DETERMINATION OF THE DROUGHT TOLERANCE IN CAMELLIA SINENSIS (L) O. KUNTZE, USING CATECHIN LEVELS IN VARIOUS CLONES IN KERICHO COUNTY

By

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March 2014 **DECLARATION**

I declare that this thesis is my own work and has not been submitted in any form for another degree to any university.

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DEDICATION

Affectionately dedicated

To

My parents, teachers and my family

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of variance
BBK	Brooke Bond Kenya
C	Catechin
CG	Catechin gallate
CL	Coumarate-Ligase
CoA	Co-enzyme A
CTC	Cutting, Tearing and Crushing
DSI	Drought Susceptibility Index

DMRT	Duncan Multiple Range Test
EC	Epicatechin
ECG	Epicatechin gallate
EGC	Epigallocatechin
EGCg	Epigallocatechin gallate
GC	Gallocatechin
GCG	Gallocatechin gallate
LWP	Leaf water potential
MPa	Megapascals
NPK	Nitrogen Phosphorus and Potassium
PAL	Phenylalanine Ammonialyase
SAS	Statistical Analysis System
SFS	Swazi Fertilizer Studies
SMC	Soil moisture content
TCC	Total catechin content
TDR	Time-domain Reflectometry
TRFK	Tea Research Foundation of Kenya

TRI	Tea Research Institute of Sri Lanka

ABSTRACT

The potentiality for use of catechin levels in selection for drought tolerance ability in clones of tea *Camellia sinensis* (L) O. Kuntze was studied. The study reported herein was carried out in Tea Research Foundation of Kenya (TRFK) between June 2011 and March 2012. Ten clones of tea known to vary in drought tolerance were screened. The experiment was conducted in an open field over three seasons. The treatments were arranged in a completely randomized design and replicated three times. Catechin levels in tea shoots were analyzed and a regression analysis done. The drought tolerant clones namely; SFS150, TRFK 303/577, and susceptible clones; TRFK 6/8, TRFK 12/9, TRFK 301/4, TRFK 31/11, S15/10, TRFK 7/9, TRFK 31/8, and BBK 35 were selected based on physiological data and field performance. Clones were evaluated in three sequential seasons; cold and wet (June-August 2011), warm and wet (October–December 2011) and dry and hot (January–March 2012) under field conditions. A highly positive correlation was observed between an increase in soil moisture deficit and a decrease in catechin contents. During the cold and wet season, the effect of soil water content on catechin level was not clearly expressed. However, significant clone × moisture treatment interactions ($p \leq 0.05$) were found for all clones during the dry and hot season. Under soil moisture stress, reduced catechin contents were found in all the ten clones. There was substantial clonal variation in response to soil water deficit. During the dry period, the drought-susceptible clones showed a relatively higher decline in catechin levels compared with the drought-tolerant clone. The catechin decreasing effect of water stress was clearly observed in the drought susceptible clones compared to the tolerant ones, suggesting that tolerance to soil moisture stress and accumulation of catechins are strongly correlated. The results indicate that declining soil moisture content (SMC) reduced catechin levels. This technique of analyzing catechin levels may help in skipping the time-consuming field tests. Thus, instead of taking several years to identify a clone that is drought tolerant, the breeder could just analyze catechin levels. Using this biochemical response could act as a substitute for or to assist in phenotypic selection, in a way which may make it more efficient, effective, reliable and cost effective compared to the conventional selection procedures.

CHAPTER ONE

INTRODUCTION

1.1 Background

1.1.1 Drought tolerance

Due to the frequent episodes of drought in tea growing regions, the demand for drought tolerant cultivars is set to become even much higher as effect of water stress on productivity of tea continues to be felt. The challenge for plant physiologists is to study detrimental effects of drought to establish selection criteria for tolerant genotypes. Difficulties in the past have included the identification of biochemical characteristics that are correlated with drought stress that could be used as indicators of drought tolerance (Navaratne, 1992; Khan and Mukhtar, 2007; Borland *et al*., 2009). Physiologists are able to measure various plant characteristics that correlate with drought tolerance, such as water use efficiency, root characteristics, leaf water potential, and stomatal characteristics (Robredo *et al*., 2007; Borland *et al*., 2009) but these tests are either too tedious or too time consuming for plant breeders to evaluate a wide range of clones.

1.1.2 Environmental conditions for tea growing

Tea (*Camellia sinensis* (L) O. Kuntze) is widely cultivated as a rain fed plantation crop and its productivity is greatly influenced by environmental conditions. For optimum production, it requires well-distributed annual rainfall of between 1400-2500 mm and a temperature range of 18-25 ºC (Nissanka, 2007). However, most of the tea growing areas

in Kenya experience less than adequate rainfall, which at times is poorly distributed (Jaetzold and Schmidt, 1983).

Although, tea growing areas of Kenya have a bimodal rainfall pattern that normally brings sufficient amount of rainfall, irregular distribution within a year causes moisture stress on tea plants mainly during the period December-March, during which potential soil water deficit close to 400 mm has been recorded (Ng'etich and Bore, 1998), resulting in a considerable loss of crop (Bore, 2010) and increase in deaths. Fluctuations in productivity of tea, in the recent past have been attributed to adverse weather conditions (De Costa *et al*., 2007; Subair, 2010). Drought episodes are remarkably aggravated by high solar radiation, high saturation vapour pressure deficit, and temperature (Lawlor and Tezara, 2008). Water stress as a result of drought brings about many physiological, biochemical and morphological changes in plants amongst them a reduction of leaf water potential, photosynthesis and stomatal conductance (Bota *et al*., 2004). Proline and abscisic acid (ABA) accumulate in higher concentrations in response to water stress, which leads to maintenance of turgor potential (Morgan, 1984; Mayer, 2006). The fact that different forms of stress generally affect the content of various plant secondary metabolites including polyphenols is generally accepted, but variation in tea polyphenolics over different seasons has yet to be quantitatively evaluated. Thus, efforts aimed at understanding the various biochemical responses, which are usually associated with osmotic adjustment, could be of great significance in evaluating water stress tolerance mechanisms and to select water stress tolerant cultivars.

1.1.3 Criteria for selection of tea cultivars

One of the major factors which have hindered rapid cultivar improvement of tea is lack of proper criterion for clonal selection (Magambo, 1983). Traditionally, selection against drought resistance has been centered on plant morphological features such as rooting depth and leaf features (Nainanayake, 2004; Malec, 2006). Physiological parameters such as transpiration rate (proportional to leaf water potential), photosynthetic rate and stomatal conductance are generally used as indices for screening tea clones for drought sensitivity (Kramer, 1983; Bota *et al.*, 2004). However, owing to the long cycle of tea breeding that approach seems not desirable. Recently, some polyphenolic compounds (Cheruiyot *et al.*, 2008), have been reported to be useful indicators for drought tolerance and can be incorporated into drought screening procedure in tea selection thus, shortening the time required to identify clones with desired traits.

Reduced crop yield has become a common phenomenon in the wake of changing trends in global climates with prolonged and severe periods of water stress witnessed in tea growing areas. Indeed, it has become imperative to lay emphasis on the screening process in order to identify adaptable clones that will ensure adequate and sustainable productivity in the tea industry in spite of the challenges posed by changes in world climate.

The postulated positive correlation between catechin levels and drought tolerance in tea will be investigated in order to ascertain whether this could be used as a criterion for clonal selection.

1.2 Hypotheses

i. The catechin levels in different clones of tea do not vary significantly with seasons.

ii. The level of catechins in tea leaves do not vary with the plant water status.

iii. There is no correlation between the soil water content and the levels of catechins in tea leaves.

1.3 Objectives of the study

1.3.1 Broad objective

The objective of the research project was to determine the effects of soil water deficit on the catechin levels of mature, field-grown tea clones at the Tea Research Station of Kenya (TRFK).

1.3.2 Specific objectives

The specific objectives of the research project were;

i. To determine the levels of catechins in various tea clones in different seasons.

ii. To determine the effect of soil water content on catechin levels in various tea clones in different seasons.

iii. To determine the effect of leaf water potential on catechin levels in tea.

1.4 Statement of the problem

Lack of proper selection criteria for drought tolerant genotypes has hampered rapid cultivar improvement of tea. Traditionally, selection against drought resistance has been centered on plant morphological features such as rooting depth and leaf features and physiological parameters such as transpiration rate, photosynthetic rate and stomatal conductance. However, such approach seems not desirable because the selection cycle takes a relatively long time (upto 10 years) to ascertain the drought tolerance ability of the tea clone. Therefore, there is a need to establish a selection criterion that can shorten the selection cycle to a few months or years.

1.5 Justification of the study

Most criteria hitherto applied in the selection of seedlings in nurseries or mature tea in the field, have not been quite reliable in selecting drought tolerant cultivars of tea. Therefore, alternative criteria ought to be tried for screening against the effects of soil water deficit. Usually, the methods recommended by tea breeders for clonal selection included an initial phase in which seedlings are chosen on the basis of various attributes of vigor. Thus, selection of tea clones has largely been based on morphological and physiological characteristics. However, biochemical response of tea to effects of drought has received little attention. Thus, the possibility of identifying drought resistant cultivars early in the selection process using biochemical traits could be of great benefit to the tea selection and improvement programmes.

Results from a study conducted in a ‘rain-out shelter’ by Tea Research Foundation of Kenya, showed that content of polyphenols in tea leaves decline with the intensity and duration of soil moisture deficit (Cheruiyot *et al.*, 2008; TBK, 2011). This probably indicates an association between biochemical reaction in tea and water stress. The researchers recommended a further research on this area before a conclusive decision is reached on the reliability of polyphenols as a criterion for screening clones for drought tolerance.

1.6 Anticipated output

i. A new approach of selection of clones based on catechin level that is more efficient, faster, reliable and cost effective compared to the conventional procedures.

ii. The identification of a biochemical trait that can assist in the screening of tea seedlings for drought tolerance.

iii. Reference for further research following submission and possible publication in peer reviewed journal.

CHAPTER TWO

LITERATURE REVIEW

2.1 The Tea plant

Tea is a perennial evergreen plant classified in Theaceae family. In nature, the tea tree can attain a height of 20-30 m (Sanaravan, *et al*., 2005; Bonheure, 1990). However, the plant is kept as a shrub of between 0.8-1.2 m high by pruning. The major tea-growing regions of the world are South-East Asia (China, India and Sri-Lanka) and East Africa where it is grown across a wide range of altitudes ranging from 1100 m to 2200 m.

