	0.525	1.000	0.083	0.395	1.000	0.349	0.661	1.000
	mature	1.000			1.000			1.000		
Mn	3rd leaf	0.007	1.000		0.048	1.000		0.224	1.000	
	2+bud	0.039	0.007	1.000	0.001	0.324	1.000	0.215	0.299	1.000
	mature	1.000			1.000			1.000		
Cu	3rd leaf	0.340	1.000		0.213	1.000		0.035	1.000	
	2+bud	0.000	0.100	1.000	0.085	0.110	1.000	0.205	0.000	1.000
	mature	1.000			1.000			1.000		
Fe	3rd leaf	0.024	1.000		0.062	1.000		0.030	1.000	
	2+bud	0.105	0.009	1.000	0.015	0.018	1.000	0.001	0.002	1.000
	mature	1.000			1.000			1.000		
Zn	3rd leaf	0.219	1.000		0.437	1.000		0.000	1.000	
	2+bud	0.081	0.230	1.000	0.224	0.381	1.000	0.020	0.238	1.000

Table 16: Regression coefficients (r^2) analyses between different nutrients in clonal tea leaves of the same age `

		Timbilil									Kipkebe									Kangaita								
		N	P	K	Ca	Mg	Mn	Cu	Fe	Zn	N	P	K	Ca	Mg	Mn	Cu	Fe	Zn	N	P	K	Ca	Mg	Mn	Cu	Fe	Zn
Mature leaf	N	1.00									1.00									1.00								
	P	0.28	1.00								0.01	1.00								0.19	1.00							
	K	0.03	0.17	1.00							0.00	0.31	1.00							0.17	0.01	1.00						
	Ca	0.25	0.10	0.03	1.00						0.00	0.04	0.01	1.00						0.12	0.01	0.02	1.00					
	Mg	0.01	0.05	0.13	0.00	1.00					0.01	0.00	0.14	0.23	1.00					0.02	0.01	0.00	0.38	1.00				
	Mn	0.04	0.02	0.29	0.29	0.01	1.00				0.00	0.07	0.25	0.25	0.13	1.00				0.16	0.10	0.00	0.16	0.17	1.00			
	Cu	0.01	0.08	0.10	0.06	0.00	0.03	1.00			0.11	0.00	0.02	0.05	0.02	0.01	1.00			0.23	0.04	0.02	0.16	0.01	0.20	1.00		
	Fe	0.17	0.26	0.07	0.09	0.05	0.09	0.04	1.00		0.00	0.08	0.25	0.04	0.07	0.18	0.04	1.00		0.03	0.02	0.01	0.19	0.30	0.13	0.02	1.00	
	Zn	0.03	0.07	0.14	0.06	0.01	0.01	0.04	0.00	1.00	0.01	0.03	0.24	0.00	0.19	0.00	0.00	0.07	1.00	0.02	0.00	0.02	0.00	0.01	0.00	0.01	0.00	1.00
3rd leaf	N	1.00									1.00									1.00								
	P	0.06	1.00								0.07	1.00								0.02	1.00							
	K	0.01	0.00	1.00							0.04	0.09	1.00							0.05	0.41	1.00						
	Ca	0.16	0.01	0.08	1.00						0.00	0.02	0.01	1.00						0.02	0.28	0.20	1.00					
	Mg	0.02	0.09	0.32	0.04	1.00					0.01	0.13	0.15	0.07	1.00					0.00	0.24	0.46	0.17	1.00				
	Mn	0.16	0.14	0.01	0.00	0.03	1.00				0.01	0.03	0.08	0.04	0.10	1.00				0.04	0.11	0.11	0.33	0.13	1.00			
	Cu	0.06	0.00	0.06	0.24	0.08	0.04	1.00			0.05	0.06	0.01	0.08	0.11	0.16	1.00			0.04	0.16	0.00	0.07	0.00	0.27	1.00		
	Fe	0.15	0.00	0.21	0.30	0.19	0.00	0.28	1.00		0.01	0.07	0.06	0.04	0.03	0.33	0.24	1.00		0.08	0.04	0.07	0.03	0.01	0.22	0.00	1.00	
	Zn	0.01	0.03	0.28	0.04	0.03	0.00	0.16	0.02	1.00	0.00	0.25	0.10	0.00	0.00	0.12	0.01	0.05	1.00	0.02	0.00	0.02	0.01	0.00	0.00	0.06	0.07	1.00
2 +b	N	1.00									1.00									1.00								
	P	0.20	1.00								0.02	1.00								0.01	1.00							
	K	0.00	0.18	1.00							0.05	0.01	1.00							0.01	0.02	1.00						
	Ca	0.04	0.00	0.02	1.00						0.00	0.06	0.02	1.00						0.09	0.06	0.16	1.00					
	Mg	0.00	0.03	0.24	0.00	1.00					0.02	0.05	0.52	0.00	1.00					0.08	0.03	0.19	0.19	1.00				
	Mn	0.05	0.00	0.02	0.03	0.10	1.00				0.03	0.01	0.12	0.02	0.13	1.00				0.04	0.02	0.00	0.21	0.08	1.00			
	Cu	0.16	0.04	0.02	0.06	0.00	0.04	1.00			0.02	0.00	0.06	0.14	0.21	0.00	1.00			0.02	0.00	0.05	0.06	0.12	0.03	1.00		
	Fe	0.00	0.12	0.01	0.25	0.00	0.08	0.08	1.00		0.00	0.02	0.07	0.22	0.02	0.02	0.08	1.00		0.02	0.05	0.01	0.07	0.04	0.06	0.00	1.00	
	Zn	0.01	0.40	0.14	0.01	0.14	0.10	0.11	0.07	1.00	0.01	0.00	0.00	0.00	0.05	0.06	0.09	0.00	1.00	0.00	0.02	0.00	0.04	0.01	0.06	0.18	0.10	1.00

4.2 Variations in mature leaf nutrients of clone TRFK 6/8 in Eastern Africa with location of production, nitrogenous fertilizer rates and plucking intervals

The nutrients norms set in diagnostic tissue analysis advisory system in Eastern Africa for seedling tea (Tolhurst, 1976) are presented in Table 2. The limit levels were set for nitrogen, phosphorus, potassium, magnesium and zinc. Whereas deficiency of the other nutrients can also limit production; their critical levels have not been set. Setting their levels require collection of more data to understand how their uptake are influenced by biotic and abiotic factors.

Variations in levels of mature tea leaf nitrogen, phosphorus and potassium are presented in Tables 17, 18 and 19 respectively. There were significant ($P \leq 0.05$) variations in all the three nutrients with locations and nitrogenous fertilizer rates at all locations. Increase in nitrogen rates resulted in significant ($P \leq 0.05$) increase in leaf nitrogen in mature leaf levels in all locations. This shows that nitrogen deficiency can be corrected by applying nitrogen fertilizer and this has also been shown in previous studies in Kenya (Wanyoko, 1988; Owuor *et al*., 1990a; Wanyoko *et al*., 1997). The variations with location demonstrate that setting a single norm for mature leaf to serve as a guide for clone TRFK 6/8 in the Eastern Africa tea growing regions can be problematic.

Mature leaf nitrogen level below 3.0% is considered deficient while level below 3.5% is mildly deficient for seedling tea (Tolhurst, 1976; Owuor and Wanyoko, 1983; Othieno, 1988). For mean site data, there was mild nitrogen deficiency up to 150 kg N/ha/year in Changoi and at control at Timbilil (Table 17). Overally, Changoi and Timbilil had the lowest mean nitrogen levels. Indeed the level of nitrogen at the control at Timbilil was showing mild deficiency. Even without application of nitrogen (control) the mature leaf nutrients levels at Sotik, Ngwazi, Maruku, Katoke, Kitabi, and Mulindi were at levels considered adequate despite the fact that at the time of

Table 17: Variations in mature leaf N levels (%) of TRFK 6/8 with location, N rates and plucking frequency

Site (S)	Plucking Frequency (PF) (days)	N-rates (N) in kg N/ha/yr 0	75	150	225	300	PF mean	Site Mean	C.V. %
	7	2.97	3.48	3.61	3.87	3.52	3.49		
Changoi	14	3.45	3.58	3.55	3.75	3.59	3.58	3.53	4.90
	21	3.36	3.31	3.22	3.34	4.35	3.52		
Mean N-rate		3.26	3.46	3.46	3.65	3.82			
LSD, P ≤ 0.05				0.23			NS		
	7	3.36	3.78	3.33	3.85	3.54	3.57		
Sotik	14	3.88	3.67	3.88	3.38	3.95	3.75	3.70	4.57
	21	3.64	3.44	3.85	4.05	3.95	3.78		
Mean N-rate		3.62	3.63	3.68	3.76	3.81			
LSD, P ≤ 0.05				NS			0.27		
	7	3.33	3.55	3.67	3.44	3.59	3.52		
Timbilil	14	3.43	3.46	3.58	3.76	3.62	3.57	3.56	3.05
	21	3.66	3.56	3.41	3.60	3.74	3.59		
Mean N-rate		3.47	3.52	3.55	3.60	3.65			
LSD, P ≤ 0.05				0.14			NS		
	7	4.92	4.69	4.74	4.82	4.84	4.80		
Ngwazi	14	4.55	4.66	4.71	4.63	4.72	4.65	4.73	3.09
	21	4.55	4.68	4.68	4.93	4.80	4.73		
Mean N-rate		4.67	4.69	4.71	4.79	4.79			
LSD, P ≤ 0.05				NS			NS		
	7	4.26	4.32	4.28	4.51	4.29	4.33		
Maruku	14	4.18	4.27	4.31	4.25	4.64	4.33	4.34	3.07
	21	4.34	4.26	4.36	4.46	4.36	4.36		
Mean N-rate		4.26	4.28	4.32	4.41	4.43			
LSD, P ≤ 0.05				0.17			NS		
	7	4.88	5.11	5.27	5.28	5.51	5.21		
Katoke	14	5.01	4.77	5.11	4.98	5.16	5.00	5.07	3.33
	21	4.81	5.26	4.85	5.08	5.03	5.01		
Mean N-rate		4.90	5.05	5.08	5.11	5.23			
LSD, P ≤ 0.05				0.22			NS		
	7	4.16	4.23	4.28	4.33	4.48	4.30		
Kitabi	14	4.15	4.24	4.27	4.32	4.44	4.28	4.29	3.24
	21	4.16	4.23	4.29	4.34	4.47	4.30		
Mean N-rate		4.16	4.24	4.28	4.33	4.46			
LSD, P ≤ 0.05				0.21			NS		
	7	4.13	4.37	4.40	4.48	4.63	4.40		
Mulindi	14	4.10	4.37	4.39	4.50	4.61	4.39	4.40	3.46
	21	4.15	4.37	4.43	4.48	4.62	4.41		
Mean N-rate		4.12	4.37	4.41	4.49	4.62			
LSD, P ≤ 0.05				0.19			NS		
All 8 sites means:	7	4.00	4.19	4.20	4.32	4.30	4.20		
	14	4.09	4.13	4.23	4.20	4.34	4.20		3.86
	21	4.08	4.14	4.14	4.29	4.42	4.21		
	N-rate	4.06	4.15	4.19	4.27	4.35			
LSD,P ≤ 0.05					0.09		NS	0.09	

Interactions: SxN = NS, SxPF = 0.13, NxPF = 0.12, SxNxPF = 0.26

NS = Not significant

sampling, the control plots had been without fertilizer application for three years. This may be attributed to recycling of plant tissue nitrogen during pruning. The results suggest that the nutrients norm set for seedling tea might have been only a good indicator of deficiency at Changoi and Timbilil. These results indicate that the mature leaf nitrogen norms set for seedling tea may not be appropriate for clonal tea, especially in the different locations. Plucking intervals however, did not influence mature leaf nitrogen levels. In all locations, increasing nitrogen rates increased mature leaf nitrogen levels. This indicates that nitrogen deficiency can be managed by application of nitrogenous fertilizer, although the appropriate rates may vary with locations.

Increasing rates of nitrogenous fertilizer had varying influences on mature leaf phosphorus at different sites. At Changoi, Sotik, Timbilil, Ngwazi, Kitabi and Mulindi, there were no significant changes in mature leaf phosphorus with higher rates on nitrogenous fertilizer. But at Katoke and Maruku there was significant ($P \leq 0.05$) response in mature leaf phosphorus to nitrogenous fertilizer application. The response however, appeared quadratic with low levels at control and at the highest nitrogen rate. That trend was repeated for the mean data for all locations. The increase observed is attributed to the additional phosphorus applied in the nitrogenous fertilizer. However, these data are at variance with previous data from Kericho, where application of high rates of nitrogenous fertilizer reduced mature leaf phosphorus levels (Owuor *et al.,* 1990a; Wanyoko *et al.,* 1992). The decline at the highest nitrogenous fertilizer level could be due to soil pH reduction (Wanyoko *et al.,* 1992) causing phosphorous fixation in the soil. There were significant variations in mature leaf mean phosphorus with locations (Tables 18) showing that soils in the various locations had varying abilities to supply the nutrient. However, the levels of phosphorus were high and well above what is considered deficient demonstrating the soils nutritional status of the

Table 18: Variations in mature leaf P levels (%) of TRFK 6/8 with location, N rates and plucking frequency

Site (S)	Plucking Frequency (PF) (days)	N-rates (N) in kg N/ha/yr 0	75	150	225	300	PF Mean	Site mean	C.V.%
Changoi	7	0.22	0.27	0.26	0.26	0.23	0.25	0.24	8.19
	14	0.25	0.22	0.27	0.23	0.22	0.24		
	21	0.23	0.24	0.22	0.22	0.22	0.23		
	Mean N-rate	0.23	0.24	0.25	0.24	0.22			
	LSD,P ≤ 0.05			NS			NS		
Sotik	7	0.18	0.19	0.19	0.19	0.18	0.19	0.19	8.62
	14	0.18	0.22	0.19	0.20	0.16	0.19		
	21	0.20	0.19	0.20	0.18	0.21	0.20		
	Mean N-rate	0.18	0.20	0.19	0.19	0.18			
	LSD, P ≤ 0.05			NS			NS		
Timbilil	7	0.18	0.19	0.19	0.17	0.17	0.18	0.18	8.80
	14	0.18	0.18	0.18	0.19	0.18	0.18		
	21	0.18	0.17	0.19	0.19	0.16	0.18		
	Mean N-rate	0.18	0.18	0.19	0.18	0.17			
	LSD, P ≤ 0.05			NS			NS		
Ngwazi	7	0.32	0.31	0.33	0.33	0.30	0.32	0.32	4.96
	14	0.36	0.31	0.34	0.31	0.31	0.33		
	21	0.29	0.33	0.32	0.31	0.30	0.31		
	Mean N-rate	0.32	0.32	0.33	0.32	0.30			
	LSD, P ≤ 0.05			NS			0.02		
Maruku	7	0.36	0.29	0.34	0.27	0.22	0.30	0.29	5.69
	14	0.25	0.26	0.32	0.32	0.27	0.28		
	21	0.24	0.32	0.27	0.31	0.24	0.28		
	Mean N-rate	0.28	0.29	0.31	0.30	0.24			
	LSD, P ≤ 0.05			0.02			NS		
Katoke	7	0.28	0.36	0.33	0.31	0.30	0.32	0.30	6.89
	14	0.22	0.33	0.34	0.30	0.24	0.29		
	21	0.26	0.32	0.27	0.29	0.24	0.28		
	Mean N-rate	0.25	0.34	0.31	0.31	0.26			
	LSD, P ≤ 0.05			0.02			0.03		
Kitabi	7	0.20	0.23	0.20	0.20	0.18	0.20	0.21	9.30
	14	0.23	0.19	0.21	0.20	0.19	0.21		
	21	0.20	0.22	0.21	0.21	0.20	0.21		
	Mean N-rate	0.21	0.21	0.21	0.20	0.19			
	LSD, P ≤ 0.05			NS			NS		
Mulindi	7	0.19	0.18	0.21	0.18	0.18	0.19	0.19	8.06
	14	0.19	0.20	0.17	0.18	0.15	0.18		
	21	0.18	0.21	0.21	0.20	0.19	0.20		
	Mean N-rate	0.19	0.20	0.19	0.19	0.17			
	LSD, P ≤ 0.05			NS			NS		
All 8 sites means:	7	0.24	0.25	0.26	0.24	0.22	0.24		7.46
	14	0.23	0.24	0.25	0.24	0.22	0.24		
	21	0.22	0.25	0.38	0.24	0.22	0.23		
	N rate	0.23	0.25	0.25	0.24	0.22			
	LSD, P ≤ 0.05			0.01			0.01	0.01	

Interactions: SxN = 0.02, SxPF = 0.01, NxPF = 0.01, SxNxPF = 0.03

NS = Not significant

nutrients was high as the levels were above what is considered deficient for mature seedling tea leaf (Tolhurst, 1976; Owuor and Wanyoko, 1983; Othieno, 1988). Even where fertilizer had not been applied, the levels were higher than the deficient limit level. This shows that soils on which these tea plants were planted may have had adequate phosphorus supply. There were significant interactions between location and nitrogen rates indicating that the observed responses were unique and did not follow the same pattern. There was no response on mature leaf phosphorus levels to plucking frequency except at Ngwazi and Katoke and in the means for all sites.

The amounts of leaf potassium monitored at all sites were high (Table 19) and supported the earlier observations (Willson, 1975c) that Eastern African soils are rich in the nutrient and there is little likelihood of obtaining tea yield response to potassium (Kamau *et al.,* 1998). Although there were no significant variations in mature leaf potassium levels with increasing rates of nitrogenous fertilizer (Table 19), the general trend was that there was decline in clone TRFK 6/8 potassium levels. This is despite the concurrent increase in applied potassium with increase in nitrogenous fertilizer rates. The decrease in mature leaf potassium with rise in nitrogenous fertilizer can be attributed to leaching triggered by excess ammonium ions in the NPK fertilizer (Owuor *et al.,* 1987d). There is normally antagonistic soil availability relationship between nitrogen and potassium in the soil (Mulder, 1953; Brady and Weil, 1996). These results suggest that application of potassium and nitrogen may need to be staggered so that at any time there is only availability of one of the nutrients. Such applications can be staggered by at least three months for a perennial crop like tea (Owuor *et al.*, 1993; Kamau *et al.*, 1999). There were no significant responses in mature leaf potassium to intervals of harvesting, except at Mulindi, and in all sites means. However, the response appeared sporadic. There were also significant ($P \leq 0.05$) interactions effects between locations and nitrogen rates and locations and plucking intervals, demonstrating

Table 19: Variations in mature leaf K levels (%) of TRFK 6/8 with location, N rates and plucking frequency

Site (S)	Plucking Frequency (PF) (days)	N-rates (N) in kg N/ha/yr 0	75	150	225	300	PF Mean	Site Mean	C.V.%
	7	1.43	1.82	1.62	1.70	1.59	1.63		
Changoi	14	1.47	1.61	1.88	1.61	1.51	1.62	1.61	2.27
	21	1.43	1.68	1.52	1.69	1.62	1.59		
Mean N-rate		1.44	1.70	1.67	1.67	1.57			
LSD, P ≤ 0.05				0.05			NS		
	7	1.81	1.62	1.72	1.52	1.62	1.66		
Sotik	14	1.67	1.69	1.62	1.55	1.55	1.62	1.62	2.86
	21	1.70	1.56	1.54	1.65	1.46	1.58		
Mean N-rate		1.72	1.62	1.62	1.58	1.54			
LSD, P ≤ 0.05				0.06			NS		
	7	1.62	1.70	1.66	1.63	1.45	1.61		
Timbilil	14	1.54	1.51	1.60	1.43	1.59	1.53	1.57	2.67
	21	1.65	1.53	1.46	1.61	1.61	1.57		
Mean N-rate		1.60	1.58	1.57	1.56	1.55			
LSD, P ≤ 0.05				NS			0.07		
	7	1.39	1.43	1.43	1.40	1.38	1.41		
Ngwazi	14	1.41	1.42	1.40	1.39	1.39	1.40	1.41	1.63
	21	1.41	1.43	1.40	1.40	1.41	1.41		
Mean N-rate		1.40	1.43	1.41	1.40	1.39			
LSD, P ≤ 0.05				0.03			NS		
	7	1.35	1.31	1.35	1.27	1.27	1.31		
Maruku	14	1.31	1.35	1.32	1.30	1.27	1.31	1.32	1.97
	21	1.32	1.36	1.35	1.34	1.31	1.34		
Mean N-rate		1.33	1.34	1.34	1.30	1.28			
LSD, P ≤ 0.05				0.03			NS		
	7	1.22	1.26	1.25	1.22	1.27	1.24		
Katoke	14	1.26	1.26	1.25	1.26	1.19	1.24	1.25	1.50
	21	1.28	1.27	1.25	1.25	1.24	1.26		
Mean N-rate		1.25	1.26	1.25	1.24	1.23			
LSD, P ≤ 0.05				0.02			NS		
	7	1.26	1.26	1.26	1.22	1.22	1.24		
Kitabi	14	1.22	1.26	1.23	1.23	1.22	1.23	1.23	1.29
	21	1.25	1.24	1.21	1.23	1.22	1.23		
Mean N-rate		1.24	1.25	1.23	1.23	1.22			
LSD, P ≤ 0.05				0.02			NS		
	7	1.22	1.22	1.22	1.21	1.19	1.21		
Mulindi	14	1.19	1.22	1.21	1.19	1.20	1.20	1.21	1.26
	21	1.27	1.24	1.21	1.20	1.19	1.22		
Mean N-rate		1.23	1.22	1.21	1.20	1.19			
LSD, P ≤ 0.05				0.02			NS		
All 8 sites means:	7	1.41	1.45	1.44	1.40	1.37	1.42		
	14	1.38	1.42	1.44	1.37	1.37	1.40		2.21
	21	1.41	1.41	1.37	1.42	1.38	1.40		
	N-rate	1.40	1.43	1.42	1.40	1.37			
LSD, P ≤ 0.05				0.01			0.02	0.02	

Interactions: SxN = 0.03, SxPF = 0.02, NxPF = 0.02, SxNxPF = 0.05

NS = Not significant

that extents of the responses varied from site to site. This indicates that factors controlling the absorption of nutrients were varying from site to site.

Mature leaf magnesium levels of clone TRFK 6/8 significantly ($P \leq 0.05$) changed with location of production (Table 20). The levels were particularly low at Katoke and Kitabi, where the levels were equivalent to borderline levels for seedling tea (Tolhurst, 1976; Othieno, 1988). In other areas, the levels were equivalent to adequate supply for seedling tea. At all locations, the clone TRFK 6/8 mature leaf magnesium levels declined with increase in nitrogenous fertilizer rates (Table 20) and this reached significant levels at Timbilil, Maruku, Katoke and Kitabi. Similar decline in mature leaf magnesium with increase in nitrogenous fertilizer rates had been observed in earlier studies (Willson, 1975a; Wanyoko *et al.,* 1992; 1997). The magnesium levels did not change due to plucking intervals except at Maruku.

The zinc levels (Table 21) were much higher than the set limits levels for seedling tea (Tolhurst, 1976; Owuor and Wanyoko, 1983; Othieno, 1988). Thus, the set limits set for seedling tea seem inappropriate for clone TRFK 6/8 at all locations in Eastern Africa. There were significant ($P \leq 0.05$) differences in mature leaf zinc levels with location of production, possibly demonstrating the zinc reserves in the soils were widely varying with locations. These tea areas were unlikely to suffer from zinc deficiency. Generally, increasing rates of nitrogenous fertilizer application increased the mature leaf zinc levels, reaching significant ($P \leq 0.05$) levels at all locations except at Maruku and Katoke. Similar responses have been recorded in Kericho (Owuor *et al*., 1993). However, plucking intervals had no significant influence on mature leaf zinc level.

Table 20: Variations in mature leaf Mg levels (%) of TRFK 6/8 with location, N rates and plucking frequency

Site (S)	Plucking Frequency (PF) (days)	N-rates (N) kg N/ha/yr 0	75	150	225	300	PF mean	Site Mean	C.V.%
	7	0.19	0.19	0.16	0.18	0.17	0.18		
Changoi	14	0.18	0.16	0.18	0.19	0.18	0.18	0.17	9.70
	21	0.18	0.17	0.17	0.13	0.14	0.16		
Mean N-rate		0.18	0.17	0.17	0.17	0.16			
LSD, P ≤ 0.05				NS			NS		
	7	0.17	0.19	0.18	0.19	0.19	0.18		
Sotik	14	0.17	0.17	0.19	0.16	0.17	0.17	0.18	11.23
	21	0.21	0.17	0.16	0.17	0.16	0.18		
Mean N-rate		0.18	0.18	0.18	0.17	0.17			
LSD, P ≤ 0.05				NS			NS		
	7	0.20	0.17	0.13	0.17	0.11	0.16		
Timbilil	14	0.19	0.13	0.19	0.15	0.13	0.16	0.17	13.03
	21	0.13	0.17	0.20	0.15	0.16	0.16		
Mean N-rate		0.18	0.16	0.17	0.16	0.13			
LSD, P ≤ 0.05				0.03			NS		
	7	0.20	0.25	0.21	0.22	0.22	0.22		
Ngwazi	14	0.21	0.20	0.23	0.20	0.21	0.21	0.21	7.73
	21	0.20	0.20	0.20	0.21	0.21	0.20		
Mean N-rate		0.21	0.22	0.21	0.21	0.22			
LSD, P ≤ 0.05				NS			NS		
	7	0.15	0.11	0.11	0.12	0.13	0.13		
Maruku	14	0.14	0.16	0.13	0.13	0.14	0.14	0.14	10.19
	21	0.19	0.15	0.15	0.15	0.13	0.15		
Mean N-rate		0.16	0.14	0.13	0.13	0.13			
LSD, P ≤ 0.05				0.02			0.02		
	7	0.10	0.13	0.09	0.10	0.07	0.10		
Katoke	14	0.13	0.12	0.14	0.09	0.08	0.11	0.11	13.39
	21	0.15	0.10	0.10	0.10	0.12	0.11		
Mean N-rate		0.13	0.12	0.11	0.10	0.09			
LSD, P ≤ 0.05				0.02			NS		
	7	0.12	0.09	0.13	0.10	0.11	0.11		
Kitabi	14	0.12	0.10	0.11	0.10	0.10	0.11	0.11	11.77
	21	0.11	0.11	0.10	0.10	0.10	0.10		
Mean N-rate		0.12	0.10	0.11	0.10	0.10			
LSD, P ≤ 0.05				0.02			NS		
	7	0.14	0.15	0.17	0.15	0.15	0.15		
Mulindi	14	0.18	0.16	0.15	0.15	0.16	0.16	0.16	8.65
	21	0.14	0.16	0.17	0.18	0.15	0.16		
Mean N-rate		0.16	0.16	0.16	0.16	0.15			
LSD, P ≤ 0.05				NS			NS		
All 8 sites means:	7	0.16	0.16	0.15	0.15	0.14	0.15		
	14	0.17	0.15	0.17	0.15	0.15	0.16		10.71
	21	0.16	0.15	0.16	0.15	0.15	0.15		
	N-rate	0.16	0.16	0.16	0.15	0.15			
LSD, P ≤ 0.05				0.01			NS	0.01	

Interactions: SxN = 0.02, SxPF = 0.01, NxPF = 0.01, SxNxPF = 0.03

NS = Not significant

Table 21: Variations in mature leaf Zn levels (ppm) of TRFK 6/8 with location, N rates and plucking frequency

Site (S)	Plucking Frequency (PF) (days)	N-rates (N) kg N/ha/yr 0	75	150	225	300	PF mean	Site (S) Mean	C.V.%
	7	16.00	16.00	19.67	20.67	19.67	18.40		
Changoi	14	16.00	18.00	17.00	19.33	19.67	18.00	17.98	10.88
	21	15.67	18.33	17.67	17.67	18.33	17.53		
Mean N-rate		15.89	17.44	18.11	19.22	19.22			
LSD, P ≤ 0.05				2.56			NS		
	7	19.00	21.67	20.67	28.00	31.33	24.13		
Sotik	14	18.00	20.00	21.00	27.67	28.00	22.93	23.62	7.59
	21	18.67	21.00	22.33	28.00	29.00	23.80		
Mean N-rate		18.56	20.89	21.33	27.89	29.44			
LSD, P ≤ 0.05				2.35			NS		
	7	21.33	21.00	20.33	22.67	23.00	21.67		
Timbilil	14	22.00	21.00	22.33	22.00	21.67	21.80	21.51	1.21
	21	20.33	20.33	22.33	21.00	21.33	21.07		
Mean N-rate		21.22	20.78	21.67	21.89	22.00			
LSD, P ≤ 0.05				1.21			NS		
	7	29.00	29.33	27.67	34.00	37.00	31.40		
Ngwazi	14	29.33	34.00	30.67	30.00	33.67	31.53	31.82	8.55
	21	31.00	29.67	35.00	34.00	33.00	32.53		
Mean N-rate		29.78	31.00	31.11	32.67	34.56			
LSD, P ≤ 0.05				3.56			NS		
	7	44.33	44.33	48.00	44.00	45.67	45.27		
Maruku	14	44.33	44.67	42.00	45.00	46.00	44.40	45.36	4.97
	21	45.33	45.33	44.67	48.00	48.67	46.40		
Mean N-rate		44.67	44.78	44.89	45.67	46.78			
LSD, P ≤ 0.05				NS			NS		
	7	38.00	42.33	34.67	44.33	43.67	40.40		
Katoke	14	43.67	40.00	46.00	42.33	45.00	43.40	42.13	6.70
	21	38.00	40.33	46.33	43.00	45.33	42.60		
Mean N-rate		39.89	40.89	42.33	43.22	44.33			
LSD, P ≤ 0.05				3.69			4.44		
	7	28.33	27.00	28.33	28.67	31.00	28.67		
Kitabi	14	26.67	29.33	28.67	30.00	29.33	28.80	28.49	5.10
	21	26.33	27.67	29.00	27.33	29.67	28.00		
Mean N-rate		27.11	28.00	28.67	28.67	30.00			
LSD, P ≤ 0.05				1.90			NS		
	7	31.67	31.67	32.67	34.67	33.33	32.80		
Mulindi	14	32.33	32.00	32.33	32.33	33.67	32.53	32.91	3.89
	21	32.67	33.67	34.00	32.00	34.67	33.40		
Mean N-rate		32.22	32.44	33.00	33.00	33.89			
LSD, P ≤ 0.05				NS			NS		
All 8 sites means:	7	28.46	29.17	29.00	32.13	33.08	30.34		
	14	29.04	29.88	30.00	31.08	32.13	30.43		6.74
	21	28.50	29.54	31.42	31.38	32.50	30.67		
	N-rate	28.67	29.53	30.14	31.53	32.53			
LSD, P ≤ 0.05					0.95		NS	1.02	

Interactions: SxN = 1.98, SxPF = 1.61, NxPF = 1.37, SxNxPF = 3.39

NS = Not significant

Diagnostic mature leaf limit levels for calcium, manganese, copper and iron have not been set. But these nutrients are beneficial for proper growth of plants. The changes in these nutrients with locations, nitrogenous fertilizer rates and plucking intervals for clone TRFK 6/8 are presented in Tables 22, 23, 24 and 25, respectively. All the mature leaf contents of these nutrients changed ($P \leq 0.05$) with location of production, further emphasising how the nutrients reserves in the soils are variable. While calcium levels declined ($P \leq 0.05$), the levels of manganese, copper, and iron increased ($P \leq 0.05$) with rise in nitrogenous fertilizer rates at all sites. Similar results for calcium had been observed in earlier studies (Willson, 1975b; Owuor *et al.*, 1988b; Wanyoko *et al*., 1990; Kebeney *et al*., 2010). These patterns follow closely those observed in the soils where available soil calcium levels decline (Dogo *et al*., 1994; Kamau *et al.,* 1998, 2008; Kebeney *et al*., 2010) while manganese levels rise (Dogo *et al*., 1994; Kamau *et al,*. 1998, 2008; Kebeney *et al*., 2010). There were significant interactions except between site x plucking frequency for manganese and site x nitrogen-rate for copper indicating differences in patterns of change in the observed variations of mature leaf calcium, manganese, copper and iron. The changes in mature leaf copper and iron due to nitrogen levels are reported for Eastern African teas for the first time. Generally, the mature leaf calcium, manganese, copper and iron were not influenced by harvesting intervals.