Commercially grown teas are hybrids of two main types, the Assam-type with relatively large leaves and China-type with small semi-erect leaves (Carr and Stephens, 1992). The China-type (*Camellia sinensis* var. *sinensis*) is the most predominant type (Van der Vossen and Wessel, 2000). Following extensive selection and hybridization, most commercial tea cultivars display vegetative characteristics intermediate between these two main types, with Banerjee (1992) identifying nine different morphologically-different hybrids. Var. *sinensis* originated under open conditions in the cool, humid tropics and therefore suitable for growing in marginal areas of subtropics because it is more resistant to drought than the Assam-type (*Camellia sinensis* var. *assamica*) which is a tropical variety sensitive to dry and cold weather conditions (Carr and Stephens, 1992).

Because of the distinct difference in the ecology of their origins, the two main varieties and their hybrids exhibit considerable variation in their adaptability. For example, var. *sinensis* is known to be a hardier ecotype than "*assamica"* tea being resistant to both cold and hot drought conditions (Mondal *et al*., 2004). This variation in their ecophysiology can be used to develop cultivars specifically suited to different climates.

2.2 Tea industry in Kenya

The tea industry plays a significant role in the economy of Kenya where it is the second leading foreign exchange earner amongst agricultural commodities after horticultural crops (TBK, 2004). It contributes approximately 25.8 % of the total export earnings, which is equivalent to 4 % of the Gross Domestic Product (GDP) of Kenya (TBK, 2004). In 2007, export from the country topped 360 million kilograms of made tea, which earned the country over 43 billion Kenya Shillings in foreign exchange (TBK 2008). The tea sub-sector also provides a source of livelihood to over 3 million people besides providing support to other sectors of the economy such as infrastructure development (TBK, 2004).

Since the introduction of tea in Kenya in early 20th century, the acreage under cultivation has steadily risen, with the land area under the crop totaling 147,080 hectares by 2007 (TBK, 2008). Tea growing areas in Kenya are divided into two regions defined by the Great Rift Valley, which is a natural geographical feature that divides the country almost asymmetrically into two major blocks. The East of Rift block comprises Mt. Kenya region, Nyambene hills, and the Aberdare highlands whereas the escarpment around Kericho, Mau highlands, Nandi Hills and Kisii highlands form the West Rift block (fig. 1).

Over the years tea production has increased through the development and release of clonal teas with high yield and better beverage quality attributes. However, emerging reports indicate that this growth in the sector may not be sustained probably due to the abiotic stress factors (Kamunya, 2010; Cheserek, 2011). The changing global climatic

trends accompanied by unprecedented weather pose a great challenge to this development. Prolonged drought periods are quite common nowadays, for instance in 1997, 2000, 2003, and most recently between mid-December, 2011 and late March, 2012 when tea yield losses of over 60 % were recorded (TBK, 2004, 2008; TRFK, 2012). It is currently held that integrating both biotechnological methods and conventional breeding approaches could result in improved cultivars tolerant to important abiotic stress factors (Kamunya, 2010). More importantly, the integration of efficient and effective selection techniques linked to important biochemical traits hold key to the development of drought tolerant clones that will ensure sustainable production.

2.3 Effects of soil moisture stress on tea

Water stress is a major environmental constraint affecting growth and production of tea. Its damage to tea plants has become a frequent phenomenon (Othieno *et al*., 1992; Cheserek, 2011). Under prolonged dry weather conditions the growth of tea plants is adversely affected by water stress (Wijeratne, 2006; Taiz and Zeiger, 2006). This is created by lack of soil moisture, high air temperature and high saturation vapor pressure deficit in the air (Wijeratne, 1996; Raj Kumar *et al*., 2010).

Reduction of shoot extension, and leaf area expansion of woody plants including tea, has been reported to be associated with soil water deficits (Fordham, 1969; Carr, 2000; Cramer *et al*., 2007; Burt, 2007). Stephens and Carr (1993) showed that the length of a pluckable shoot with three leaves and a bud during the dry season of Tanzania was reduced from 130 mm in fully-irrigated and well-fertilized tea to 15 mm in un-irrigated

tea. Due to slow growth of shoots under soil water stress tea yields are greatly reduced (Carr, 2000; Carr and Stephens, 1992). This slow growth under environmental stress is a result of low shoot water potential that affects cellular turgor (Squire, 1977; Carr and Stephens, 1992). In many tea-growing regions, seasonal fluctuation in tea yield has been related to the soil moisture level (Carr, 2000). Water stress delays or stops bud dormancy break leading to accumulation of dormant buds in the tea bush. Soil moisture deficits not only limit tea production but may also lead to plant death (Carr and Stephens, 1992; Wijeratne, 2006; Cramer *et al*., 2007). Thus, it is a severe environmental constraint to plant productivity. Drought-induced loss in crop yield probably exceeds losses from all other causes (Ng'etich, 1997), since both the severity and duration of the stress are critical.

Drought tolerant clones are better able to survive drought episodes through a more conservative use of water. They may be of greater value than clones selected for higher quality particularly under low-input conditions typical of many farming systems of drought-prone regions (Karunarathne *et al*., 2004). Internal water deficit is initiated when low water potential develops and cell turgor begins to fall below its maximum value (Kozlowski and Pallardy, 2007). Maintenance of higher internal water status at low soil moisture is an important survival mechanism in many plant species (Reynolds *et al*., 2007; Warren, 2007).

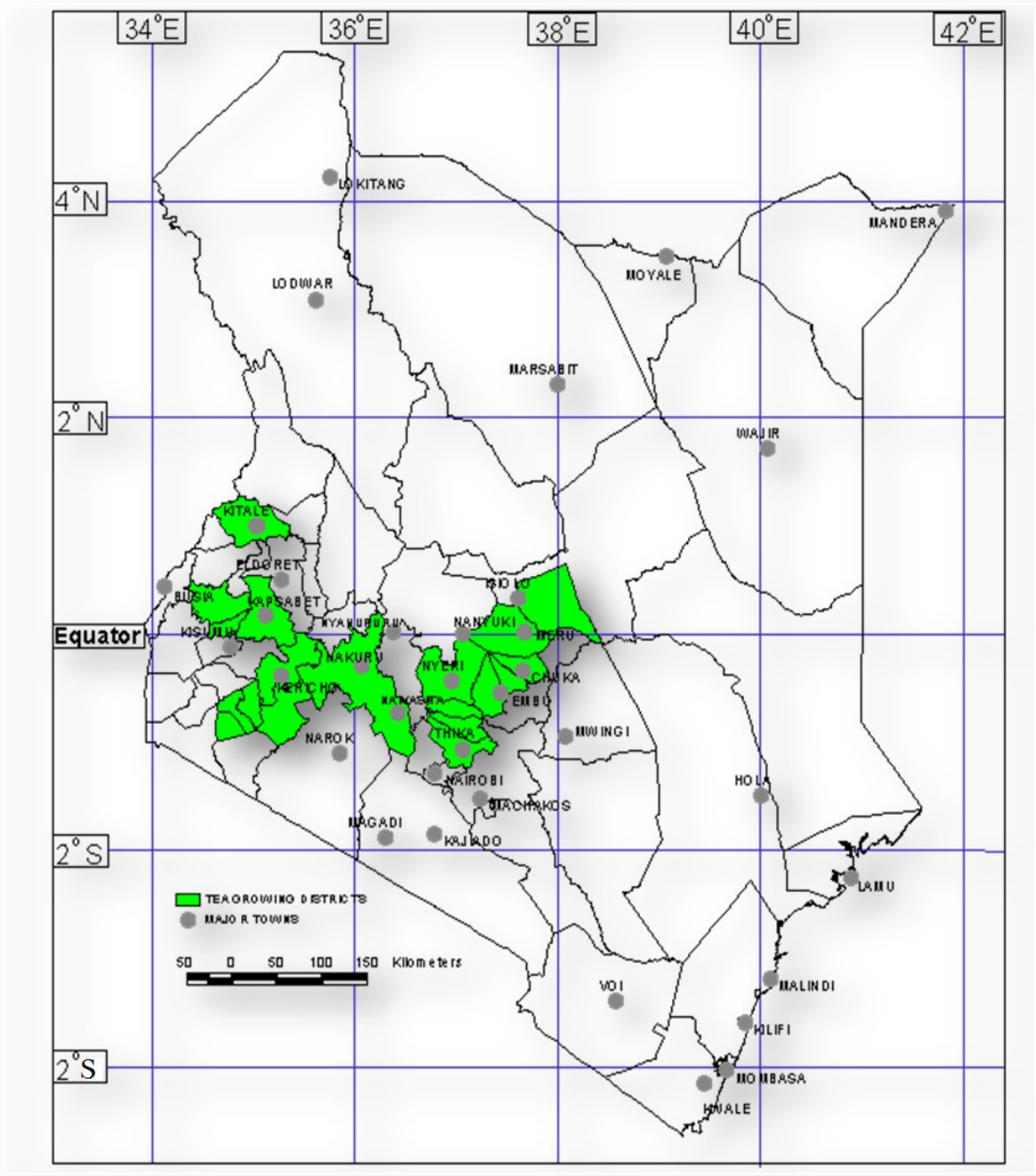

Figure 2.1: Tea growing regions of Kenya. (TRFK, 2012)

Tea cultivars more tolerant to drought generally differ morphologically and/or physiologically, with mechanisms allowing greater production under limited water supply. These mechanisms involve maximization of water uptake by deep, dense root systems and/or minimization of water loss by stomatal closure and reduction of leaf area (Warren, 2007; Cramer *et al*., 2007). DaMatta *et al*. (2003) found that the better crop yield of a drought-tolerant clone, compared with a drought-sensitive one, was associated with maintenance of leaf area and higher tissue water potentials, as a consequence of smaller stomatal conductance, which would result in less carbon isotope discrimination.

Drought tolerant cultivars regulate the stomatal function by having some carbon fixation during moisture stress conditions thus improving water use efficiency (Damayanthi *et al.*, 2010). The maintenance of higher water status with higher stomatal conductance observed in many plants usually occurs together with their deep rooting ability which allows extraction of water from deep soil layers. Some trees maintained a constant evapotranspiration rate even during dry periods due to deep rooting (Williams *et al*., 2001; Reynolds *et al*., 2007). According to Wijeratne (2006), some drought tolerant tea cultivars exhibit deeper roots.