Generally, the amount of mature leaf nutrients significantly ($P \leq 0.05$) varied with location of production (Tables 17-25). The results indicate that due to variations in environmental factors at the sites, even with application of the same agronomic inputs, the level of the nutrients will be different. This in part could be due to variations in the levels of micronutrients in the soils at different locations, especially for the nutrients not supplied and/or due to past agronomic inputs in the fields. It can also be partly attributed to soil characteristics like pH and organic matter variations.

Table 22: Variations in mature leaf Ca levels (%) of TRFK 6/8 with location, N rates and plucking frequency

Site (S)	Plucking Frequency (PF) (days)	N-rates (N) kg N/ha/yr 0	75	150	225	300	PF mean	Site (S) Mean	C.V. %
	7	1.15	1.24	1.09	1.04	0.92	1.09		
Changoi	14	1.18	1.11	1.03	1.21	1.11	1.13	1.10	12.92
	21	1.17	1.05	1.06	1.12	1.03	1.09		
Mean N-rate		1.16	1.13	1.06	1.12	1.02			
LSD, P ≤ 0.05				NS			NS		
	7	1.68	1.89	1.79	1.69	1.54	1.72		
Sotik	14	1.67	1.65	1.77	1.81	1.71	1.73	1.71	4.17
	21	1.90	1.73	1.52	1.67	1.65	1.70		
Mean N-rate		1.75	1.76	1.70	1.73	1.63			
LSD, P ≤ 0.05				0.09			NS		
	7	0.89	0.73	0.84	0.80	0.73	0.80		
Timbilil	14	0.79	0.81	0.82	0.74	0.70	0.77	0.81	4.94
	21	0.96	0.98	0.68	0.83	0.84	0.86		
Mean N-rate		0.88	0.84	0.80	0.79	0.76			
LSD, P ≤ 0.05				0.04			NS		
	7	0.38	0.41	0.41	0.41	0.43	0.41		
Ngwazi	14	0.39	0.41	0.41	0.43	0.41	0.41	0.40	6.01
	21	0.38	0.40	0.42	0.42	0.42	0.41		
Mean N-rate		0.38	0.40	0.41	0.42	0.42			
LSD, P ≤ 0.05				0.05			NS		
	7	0.21	0.27	0.22	0.16	0.17	0.21		
Maruku	14	0.21	0.17	0.19	0.21	0.21	0.20	0.20	6.08
	21	0.28	0.16	0.17	0.19	0.13	0.19		
Mean N-rate		0.24	0.20	0.19	0.19	0.17			
LSD, P ≤ 0.05				0.02			0.02		
	7	0.32	0.28	0.33	0.36	0.33	0.33		
Katoke	14	0.35	0.37	0.39	0.34	0.18	0.33	0.33	5.53
	21	0.38	0.39	0.31	0.32	0.34	0.35		
Mean N-rate		0.35	0.35	0.34	0.34	0.29			
LSD, P ≤ 0.05				0.02			NS		
	7	0.46	0.54	0.32	0.35	0.32	0.40		
Kitabi	14	0.37	0.49	0.41	0.34	0.27	0.38	0.42	6.25
	21	0.54	0.39	0.58	0.47	0.36	0.47		
Mean N-rate		0.46	0.47	0.44	0.39	0.32			
LSD, P ≤ 0.05				0.03			0.04		
	7	0.71	0.63	0.68	0.62	0.59	0.65		
Mulindi	14	0.71	0.66	0.61	0.63	0.53	0.63	0.62	3.99
	21	0.55	0.62	0.65	0.60	0.56	0.60		
Mean N-rate		0.66	0.63	0.65	0.62	0.56			
LSD, P ≤ 0.05				0.03			0.04		
All 8 sites means:	7	0.73	0.75	0.71	0.68	0.63	0.70		
	14	0.71	0.71	0.70	0.71	0.64	0.69		7.49
	21	0.77	0.72	0.67	0.70	0.67	0.70		
	N-rate	0.74	0.73	0.69	0.70	0.64			
LSD, P ≤ 0.05				0.03			NS	0.03	

Interactions: SxN = 0.06, SxPF = 0.05, NxPF = 0.04, SxNxPF = 0.10

NS = Not significant

Table 23: Variations in mature leaf Mn levels (%) of TRFK 6/8 with location, N rates and plucking frequency

Site (S)	Plucking Frequency (PF) (days)	N-rates (N) kg N/Ha/yr 0	75	150	225	300	PF mean	Site Mean	C.V.%
	7	0.34	0.41	0.44	0.40	0.45	0.41		
Changoi	14	0.43	0.41	0.43	0.37	0.41	0.41	0.41	6.84
	21	0.36	0.40	0.42	0.49	0.45	0.42		
Mean N-rate		0.38	0.40	0.43	0.42	0.44			
LSD, P ≤ 0.05				0.04			NS		
	7	0.30	0.28	0.29	0.30	0.30	0.29		
Sotik	14	0.26	0.30	0.30	0.31	0.29	0.29	0.29	9.26
	21	0.25	0.25	0.32	0.28	0.31	0.28		
Mean N-rate		0.27	0.28	0.30	0.30	0.30			
LSD, P ≤ 0.05				NS			NS		
	7	0.23	0.27	0.24	0.26	0.25	0.25		
Timbilil	14	0.24	0.24	0.22	0.27	0.24	0.24	0.25	8.62
	21	0.25	0.26	0.21	0.25	0.27	0.25		
Mean N-rate		0.24	0.25	0.23	0.26	0.26			
LSD, P ≤ 0.05				0.03			NS		
	7	0.17	0.17	0.16	0.16	0.18	0.17		
Ngwazi	14	0.16	0.17	0.16	0.17	0.18	0.17	0.17	6.69
	21	0.14	0.17	0.17	0.16	0.18	0.17		
Mean N-rate		0.16	0.17	0.16	0.17	0.18			
LSD, P ≤ 0.05				0.01			NS		
	7	0.14	0.16	0.16	0.15	0.16	0.15		
Maruku	14	0.11	0.15	0.15	0.14	0.15	0.14	0.15	9.75
	21	0.14	0.15	0.13	0.14	0.16	0.15		
Mean N-rate		0.13	0.15	0.15	0.14	0.16			
LSD, P ≤ 0.05				0.02			NS		
	7	0.13	0.13	0.13	0.16	0.15	0.14		
Katoke	14	0.14	0.15	0.14	0.15	0.17	0.15	0.15	9.74
	21	0.13	0.16	0.12	0.15	0.16	0.15		
Mean N-rate		0.14	0.15	0.13	0.16	0.16			
LSD, P ≤ 0.05				0.02			NS		
	7	0.13	0.13	0.14	0.13	0.14	0.13		
Kitabi	14	0.14	0.12	0.14	0.13	0.13	0.13	0.13	5.92
	21	0.13	0.13	0.13	0.13	0.13	0.13		
Mean N-rate		0.13	0.13	0.14	0.13	0.13			
LSD, P ≤ 0.05				NS			NS		
	7	0.19	0.21	0.21	0.21	0.23	0.21		
Mulindi	14	0.26	0.22	0.18	0.20	0.21	0.22	0.21	7.75
	21	0.18	0.20	0.21	0.21	0.23	0.20		
Mean N-rate		0.21	0.21	0.20	0.21	0.22			
LSD, P ≤ 0.05				NS			NS		
All 8 sites means:	7	0.20	0.22	0.22	0.22	0.23	0.22		
	14	0.22	0.22	0.22	0.22	0.22	0.22		8.88
	21	0.20	0.22	0.21	0.23	0.24	0.22		
	N-rate	0.21	0.22	0.22	0.22	0.23			
LSD, P ≤ 0.05				0.01			NS	0.01	

Interactions: SxN = 0.02, SxPF = NS, NxPF = 0.01, SxNxPF = 0.03

NS = Not significant

Table 24: Variations in mature leaf Cu levels (ppm) of TRFK 6/8 with location, N rates and plucking frequency

Site (S)	Plucking Frequency (PF) (days)	N-rates (kg N/ha/yr) 0	75	150	225	300	PF mean	Site Mean	C.V.%
	7	9.33	10.67	11.67	12.67	13.00	11.47		
Changoi	14	11.67	11.00	10.33	10.33	13.00	11.27	11.29	17.61
	21	10.67	10.67	11.33	12.33	10.67	11.13		
Mean N-rate		10.56	10.78	11.11	11.78	12.22			
LSD, P ≤ 0.05				2.60			NS		
	7	9.33	10.00	9.33	10.33	10.67	9.93		
Sotik	14	9.33	9.00	10.00	10.00	10.33	9.73	9.91	11.02
	21	9.00	9.00	11.00	11.00	10.33	10.07		
Mean N-rate		9.22	9.33	10.11	10.44	10.44			
LSD, P ≤ 0.05				1.43			NS		
	7	7.67	7.33	8.00	7.67	10.33	8.20		
Timbilil	14	7.67	7.33	8.00	8.67	7.33	7.80	7.84	10.73
	21	6.67	8.33	7.00	7.67	8.00	7.53		
Mean N-rate		7.33	7.67	7.67	8.00	8.56			
LSD, P ≤ 0.05				1.10			NS		
	7	8.67	9.00	9.00	8.33	9.00	8.80		
Ngwazi	14	8.33	9.00	9.00	9.00	9.00	8.87	9.00	10.08
	21	9.00	8.33	8.67	10.00	10.67	9.33		
Mean N-rate		8.67	8.78	8.89	9.11	9.56			
LSD, P ≤ 0.05				NS			NS		
	7	17.67	19.00	19.00	16.67	18.67	18.20		
Maruku	14	18.00	17.33	17.00	20.00	20.00	18.47	18.56	6.68
	21	18.67	18.33	20.00	19.67	18.33	19.00		
Mean N-rate		18.11	18.22	18.67	18.78	19.00			
LSD, P ≤ 0.05				NS			NS		
	7	15.33	14.33	15.00	13.00	14.67	14.47		
Katoke	14	13.00	13.00	13.33	14.67	15.00	13.80	14.20	7.97
	21	13.33	15.00	14.00	15.00	14.33	14.33		
Mean N-rate		13.89	14.11	14.11	14.22	14.67			
LSD, P ≤ 0.05				NS			NS		
	7	8.00	10.67	9.33	8.67	12.67	9.87		
Kitabi	14	10.67	9.00	15.33	13.00	11.67	11.93	11.07	13.69
	21	10.67	12.00	8.00	13.67	12.67	11.40		
Mean N-rate		9.78	10.56	10.89	11.78	12.33			
LSD, P ≤ 0.05				1.98			2.38		
	7	6.00	6.33	7.67	8.00	7.33	7.07		
Mulindi	14	8.67	8.00	9.67	7.00	8.00	8.27	7.78	16.94
	21	6.00	8.33	6.33	9.33	10.00	8.00		
Mean N-rate		6.89	7.56	7.89	8.11	8.44			
LSD, P ≤ 0.05				NS			NS		
All 8 sites means:	7	10.25	10.92	11.13	10.67	12.04	11.00		
	14	10.92	10.46	11.58	11.58	11.79	11.27		12.97
	21	10.50	11.25	10.79	12.33	11.87	11.35		
	N-rate	10.56	10.88	11.17	11.53	11.90			
LSD, P ≤ 0.05				0.67			NS	0.72	

Interactions: SxN = NS, SxPF = 1.14, NxPF = 0.97, SxNxPF = 2.40

NS = Not significant

Table 25: Variations in mature leaf Fe levels (ppm) of TRFK 6/8 with location, N rates and plucking frequency

Site (S)	Plucking Frequency (PF) (days)	N-rates (N) kg N/ha/yr 0	75	150	225	300	PF mean	Site mean	C.V.%
	7	96.67	123.67	156.33	172.33	185.00	146.80		
Changoi	14	102.00	109.00	147.33	142.67	190.00	138.20	141.82	6.99
	21	97.33	110.67	159.67	154.00	180.67	140.47		
Mean N-rate		98.67	114.44	154.44	156.33	185.22			
LSD, P ≤ 0.05				12.98			NS		
	7	151.33	145.67	148.67	156.67	176.67	155.80		
Sotik	14	145.67	157.00	158.00	162.67	167.00	158.07	155.11	6.85
	21	135.33	148.67	160.00	150.67	162.67	151.47		
Mean N-rate		144.11	150.44	155.56	156.67	168.78			
LSD, P ≤ 0.05				13.90			NS		
	7	154.67	179.00	185.67	181.33	201.67	180.47		
Timbilil	14	161.67	177.33	181.67	188.33	182.67	178.33	180.04	6.04
	21	174.67	183.00	178.33	182.67	188.00	181.33		
Mean N-rate		163.67	179.78	181.89	184.11	190.78			
LSD, P ≤ 0.05				14.24			NS		
	7	76.33	76.67	74.33	77.67	87.67	78.53		
Ngwazi	14	78.33	78.67	86.33	89.33	82.33	83.00	80.49	6.58
	21	73.33	75.00	76.00	85.00	90.33	79.93		
Mean N-rate		76.00	76.78	78.89	84.00	86.78			
LSD, P ≤ 0.05				6.93			NS		
	7	78.33	75.33	87.00	80.33	88.33	81.87		
Maruku	14	82.33	81.33	80.00	81.33	89.33	82.87	82.20	7.29
	21	84.33	88.33	78.67	87.00	71.00	81.87		
Mean N-rate		81.67	81.67	81.89	82.89	82.89			
LSD, P ≤ 0.05				NS			NS		
	7	134.00	146.00	145.00	132.33	140.67	139.60		
Katoke	14	128.00	127.67	128.33	133.67	136.00	130.73	135.31	4.69
	21	136.67	130.00	131.00	139.00	141.33	135.60		
Mean N-rate		132.89	134.56	134.78	135.00	139.33			
LSD, P ≤ 0.05				NS			9.97		
	7	129.33	127.00	162.67	166.67	184.33	154.00		
Kitabi	14	161.00	164.67	142.33	195.33	190.67	170.80	156.18	4.33
	21	138.33	140.33	165.33	130.67	144.00	143.73		
Mean N-rate		142.89	144.00	156.78	164.22	173.00			
LSD, P ≤ 0.05				8.85			NS		
	7	77.33	71.00	79.00	93.33	100.00	84.13		
Mulindi	14	75.33	102.00	99.33	89.33	88.00	90.80	87.58	7.56
	21	68.33	91.33	87.67	87.00	104.67	87.80		
Mean N-rate		73.67	88.11	88.67	89.89	97.56			
LSD, P ≤ 0.05				8.67			NS		
All 8 sites means:	7	112.25	118.04	129.83	132.58	145.54	127.65		
	14	116.79	124.71	127.92	135.21	140.75	129.10		6.88
	21	113.54	120.92	129.58	127.00	135.33	125.28		
	N-rate	114.19	121.22	129.11	131.64	140.54			
LSD, P ≤ 0.05				4.05			NS	4.37	

Interactions: SxN = 8.46, SxPF = 6.87, NxPF = 5.84, SxNxPF = 14.46

NS = Not significant

There were large variations in the mean values for sites for nitrogen, potassium, calcium, manganese, zinc, copper and iron. These large variations maybe the cause in part of the variations in yields of clonal tea due to locations observed in the past (Wachira *et al.*, 2002; Owuor *et al.*, 2009; 2010a). These results further reveal that the nutrients norms set even in a single cultivar may vary with location.

These results demonstrate that diagnostic limits set for seedling tea may not be suitable for clone TRFK 6/8. There is therefore need to develop tissue analysis diagnostic norms for clonal tea and these needs to be region specific as the nutrients levels widely vary with location of production. Deficiency of nitrogen can be conveniently cured through application of nitrogen fertilizer as there is response in mature leaf to nitrogen application. However, such application triggers decline in potassium levels in the leaf. The application of the two nutrients need to be staggered so that at any time the plant is exposed to only one nutrient in high amounts. However, it is necessary to establish conditions for applying nitrogen and potassium for optimal uptake by the plant. Continuous application of high rates of nitrogenous fertilizers could trigger deficiency of potassium, calcium, and magnesium while causing very high levels (toxic) of manganese. However plucking intervals had minimal effects on mature leaf nutrients levels.

4.3 Variations in levels of caffeine and flavan-3-ols in green tea leaf with location of production, season and genotype

Previous studies have shown that the green leaf flavan-3-ols are indicators of plain black tea quality potential of clonal teas. For instance, Obanda *et al*., (1997) found ECG, EGCG and caffeine content of green leaf to be indicators of plain black tea quality. It has also been suggested that higher EGCG and ECG content in fresh leaf might lead to the formation of higher theaflavin-

digallates in the plain black tea (Madanhire, 1995), a parameter associated with Kenyan plain black tea quality (Owuor and Obanda, 1997, 2007). In Central and Southern Africa, green leaf EC significantly predicted black tea quality (Wright *et al*., 2000). There existed a large difference between flavan-3-ols in green leaves of Kenya clonal teas (Owuor and Obanda, 2007) and those on tea clones produced in Central and Southern Africa (Wright *et al*., 2000). From the higher total theaflavins in Kenyan black teas than in the Central African ones (Owuor *et al*., 1986), it had been assumed that Kenyan green tea leaves had higher levels of flavan-3-ols than those from Central Africa, however, Central African tea clones were found to have, on average, about twice the amounts of flavan-3-ols (Wright *et al*., 2000) compared to the Kenya tea clones. Thus, the distribution of the individual flavan-3-ols in green leaf may be more critical to theaflavins formation than total flavan-3-ols. Factors that influence the levels of flavan-3-ols in green tea leaf are unkown making it impossible to optimize cultural and agronomic activities/inputs to enhance tea quality. Although clonal variations in the flavan-3-ols levels have been shown for tea grown at one site (Owuor and Obanda, 1995; Wright *et al*., 2000), it is not known how the flavan-3-ols vary with geographical location of production and season for tea grown in Kenya. Consequently, clonal selection/breeding research has been centralized in one location in the belief that a superior clone maintains it attributes in all tea growing areas in Kenya. But plain black tea quality of some cultivars varied with geographical location of production in Kenya and the extent of the changes varied with caltivars (Owuor *et al*., 2010b), plucking intervals (Owuor *et al*., 2009) and nitrogenous fertilizer rates (Owuor *et al*., 2010a). Caffeine is regarded as an important constituent of tea, bestowing mood and cognitive-enhancing properties (Bokuchava and Skobeleva, 1969; Chow and Kramer, 1990). Caffeine correlates positively with plain black tea quality (Millin *et al*., 1969; Obanda *et al*., 1997; Wright *et al*., 2000). It contributes towards the briskness of plain black

tea (Deb and Ullah, 1968) as it complexes with the polyphenols in tea, mainly theaflavins (Roberts, 1962) in a process that leads to 'cream' formation. The complex modifies the taste making plain black tea taste brisker (Sanderson *et al.,* 1976). In Kenya, caffeine is used as an important quality parameter for the evaluation of plain black tea quality (Owuor *et al*., 1986; Owuor and Chavanji, 1988).

Representative HPLC chromatogram (278nm) for green leaf caffeine and catechins found in this study are shown in Figure 8. The chromatograms indicate good baseline resolution for the individual flavan-3-ols and caffeine.

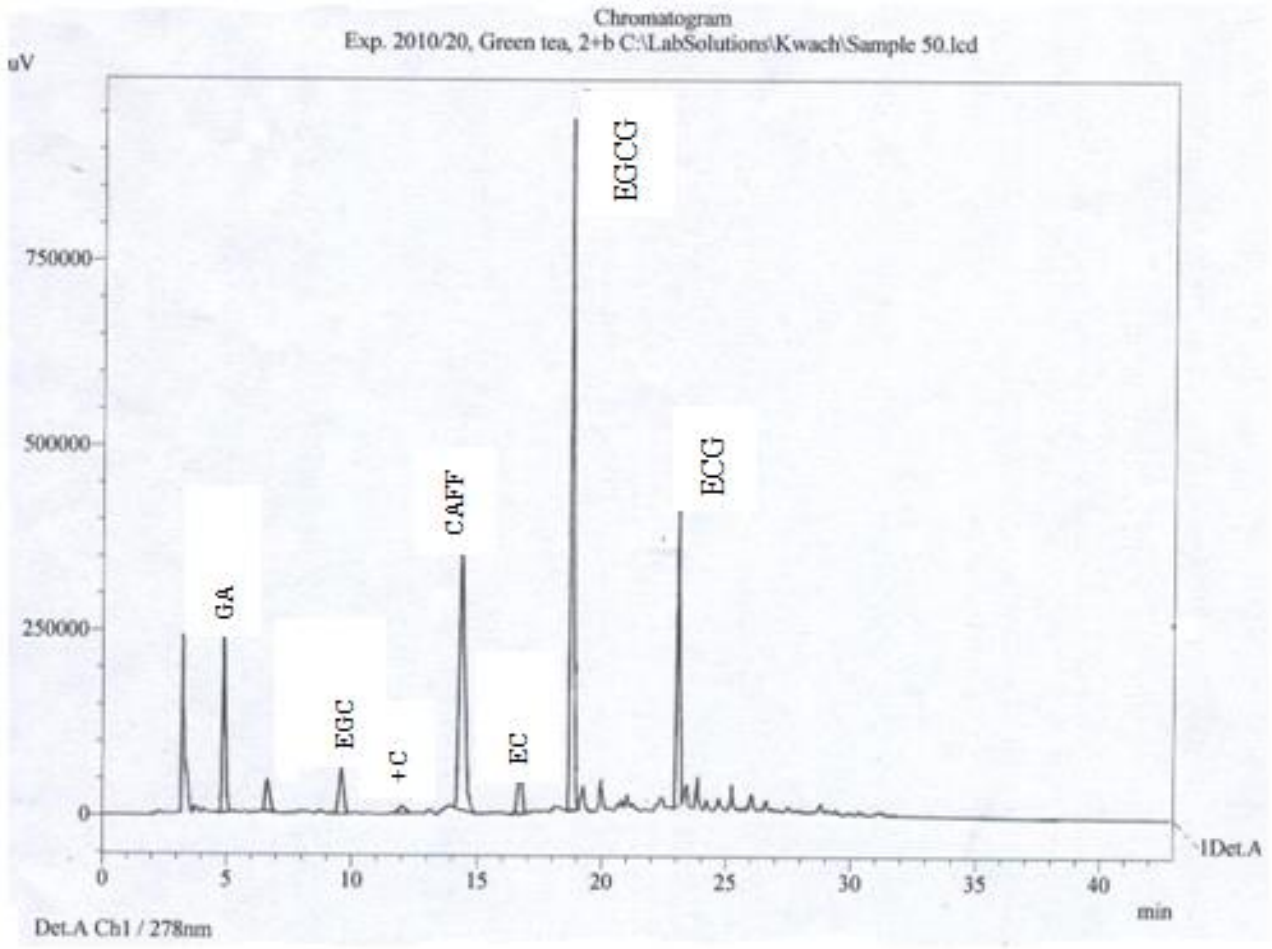

Figure 8: HPLC chromatogram (278nm) for caffeine and flavan-3-ols

4.3.1 Variations in leaf caffeine with genotype, season and location

The effects of genotype, location of production and season on green young leaf (2+bud) caffeine are presented in Table 26. Its levels varied significantly ($P \leq 0.05$) with clones both at individual locations and mean of locations. Clones AHP S15/10, BBK 35, TRFK 12/19, TRFK 31/8, TRFK 12/12, TRFK 54/40 recorded significantly ($P \leq 0.05$) higher amounts in all sites than the other clones, suggesting large genetic differences in the clones used (Magoma *et al*., 2000), leading to differences in quality potentials as observed in earlier studies (Owuor and Chavanji, 1986; Owuor *et al*., 1987b). This observation supports the suggestion that genetic factors might influence caffeine contents of plain black tea (Owuor, 1987b).

The amounts of caffeine was also significantly ($P \leq 0.05$) influenced by location of production. Kangaita clones recorded significantly lower ($P \leq 0.05$) caffeine contents than Kipkebe and Timbilil plants whose caffeine levels were comparable. This may imply that quality of plain black tea, as measured by "cream" formation, may be higher for Timbilil and Kipkebe grown clones as compared with Kangaita grown clones, showing locational influence on plain black tea quality. However, the results also indicate that even a single clone would not replicate leaf caffeine contents in different locations. These results are consistent with earlier studies (Owuor *et al*., 1987b; Dev Choudhury *et al.,* 1991) that reported influence of location on plain black tea caffeine content.

In variance with earlier reports (Owuor, 1987b; Dev Choudhury *et al.,* 1991), there was no seasonal effect on leaf caffeine content. This could imply that the two seasons used in this study did not lead to variations in shoot growth rate which would affect caffeine levels (Cloughley, 1982). These results are not surprising. With climate change, the weather patterns, especially rainfall distribution has changed drastically. As a result, there were sporating rainfall incidences

Table 26: Variations in young leaf caffeine levels (mg/g DM) with location, season and genotype

Site (S)	Season (Se)	Clone (C)										Mean season	Mean site
		TRFK 6/8	TRFK 7/9	TRFK 303/577	AHP S15/10	BBK 35	TRFK 12/19	TRFK 54/40	TRFK 303/1199	TRFK 31/8	TRFK 12/12		
Kangaita	Dry	38.1	33.1	31.8	43.6	37.3	34.3	40.4	30.6	27.0	40.9	35.7	
	Wet	28.4	32.1	29.8	40.4	43.9	40.0	37.0	23.0	32.1	35.2	34.2	35.0
	Mean clone	33.2	32.6	30.8	42.0	40.6	37.2	38.7	26.8	29.6	38.1		
	CV (%)					14.4							
	LSD P ≤ 0.05					6.6						NS	
Kipkebe	Dry	36.8	35.0	40.6	38.9	37.7	42.1	40.6	35.7	41.0	32.7	38.1	
	Wet	36.6	40.3	39.2	44.5	46.7	43.7	40.0	38.7	40.4	39.4	41.0	39.5
	Mean clone	36.7	37.7	39.9	41.8	42.2	42.9	40.3	37.2	40.7	36.1		
	CV (%)					7.5							
	LSD P ≤ 0.05					3.9						NS	
Timbilil	Dry	34.3	40.9	32.5	33.5	46.4	37.8	38.4	50.6	50.0	44.3	40.9	
	Wet	41.2	39.1	34.9	37.6	40.6	44.8	40.6	50.8	48.4	53.5	43.2	42.0
	Mean clone	37.8	40.0	33.7	35.5	43.5	41.3	39.5	50.7	49.2	48.9		
	CV (%)					10.0							
	LSD P ≤ 0.05					5.5						NS	
All 3 sites means:	Dry	36.4	36.3	35.0	38.7	40.5	38.1	39.8	39.0	39.3	39.3	38.2	
	Wet	35.4	37.2	34.6	40.8	43.7	42.8	39.2	37.5	40.3	42.7	39.4	
	Clone	35.9	36.8	34.8	39.7	42.1	40.5	39.5	38.2	39.8	41.0		
	CV (%)					9.1							
LSD P ≤ 0.05						3.1						NS	3.2
Interactions:	SxC					5.0							
	SxSe					4.6							
	CxSe					NS							
	SxCxSe					7.1							

NS = Not significant

in the season that is traditionally considered dry. The seasonal variations were therefore not large enough to cause changes in caffeine levels. In addition, the growth rate of tea in Kenya does not undergo such large seasonal variations (Ng'etich *et al*., 2001) as tea is grown almost along the equator. In countries further away from the equator (Malec and Vigo, 1988), significant variations in caffeine levels due to seasons were recorded.

There were significant ($P \leq 0.05$) interactions effects between clones and location of production, demonstrating that the magnitudes of the responses of the different clones in caffeine production vary differently from location to location. Thus a clone that is producing high amounts of caffeine in one location may not produce high levels at a different location. Significant interaction effect between location of production and season suggest that the magnitude of changes in caffeine levels at different locations in a single clone varies from season to season.

4.3.2 Variations in total polyphenols levels with genotype, season and location

Provided polyphenol oxidase activity is not limiting, total polyphenols (Obanda *et al*., 1997, 1999), influence the resultant plain black tea quality. Changes in total flavan-3-ols levels due to location of production, cultivars and season of production are presented in Table 27. Location of production, cultivars and season caused significant ($P \leq 0.05$) variations in total flavan-3-ols. The total polyphenols levels were very high and in most cases above 30% reported in previous studies (Harbowy and Balentine, 1997). Total polyphenols amounts significantly ($P \leq 0.05$) varied with clones at all individual sites and in all sites pooled together (Table 27) indicating the influence of genetic variations in resultant plain black tea quality. The generally high total polyphenols levels and lack of significant variations with location of production

demonstrate the general ability of the clones to make high quality plain black tea in all locations, however the significant interactions between clones and sites may be an indication that a single clone may not replicate plain black tea quality potential in all growing locations due to interaction effects. Although overall leaf produced during dry season had higher ($P \leq 0.05$) levels of total polyphenols, at individual locations the patterns were the same.

4.3.3 Variations in dihydroxyflavan-3-ols with genotype, season and location

Oxidation products of green tea leaf catechins, mainly theaflavins and thearubigins (Takino *et al.*, 1964; Brown *et al.*, 1966, 1969; Deb and Ullah, 1968), residual green leaf catechins and caffeine are responsible for plain black tea quality. The formation of a single theaflavin molecule requires a dihydroxyflavan-3-ol (simple catechin) and a trihydroxyflavan-3-ol (Nakagawa and Torii, 1965; Robertson, 1983a). The simple catechins in green leaf include (+) catechin (+)C, epicatechin (EC) and epicatechin gallate (ECG) (Robertson, 1983b).

Variations in young green leaf (+)C, EC and ECG with clones, location of production and season are presented in Tables 28, 29 and 30 respectively. The amounts of the simple catechins were in the order ECG>EC>(+)C. This order was slightly different from that of Central and Southern African clonal teas, where EC was the dominant flavan-3-ol (Wright *et al.*, 2000). The simple catechins showed significant variations ($P \leq 0.05$) with clones both at individual locations and in all production locations. Similar variations with clones were noted in total simple catechins (Table 31), where TRFK 31/8 recorded the lowest total simple catechin, TRFK 7/9, TRFK 303/577, S15/10 and TRFK 54/40 had moderate amounts while the other clones had significantly ($P \leq 0.05$) high amounts of above 50.0 mg/g on dry matter (DM) basis for all sites.