Mature tea bushes with well developed root systems withstand drought better than young tea plantations (Hare *et al*., 1998). However, under critical water deficit, tea bushes are prone to drought effects. Although, irrigation is a viable solution during dry periods, there

are practical limitations such as lack of water resources and high cost. Therefore, the use of drought tolerant tea clones with higher water use efficiency in drought prone regions is of utmost importance. Maintenance of high leaf water potential during drought helps to continue appreciable rates of photosynthesis and other metabolic processes, and consequently high instantaneous water use efficiency (Handique and Manivel, 1986). With the progression of drought, generally there is an increase in total soluble secondary metabolites in leaves. The degree of the increase varies in different tea cultivars. Decrease of polyphenol content with desiccation is a positive character in drought tolerance of tea (Rajagopal *et al*., 2004). Osmotic adjustments delay stomatal closure during drought. High rates of gas exchange during drought have been reported in plants that adjust osmotically (Abrams *et al*., 1990; Damayanthi *et al.*, 2010). Certain plants maintained a comparatively higher water status and higher stomatal conductance, and also high total soluble sugar contents, indicating their ability to withstand drought by osmotic adjustments and probably by extracting water with their deep root systems (Barman *et al*., 1993; Tezara *et al*., 2002; Damayanthi *et al.*, 2010).

Reduction of leaf water potential leads to a reduction of stomatal conductance which progressively inhibits photosynthesis (Tezara *et al*., 2002). Decrease of photosynthesis due to water stress is a consequence of both stomatal closure causing increased constraints on carbon dioxide diffusion and decreased chloroplast activity (Ogren and Oquist, 1985). With the depletion of leaf water potential, stomatal conductance and transpiration rate also decline and the degree of reduction varies among plants (Barman *et al*., 1993). The percentage reduction of transpiration rate during the moisture stress

period is highest in drought susceptible cultivars. With the progression of the drought, resistant tea cultivars maintained higher transpiration rates compared to drought susceptible cultivars (Damayanthi *et al.*, 2010).

2.4 Response of tea to water deficits

Water stress negatively affects many plant processes, such as photosynthesis, transpiration, and metabolite accumulation (Bota *et al.*, 2004; Sivilotti *et al*., 2005; Ohashi *et al*., 2006), and causes substantial reductions in plant productivity (Reddy *et al*., 2010). Plant response to water stress includes morphological and biochemical changes, resulting in acclimation in non-severe cases, and damage and loss of plant parts, in severe cases (Chaves *et al.*, 2002). In response to stress factors plants are known to accumulate organic osmolytes, such as proline, glycine betaine, non-reducing sugars and polyols (Sabry *et al*., 1995; Hare *et al*., 1998). The role of these organic compounds is not clearly defined, but it is generally accepted that they contribute to ameliorating stress in the plant (Slama *et al*., 2006; Upadhyaya and Panda, 2013). Most of the stress-related organic compounds are secondary plant metabolites. Tea plants contain large amounts of polyphenols particularly of flavonol class. The anti-oxidant properties of total polyphenols are attributed to the chelating properties (Winkel 2001). Most of the total polyphenols chelate iron (Fe^{2+}) has good scavenging capacity (Peterson, *et al*., 2004). For good scavenging activity, a catechol moiety on ring B is required for the completion of oxidation process. The chelating could thus raise the scavenging activity to the level of the most active scavengers, possibly by site-specific scavenging. Therefore anti-

oxidative capacity of flavonoids increased as their Fe^{2+} chelating activities increased (Khan and Mukhtar, 2007)).

The osmotic adjustment in response to soil water deficit enables a given genotype of tea to tolerate longer periods of water stress during a drought. Wijeratne (1994) and Karunarathne *et al*. (2004) have shown that a relatively drought resistant tea genotype had a greater capability for osmotic adjustment than a relatively drought susceptible genotype. Water stress triggers varying responses in cellular metabolic activities of tea, which may be reflected in growth rate and yield of the crop, depending on the clone (Duan, 1992; Wijeratne, 1998; Subair, 2010).

At cellular levels, water stress affects the enzyme activities as well as the structural integrity, as water is essential for enzyme function. Such variations observed among different tea cultivars are due to an array of morphological, or physiologica1/biochemical adaptations (Tardieu, 2005; Lawlor and Tezara, 2008). One of the adaptations of the water stress tolerant cultivars of plants is the dominance of a defense system of anti-oxidant enzymes to protect the cell membrane against lipid peroxidation, the most damaging cellular response observed in response to water stress. Some of the observed effects of water stress may be the result of a stress-induced impairment of the biosynthetic machinery required for photosynthetic assimilation of carbon and/or its conversion to metabolically useable forms.

In other cases, stress-induced changes may reflect adaptations for stress tolerance. Osmotic adjustment is an important mechanism of a plant's tolerance to a drought environment (Ludlow *et al*., 1990; Upadhyaya and Panda, 2013). Wijeratne *et al*. (1998) showed that the drought-tolerant genotypes had a lower osmotic potential at full turgor and higher apoplastic water content than the drought-susceptible genotype. The lower osmotic potential allows the drought-tolerant genotype to absorb water from drier soils because of the greater water potential gradient between soil and plant. This was confirmed by the lower soil water content and soil water potential at permanent wilting point of the drought-tolerant genotype. Furthermore, the higher apoplastic water content also allows a plant to better tolerate drought by transferring apoplastic water to the cytoplasm during periods of water deficits.

2.5 Water relations

The water status of a plant is determined by the balance between water absorption by its root system and water loss through transpiration (Liyanapatabendi *et al*., 2007). Thus, the plant growth is controlled directly by plant water status and only indirectly by atmospheric and soil water deficits (Kramer, 1983). Injurious plant water stress however, results from low leaf water potential which develops over long periods of time because of decreasing soil water supply. Cramer *et al*., (2007) reported that plant water status could be reliably indexed from the leaf water potential. The leaf water potential varies inversely with diurnal trends of atmospheric vapour pressure deficits and soil moisture content.

Whenever the water potential within the plant cells is less than that of the surrounding soil water, movement of water from the soil into the roots will occur. However, under conditions of marked internal water stress, the tension generated in the xylem vessels will be propagated across the whole plant to the peripheral root cells hence, water potentials of greater negativity could develop in peripheral root cells than would otherwise be possible (Huang *et al*., 2000; Davies and Zhang, 2007). During the gradual development of a drought, Stephens and Carr (1991) observed that clonal tea develops water stress earlier than seedling tea. This was partly attributed to the variation in their root systems. Earlier development of water stress was indicated by the faster decline of shoot xylem water potential and earlier stomatal closure. However, the results of Carr (1977) suggested that seedlings were more susceptible to extreme dry conditions (a potential soil water deficit of 300 mm) than clonal tea, which had deep root systems.

In a study to investigate osmotic adjustments and associated water relation in clonal tea that was carried out in different tea growing agro climatic regions of Sri Lanka, the seedlings had a significantly deeper root system than clones. However, in a similar study carried out using mature, field-grown comparison trial of different seedling progenies and clones the overall mean root depth was higher in clones than in seedlings (Liyanapatabendi *et al*., 2007). Therefore, depth of the root system probably has a greater influence in determining the drought tolerance of a given genotype than its method of propagation (whether seedling or clonal). Knowledge of water relations in crops may be useful, in using anti-transpirants, in designing more effective irrigation and in breeding more drought tolerant varieties (Squire, 1978; Pinheiro, 2004)

2.5.1 Environmental stress physiology of tea

The most important agro – meteorological factors thought to affect tea production in Kenya are; soil moisture, atmospheric vapour pressure deficits, radiation and temperature Wijeratne *et al*. (2007). All these tend to change mainly with changes in rainfall. However, the lack of knowledge of the mechanism by which these factors influence tea production has severely limited the development of methods to control these variables (Tanton, 1979). Therefore, there is need to understand how they cause fluctuations in clonal tea productivity in order to institute more efficient management practices and reduce the yield fluctuations.

2.5.2 Temperature

Generally, tea grows well within air temperature range of about 18-25 ºC. Air temperatures below 13 ºC and above 30 ºC have been found to reduce shoot growth (Carr, 1972; Watson, 1986; Mohotti and Lawlor, 2008). The rate of shoot initiation in tea increases linearly with rise in temperature from the base (threshold) temperature to an optimum temperature and thereafter decreases linearly with further increases in temperature up to the maximum or ceiling temperature (Squire, 1990; Roberts *et al*., 1997).

The base (threshold) temperature for tea shoot extension has been found to vary from 7 ºC (Obaga *et al*., 1988) to about 15 ºC with 12.5 ºC being the average (Lawlor and

Tezara, 2008). Stephens and Carr (1993) showed that base temperature for shoot extension (7 ºC) of tea growing with adequate water and nutrients was 2-3 ºC higher than that for shoot development. In contrast, Squire *et al*. (1993) observed similar base temperature for both these processes in tea growing in the Kenyan highlands. Moreover, Stephens and Carr (1990) observed 12.5 º C base temperatures for shoot extension during a warming phase than during a cooling phase.

Although, optimum temperature for shoot growth of tea has not been clearly defined, Lawlor and Tezara (2008) reported the optimum temperature to be in the range between 18-30 ºC and ceiling temperature to be in the range 35-40 ºC. However, Tanton (1992) implied that the upper temperature limit for shoot growth could be as high as 36 ºC in the absence of other stress factors limiting shoot growth. Wijeratne and Fordham (1996) reported that shoot extension rate and weight per shoot decreased when air temperature rose above 26 ºC. Shoot population density also decreased linearly above air temperature range of 25-29 ºC (Wijeratne, 1994). In addition to air temperature, soil temperature also influences the growth of the tea plant (Carr and Stephens, 1992), especially in situations where growth of tea is limited by low soil temperature (Carr, 1970; 1972).

Magambo and Othieno (1983) reported that high soil temperature during the day time combined with low soil temperature during the night induced early flowering of tea and reduced its vegetative growth. Othieno (1982) showed that the diurnal variation of soil temperature in a young tea field with incomplete canopy cover differed under different types of mulches depending on their heat absorptivity. However, these variations disappeared when the canopy cover of tea increased above 60%. Tanton (1992) also

reported a close relationship between soil temperature and yields of young tea. The mulches that allowed higher soil temperature levels showed higher tea yields (Othieno, 1982).

2.5.3 Saturated atmospheric vapour pressure deficits

Tea is one of the plant species which has been shown to be highly sensitive to atmospheric vapour pressure deficit of the growing environment (Odhiambo *et al*., 1993). Tea plants benefit from high atmospheric humidity (Handique and Manivel, 1986; De Costa *et al*., 2007). In China (Huang, 1989) reported that high atmospheric humidity was favourable for the tea plants during the growth period. During the dry periods of many tea growing regions of the world, vapour pressure deficit could rise to levels which would decrease stomatal conductance, shoot water potential and the rates of shoot initiation and extension (Squire and Callander, 1981). Squire (1979) reported that the hot and dry seasons in the tea growing regions were characterized by low yields. Even when the tea was irrigated; the yields never rose to the levels obtained in the hot and wet season because yield was restricted by high saturated vapour pressure deficits. When the saturation vapour pressure deficits increased above 23 MPa the yield of tea progressively decreased (Tanton, 1982). Othieno (1978) and Ng'etich (1995) reported that growth rates and yields of tea are often reduced in the dry periods in East Africa when the potential soil moisture deficit reached 400 mm and the saturated vapour pressure deficits were upto 25 MPa.

Vapour pressure deficit influences these key processes of yield formation of tea even during periods when the soil is wet. Anandacoomaraswamy *et al*. (2000) showed that high humidity reduced air temperature around the tea bush, improved the plant water balance and favourably affected many physiological processes resulting in high production. Furthermore, studies have shown that the linear relationship between shoot extension rate and temperature breaks down at higher vapour pressure deficit (Squire, 1979; Squire and Callander, 1981). During wet periods with frequent rain, tea leaf water potential has an inverse, linear relationship with vapour pressure deficit (Williams, 1971; Squire, 1976, 1979). This probably operates through the influence of vapour pressure deficit on transpiration, which increases with increasing vapour pressure deficit causing a decrease in leaf water potential.

2.5.4 Soil water availability

Drought episodes are remarkably aggravated by both high solar radiation and temperature, so drought should be accounted for as a multidimensional stress (DaMatta, 2003). As discussed by Mullet and Whitsitt (1997), one approach to improve crop performance in water-limited environments is to select for genotypes that have improved performance in these environments. This approach has proved partially successful, but difficult to accomplish due to the variability of rainfall and the polygenic nature of drought tolerance. A complementary approach to improve plant performance for drought-prone regions involves the identification and selection of traits that contribute to drought tolerance or water use efficiency.

2.5.5 Evapotranspiration

Evapotranspiration is almost equal to transpiration for a well-maintained tea canopy that covers the ground almost completely allowing very little solar radiation to penetrate down to the soil surface (Medrano *et al*., 1998; Pinheiro *et al*., 2004). A study by Anandacoomaraswamy *et al*. (2000) showed that both hourly and daily transpiration rates were highly sensitive to soil water availability. When daily transpiration rate was maintained at a maximum of 1.6 litres per plant per day the soil water content decreased from field capacity 44 % (same as field capacity) down to 33 %. Within this range of soil water content, maximum hourly transpiration rates of 0.53-0.93 litres/ plant per hour were maintained during the period between 10.00 am and 2.00 pm. When the soil water content decreased below 33 %, daily and hourly transpiration rates declined rapidly down to 0.71 litres/plant per day and 0.27-0.53 litres/plant per hour respectively, near permanent wilting point (15 %). In this instance, the reduction of transpiration rate with decreasing soil water content was probably caused by gradual stomatal closure and consequent reduction of stomatal conductance.

2.6 Effect of soil moisture stress on tea productivity

Most of the tea growing regions of the world experiences soil water deficit of varying magnitudes and durations. As the soils get drier, the availability of water at the soil-root interface decreases resulting in the reduction in the absorption ability of soil water by the plants (Fitter and Hay, 1981; Upadhyaya and Panda, 2013).

Water stress resulting from reduced water availability reduces crop growth and productivity (Hsiao, 1973). Due to inadequate and poorly distributed rainfall in most tea growing regions in Kenya, tea plants are subjected to dry periods varying in severity and duration, as a result of which there is severe yield reduction and poor crop distribution throughout the year (Othieno, 1978). The length of time during which the crop stays under moisture stress and the total magnitude of the stress are important factors which affect crop growth and yields (Stephens and Carr, 1989). Carr (1971) reported in Tanzania that soil water deficits in excess of 100 mm over a whole dry period lasting about five months significantly reduced yields.

The change which arises in the potential soil moisture deficits with time demonstrate how differences in water stress develops over time under different regimes of soil moisture (Stephens and Carr, 1989). Othieno (1978) reported that there was variation in clonal response to water stress in tea during the dry season. The clones which were more susceptible to water stress showed a greater demand for water than those which showed less susceptibility. The physiological processes which are important in tea adaptations and tolerance to drought however, are not well understood. This has caused difficulties in the selection of more tolerant clones. The effects of water deficits on tea yield can be predicted by examining the effects on the principal yield components viz. shoot density and shoot extension growth. Wijeratne *et al*. (2007) observed that the rate of shoot production, which primarily determines shoot density, decreased when the average midday shoot water potential fell below -0.6 to -0.7 MPa. Squire and Callander (1981)

observed this limiting shoot water potential to be -0.8 MPa. Shoot water potential could fall below the limiting value due to an increase of soil water deficit during prolonged rainless periods. However, the limiting shoot water potential could be reached even when tea is growing on a wet soil, if the vapour pressure deficit increases beyond a threshold (Williams, 1971). Wijeratne and Fordham (1996) found that shoot water potential of tea decreased linearly with soil water deficit rising above 30-40 mm at low altitudes in Sri Lanka.

2.7 Plant water potential

Different physiological processes vary in their sensitivity to reduced plant water potential caused by water deficits. Cell expansion can be inhibited at plant water potential of approximately –0.4 to –0.8 MPa (Plaut and Meiri, 1994), while photosynthetic CO_2 fixation is much less sensitive to changes in plant water potential and can be reduced at plant water potential of –1 to –2 MPa (Hsiao and Acevedo, 1974). In tea, midday plant water potential is stabilized by effective stomatal control of leaf water status under a wide range of evaporative demands (Wijeratne, 2004). For plants that have been moderately water stressed, midday plant water potential can be higher than that of well-watered plants due to stomatal closure (Levy, 1980).

Water potential of tea declined from –2.6 to –2.8 MPa under water stress where soil water content reached minimum of 7.6 % compared to 15.5 % for well watered soils (Othieno, 1982). Predawn plant water potential differences were more significant among water deficit treatments than midday plant water potential in peach trees (Girona *et al*., 2009).

With decreasing soil water content, water uptake in trees during the afternoon and night becomes increasingly less resulting in low pre-dawn plant water potential (Braun *et al*., 1989). Maintaining positive plant water potential is essential for the continuation of many processes in the plant such as cell expansion, stomatal movement, and enzymatic reactions (Nagarajah, 1989). Osmotic adjustment may occur in different parts of the plant and varies depending on the level of stress, and variety, season and growth stage.

2.8 Flavonoids functions in plants

Flavonoids, including catechins and anthocyanins are a major class of phenolic secondary metabolites that bestow a multitude of adaptive benefits to the plant that produces them (Edreva, *et al*., 2008; Noori *et al*., 2010). They play a major role in the adaptation of plants to the changing environment and in overcoming stress constraints. However, over the years, more attention has been focused on the contribution of the flavonoids to human health than their natural role in plant survival and productivity.

There are several known flavonoids of diverse physiological functions in plants (Harborne, 1976; Koes *et al*., 1994; Dai and Mumper, 2010). Flavonoids are suggested to have many functions like flower, fruit, and seed pigmentation, protection against UV light, defence against pathogenic micro-organisms, role in plant fertility and germination of pollen and acting as signal molecules in plant-microbe interactions (Olsen *et al*., 2007). They are beneficial to the plant itself as physiologically active compounds, stress protecting agents, attractants or feeding deterrents (Treutter, 2006; Gill and Tuteja, 2010). Coberly and Rausher (2003) found that flavonoids function in the plant stress response.

Also they are reported to be induced by high light, cold stress, drought stress and heavy metals (Warren and Mackenzie, 2001). Many flavonoid biosynthetic genes are induced under stress conditions. It has been found that there is considerable increase in flavonoid levels following biotic and abiotic stresses, such as, drought, metal toxicity and nutrient deprivation (Winkel, 2001).

Changes in levels of flavonoids in plants under a variety of abiotic stress conditions have been reported, though there are only a few instances of unambiguous evidence of their role in abiotic stress tolerance. Caldwell and Flint (1994) reported that through their accumulation in the upper epidermal cells, flavonoids effectively protect the sensitive inner cells from the damaging effects of ultraviolet light by absorbing the radiation and this epidermal absorption presumably serves to protect organelles such as chloroplast.

Low temperatures have been reported to have a significant impact on the synthesis of anthocyanins in plants (Malec, 2006; Khan and Mukhtar, 2007). The levels of anthocyanins show a dramatic increase during cold acclimation (Christie *et al*., 1994). Low temperatures have also been shown to enhance the anthocyanins levels in sorghum (Seigler, 1998; Wink, 2010) Cold stress and light seem to act in a synergistic fashion leading to the accumulation of anthocyanins pathway genes. Generally, this observation shed light on the impact of environmental stress factors on flavonoids biosynthesis.

2.8.1 Response of total polyphenol content in tea to seasonal variations

Extraordinarily large amounts of these polyphenolic substances are found in leaves of tea plants (Friedman *et al*., 2006; Zaveri, 2006; Cherotich *et al*., 2013). The most significant of these polyphenols are the flavonoid monomers: catechins (C) (flavan-3-ols) and catechin gallate (CG) which constitute up to 30% (w/w) of the dry weight of the tea material (Zaveri, 2006; Kim, *et al*., 2014).