Table 27: Variations in clonal total polyphenols levels (%GAE) with location, season and genotype

Site (S)	Season (Se)	Clone (C)										Mean season	Mean site
		TRFK 6/8	TRFK 7/9	TRFK 303/577	AHP S15/10	BBK 35	TRFK 12/19	TRFK 54/40	TRFK 303/1199	TRFK 31/8	TRFK 12/12		
Kangaita	Dry	29.31	28.18	30.47	28.95	30.67	32.12	20.21	32.01	29.34	33.90	30.52	29.25
	Wet	29.25	26.07	37.41	27.67	36.84	28.94	28.04	30.33	27.19	28.10	27.99	
	Mean clone	29.28	27.13	38.94	28.31	38.76	30.53	29.13	31.17	28.27	31.00		
	CV (%)					6.02							
	LSD P ≤ 0.05					1.51						1.26	
Kipkebe	Dry	29.22	29.78	25.30	28.39	31.23	30.01	31.69	31.15	28.95	31.14	29.69	28.84
	Wet	27.35	27.94	28.15	27.39	28.63	27.54	29.16	30.26	27.38	26.18	28.00	
	Mean clone	28.29	28.86	26.73	27.89	29.93	28.78	30.43	30.71	28.16	28.66		
	CV (%)					5.73							
	LSD P ≤ 0.05					1.41						NS	
Timbilil	Dry	28.14	28.73	32.15	30.16	32.22	31.23	31.27	30.98	30.32	31.85	30.71	28.68
	Wet	29.21	25.60	30.73	27.21	27.09	24.69	25.61	28.13	21.88	26.46	26.66	
	Mean clone	28.68	27.16	31.44	28.69	29.65	27.96	28.44	29.56	26.10	29.15		
	CV (%)					6.67							
	LSD P ≤ 0.05					1.63						NS	
All 3 sites means:	Dry	28.89	28.90	29.31	29.17	31.37	31.12	27.72	31.38	29.54	96.89	30.30	
	Wet	28.60	26.54	32.10	27.42	30.85	27.06	27.60	29.57	25.48	26.91	27.55	
	Clone	28.75	27.72	29.04	28.30	29.45	29.09	29.33	30.48	27.51	29.61		
	CV (%)					6.49						2.33	NS
LSD P ≤ 0.05						0.93							
Interactions:	SxC					1.49							
	SxSe					1.36							
	CxSe					1.31							
	SxCxSe					2.11							

NS = Not significant

The significant variations in simple catechins composition suggest that there were genetic differences between the clones (Magoma *et al*., 2000) in part explaining the previously observed differences in plain black tea quality, especially theaflavins for clones (Owuor *et al*., 2010b). Levels of EC in Central and Southern African clonal teas correlated linearly (Wright *et al*., 2000) but inversely (Owuor and Obanda, 2007) for Kenyan clonal teas with plain black tea quality. Thus there were clones with potential to produce low or high quality plain black teas as predetermined by the EC levels.

Although the variations in individual dihydroxyflavan-3-ols were ($P \leq 0.05$) affected by location of production (Tables 28, 29, 30), total dihydroxyflavan-3-ols were not affected by location of production (Table 31). The EC levels were higher ($P \leq 0.05$) at Kangaita than other sites, however the levels of (+)C and ECG were higher at Timbilil and Kipkebe. Since levels of (+)C was much lower in all clones, when the simple flavan-3-ols were summed up, there was no significant difference in the levels due to location of production. Thus differences in the total dihydroxyflavan-3-ols in clones at different locations can not explain the overall quality differences observed in plain black tea quality parameters (Owuor *et al*., 2010b). Season of production did not influence the individual or total dihydroxyflavan-3-ols levels.

Significant ($P \leq 0.05$) interactions effects between location of production and clones in the levels of (+)C (Table 28), EC (Table 29), ECG (Table 30) and total dihydroxyflavan-3-ols (Table 31) were recorded, demonstrating that the extents of formation of these metabolites in different clones varied in magnitudes from location to location. The interactions effects between locations of production and seasons were also significant ($P \leq 0.05$) for (+)C, EC and total dihydroxyflavan-3-ols. The magnitudes of the variations of these parameters in different seasons therefore vary with location of production. These metabolites would therefore influence plain

Table 28: Variations in young leaf (+) Catechin levels (mg/g DM) with location, season and genotype

Site (S)	Season (Se)	Clone (C)										Mean season	Mean site
		TRFK 6/8	TRFK 7/9	TRFK 303/577	AHP S15/10	BBK 35	TRFK 12/19	TRFK 54/40	TRFK 303/1199	TRFK 31/8	TRFK 12/12		
Kangaita	Dry	5.4	3.7	5.0	1.3	3.8	5.2	2.8	2.8	2.4	4.1	3.7	
	Wet	5.2	5.3	3.8	3.6	5.3	3.5	2.2	1.9	2.4	3.3	3.7	3.7
	Mean clone	5.3	4.5	4.4	2.5	4.5	4.3	2.5	2.4	2.4	3.7		
	CV (%)					19.9							
	LSD P ≤ 0.05					1.0						NS	
Kipkebe	Dry	7.4	4.5	5.1	3.5	6.5	6.6	4.2	5.0	3.6	3.0	4.9	
	Wet	3.9	1.9	3.6	4.7	4.2	4.7	4.0	3.8	3.9	2.6	3.7	4.3
	Mean clone	5.6	3.2	4.3	4.1	5.4	5.6	4.1	4.4	3.8	2.8		
	CV (%)					17.9							
	LSD P ≤ 0.05					1.0						NS	
Timbilil	Dry	3.8	1.7	2.8	2.0	1.9	4.1	3.2	7.8	8.3	4.5	4.0	
	Wet	2.6	1.8	1.5	2.1	2.3	1.1	1.9	5.6	7.1	6.4	3.3	3.6
	Mean clone	3.2	1.7	2.2	2.1	2.1	2.6	2.6	6.7	7.7	5.4		
	CV (%)					21.8							
	LSD P ≤ 0.05					1.0						NS	
All 3 sites means:	Dry	5.5	3.3	4.3	2.3	4.1	5.3	3.4	5.2	4.8	3.9	4.2	
	Wet	3.9	3.0	3.0	3.5	3.9	3.1	2.7	3.8	4.5	4.1	3.5	
	Clone	4.7	3.1	3.6	2.9	4.0	4.2	3.1	4.5	4.6	4.0		
	CV (%)					19.8							
LSD P ≤ 0.05, Site (S)						0.6						NS	0.6
Interactions:	SxC					0.9							
	SxSe					0.9							
	CxSe					0.8							
	SxCxSe					1.3							

NS = Not significant

Table 29: Variations in young leaf epicatechin (EC) (mg/g DM) with location, season and genotype

Site	Season	Clone										Mean season	Mean site
		TRFK 6/8	TRFK 7/9	TRFK 303/577	AHP S15/10	BBK 35	TRFK 12/19	TRFK 54/40	TRFK 303/1199	TRFK 31/8	TRFK 12/12		
Kangaita	Dry	17.8	16.1	17.4	14.8	10.6	13.7	17.1	16.6	11.8	13.1	14.9	
	Wet	21.2	15.7	17.6	14.0	8.7	14.3	15.4	15.7	16.8	12.9	15.2	15.1
	Mean clone	19.5	15.9	17.5	14.4	9.7	14.0	16.3	16.2	14.3	13.0		
	CV (%)					10.0							
	LSD P ≤ 0.05					2.0						NS	
Kipkebe	Dry	10.1	10.4	19.6	12.8	15.5	17.1	9.9	11.6	15.0	17.0	13.9	
	Wet	11.3	11.1	16.0	12.9	13.8	16.4	10.1	13.4	14.1	10.5	13.0	13.4
	Mean clone	10.7	10.7	17.8	12.9	14.6	16.8	10.0	12.5	14.6	13.8		
	CV (%)					9.4							
	LSD P ≤ 0.05					1.7						NS	
Timbilil	Dry	12.8	19.3	14.9	14.4	13.6	10.5	9.3	19.8	15.6	13.5	14.4	
	Wet	13.4	18.0	12.1	14.4	12.5	7.3	10.6	15.8	12.5	13.4	13.0	13.7
	Mean clone	13.1	18.6	13.5	14.4	13.1	8.9	9.9	17.8	14.0	13.4		
	CV (%)					12.5							
	LSD P ≤ 0.05					2.2						NS	
All 3 sites means:	Dry	13.6	15.3	17.3	14.0	13.2	13.8	12.1	16.0	14.1	14.5	14.4	
	Wet	15.3	14.9	15.2	13.8	11.7	12.7	12.0	15.0	14.5	12.3	13.7	
	Clone	14.4	15.1	16.3	13.9	12.5	13.2	12.1	15.5	14.3	13.4		
	CV (%)					10.7							
LSD P ≤ 0.05						1.1						NS	1.2
Interactions:	SxC					1.8							
	SxSe					1.7							
	CxSe					1.6							
	SxCxSe					2.6							

NS = Not significant

Table 30: Variations in young leaf epicatechin gallate (ECG) (mg/g DM) with location, season and genotype

Site (S)	Season (Se)	Clone (C)										Mean season	Mean site
		TRFK 6/8	TRFK 7/9	TRFK 303/577	AHP S15/10	BBK 35	TRFK 12/19	TRFK 54/40	TRFK 303/1199	TRFK 31/8	TRFK 12/12		
Kangaita	Dry	26.3	35.3	25.0	23.6	25.8	27.5	30.3	33.3	24.3	26.4	27.8	
	Wet	24.7	29.6	25.9	25.2	25.3	29.2	29.4	32.7	29.6	26.3	27.8	27.8
	Mean clone	25.5	32.5	25.4	24.4	25.5	28.3	29.8	33.0	27.0	26.4		
	CV (%)					9.6							
	LSD P ≤ 0.05					3.5						NS	
Kipkebe	Dry	32.9	27.4	30.4	37.9	40.8	32.3	35.2	36.0	29.9	29.8	33.3	
	Wet	27.2	25.3	33.8	34.5	40.6	36.1	30.7	30.9	27.5	24.1	31.1	32.2
	Mean clone	30.1	26.4	32.1	36.2	40.7	34.2	33.0	33.5	28.7	27.0		
	CV (%)					12.3							
	LSD P ≤ 0.05					5.2						NS	
Timbilil	Dry	27.3	34.7	35.2	36.4	22.4	24.9	25.2	37.3	42.7	43.9	33.0	32.7
	Wet	29.0	37.2	35.6	36.0	23.8	19.5	23.1	42.1	41.4	36.5	32.4	
	Mean clone	28.2	35.9	35.4	36.2	23.1	22.2	24.1	39.7	42.1	40.2		
	CV (%)					9.9							
	LSD P ≤ 0.05					4.2						NS	
All 3 sites means:	Dry	28.8	32.5	30.2	32.6	29.7	28.2	30.2	35.5	32.3	33.4	31.4	
	Wet	27.0	30.7	31.8	31.9	29.9	28.3	27.7	35.2	32.8	29.0	30.4	
	Clone	27.9	31.6	31.0	32.3	29.8	28.2	29.0	35.4	32.6	31.2		
	CV (%)					10.6							
LSD P ≤ 0.05						2.5						NS	2.6
Interactions:	SxC					4.0							
	SxSe					NS							
	CxSe					NS							
	SxCxSe					NS							

NS = Not significant

Table 31: Variations in clonal total simple flavan-3-ols levels (mg/g DM) with location, season and genotype

Site (S)	Season (Se)	Clone (C)										Mean season	Mean site
		TRFK 6/8	TRFK 7/9	TRFK 303/577	AHP S15/10	BBK 35	TRFK 12/19	TRFK 54/40	TRFK 303/1199	TRFK 31/8	TRFK 12/12		
Kangaita	Dry	49.6	41.3	50.1	43.6	58.5	59.8	48.6	58.2	39.3	69.8	51.9	
	Wet	51.2	41.1	47.0	42.5	50.0	53.3	45.6	54.4	37.2	58.8	48.1	50.0
	Mean clone	50.4	41.2	48.6	43.1	54.3	56.6	47.1	56.3	38.2	64.3		
	CV (%)					8.9							
	LSD P ≤ 0.05					5.8						NS	
Kipkebe	Dry	55.2	40.2	52.7	50.4	54.1	49.3	49.8	53.0	39.4	66.6	51.1	
	Wet	50.5	39.2	50.4	42.4	52.1	44.8	37.3	49.2	27.9	61.0	45.5	48.2
	Mean clone	52.9	39.7	51.5	46.4	53.1	47.0	43.5	51.1	33.7	63.8		
	CV (%)					9.5							
	LSD P ≤ 0.05					6.0						NS	
Timbilil	Dry	47.6	47.0	43.5	42.9	62.9	54.4	44.5	52.8	39.0	61.8	49.7	
	Wet	47.1	46.4	43.8	37.6	58.6	46.3	44.4	52.6	34.3	56.3	46.7	48.3
	Mean clone	47.3	46.7	43.7	40.3	60.7	50.3	44.5	52.7	36.7	59.1		
	CV (%)					8.2							
	LSD P ≤ 0.05					5.2						NS	
All 3 sites means:	Dry	50.8	42.8	48.8	45.6	58.5	54.5	47.6	54.7	39.2	66.1	50.9	
	Wet	49.6	42.2	47.1	40.8	53.6	48.1	42.4	52.1	33.1	58.7	46.8	
	Clone	50.2	42.5	47.9	43.2	56.0	51.3	45.0	53.4	36.2	62.4		
	CV (%)					8.8							
LSD P ≤ 0.05						3.2						NS	NS
Interactions:	SxC					5.2							
	SxSe					4.8							
	CxSe					4.6							
	SxCxSe					7.4							

NS = Not significant

black tea quality in different clones to different extents and in unpredictable manner. This observation emphasizes the importance of testing clones in regions of intended production before a cultivar is released for general use.

The interactions effects between seasons and clones were significant for (+)C, EC and total dihydroxyflavan-3-ols suggesting that the clones used in this study may have different stabilities in the production of the metabolites. Thus, black tea quality from these clones may vary from one location to another even for a single clone explaining the earlier reports on black tea that genotype selected in one location for high quality, did not necessarily retain the quality potential in new growing regions (Owuor *et al*., 2008b, 2010a, 2010b).

4.3.4 Variations in trihydroxyflavan-3-ols with genotype, season and location

Trihydroxyflavan-3-ols have lower redox potentials than dihydroxyflavan-3-ols (Bajaj *et al*., 1987) and are therefore oxidized faster during fermentation phase of black tea processing. The trihydroxyflavan-3-ols in green tea leaf include GA, EGC and EGCG. Total trihydroxyflavan-3-ols and EGCG levels in green leaf had linearly correlated with plain black tea quality (Owuor *et al*., 2006; Owuor and Obanda, 2007), showing they are good indicators of plain black tea quality in Kenya.

Variations in GA, EGC and EGCG composition of green young tea leaf with clones, location of production and season are given in Tables 32, 33 and 34 respectively. The order of gallocatechins amounts was EGCG>EGC>GA. All the trihydroxyflavan-3-ols levels varied ($P \leq 0.05$) with location of production. Kipkebe and Timbilil had higher ($P \leq 0.05$) amounts of GA, Kangaita had highest ($P \leq 0.05$) EGC and Timbilil had highest ($P \leq 0.05$) EGCG. This indicated that even with the use of one cultivar, it is difficult to produce plain black tea of the same quality

in different locations of production (Owuor *et al*., 2008b), even within Kenya (Owuor *et al*., 2010a, 2010b). The trihydroxyflavan-3-ols levels also varied ($P \leq 0.05$) with clones. TRFK 6/8, TRFK 303/577, AHP S15/10, TRFK 12/19, TRFK 54/40, TRFK 303/1199 and TRFK 31/8 recorded comparable total gallocatechins for all sites, however the amounts were significantly ($P \leq 0.05$) higher than the other clones. Again, this demonstrated the varying potentials of the cultivars to produce plain black teas with different quality (Owuor *et al.*, 2010b). There were significant ($P \leq 0.05$) interactions effects between trihydroxyflavan-3-ols levels in clonal tea leaves and location of production, suggesting that patterns and extents of their variations in different clones changed with locations of production. The results suggest that for production of high quality plain black teas, new cultivars must be pretested in areas of intended production to ensure only cultivars with potential for production of high quality plain black teas are released to specific areas.