Polyphenols are potential quality indicators and tea leaves are rich source of polyphenols, which are potent antioxidants in nature (Heim, *et al*., 2002; Zaveri, 2006; Khan and Mukhtar, 2007; Anesini and Filip, 2008; Kim, *et al*., 2014). Kottur *et al*., (2010) reported that polyphenols content in tea leaves are profoundly affected by season. For example, Assam tea produced best quality tea during winter followed by autumn tea. The summer tea was the worst in terms of quality possibly due to high temperature, low soil moisture levels, and high temperatures which apparently produced higher tannins and lower concentration of polyphenols (Kottur *et al*., 2010; Cherotich *et al*., 2013).

The polyphenol content of tea has also been reported to be influenced by seasonal, genetic, and agronomic factors, the highest levels produced when shoot growth rates are most rapid (Cloughley, 1982). Polyphenol decreases progressively with season in freshly plucked tea shoots early in the year. Early monsoon (increasing plucking period) favors the production of tea with low polyphenol content whilst monsoon (peak plucking period) favours the production of tea with high polyphenol content. Late monsoon favours the production of tea with low polyphenol contents (Wood *et al.*, 2006).

2.8.2 Biosynthesis of flavonoids

Tea has a unique ability to divert large amounts of carbon from aromatic amino acid metabolism to biosynthesis of flavonoids (Fig. 2.2) through the phenylpropanoid pathway (Magoma *et al*., 2000; Winkel, 2001). Phenylpropanoids are derived from cinnamic acid (Molla, 1981; Wood *et al*., 2006). The enzyme phenylalanine ammonialyase (PAL) catalyses the deamination of phenylalanine to cinnamic acid, which is further, modified by hydroxylase (Dixon *et al*., 2005). The enzyme 4-Coumaryl CoA ligase (4CL) catalyses the formation of CoA esters of cinnamic acid and these activated intermediates are used in the biosynthesis of diverse compounds via specific branch pathways, such as those leading to catechin biosynthesis (fig. 2) (Winkel, 2001).

Declining soil moisture content triggers the levels of stress related flavonoids biogenesis to decrease to some extent during stress conditions as a response mechanism to alleviate such conditions. The decline of catechin contents, leading to osmotic adjustment, plays a major role leading to drought tolerance (Cheruiyot *et al.*, 2008*).* Some of these compounds may be useful indicators of the extent to which plants can tolerate water stress and hence, aid in selection for drought tolerance in tea.

2.8.3 Factors affecting flavonoids biosynthesis

There are many factors that have been reported to affect flavonoids biosynthesis in plants, including light, temperature, soil type, water, nutritional status, and plant growth

regulators (Keller and Hrazdina 1998; Nainanayake, 2004; Lamina *et al*., 2006; Das and Mondal, 2012). Nutrient availability has a fundamental influence on plant growth (Russell, 1961; Marschner, 1995; Keller *et al*., 1998) and has been shown to affect the

Fig. 2.2: Biosynthesis of major groups of flavonoids

(Source: Seigler, 1998)

flavonoids composition of plant tissues. For instance, both low and excessively high levels of nitrogen fertilizer have been shown to decrease color in grape berries (Kliewer, 1977; Keller and Hrazdina 1998; Delgado, 2009), while high potassium has been reported to increase color in grapes (Morris *et al*., 1983; Jackson and Lombard 1993; Das and Mondal, 2012). The most likely mechanism for decreasing phenolic content at high nutrient levels is excessive vigor (very high activity related to growth). Vigor has also been reported to impact upon the tannin content and composition of grape skins (Dan and Lee, 2004; Jeong *et al*., 2004). Cortell *et al*. (2005) reported that in the berry skin, pro-anthocyanidins was higher in low-vigor vines, with an increase in the proportion of epigallocatechin subunits in pro-anthocyanidin polymers.

Physical characteristics can also affect flavonoids accumulation (Jackson and Lombard, 1993; McDonald *et al*., 1998). Soil characters like the parent material and the age of the soil that largely determine the micronutrient pool, structure, and texture of soil have a significant effect on plant growth (Russell, 1961; Northcote, 1992; Marshner, 1995). However, the major consequence of soil type is the capacity of the soil to hold water (Russell, 1961; Northcote, 1992). Reports suggest water deficit increases tannin and anthocyanin content in grapes (Nadal and Arola, 1995; Dry *et al*., 1998). In grape cell cultures, anthocyanin biosynthesis is extremely sensitive to osmotic stress (Do and

Cormier, 1991; Suzuki, 1995). Osmotic stress results in increased anthocyanin accumulation. Auxins and cytokinins, plant growth regulators that are used to manage plant production have been shown to increase anthocyanin biosynthesis in plants (Nakamura *et al*., 1980; Ozeki and Komamine, 1981; Deikman and Hammer, 1995). Despite the variability conferred by any or all of these parameters, the greatest influences on the flavonoid content of any cultivar are site and season (Bakker *et al*., 1986; Gonzalez-San Jose *et al*., 1990; McDonald *et al*., 1998; de Freitas and Glories, 1999; Guidoni *et al*., 2008). For a given site, it is assumed that characteristics such as soil will remain invariant, nutrition will be adequate, and agronomic practices will not vary greatly from year to year. Thus, the primary seasonal difference will be climatic, predominantly sunlight and temperature.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Site

The experiment was located in Field 12C (Appendix iii) at Timbilil estate, Tea Research Foundation of Kenya (0^0 22’ S; 35^0 21’ E), altitude; 2178 m above mean sea level. The topography of the area is steeply dissected with an average slope of 30 degrees (Carr, 1972; Callander and Woodhead, 1981).

3.1.1 Physicochemical characteristics of soil in the study area

The soils of the study area is a fine mixture of clay of kaolinite type (75-85%) and organic matter (30 %) (Othieno, 1973; Carr, 1974) hence, it has many properties in common with those of other tea growing areas of Kenya. It is highly weathered, leached and acidic, pH 4.5 soil conditions in which tea grows best (Othieno, 1973; Carr, 1974). The soil acidity is increased by extended application of nitrogenous fertilizers (Hoshina *et al*., 1983; Stephens and Carr, 1991). The soils are deep and well drained with crumby surface soil structure grading to a moderate aggregate structure in the sub-soil with many pore spaces (50 %) making it ideal for tea growth (Watson, 1986). The surface soil colour is dark brown grading to strong brown in the moist sub soil.

3.1.2 Climatic conditions of the study area

The study site is located in the highlands West of Rift valley with an annual average rainfall of 2000 mm. This area normally experiences seasonal dry spells from mid December to late March characterized by cool nights and hot days (Fig 3.1). Usually between the months of April and November the region receives an average of 2300 mm, well distributed rainfall (Fig 3.2). The experiment was carried out in an open field with mature tea over a period of three seasons *viz*.wet and cool, warm and wet, and dry and hot seasons.

Figure 3.1: The Temperature patterns at the experimental site, TRFK, over the period of trial (June, 2011 to March, 2012). Source: Annual Report of Tea Research Foundation of Kenya, (2011/2012).

Figure 3.2: The rainfall pattern at the experimental site, TRFK, over the period of trial (June, 2011 to March, 2012). Source: Annual Report of Tea Research Foundation of Kenya (2012)

The monthly mean temperatures, rainfall averages, saturation vapour pressure deficits, evapotranspiration rates are shown in Appendix v. Weather conditions in 2011 were generally favorable for growth of tea. However, in the first two calendar months of 2012 temperatures were quite high and there was not enough precipitation. During that period there were 28 days with temperatures over 27º C [data not shown]. From early December, 2011 to 25th February 2012, an average of 0.9 mm/month of rainfall was recorded in Timbilil estate, TRFK. Therefore, tea suffered severe drought stress over that season. On 27th, 28th and 29th February 2012, there were light showers that brought 7.4, 9.4 and 2.3 mm precipitation, respectively (Fig. 3.3).

3.1.3 Air temperature and the atmospheric vapour pressure deficit

The air temperature (Fig. 3.3) increased with the increase in vapour pressure deficit from 22 °C in the cool and rainy period (June-August) to 27 °C in the dry and hot season (January-March). During the same period catechin content dropped by an average of 45 % in susceptible clones and by12 % in drought tolerant clones

Figure 3.3: The relationship between air temperature and the atmospheric vapour pressure deficit in the field during the study period

3.1.4 Seasonal variation of atmospheric vapour pressure deficit

The vapour pressure deficit (VPD) of air (Fig. 3.4) averaged 5.7 bars in the wet and cold period, increasing to about 6.45 bars during warm and wet season and further increasing to 10.5 bars in the dry and hot season.

Figure 3.4: Seasonal variation of atmospheric vapour pressure deficit

3.2 Plant materials

For the purpose of this study, ten contrasting cultivars of tea in terms of drought tolerance and superior quality attributes were selected from the existing tea bushes (average age 28 years) (Table 3.1). The selected clones are true representative of clones grown across Kericho County. The ten cultivars were drought tolerant clones: SFS 150 and TRFK 303/577[control], and drought susceptible clones: TRFK 6/8, TRFK 301/4, TRFK 12/19, TRFK 31/11, BBK 35, S15/10, TRFK 7/9, and TRFK 31/8 [control].

Table 3.1: The drought response and quality attributes of the ten clones **(**TBK, 2004)

Clone	**Drought response**	**Black tea Quality**
TRFK 31/8	Fairly good	Medium
S15/10	Poor	Medium
TRFK 12/19	Fairly good	High
TRFK 7/9	Fairly good	High
BBK35	Fairly good	High
SFS 150	Tolerant	Medium
TRFK 303/577	Tolerant	Medium
TRFK 301/4	Moderately affected	Plain quality
TRFK 6/8	Fairly good	Very high
TRFK 31/11	Poor	High

3.3 Experimental design and treatments

The experiment was superimpossed on Field 12C with mature, fully grown tea bushes established in 1983 in what was a virgin forest land. The exprimental area is arranged in 50 randomized blocks each measuring 225.5 m^2 surrounded by single guard row. The effective plot area consisted of 100 bushes with spacing of 1.2 m between rows and 0.75

m between plants. For the purpose of the present study the effective plot area was subdivided to form three replicates each having 30 plants (Appendix iv). The measurements were carried out from the onset of rains in June, 2011 and continued until the end of the rains in November, 2011. The same was also done from January through March, 2012 when a prolonged dry spell was experienced. Soil moisture content (SMC) was measured in 30 representative sample areas that were selected randomly from each treatment. Changes in biochemical parameters over time were also studied during this period. The factorial arrangements of the experiment were as follows;

		C_7R_3
$C_1R_1P_{13}$	$C_4R_2P_{21}$	P_{26}
		C_8R_1
$C_1R_2P_{15}$	$C_4R_3P_{18}$	P_{19}
		C_8R_2
$C_1R_3P_{27}$	$C_5R_1P_2$	P_1
		C_8R_3
$C_2R_1P_4$	$C_5R_2P_9$	P_{28}
		C_9R_1
$C_2R_2P_{29}$	$C_5R_3P_{16}$	P_{30}
		C_9R_2
$C_2R_3P_{11}$	$C_6R_1P_{17}$	P_{23}
		C_9R_3
$C_3R_1P_{25}$	$C_6R_2P_{20}$	P_7
		$C_{10}R_1$
$C_3R_2P_{14}$	$C_6R_3P_{24}$	P_{12}
		$C_{10}R_2$
$C_3R_3P_3$	$C_7R_1P_6$	P_5
		$C_{10}R_3$
$C_4R_1P_8$	$C_7R_2P_{10}$	P_{22}

Where,
C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, and C_{10} are clones: TRFK 31/8, S15/10, TRFK 7/9, TRFK 6/8, TRFK 12/19, TRFK 31/11, TRFK 301/4, TRFK 303/577, BBK35 and SFS150 respectively.
R_1 – R_3 – Replicates
P_1- P_{30} – Plot numbers

3.4 Biometric measurements

For the biometric measurements, mature, fully grown and healthy leaves on the plucking table were selected. The physiological and biochemical parameters that were evaluated were; total catechin content and leaf water potential. Soil moisture content was also measured to study the responses of tea plants to drought effects. All the measurements were made between 9.00 a.m. and 2.00 pm at intervals of 1 hour.

3.5 Total catechin content (TCC)

3.5.1 Sample collection

Leaves weighing 300g (the terminal two leaves and bud) were randomly collected from each plot during the cold June to August and wet September to November seasons when the conditions are relatively warm and wet. The last sampling was done towards the end of the dry and hot period December-March when tea plants were experiencing severe water stress (Plate 3.1).

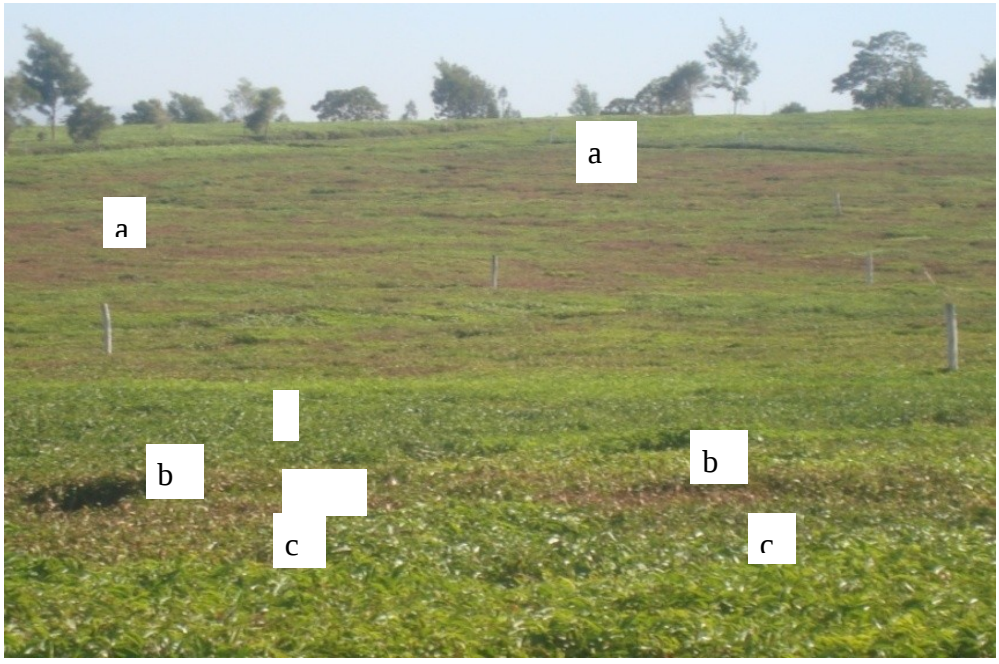

Plate 3.1: A tea field in TRFK's Timbilil estate, Kericho showing effects of drought stress (January – March, 2012). Further backgrounds (a) are bushes of TRFK6/8, TRFK 31/11, TRFK 31/8 and S15/10 all drought-susceptible; the browning leaves are due to severe effects of water stress. In the foreground (b) are clone SFS150 adjacent to TRFK 303/577 both drought tolerant, showing little effect of drought. The area labeled (c) is a guard row of drought susceptible clone between the adjacent blocks.

3.5.2 Preparation of the extracts

Sampled fresh green leaves from each plot were carefully steamed in a pressure cooker for one minute then placed in the withering bay and left to dry for two hours. The dried samples were processed using crush, tear and curl (CTC) then ground using a blender into fine powder, then sealed in paper bags (with aluminium foil lining) and safely stored in dark dry environment of 4 ºC awaiting the analysis.

3.5.3 Analysis of catechins

Estimation of catechins was determined according to the procedures of Folin Ciocalteu method (Piendl and Biendl, 2000). Fresh leaves (0.5 g) were homogenized in 5 ml of 70% methanol using a chilled pestle and mortar with subsequent centrifugation at 4000 revolutions per minute for 20 minutes. From the solution 10 ml was pipetted and mixed with Gallic acid standard solutions (0.1 ml) and 50 g anhydrous Gallic acid transferred into the reagent tube. Folin Ciocalteu phenol reagent was then added to each tube. Within five minutes from adding Folin Ciocalteu phenol reagent, 5.0 ml of sodium carbonate solution was added to stabilize the material and allowed to stand for 2 hours at room temperature for completion of the reaction. The amount of catechins in the test sample was calculated from a standard curve (Fig. 3.5) generated using Gallic acid and then expressed as the amount of Gallic acid equivalent. A best-fit linear calibration graph from the mass of anhydrous gallic acid standards was constructed against the Gallic acid

standard optical densities (table 3.2). The contents of catechins in the leaf were then expressed as percentages of the mass of sample dry matter.

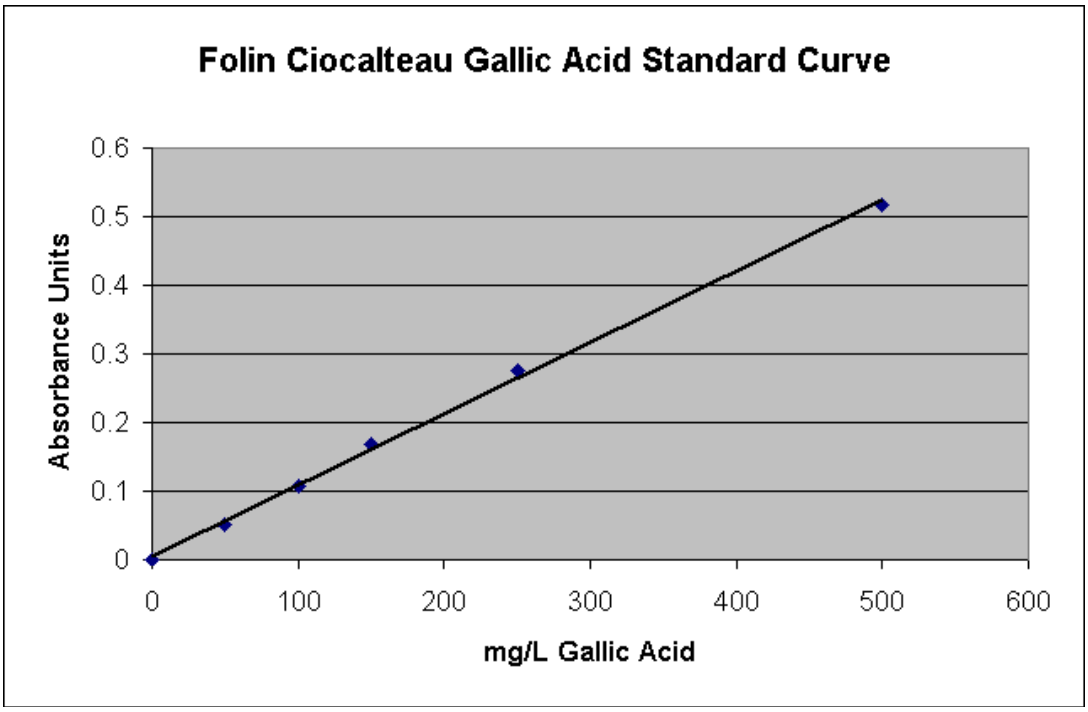

Fig. 3.5: Calibration curve of gallic acid

	Control (mg)	Added (mg)	Found ± SD (mg)	Recovery (%)	Precision RSD (%)	Robustness RSD (%)				
					Flow rate (ml/min)	AcOH conc. (%, v/v)	Column Temp. (°C)			
					0.75	0.85	2.95	3.05	29	31

Gallic acid	43.86	50	91.34 ± 2.26	97.31						
		150	195.59 ± 7.48	100.89	1.43	2.31	2.55	2.13	0.84	0.65
		250	292.86 ± 10.88	99.66						
		50	192.11 ± 5.57	100.81						

(RSD), Relative standard deviation, **SD,** Standard deviation, AcOH, acetic acid concentration

Table 3.2: Optical density (absorbance) readings of Gallic acid

3.6 Soil moisture content (SMC)

Soil moisture content of the root zone at 60 cm depth was measured along with physiological parameters of tea, leaf water potential and catechin levels. Soil was augured at 60 cm depth and the soil moisture content was determined using time-domain reflectometry (TDR) soil moisture meter (Trime FM-2, Eijkelkamp Agrisearch Equipment Giesbeek, and the Netherlands) (Plate 3.2)

3.7 Leaf water potential (LWP)

Leaf water potential was determined periodically in different seasons that is during wet and cold season (May-August), hot and wet season (October-December), 2011 and then again during the dry and hot period between January and March, 2012. Leaf water potential was measured between 10.00 am and 2.00 pm at an interval of 1 hour on the entire leaf by observing the presence of water on the cut surface of the leaf petiole using a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, California) (plate 3.3).