Except for EGC, there were no significant variations in the trihydroxy-flavan-3-ols levels (Table 32, 34 and 35) with season of production. The results are at variance with those reported in China (Chen *et al*., 2010), Turkey (Erturk *et al*., 2010) and Australia (Yao *et al*., 2005). In Kenya seasonal variations had been observed in plain black tea quality (Owuor, 1992, 1994). However, such variations were much lower than those repoted further away from the equator in Northeast India (Sud and Baru, 2000), China (Wang *et al*., 2011), Turkey (Erturk *et al*., 2010) and Australia (Yao *et al*., 2005). In Kenya, tea is grown near the equator with little seasonal variations in temperatures and sunshine hours. Plain black tea quality in Kenya can be more stable and uniform with less seasonal variability compared to countries further away from the equator.

Table 32: Variations in young leaf gallic acid (GA) (mg/g DM) with location, season and genotype

Site (S)	Season (Se)	Clone (C)										Mean season	Mean site
		TRFK 6/8	TRFK 7/9	TRFK 303/577	AHP S15/10	BBK 35	TRFK 12/19	TRFK 54/40	TRFK 303/1199	TRFK 31/8	TRFK 12/12		
Kangaita	Dry	7.0	7.2	7.1	9.7	7.3	7.3	10.3	8.2	7.1	8.4	8.0	
	Wet	6.0	7.8	7.5	9.4	9.6	8.7	8.2	8.4	7.1	9.6	8.2	8.1
	Mean clone	6.5	7.5	7.3	9.5	8.5	8.0	9.2	8.3	7.1	9.0		
	CV (%)					11.2							
	LSD P ≤ 0.05					1.2						NS	
	Dry	8.6	8.8	10.0	10.6	8.7	6.0	9.6	7.3	13.7	10.1	9.3	
Kipkebe	Wet	8.7	9.4	10.9	10.4	10.8	8.0	9.0	6.9	10.2	12.8	9.7	9.5
	Mean clone	8.7	9.1	10.4	10.5	9.8	7.0	9.3	7.1	12.0	11.4		
	CV (%)					15.6							
	LSD P ≤ 0.05					1.9						NS	
Timbilil	Dry	11.5	11.1	6.6	6.9	10.0	7.2	7.6	9.7	10.1	10.0	9.1	
	Wet	13.7	9.1	8.0	7.9	9.4	9.9	8.1	11.6	11.0	10.3	9.9	9.5
	Mean clone	12.6	10.1	7.3	7.4	9.7	8.6	7.9	10.6	10.5	10.2		
	CV (%)					16.3							
	LSD P ≤ 0.05					2.0						NS	
All 3 sites means:	Dry	9.0	9.0	7.9	9.1	8.7	6.8	9.2	10.3	9.5	8.8		
	Wet	9.5	8.8	8.8	9.2	9.9	8.9	8.4	9.4	10.9	9.3		
	Clones	9.3	8.9	8.3	9.1	9.3	7.9	8.8	8.7	9.9	10.2		
	CV (%)					14.7							
LSD P ≤ 0.05						1.0						NS	1.0
Interactions:	SxC					1.6							
	SxSe					NS							
	CxSe					1.4							
	SxCxSe					2.3							

NS = Not significant

Table 33: Variations in young leaf epigallocatechin (EGC) (mg/g DM) with location, season and genotype

Site (S)	Season (Se)	Clone (C)										Mean season	Mean site
		TRFK 6/8	TRFK 7/9	TRFK 303/577	AHP S15/10	BBK 35	TRFK 12/19	TRFK 54/40	TRFK 303/1199	TRFK 31/8	TRFK 12/12		
Kangaita	Dry	56.4	39.0	56.5	48.4	44.5	53.7	53.6	57.7	34.3	43.6	48.8	
	Wet	57.6	45.3	42.5	31.4	24.9	31.8	30.7	51.0	46.7	39.8	40.2	44.5
	Mean clone	57.0	42.2	49.5	39.9	34.7	42.8	42.2	54.4	40.5	41.7		
	CV (%)					12.8							
	LSD P ≤ 0.05					7.5						NS	
Kipkebe	Dry	32.4	36.1	44.5	48.3	42.8	52.4	41.6	33.4	44.6	56.4	43.2	
	Wet	33.2	31.6	29.5	16.2	28.1	36.5	16.2	29.9	33.8	20.4	27.5	35.4
	Mean clone	32.8	33.8	37.0	32.3	35.5	44.4	28.9	31.7	39.2	38.4		
	CV (%)					15.9							
	LSD P ≤ 0.05					7.3						NS	
Timbilil	Dry	64.5	46.8	45.2	44.4	45.7	56.4	40.1	50.5	36.8	41.8	47.2	
	Wet	33.9	46.0	34.3	37.6	38.1	12.9	33.5	20.1	16.0	17.9	29.0	38.1
	Mean clone	49.2	46.4	39.7	41.0	41.9	34.7	36.8	35.3	26.4	29.8		
	CV (%)					15.4							
	LSD P ≤ 0.05					7.7						NS	
All 3 sites means:	Dry	51.1	40.6	48.7	47.0	44.3	54.2	45.1	47.2	38.6	47.3	46.4	
	Wet	41.6	41.0	35.4	28.4	30.4	27.1	26.8	33.7	32.2	26.0	32.3	
	Clone	46.3	40.8	42.1	37.7	37.4	40.6	36.0	40.4	35.4	36.6		
	CV (%)					14.4							
LSD P ≤ 0.05						4.3						10.7	4.4
Interactions:	SxC					6.9							
	SxSe					6.2							
	CxSe					6.0							
	SxCxSe)					9.7							

NS = Not significant

Table 34: Variations in young leaf epigallocatechingallate (EGCG) (mg/g DM) with location, season and genotype

Site	Season	Clone										Mean season	Mean site
		TRFK 6/8	TRFK 7/9	TRFK 303/577	AHP S15/10	BBK 35	TRFK 12/19	TRFK 54/40	TRFK 303/1199	TRFK 31/8	TRFK 12/12		
Kangaita	Dry	80.2	80.0	81.0	78.8	95.2	89.8	88.2	102.9	68.1	85.9	85.0	
	Wet	77.7	88.4	76.5	82.4	83.6	92.5	90.7	97.6	83.9	92.7	86.6	85.8
	Mean clone	79.0	84.2	78.8	80.6	89.4	91.2	89.5	100.2	76.0	89.3		
	CV (%)					8.0							
	LSD P ≤ 0.05					9.0						NS	
Kipkebe	Dry	110.2	96.1	79.0	109.1	97.9	93.6	111.9	105.1	90.8	96.5	99.0	
	Wet	87.8	95.3	92.8	92.2	88.8	101.0	77.0	92.3	95.3	85.7	90.8	94.9
	Mean clone	99.0	95.7	85.9	100.7	93.4	97.3	94.4	98.7	93.0	91.1		
	CV (%)					7.1							
	LSD P ≤ 0.05					8.7						NS	
Timbilil	Dry	96.4	93.8	105.1	107.5	87.3	100.1	102.8	92.4	105.1	97.8	98.8	
	Wet	99.6	104.0	89.8	104.5	98.4	68.0	97.9	107.1	76.6	77.3	92.3	95.6
	Mean clone	98.0	98.9	97.5	106.0	92.8	84.1	100.3	99.8	90.3	87.5		
	CV (%)					6.5							
	LSD P ≤ 0.05					8.1						NS	
All 3 sites means:	Dry	95.6	90.0	88.4	98.5	93.5	94.5	101.0	100.1	88.0	93.4	94.3	
	Wet	88.4	95.9	86.4	93.0	90.3	87.2	88.5	99.0	85.3	85.2	89.9	
	Clone	92.0	92.9	87.4	95.8	91.9	90.8	94.8	99.6	86.6	89.3		
	CV (%)					7.2							
LSD P ≤ 0.05, Site (S)						5.0						NS	5.2
Interactions:	SxC					8.0							
	SxSe					7.3							
	CxSe					7.0							
	SxCxSe)					11.3							

NS = Not significant

Table 35: Variations in clonal total gallocatechins level (mg/g DM) with location, season and genotype

Site (S)	Season (Se)	Clone (C)										Mean season	Mean site
		TRFK 6/8	TRFK 7/9	TRFK 303/577	AHP S15/10	BBK 35	TRFK 12/19	TRFK 54/40	TRFK 303/1199	TRFK 31/8	TRFK 12/12		
Kangaita	Dry	152.8	144.3	157.3	148.9	147.5	163.4	154.9	167.8	162.1	164.6	156.4	
	Wet	143.7	118.9	127.1	133.0	117.2	137.6	133.8	153.8	134.0	122.0	132.1	144.2
	Mean clone	148.3	131.6	142.2	141.0	132.4	150.5	144.3	160.8	148.0	143.3		
	CV (%)					6.5							
	LSD P ≤ 0.05					12.2						NS	
Kipkebe	Dry	122.7	151.8	174.9	153.5	166.0	163.2	165.4	159.0	161.3	151.1	156.9	
	Wet	142.5	119.5	157.4	132.7	116.0	103.3	119.4	131.6	89.6	102.3	121.4	140.8
	Mean clone	132.6	135.7	166.2	143.1	141.0	133.3	142.4	145.3	125.5	126.7		
	CV (%)					7.8							
	LSD P ≤ 0.05					14.2						NS	
Timbilil	Dry	143.8	149.2	111.4	137.7	145.7	150.3	169.4	156.3	156.6	153.5	147.4	
	Wet	127.6	132.5	136.0	137.9	132.1	129.6	147.4	152.7	140.1	106.1	134.2	139.2
	Mean clone	135.7	140.9	123.7	137.8	138.9	140.1	158.4	154.5	148.4	129.8		
	CV (%)					4.8							
	LSD P ≤ 0.05					8.7						NS	
All 3 sites means:	Dry	139.8	148.4	147.9	146.7	153.1	159.0	163.2	161.0	160.0	156.4	153.6	
	Wet	137.9	123.6	140.2	134.5	121.8	123.5	133.5	146.0	121.2	110.1	129.3	
	Clone	138.9	136.0	144.0	140.6	137.4	141.3	148.4	153.5	140.6	133.3		
	CV (%)					6.5							
LSD P ≤ 0.05						6.9						NS	NS
Interactions:	SxC					11.1							
	SxSe					10.2							
	CxSe					9.8							
	SxCxSe					15.8							

NS = Not significant

4.3.5 Variations in sum of flavan-3-ols levels, ratio of gallo/simple flavan-3-ols and ratio of gallated/non gallated flavan-3-ols with genotypes, location of production and season

Total catechins in green tea leaf is the sum of EGC, (+)C, EC, EGCG and ECG (ISO 14502-2, 2005). The ratio of trihydroxyflavan-3-ols to dihydroxyflavan-3-ols influenced the amounts of theaflavins produced (Owuor *et al*., 2006). Since the trihydroxy-flavan-3-ols have lower redox potentials (Bajaj *et al*., 1987), a high trihydroxy-flavan-3-ols to dihydroxy-flavan-3-ols ratio ensures that high amounts of theaflavins are formed (Owuor and Obanda, 2007). The total theaflavins produced is largely influenced by the ratio of trihydroxy to dihydroxy flavan-3-ols present in tea leaves (Wright *et al*., 2000; Owuor and Obanda, 2007). In black tea processing, the oxidation of flavan-3-ol gallic acid esters lead to production of theaflavin gallic acid esters, which are more astringent than theaflavins (Sanderson *et al*., 1976). Although black tea have same total theaflavins contents, black teas with higher amounts of theaflavin gallic acid esters are of higher quality (Owuor and Obanda, 1995).

Total catechins (Table 36) varied ($P \leq 0.05$) with clones at all individual sites, except in Kangaita and for all sites pooled together. There were no seasonal effects, however unlike total polyphenols, total catechins were significantly ($P \leq 0.05$) influenced by location of production. This may indicate that location of production affects the composition of leaf by individual catechins and not total polyphenolic content.

The variations of trihydroxyflavan-3-ols to dihydroxyflavan-3-ols ratios with locations, clones and seasons are presented in Table 39. There were no significant variations in their ratios with geographical areas of production, suggesting that the parameter may be stable to variations in growth factors closer to the equator. The clones varied in their trihydoxy to dihydroxyflavan-

3-ols ratios, further emphasizing the variations in the plain black tea quality potential due to genetic differences.

The changes in total flavan-3-ol gallic acid esters, simple (non-ester) flavan-3-ols, the ratio of trihydoroxy to dihydroxyflavan-3-ols and the ratio of flavan-3-ol gallic acid esters to simple flavan-3-ols are presented in Tables 37, 38, 39 and 40 respectively. The total flavan-3-ol gallic acid esters varied ($P \leq 0.05$) with clones but not with location of production and season (Table 37). But for total simple flavan-3-ols, whereas the levels did not vary with seasons, their levels were influenced ($P \leq 0.05$) by location of production and clones. However, the ratio of flavan-3-ol gallic acid esters to simple flavan-3-ols is more critical to plain black tea quality than total flavan-3-ols alone (Owuor and Obanda, 1995, 2007). The ratio of flavan-3-ol gallic acid esters to simple flavan-3-ols significantly varied ($P \leq 0.05$) with location of production, clones and seasons (Table 40). Except for seasonal differences at the individual sites, at every location there were significant ($P \leq 0.05$) differences in the ratio due to clones and location of production. The result demonstrate the potential of different clones to produce plain black teas of differerent qualities, and that such quality will vary with location of production as had been observed in plain black tea (Owuor *et al*., 2008b, 2010a, 2010b). There were significant interactions effects between location of production and clones, demonstrating that the extents or magnitudes of the changes in the ratio varied fron location to location in different clones. It is therefore not possible to produce same plain black tea quality even using one cultivar when production sites are varied. Tea cultivars must be evaluated for quality in the intended areas of production before extensive cultivation, even within one country. There were also significant ($P \leq 0.05$) interactions effects between location of production and seasons. This was because the magnitude of changes in the environmental factors that influence growth and production of plant metabolites vary in different

locations and seasons. There were also significant ($P \leq 0.05$) interactions effects between seasons and clones.

In countries further away from the equator, seasons of production have large influence on black tea quality (Erturk *et al*., 2010) due to large seasonal variations in flavan-3-ols (Wang *et al*., 2011). These changes have been attributed to variations in growth patterns (Mathews and Stephens, 1998) caused by large variations in temperatures and sunshine hours. Indeed the flavan-3-ols levels increased with rise in temperatures (Wang *et al*., 2011). In Kenya however, tea is grown close to the equator where variations in seasonal temperature and sunshine hours are minimal. Consequently seasonal changes in plain black tea quality parameter are low (Owuor, 1992, 1994) compared to sub tropical countries.