Two mature leaves (2nd and 3rd) and a bud were randomly selected from each plot for leaf water potential measurements. The leaf was enclosed in a reflective plastic bag for 1 hour to suppress transpiration and allow stem water potential to equilibrate with leaf water potential (Shackel *et al*., 1997). The leaf was cut (in slanting manner) and enclosed in the pressure chamber with the cut end protruding through a rubber stopper which is used to seal the chamber. The pressure in the chamber was gradually increased until the sap appeared at the end of the xylem vessels. After the pressure was recorded, the sap was released through an outlet valve and the sample removed (Turner, 1981; Phene *et al*., 1990).

3.8 Saturated vapour pressure deficit

The readings for atmospheric vapour pressure deficit were taken by the micro-logger situated at about 25 cm above the plucking table. The wet and dry bulb thermometers were connected to the thermocouple sensors which were connected to the logger on the other end. The readings were taken at 30 min intervals between 9.00 am and 2.00 pm.

Plate 3.2: Time-domain Reflectometry (TDR) soil moisture meter (Model E20/20N TDR)

Plate 3.3: Pressure chamber: Measuring the xylem water potential of tea shoots (Model 1515D)

3.9 Meteorological data

Meteorological data were recorded using instruments installed in TRFK weather station next to the experiment. All instruments were mounted at least 1m above the soil. Hourly data were recorded, from which minimum and maximum temperatures, cumulative total radiation and total rainfall were derived on a daily basis. The total solar irradiance during the period of study was estimated using Gunn Bellani Pyronometer placed at 20cm above the plucking table. The rate of evaporation was also monitored during the study period using Class 'A' pan.

3.10 Statistical analysis

Regression analysis was done using GenStat (GenStat 5 release 4.2), and correlation analysis was performed with SAS (Ver.8.1 e). Principal components analysis (PCA) was used draw out the effects and possible interactions of the soil moisture content and catechin levels. All one-way ANOVAs were accompanied by mean separation by Duncan Multiple Range Test (DMRT) using the SAS version 8.0 statistical packages (SAS Institute Inc: 1999).

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 RESULTS

4.1.1 Seasonal variation of Catechins

Regression analysis (Table 4.1) indicated the range in which catechin levels fluctuated in each clone when subjected to varying soil water content. Clones TRFK 31/8 and TRFK 6/8 had 3.58 % and 2.74% ranges respectively, which were low compared to clone TRFK 31/11 and SFS150 with a high of 10.45 % and 12.68 % respectively.

Table 4.1: Catechin estimates of fluctuation ranges (%). Obtained from Regression Analysis ($P \leq 0.05$)

Clone	Fluctuation range (%)	Lowest limit attained (%)
TRFK 31/8	3.58	12.51
TRFK 301/4	6.72	17.69

TRFK31/11	10.45	14.53
TRFK 303/577	6.19	12.36
TRFK 6/8	2.74	14.93
TRFK 7/9	5.07	15.88
BBK35	6.08	13.62
SFS150	12.68	19.70
S15/10	8.35	13.73
TRFK 12/19	5.49	12.36

Correlation studies (Table 4.2, 4.3 & 4.4) between soil moisture content and catechin content revealed that soil moisture content showed a significant correlation value ($P \leq 0.05$) with the catechin content. The study showed that the catechin concentration in tea leaves increased as water stress built up, this trend was consistently observed moving from the wet season to the dry seasons. The amount of moisture received by the tea plants affected the catechin content significantly. For instance, a low correlation between soil moisture content and catechins was reported during the cool and wet season, and warm and wet season as well, however, the correlation between the catechin content was relatively high during the dry and hot season (Table 4.2, 4.3 & 4.4). Therefore the total catechins positively correlated with soil moisture content ($P \leq 0.05$). With the initially high soil moisture content, catechin content did not vary significantly among clones during the period cool and wet and again during warm and wet seasons. However, with the depletion of soil moisture, the catechin content declined significantly. The catechin levels at the end of the dry and hot period differed significantly ($P \leq 0.05$) among the ten clones. The interaction among the soil moisture content and clone were also significant (Table 4.6). Catechin content in leaves of tea remained constant from June-November period (that

period was relatively wet, and the tea plants were not experiencing any stress) and gradually decreasing from December reaching the lowest levels towards the end of January-March season (dry and hot). Though, there was a general decreased in catechin content in all clones during dry and hot season, there was less fluctuation in drought tolerant clones; TRFK 303/577 and SFS 150 (Table 4.5).

Table 4.2: Correlation analysis between total catechins and soil moisture content during cool and wet season

Clone	**Cool and wet season**	**R^2 Value**
	Regression Equation	
TRFK31/8	y = -3.6357x + 59.213	R^2 = 0.4009
TRFK301/4	y = -9.4223x + 71.148	R^2 = 0.6601
TRFK311/11	y = -3.2983x + 59.451	R^2 = 0.4161
TRFK303/577	y = -8.02x + 66.007	R^2 = 0.5959
TRFK6/8	y = -1.532x + 55.085	R^2 = 0.3472
TRFK 7/9	y = -5.8183x + 64.877	R^2 = 0.63
BBK35	y = -4.9911x + 59.074	R^2 = 0.6188
SFS150	y = -8.9729x + 67.47	R^2 = 0.5044
S15/10	y = -12.425x + 77.202	R^2 = 0.5845
TRFK 12/19	y = -5.5714x + 63.07	R^2 = 0.6089

Table 4.3: Correlation analysis between total catechins and soil moisture content during warm and wet season

Clone	warm and wet season	R^2 Value
	Regression Equation	
TRFK31/8	y = -4.9731x + 66.739	R^2 = 0.7612
TRFK301/4	y = -4.64x + 60.16	R^2 = 0.4369
TRFK311/11	y = -0.6811x + 54.227	R^2 = 0.527
TRFK303/577	y = -7.208x + 69.868	R^2 = 0.525
TRFK6/8	y = -7.2554x + 67.374	R^2 = 0.7312
TRFK 7/9	y = -9.8131x + 71.726	R^2 = 0.7762
BBK35	y = -9.6654x + 73.157	R^2 = 0.6405
SFS150	y = -7.194x + 62.817	R^2 = 0.5762
S15/10	y = -6.2151x + 71.325	R^2 = 0.694
TRFK 12/19	y = -11.675x + 77.656	R^2 = 0.6803

Table 4.4: Correlation analysis between total catechins and soil moisture content during dry and hot season

Clone	Dry and hot season	R^2 Value
	Regression Equation	
TRFK31/8	y = -3.6477x + 41.739	R^2 = 0.6258
TRFK301/4	y = -5.6043x + 39.83	R^2 = 0.6978
TRFK311/11	y = -2.76x + 36.017	R^2 = 0.7132
TRFK303/577	y = -2.2223x + 33.591	R^2 = 0.8236
TRFK6/8	y = -1.5337x + 33.838	R^2 = 0.5052
TRFK 7/9	y = -3.1211x + 31.717	R^2 = 0.4086
BBK35	y = -3.4697x + 32.525	R^2 = 0.5385
SFS150	y = -5.3066x + 39.758	R^2 = 0.8169
S15/10	y = -5.3983x + 38.691	R^2 = 0.7618
TRFK 12/19	y = -3.3111x + 35.967	R^2 = 0.6879

The results of this study indicate that declining soil water content decreases catechin content (Table 4.5). The results also show that drought-tolerant clones have high catechin content with restricted fluctuation to changes in soil water content. Water stress lowered catechin levels content in tea leaves, and there were significant correlations ($P \leq 0.05$) between soil water content and catechin content. This was expected, because water is one of the raw materials in photosynthesis, and it directly impacts on organic synthesis for both growth and secondary metabolites. Of significance was the potential amount and extent of variation of catechin content in each clone, which might provide a basis for clonal selection, improvement of tea for better yield and quality.

Clones TRFK 303/577 and TRFK SFS 150 had significantly different catechin content than the other clones in the study (Fig. 4.1, 4.2, & 4.3). Similarly, the same clones had higher leaf water potential, indicating that they were more tolerant to water stress. Given the close correlation these results suggest an association of catechin contents with water

stress in tea. This observation agrees with results of Khan and Mukhtar (2007) who noted increased polyphenols in light and water-stress resistant safflower and cucumber seedlings as compared to those which responded weakly to the stresses.

Further investigation of flavonoid contents in safflower revealed that a strong antioxidant was responsive to both light and water stress while a weak antioxidant remained unchanged. Besides absolute amounts of catechins, results of this study show varied fluctuation of catechin content with changes in soil moisture content, and suggest that clones with more stable catechin contents are more tolerant to water stress (Table 4.5). This implies that tea cultivars which have less fluctuation in catechin content are less affected by changes in soil moisture content and may reflect tolerance to drought.

Table 4.5: Soil moisture content and catechins analysis of variance in three different seasons at Timbilil estate, Kericho

	Season I [Cool & wet] June-August		Season II [Warm &wet] Sept-November		Season III [Dry & hot] Dec-February	
Clone	SMC (%)	Catechins (%)	SMC (%)	Catechins (%)	SMC (%)	Catechins (%)
TRFK31/8	54.64	38	58.81	40	33.61	24
TRFK301/4	57.67	15	60.59	13	31.43	9
TRFK31/11	53.15	43	58.33	40	31.38	21
TRFK303/577	52.87	23	54.84	23	30.96	21
TRFK6/8	52.45	47	56.02	48	30.94	26
TRFK 7/9	56.69	33	58.95	30	28.25	13
BBK35	52.19	30	54.29	30	27.76	13
SFS150	54.13	18	56.09	16	30.37	12

S15/10	55.76	21	59.66	19	29.93	10
TRFK 12/19	55.14	32	55.61	32	31.09	18

Data are means of three measurements collected in the same season. Measurements were made between 10.00 am and 1.00 pm at intervals of an hour.

Figure 4.1: Principal Component Analysis of catechins for ten clones during dry and hot season

Figure 4.2: Principal Component Analysis of catechins for ten clones during warm and wet season

Figure 4.3: Principal Component Analysis of catechins for ten clones during cool and wet season

4.1.2 Variation of catechin content under different seasons

Catechin content of the ten clones varied from 15 to 47%, 13 to 48%, and 9 to 26% during cool and wet, warm and wet and dry and hot seasons respectively (Table 4.5). Although on the average clone TRFK 6/8 and TRFK 31/11 contained more catechins than that of the other clones the differences were not statistically significant (P>0.05) among the clones (Table 4.6). This is partly due to the large deviation in catechin contents among the individual clones. Some clones in the same season had either extremely low or high catechin content.