Table 36: Variations in young leaf total catechins levels (mg/g DM) with location, season and genotype

Site (S)	Season (Se)	Clone (C)										Mean season	Mean site
		TRFK 6/8	TRFK 7/9	TRFK 303/577	AHP S15/10	BBK 35	TRFK 12/19	TRFK 54/40	TRFK 303/1199	TRFK 31/8	TRFK 12/12		
Kangaita	Dry	186.1	172.1	194.5	179.9	195.8	213.2	188.4	208.9	183.4	227.4	195.0	183.1
	Wet	186.4	151.3	166.0	168.3	158.5	183.4	170.2	194.2	162.5	171.3	171.2	
	Mean clone	186.3	161.7	180.2	174.1	177.1	198.3	179.3	201.6	173.0	199.4		
	CV (%)					6.2							
	LSD P ≤ 0.05					14.8						18.6	
Kipkebe	Dry	185.1	190.5	145.9	175.1	203.6	192.8	205.4	204.7	181.9	201.4	188.6	179.8
	Wet	166.1	170.7	174.4	164.5	175.5	168.5	178.0	194.7	165.7	151.4	171.0	
	Mean clone	175.6	180.6	160.2	169.8	189.6	180.7	191.7	199.7	173.8	176.4		
	CV (%)					5.8							
	LSD P ≤ 0.05					13.5						NS	
Timbilil	Dry	174.2	180.0	213.3	193.0	211.6	202.8	202.6	203.2	195.9	208.4	198.5	177.8
	Wet	184.3	147.7	198.9	163.3	160.5	137.9	143.4	173.3	108.9	153.6	157.2	
	Mean clone	179.3	163.8	206.1	178.2	186.1	170.4	173.0	188.3	152.4	181.0		
	CV (%)					6.9							
	LSD P ≤ 0.05					16.0						NS	
All 3 sites means:	Dry	181.8	180.9	184.6	182.7	203.7	202.9	198.8	205.6	187.1	212.4	194.0	
	Wet	178.9	156.6	179.8	165.4	164.8	163.3	163.7	187.4	145.7	158.8	166.5	
	Clone	180.4	168.7	182.2	174.0	184.2	183.1	181.3	196.5	166.4	185.6		
	CV (%)					6.7							
LSD P ≤ 0.05						9.1						22.7	NS
Interactions:	SxC					14.6							
	SxSe					13.3							
	CxSe					12.8							
	SxCxSe					20.6							

NS = Not significant

Table 37: Variations in clonal total trihydroxyflavan-3-ols levels (mg/g DM) with lacation, season and genotype

Site (S)	Season (Se)	Clone (C)										Mean season	Mean site
		TRFK 6/8	TRFK 7/9	TRFK 303/577	AHP S15/10	BBK 35	TRFK 12/19	TRFK 54/40	TRFK 303/1199	TRFK 31/8	TRFK 12/12		
Kangaita	Dry	106.5	107.6	121.0	119.2	126.6	137.1	125.2	141.1	122.2	149.2	125.6	
	Wet	102.4	102.4	117.6	112.3	109.4	125.9	118.3	128.5	109.6	129.7	115.6	120.6
	Mean clone	104.5	105.0	119.3	115.8	118.0	131.5	121.8	134.8	115.9	139.5		
	CV (%)					6.0							
	LSD P ≤ 0.05					9.5						NS	
Kipkebe	Dry	115.3	121.0	136.2	143.1	147.0	147.1	126.2	140.3	125.0	147.8	134.9	
	Wet	118.0	108.9	130.3	115.0	126.7	107.7	109.8	125.4	87.5	118.0	114.7	123.6
	Mean clone	116.7	115.0	133.2	129.1	136.8	127.4	118.0	132.9	106.3	132.9		
	CV (%)					8.1							
	LSD P ≤ 0.05					13.3						NS	
Timbilil	Dry	106.0	117.3	92.5	123.5	138.7	141.1	123.7	143.9	127.9	141.7	125.6	
	Wet	102.4	121.7	113.5	120.6	129.4	123.2	128.7	140.6	121.0	113.8	121.5	124.8
	Mean clone	104.2	119.5	103.0	122.1	134.1	132.2	126.2	142.2	124.5	127.7		
	CV (%)					5.7							
	LSD P ≤ 0.05					9.2						NS	
All 3 sites means:	Dry	109.3	115.3	116.6	128.6	137.4	141.8	125.0	141.8	125.0	146.2	128.7	
	Wet	107.6	111.0	120.5	116.0	121.8	118.9	118.9	131.5	106.0	120.5	117.3	
	Clone	108.5	113.1	118.5	122.3	129.6	130.4	122.0	136.6	115.5	133.4		
	CV (%)					6.8							
	LSD P ≤ 0.05, Site (S)					6.3						NS	NS
Interactions:	SxC					10.2							
	SxSe					9.3							
	CxSe					9.0							
	SxCxSe					NS							

NS = Not significant

Table 38: Variations in clonal total non gallated flavan-3-ols levels (mg/g DM) with location, season and genotype

Site (S)	Season (Se)	Clone (C)										Mean season	Mean site
		TRFK 6/8	TRFK 7/9	TRFK 303/577	AHP S15/10	BBK 35	TRFK 12/19	TRFK 54/40	TRFK 303/1199	TRFK 31/8	TRFK 12/12		
Kangaita	Dry	79.6	64.5	73.5	60.7	69.2	76.1	63.3	67.8	61.2	78.2		
	Wet	84.0	48.9	48.4	56.0	49.1	57.6	51.9	65.7	52.9	41.6	69.4	62.5
	Mean clone	81.8	56.7	60.9	58.4	59.1	66.8	57.6	66.8	57.1	59.9	55.6	
	CV (%)					11.5							
	LSD P ≤ 0.05					1.1						NS	
Kipkebe	Dry	58.9	59.0	77.1	49.9	64.6	55.7	76.4	62.9	70.0	60.6	63.5	
	Wet	66.3	38.8	68.6	48.4	33.8	30.3	33.5	47.9	21.4	35.6	42.5	56.2
	Mean clone	62.6	48.9	72.9	49.1	49.2	43.0	55.0	55.4	45.7	48.1		
	CV (%)					11.3							
	LSD P ≤ 0.05					7.8						NS	
Timbilil	Dry	79.1	73.2	53.5	51.6	64.9	51.7	81.7	60.8	53.9	59.7	63.0	
	Wet	63.7	49.0	60.9	43.9	46.1	45.3	49.3	54.2	44.7	37.6	49.5	53.0
	Mean clone	71.4	61.1	57.2	47.7	55.5	48.5	65.5	57.5	49.3	48.7		
	CV (%)					9.9							
	LSD P ≤ 0.05					7.3						NS	
All 3 sites means:	Dry	72.5	65.6	68.0	54.1	66.2	61.2	73.8	63.8	61.7	66.2	65.3	
	Wet	71.3	45.6	59.3	49.4	43.0	44.4	44.9	55.9	39.7	38.3	49.2	
	Clone	71.9	55.6	63.7	51.7	54.6	52.8	59.3	59.9	50.7	52.2		
	CV (%)					11.0							
LSD P ≤ 0.05						4.8						NS	5.0
Interactions:	SxC					7.7							
	SxSe					7.0							
	CxSe					6.7							
	SxCxSe					10.8							

NS = Not significant

Table 39: Variations in clonal gallocatechins/simple flavan-3-ols ratio with location, season and genotype

Site (S)	Season (Se)	Clone (C)										Mean season	Mean site
		TRFK 6/8	TRFK 7/9	TRFK 303/577	AHP S15/10	BBK 35	TRFK 12/19	TRFK 54/40	TRFK 303/1199	TRFK 31/8	TRFK 12/12		
Kangaita	Dry	3.11	3.50	3.15	3.42	2.54	2.75	3.21	2.89	4.13	2.37	3.11	
	Wet	2.81	2.89	2.71	3.14	2.38	2.58	2.96	2.86	3.61	2.08	2.80	2.95
	Mean clone	2.96	3.20	2.93	3.28	2.46	2.66	3.08	2.87	3.87	2.22		
	CV (%)					9.76							
	LSD P ≤ 0.05					0.38						NS	
Kipkebe	Dry	2.24	3.79	3.34	3.05	3.07	3.32	3.33	3.02	4.11	2.28	3.15	
	Wet	2.82	3.06	3.13	3.13	2.24	2.30	3.23	2.68	3.23	1.68	2.75	3.00
	Mean clone	2.53	3.43	3.23	3.09	2.66	2.81	3.28	2.85	3.67	1.98		
	CV (%)					8.65							
	LSD P ≤ 0.05					0.33						NS	
Timbilil	Dry	3.02	3.18	2.56	3.21	2.32	2.78	3.80	2.96	4.02	2.49	3.03	
	Wet	2.72	2.86	3.12	3.67	2.28	2.80	3.32	2.90	4.09	1.90	2.97	2.95
	Mean clone	2.87	3.02	2.84	3.44	2.30	2.79	3.56	2.93	4.06	2.19		
	CV (%)					5.37							
	LSD P ≤ 0.05					0.21						NS	
All 3 sites means:	Dry	2.79	3.49	3.02	3.23	2.64	2.94	3.45	2.96	4.09	2.38	3.10	
	Wet	2.78	2.94	2.99	3.31	2.30	2.56	3.17	2.81	3.64	1.89	2.84	
	Clone	2.78	3.21	3.00	3.27	2.47	2.75	3.31	2.89	3.86	2.13		
	CV (%)					8.05							
LSD P ≤ 0.05, Site (S)						0.18						NS	NS
Interactions:	SxC					0.29							
	SxSe					0.27							
	CxSe					0.25							
	SxCxSe					0.41							

NS = Not significant

Table 40: Variations in clonal gallated/non gallated catechins ratio with location, season and genotype

Site (S)	Season (Se)	Clone (C)										Mean season	Mean site
		TRFK 6/8	TRFK 7/9	TRFK 303/577	AHP S15/10	BBK 35	TRFK 12/19	TRFK 54/40	TRFK 303/1199	TRFK 31/8	TRFK 12/12		
Kangaita	Dry	1.37	1.71	1.65	1.98	1.86	1.82	1.98	2.11	2.02	1.92	1.84	
	Wet	1.22	2.09	2.43	2.02	2.23	2.20	2.29	1.99	2.08	3.14	2.17	2.01
	Mean clone	1.30	1.90	2.04	2.00	2.04	2.01	2.13	2.05	2.05	2.53		
	CV (%)					11.18							
	LSD P ≤ 0.05					0.29						NS	
Kipkebe	Dry	1.97	2.07	1.82	2.90	2.28	2.64	1.66	2.24	1.77	2.45	2.18	2.30
	Wet	1.78	2.83	1.90	2.41	3.81	3.56	3.28	2.62	4.07	3.33	2.96	
	Mean clone	1.88	2.45	1.86	2.65	3.05	3.10	2.47	2.43	2.92	2.89		
	CV (%)					10.10							
	LSD P ≤ 0.05					0.34						NS	
Timbilil	Dry	1.34	1.61	1.74	2.41	2.16	2.77	1.52	2.38	2.41	2.39	2.07	
	Wet	1.61	2.48	1.87	2.76	2.86	2.73	2.61	2.60	2.71	3.01	2.53	2.57
	Mean clone	1.48	2.05	1.80	2.59	2.51	2.75	2.07	2.49	2.56	2.70		
	CV (%)					10.04							
	LSD P ≤ 0.05					0.30						NS	
All 3 sites means:	Dry	1.56	1.80	1.74	2.43	2.10	2.41	1.72	2.24	2.07	2.25	2.03	
	Wet	1.54	2.47	2.07	2.40	3.00	2.83	2.73	2.40	2.95	3.16	2.55	
	Clone	1.55	2.13	1.90	2.41	2.53	2.62	2.22	2.32	2.51	2.71		
	CV (%)					10.36							
LSD P ≤ 0.05						0.18						0.45	0.19
Interactions:	SxC					0.29							
	SxSe					0.26							
	CxSe					0.25							
	SxCxSe					0.41							

NS= Not significant

4.4 Variations in green young tea leaf caffeine and flavan-3-ols levels of clone TRFK 6/8 with nitrogenous fertilizer rates and location of production in Eastern Africa

4.4.1 Variations in caffeine levels with location of production and nitrogenous fertilizer rates

Variations in caffeine with location of production within Eastern Africa and nitrogen rates are shown in Table 41. Clone TRFK 6/8 could not replicate leaf caffeine contents in different locations in Eastern Africa. This is consistent with the observation made on clones grown in different locations in Kenya (Table 26). Similar results were observed in Northeast India black teas (Dev Choudhury *et al*., 1991). Rwanda locations recorded the highest leaf caffeine amounts, while sites in Tanzania had the lowest. This may imply that the quality of plain black tea as measured by 'cream' formation may be largely dependent on location of production in Eastern Africa. Caffeine increased significantly ($P \leq 0.05$) with increase in nitrogen rates in all the sites. This trend had been established earlier in Kenya plain black teas at single locations (Owuor *et al*., 1987a, 1991; Owuor and Odhiambo, 1994) and in Pakistani tea growing in different agro ecological zones under different nitrogen fertilizer rate (Akhlas *et al*., 2003). In Kenyan plain black tea, in addition to caffeine, rates of nitrogenous fertilizers increased levels of those compounds imparting inferior and superior aroma to flavor black teas. However, the flavor index, the ratio of compounds imparting superior flavor to those contribut characteristics, decreased with increasing rates of nitrogenous fertilizers (Owuor *et al*., 1987d). This observation demonstrates that high nitrogenous fertilizer reduces both plain and flavory black tea quality (Owuor *et al*., 1987d, 1991, 2010a). The interactions effects between nitrogenous fertilizer rates and sites were not significant implying that the pattern of change in caffeine levels was the same in all tha tea growing locations studied.

Table 41: Variations in caffeine levels (mg/g DM) in 2 + a bud of clone 6/8 with location and N-rates

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

4.4.2 Variations in total polyphenols levels with location of production and nitrogenous fertlizer rates

Total polyphenols include flavanols, flavonols, flavonol glycosides, polyphenolic acids and depsides put together and had been reported to make up about 30% of the dry weight in tea shoot (Harbowy and Balentine, 1997). Total polyphenols influence plain black tea quality if polyphenol oxidase activity is not limiting (Obanda *et al*., 1997, 1999). Variation in total polyphenols content of green leaf of clone TRFK 6/8 in Eastern African tea growing region are shown in Table 42. The levels were generally lower than those reported earlier (Harbowy and Balentine, 1997). These levels significantly ($P \leq 0.05$) varied with location of production, with Kenyan sites recording higher values than the sites in Tanzania and Rwanda which were comparable. The total polyphenolic levels increased ($P \leq 0.05$) with nitrogenous fertilizer rates above 150 kg N/ha/yr. There were significant ($P \leq 0.05$) interactions between the rates of nitrogen and location of production indicating that the variation patterns were location specific. These results imply that even for a single clone, nitrogenous fertilizer rate that would ensure optimum plain black tea quality may be region specific in the tea growing region of Eastern Africa.

Table 42: Variations in total polyphenols levels with location of production and N rates (%GAE)

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

4.4.3 Variations in levels of dihydroxyflavan-3-ols with location of production and nitrogenous fertilizer rates

The simple catechins [(+) C, EC and ECG] varied significantly ($P \leq 0.05$) with location of production and with nitrogen rate (Table 42). These variations with location of production may be attributed to several factors including soil types, soil fertility (Bonheure and Willson, 1992), temperatures (Tanton, 1982), rainfall and rainfall distribution (Othieno *et al*., 1992) and altitudes (Owuor *et al*., 1990b, 1990c; Squire *et al*., 1993). Earlier reports (Akhlas *et al*., 2003) indicated that nitrogen rates above 180 kg/ha/yr leads to reduced EC in tea plant. Structurally catechins are carbon-based metabolites. Their production is determined by availability of carbohydrates (Bryant *et al*., 1983). The young shoot is a physiological 'sink' and has to import carbon (C) from a 'source' owing to its low photosynthetic capability (Arnold *et al*., 2004). Decrease of catechins synthesis in young shoots under low nitrogen supply therefore, could be a result of low carbohydrate availability due to reduced photosynthate import from the 'source'. In Central and Southern Africa, green leaf EC levels linearly correlated with plain black tea quality (Wright *et al*., 2000) while while the correlation was inverse with Kenyan plain black tea quality (Owuor and Obanda, 2007). Higher ECG content in fresh leaf might lead to the formation of higher

theaflavin-digallates in the plain black tea (Madanhire, 1995), a parameter associated with Kenyan plain black tea quality (Owuor and Obanda, 2007). This implies that levels of EC and ECG of green leaf may influence plain black tea quality.

Variations in dihydroxyflavan-3-ols of young green leaves of clone TRFK 6/8 with location of production and nitrogenous fertilizer rates are presented in Table 43.

Table 43: Variations in clone TRFK 6/8 dihydroxyflavan-3-ols levels (mg/g DM) with location of production and N rates

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

C.V. (%)	4.3	
LSD, P ≤ 0.05	1.5	1.5
Interaction	2.9	

Low amounts of nitrogen up to about 150 kg N/ha/yr generally reduced amounts of simple catechins and total simple catechins relative to the control; however the reduction did not reach significant levels. This demonstrates that plain black tea quality as influenced by levels of dihydroxyflavan-3-ols may not be affected significantly by location of production *per se* in Eastern Africa; however the effect may be due to significant ($P \leq 0.05$) interactions effects between nitrogenous fertilizer rates and location of production. These results indicate that the actual optimum nitrogenous fertilizer rate may vary from one location of production to another and further explain the plain black tea quality variations with locations (Owuor *et al*., 2009, 2010a) and nitrogen rates (Owuor *et al*., 1987d, 2010a; Owuor and Odhiambo, 1994) observed in the previous studies.

4.4.4 Variations in levels of trihydroxyflavan-3-ols with location of production and nitrogenous fertilizer rates

The trihydroxyflavan-3-ols in green tea leaf include gallic acid (GA), epigallocatechin (EGC) and epigallocatechin gallate (EGCG). They are oxidized faster during tea fermentation phase of black tea processing due to their lower redox potential compared to dihydroxyflavan-3-ols (Bajaj *et al*., 1987). Total trihydroxyflavan-3-ols and EGCG levels in green tea leaf are good indicators of Kenyan plain black tea quality as they correlated linearly with Kenyan plain black tea quality (Owuor *et al*., 2006; Owuor and Obanda, 2007). Gallic acid can react with a dihydroxyflavan-3-ol to yield theaflavic acid, thereby increasing total theaflavins and hence enhancing plain black tea quality (Janna, 2011). High total TFs in plain black tea, which is also a

quality parameter, has been associated with high levels of EGC in green leaf (Hilton *et al*., 1973; Robertson, 1983b).

Variations in GA, EGC and EGCG with nitrogenous fertilizer rates in the Eastern African teas growing region are presented in Table 44. Trihydroxyflavan-3-ols significantly ($P \leq 0.05$) varied with location of production. This demonstrates that even with the same cultivar, it is

Table 44: Variations in trihydroxyflavan-3-ols levels (mg/g) DM with location of production and nitrogen rates

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

Mean	136.9	143.7	133.2	66.5	96.8	94.6	77.1	
C.V. (%)				4.3				
LSD, P ≤ 0.05				4.1				NS
Interaction				7.8				

difficult to produce plain black tea of the same quality in different locations, further supporting previous observation that clonal plain black tea quality varies with location of production (Owuor *et al*., 2008b) even within the same country (Owuor *et al*., 2010a, 2010b). Sites in Kenya had significantly ($P \leq 0.05$) highest trihydroxyflavan-3-ols levels. This may imply that Kenyan plain black tea may be superior in quality as measured by theaflavin formation when compared with tea from Rwanda or Tanzania.

The trihydroxyflavan-3-ols levels also varied significantly ($P \leq 0.05$) with nitrogen rates. Generally low nitrogen levels, below 150 kg N/ha/yr increased EGCG amounts but decreased EGC amounts in green leaf. Previous studies had also reported that nitrogen fertilization above 180 kg N/ha/yr depressed (-)-EGC in young shoots whereas it either increased or decreased (-)-EGCG concentration (Hilton *et al*., 1973). The variations in total trihydroxyflavan-3-ols (Table 44) did not reach significant ($P \leq 0.05$) levels, however, the significant interactions effects between nitrogenous fertilizer rate and location of production suggests that even a single cultivar may not replicate plain black tea quality in different locations under same fertilizer regime. These results demonstrate that for high quality plain black tea procuction, an optimum nitrogenous fertilizer rate is necessary in each tea production region in Eastern Africa even for a single clone.

4.4.5 Variations in total flavan-3-ols levels, ratio of gallo to simple flavan-3-ols and ratio of gallated to non-gallated flavan-3-ols with location of production and nitrogenous fertilizer rates

The ratio of trihydroxyflavan-3-ols to dihydroxyflavan-3-ols influence the amounts of theaflavins produced during black tea manufacture (Owuor *et al*., 2006). A high trihydroxy to dihydroxyflavan-3-ols ratios ensures formation of high amounts of theaflavins (Owuor and Obanda, 2007) since the trihydroxyflavan-3-ols have lower redox potentials than dihydroxyflavan-3-ols (Bajaj *et al*., 1987).

The variations in trihydroxy to dihydroxyflavan-3-ols ratio with nitrogenous fertilizer rates on clone TRFK 6/8 in Eastern Africa are presented in Table 45.

Table 45: Variations in flavan-3-ols ratios with location of production and N rates

Catechin	N-rate kg N/ha/yr	Timbilil	Changoi	Sotik	Mulindi	Kitabi	Maruku	Katoke	Mean N-rate
Gallated /Non gallated	0	1.45	1.80	1.79	0.88	1.28	1.02	0.82	1.29
	75	1.51	1.93	1.85	1.09	1.30	1.08	0.89	1.38
	150	1.51	1.95	1.89	1.19	1.50	1.10	0.90	1.43
	225	1.46	1.59	1.74	0.96	1.28	1.15	0.83	1.29
	300	1.35	1.55	1.72	0.94	1.30	1.18	0.77	1.26
	Mean	1.46	1.76	1.80	1.01	1.33	1.11	0.84	
	C.V. (%)				7.27				
	LSD, P ≤ 0.05				0.09				0.08
	Interaction				0.16				
Gallo catechin /simple catechin	0	2.54	3.07	3.04	2.23	1.80	2.67	2.25	2.51
	75	2.69	3.26	3.18	2.83	2.15	2.82	2.45	2.77
	150	2.72	3.43	3.27	3.06	2.26	2.85	2.97	2.94
	225	2.54	2.80	3.01	2.49	2.00	3.02	2.26	2.59
	300	2.35	2.75	2.97	2.44	1.95	2.99	2.04	2.50
	Mean	2.57	3.06	3.09	2.61	2.03	2.87	2.39	
	C.V. (%)				7.23				
	LSD, P ≤ 0.05				0.17				0.16
	Interaction				0.32				

The ratios varied ($P \leq 0.05$) with location of production in variance with the observation made on clones within Kenya (Table 39) suggesting that further from the equator, locational effects become more apparent. This supports earlier observations that plain black tea quality variations nearer the equator (Owuor, 1992, 1994) are much lower than further away from the equator (Sud

and Baru, 2000; Yao *et al*., 2005; Erturk *et al*., 2010; Wang *et al*., 2011). Sites in Kenya recorded highest ratios of gallated: non gallated and gallocatechins: simple catechins followed by sites in Tanzania and then sites in Rwanda. This implies that with the high ratios recorded in Kenyan sites, quality of resultant plain black tea from these Kenyan sites may be superior to those of the other sites. Trihydroxy to dihydroxyflavan-3-ols ratio also varied ($P \leq 0.05$) with nitrogenous fertilizer rate (Table 45). The ratio of trihydroxyflavan-3-ols to dihydroxyflavan-3-ols was enhanced by low nitrogen levels but depressed by high nitrogen levels. This observation supports earlier reports (Owuor *et al*., 1997, 2000; Venkatesan and Ganapathy, 2004; Venkatesan *et al*., 2004) that high rates of nitrogenous fertilizer lower plain black tea quality.

The variations in total catechins, total flavan-3-ol gallic acid esters and simple (non-ester) flavan-3-ols and sum of different categories of catechins are shown in Table 46. The parameters all varied significantly with location of production and nitrogenous fertilizer rates demonstrating that even for a single cultivar, it may be difficult to replicate plain black tea quality potential in different growing regions in Eastern Africa under the same nitrogen fertilizer norm. The ratio of flavan-3-ol gallic acid esters to simple flavan-3-ols which is more critical to black tea quality (Owuor and obanda, 1995, 2007) is presented in Table 45. The flavan-3-ol gallic acid esters to simple flavan-3-ols ratio varied significantly ($P \leq 0.05$) with location of production and with nitrogen rate. The ratio was depressed by low nitrogen levels but enhanced by high nitrogen levels, a reverse of what was observed in the ratio of trihydroxyflavan-3-ols to dihydroxyflavan-3-ols. Further, the two plain black tea critical ratios showed significant ($P \leq 0.05$) interactions effects between location of production and fertilizer rates. These observations demonstrate the synthesis of caffeine and flavan-3-ols is affected by location of production, nitrogen fertilizer rate and the interactions effects between location of production and nitrogen fertilizer rate. A

blanket nitrogen fertilizer rate therefore is not appropriate even for a single clone grown in different locations. It is necessary to evaluate optimal fertilizer rates for clones in the intended growing region before release for extensive cultivation to ensure optimum synthesis of quality precursor compounds.

Table 46: Variations in sum of flavan-3-ols (mg/g DM) with location of production and nitrogen rates

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

CHAPTER FIVE

5 SUMMARY, CONCLUSIONS, RECOMMENDATIONS STUDY LIMITATIONS AND SUGGESTIONS FOR FUTURE STUDIES

This study was set to evaluate the suitability of seedling tea-based nutrients diagnostic limits on clonal tea in different locations in Kenya and on clone TRFK 6/8 under different nitrogenous fertilizer rates and plucking intervals in Eastern Africa. Further, it was expected to determine variations in the quality precursor compounds in the clones with location of production, genotype, nitrogen rates and season. From the study, the following summary, conclusions and recommendations may be drawn:

5.1 Summary

1. Tea plants used in the trials received the recommended fertilizer rate of 150 kg N/ha/year and did not show any visual signs of deficiency. However leaf nutrients levels quite often fell in the borderline or deficiency regions with very isolated cases of adequate levels. This may imply that the recommendations based on seedling may not be used as a guide to foliar analysis advisory system for clonal tea despite the fact that they are currently used on clonal tea in all growing locations.

2. Even with application of the same agronomic inputs, the levels of the nutrients in mature leaf of clone TRFK 6/8 were significantly ($P \leq 0.05$) different from one location to another. This in part could be due to variations in the levels of micronutrients in the soils at different locations, especially for the nutrients not supplied and/or due to past agronomic inputs in the fields. There were large variations in the mean values for sites for nitrogen, potassium, calcium, manganese, zinc, copper and iron. These large variations

may be the cause in part of the variations in the yields of clone TRFK 6/8 due to locations observed in the past. Plucking interval did not significantly influence the mature leaf nutrients of clone TRFK 6/8.

3. The amount of caffeine varied ($P \leq 0.05$) with clones and was further influenced by location of production. There was no significant ($P \leq 0.05$) seasonal effect on leaf caffeine content. Total polyphenols amounts ($P \leq 0.05$) varied with genotypes but not location of production; however there were significant ($P \leq 0.05$) interactions between clones and sites. The amounts of the simple catechins were in the order ECG>EC>(+)C, that of gallocatechins was EGCG>EGC>GA and both dihydroxyflavan-3-ols and trihydroxyflavan-3-ols showed significant variations ($P \leq 0.05$) with clones and location of production. However, there was no seasonal effect ($P \leq 0.05$) on simple and gallocatechins amounts. There were no significant variations in flavan-3-ols ratios with site. However there were significant ($P \leq 0.05$) interaction between clones and location for the ratios.

4. Caffeine levels in TRFK 6/8 increased ($P \leq 0.05$) with increase in nitrogen rates, but the magnitude of the increase varied from one location of production to another. Low amounts of nitrogen up to about 150 kg N/ha/yr reduced amounts of dihydroxyflavan-3-ols in clonal TRFK 6/8 leaves with this effect seen more in EC and ECG than in (+) C. The trihydroxyflavan-3-ols levels also varied significantly ($P \leq 0.05$) with nitrogen rates. Total flavan-3-ols and total non gallated flavan-3-ols were more depressed ($P \leq 0.05$) by low nitrogen rates than total gallated flavan-3-ols. The gallated: non gallated flavan-3-ols

ratio and the trihydroxyflavan-3-ols: dihydroxyflavan-3-ols ratio varied significantly ($P \leq 0.05$) with location of production.

5.2 Conclusions

1. The nutrients diagnostic limits based on seedling tea cannot be used to guide foliar analysis advisory system for clonal tea.

2. The mature leaf diagnostic nutrients limits set for seedling tea in are not suitable to guide foliar analysis advisory system for clone TRFK 6/8 in the Eastern Africa tea growing regions.

3. Composition of clonal green leaf by caffeine, individual and/or total flavan-3-ols and ratio of flavan-3-ols is influenced by location of production in Kenya. A high quality clone in one region therefore may not replicate black tea quality in a new location.

4. Green leaf caffeine, individual and/or total flavan-3-ols and ratio of flavan-3-ols of clone TRFK 6/8 are affected by nitrogenous fertilizer rates, location of production and interaction effects between locations and nitrogen rates.

5.3 Recommendations

1. Clonal and locational specific foliar diagnostic norms should be developed to guide foliar advisory system in tea growing regions of Kenya.

2. Mature leaf diagnostic norms for clone TRFK 6/8 should be developed for specific regions of production in Eastern Africa.

3. Each clone should be evaluated and recommended for areas of their optimal biosynthesis of quality precursor compounds in the Kenya tea growing regions to improve plain black tea quality.

4. Each tea growing region of Eastern Africa should be evaluated for optimum nitrogenous fertilizer rates that would ensure optimum plain black tea quality for clone TRFK 6/8.

5.4 Suggestions for future studies

1. There is need to establish optimal nitrogen rates for every clone in each growing region that is economical and retains plain black tea quality potential in Eastern Africa.

2. The environmental factors (edaphic or non edaphic) responsible for the observed variations in nutrients contents and plain black tea quality precursor components in the growing locations should be established.

3. There is need to assess influence of seasonal changes on forms of /and activity of polyphenol oxidase enzyme.

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7 APPENDIX

7.1 Appendix 1. Experimental layout for the clone 6/8 fertilizer trials in the sites.

1 N_{300} Pf_{7}	2 N_{0} Pf_{21}	3 N_{150} Pf_{21}	4 N_{225} Pf_{14}	5 N_{0} Pf_{7}
10 N_{300} Pf_{21}	9 N_{150} Pf_{7}	8 N_{300} Pf_{14}	7 N_{225} Pf_{21}	6 N_{75} Pf_{14}
11 N_{225} Pf_{7}	12 N_{150} Pf_{14}	13 N_{75} Pf_{21}	14 N_{0} Pf_{14}	15 N_{75} Pf_{7}
20 N_{225} Pf_{21}	19 N_{225} Pf_{14}	18 N_{150} Pf_{21}	17 N_{75} Pf_{14}	16 N_{0} Pf_{21}
21 N_{225} Pf_{7}	22 N_{300} Pf_{14}	23 N_{150} Pf_{14}	24 N_{300} Pf_{7}	25 N_{300} Pf_{21}
30 N_{75} Pf_{21}	29 N_{0} Pf_{14}	28 N_{0} Pf_{7}	27 N_{75} Pf_{7}	26 N_{150} Pf_{7}
31 N_{25} Pf_{14}	32 N_{75} Pf_{14}	33 N_{225} Pf_{21}	34 N_{150} Pf_{21}	35 N_{225} Pf_{7}
40 N_{75} Pf_{7}	39 N_{300} Pf_{7}	38 N_{300} Pf_{14}	37 N_{150} Pf_{14}	36 N_{0} Pf_{21}
41 N_{300} Pf_{21}	42 N_{150} Pf_{7}	43 N_{0} Pf_{7}	44 N_{75} Pf_{21}	45 N_{0} Pf_{14}
Note: REP 1 Plots 1-15; REP 2 Plots 16-30; REP 3 Plots 31-45. N150 Pf21 denotes; Nitrogen fertilizer application at 150 kg N/Ha/yr as NPKS and a plucking frequency of 21 days. Spacing: 1.22 m x 0.9m; Plant population per plot: 30 bushes (6*5).				