Table 4.6: Variation in catechin levels in shoots of the ten clones over the three seasons

Clone	Season I[Cool & wet] June-August	Season II[Warm & wet] September-November	Season III [Hot & dry] December-February

TRFK 31/8	0.4700	a	0.4767	a	0.2600	a
TRFK301/4	0.4267	ab	0.4033	ab	0.2433	a
TRFK311/11	0.3833	ab	0.4033	ab	0.2133	ab
TRFK303/577	0.3233	bc	0.3300	abc	0.2067	abc
TRFK 6/8	0.3200	bc	0.3167	bc	0.1767	abcd
TRFK 7/9	0.3100	bc	0.3033	bc	0.1333	bcd
BBK35	0.2300	cd	0.2967	bc	0.1300	bcd
SFS150	0.2067	cd	0.1900	cd	0.1200	cd
S15/10	0.1867	d	0.1867	cd	0.0966	d
TRFK 12/19	0.1800	d	0.1300	d	0.0900	d

The interaction means and marginal means followed by a common letter are not significantly different at 5% levels by Duncan's multiple range tests.

4.1.3 Change in Leaf water potential (Ψ_{Leaf}) in relation to catechins contents

The changes in leaf water potential (LWP) of different clones are presented in Table 4.7. The results showed that the decline in catechin content corresponds with a decline in leaf water potential. At the lowest moisture levels, the drought tolerant clones TRFK 303/577 and SFS 150 maintained significantly higher ($P \leq 0.05$) leaf water potential compared to the drought susceptible clones: TRFK 6/8, TRFK 12/9, TRFK 301/4, TRFK 31/11, S15/10, TRFK 7/9, TRFK 31/8 and BBK 35.

Table 4.7: Comparison of leaf water potential (Leaf) and catechins contents in seasons I, II and III at TRFK, Kericho

	Season I[Cool & wet] June-August		Season II[Warm & wet] September-November		Season III [Hot & dry] December-February	
Clone	Leaf (MPa)	Catechins (%)	Leaf (MPa)	Catechins (%)	Leaf (MPa)	Catechins (%)
TRFK 31/8	-4.60	38	-6.73	40	-16.03	24
TRFK301/4	-4.87	15	-6.53	13	-18.37	9
TRFK311/11	-4.40	43	-6.53	4.0	-18.93	21
TRFK303/57	-4.00	23	-6.00	33	-17.17	21

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TRFK 6/8	-3.73	47	-6.03	48	-18.73	26
TRFK 7/9	-4.80	33	-6.0	30	-17.47	13
BBK35	-4.40	30	-6.40	30	-16.50	13
SFS150	-4.67	18	-6.20	16	-15.80	12
S15/10	-5.9	21	-6.87	19	-18.97	10
TRFK 12/19	-4.27	32	-6.40	32	-16.50	18
F	s	s	s	s	s	s
CV (%)	7.54	22.83	4.76	31.23	3.04	34.23
LSD	P≤0.05		P≤0.05		P≤0.05	

s., significant; MPa., Megapascals; LSD., least significant difference; cv., coefficient of variation. Data are means of three measurements collected on the same vines. Measurements were made between 10.00 am and 1.00 pm at intervals of an hour.

4.2 DISCUSSION

This study found out that, as a consequence of water stress, catechin concentrations in tea plants decreased during the dry period, results which are in agreement with those reported by Ojeda *et al*. (2002), Flexas and Medrano (2002) and Cheruiyot *et al*. (2008) who found out that, under water stress, phenolic biosynthesis in tea is significantly limited. The results also essentially support the findings of Esteban *et al*. (2001), Roby *et al*. (2004) and Salón *et al*. (2005), who reported that a direct response on phenolic biosynthesis to water deficit by a plant can be either positive or negative, depending on the type of phenolic compound, the degree of water deficit and the period during which is applied.

The study has shown a close relationship between soil water stress and the catechin concentration of the tea. Although the drought susceptible clones showed lower catechin levels during dry period than the drought tolerant clones, the differences

were not statistically significant ($P \leq 0.05$). This is partly due to the large deviation in catechin content among the individual clones in the same group. For example the average catechin content for clone TRFK 6/8 and TRFK 31/11 was much higher than that in the rest of the clones (all droughts susceptible) within the same season. The same phenomenon was observed between the drought tolerant clones. A few clones among the drought susceptible had either extremely low or high catechin content. The variations might have been due to physiological differences such as deep rooting ability.

Ranking drought tolerance ability on the basis of catechin levels suggested that clone TRFK 303/577 was the most drought tolerant clone, followed by SFS 150. These results agree with findings by Obaga *et al*. (1988) where physiological criteria were applied to rank some of the clones used in the experiment. The pattern of variation of leaf water potential over different soil moisture regimes is similar to that described in other studies (Turner, 1981; Morgan, 1984; Stephens and Carr, 1989; Ripullone *et al*., 2007).

A study conducted to investigate osmotic adjustments and associated water relation in clonal tea in Sri Lanka provided evidence of a link between a decline in soil water potential and tea quality (proportional to levels of catechins), indicating that tea plant responded negatively to xylem cavitations and tend to regulate phenolic synthesis in a manner that prevents further decline in leaf water potential (Wijeratne, 2006; Nissanka, 2007; De Costa *et al*., 2007; Kumar and Kumar, 2010). Salleo *et al*. (2000)

and Sperry *et al*. (2002) have also reported a concomitant decline in tea quality with increasing soil moisture deficit.

In general, leaf water potential of all the clones fell to lower mean values in Jan-march, -16.0 MPa in drought tolerant and -18.5 MPa in drought susceptible clones. Leaf water potential was above -4.0 MPa, when soil water content increased from an average of 50% during the cool and wet season (June – August) to 60% in the warmer and wet September – November period during which there was a moderate increase in catechin levels (Table 3). Different trend was observed during the dry period with a sharp decrease in leaf water potential when mean values for soil moisture content reached below 35% and the saturated atmospheric pressure had fallen to 20 MPa/month. Thus, these results show that soil moisture stress associated with high saturation vapour deficits causes adverse impacts on water economy of tea plants.

Evapotranspiration increased from a minimum 119 mm/day in wet and cold period to a maximum of 136 mm/day during dry and hot period (Appendix VI). Air temperature varied seasonally between 22°C in the cool and wet and 28.5 °C in dry and hot season. Over the same period, vapour pressure deficit varied between 5.7 and 22.7 MPa, as the season progressed.

Soil water deficit increased linearly from 30.3 (wet and cold season) to 107 mm (dry and hot season) whereas radiation ranged from 15.92 to 25.12 cm-²/day (Appendix VI).

Concurrent measurements over the study period indicated that leaf water potential was substantially greater in TRFK 303/577 (drought tolerant) than in TRFK 31/11(drought susceptible).

This study also confirmed that there is a relationship between vapour pressure deficits, air temperature and radiation, where a rise in radiation caused a rise in air temperature and a more negative leaf water potential of tea leaves. This is in agreement with (William, 1971; Squire, 1979; Tanton, 1982) who showed that there is an inverse linear relationship between vapour pressure deficits as a result of high air temperature and the internal water status of the tea plant. Nogués *et al*. (1998) reported that the interaction between radiation and water stress was beneficial for *Pisum sativa* and suggested that reduced solar radiation delayed and reduced the harmful effects of water stress. Alexieva *et al*. (2001) also noted that in the *Lycopersicon esculentum* and *Triticum aestivum*, interaction between radiation and water stress to induce protective mechanism was synergistic. This could be attributed to sufficient soil water content throughout that period as shown by high soil moisture content (54 and 57 mm, respectively).

Frequent rainfall from June to November provided an adequate water supply thus, tea did not experience any stress. However, during the January-March period the high vapour pressure deficit and high soil moisture content deficits were sufficient to cause changes in the internal plant water status thus, exacerbating the effects of drought. There was clonal variation in response to the effects of high vapour pressure

deficit and high soil moisture content deficits. For instance, drought tolerant clones had the lowest percentage decrease in catechin content during the dry period, whereas the drought susceptible clones had a significant decreased. In general, the contribution of catechins to the reduction of leaf water potential with increasing water stress seems quite remarkable in drought tolerant clones compared with the susceptible clone

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

i) The levels of catechins are season dependent. The apparent seasonal variation of catechins levels is a clear indication of clonal differences which could be exploited in tea selection.

ii) Results showed that catechin concentrations were positively correlated with soil moisture content in all treatments. Under severe soil water stress, catechins concentration decreased by 48 and 25% for both droughts susceptible and tolerant clones, respectively.

iii) The drought tolerance ability of SFS 150 and TRFK 303/577 can be attributed to their ability to reduce their leaf water potentials to more negative values that enabled them to absorb water from soil with low water potentials. It was also confirmed that clone SFS 150 and TRFK 303/577 can withstand dry weather conditions better than other investigated clones.

5.2: Recommendations for tea farmers and researchers

i) The technique of clonal selection is quite easy to perform and the tea breeders could apply it to select cultivars for water stress tolerance ability as well as for quality attributes.

ii) This new approach of selecting clones based on the catechin level appears to be more efficient, reliable, fast and cost effective compared to the conventional procedures, hence could easily be adopted as a selection criteria.

5.3 Suggestions for future study

i) There is a need to repeat the study probably with the same clones in different locations across the country as responses could vary.

ii) To effectively evaluate the new screening technique for drought tolerance in tea it is suggested that growth determinants such as root length density, rate of shoot growth and stability of harvested yields during periods of prolonged water stress should be included in future study.

iii) More studies should be conducted using relatively younger plants (<7years), given that the average age for tea bushes used in the present study is 27 years hence, the differences physiological development over time could have affected the results.

iv) In view of the findings, it is of interest, therefore, to study the influence of elevated carbon IV oxide (CO_2) on the accumulation of catechins and total polyphenols in tea.

v) Although a significant correlation between catechin levels and soil moisture stress was observed, there is a need for further research in a period of more than one year.

vi) To speed up the search for an effective screening method for drought tolerance in tea aspects of molecular biology that elucidate some fundamental characteristics and mechanisms of tea should play a significant role.

vii) The general influence of elements present in soil on the tolerance ability of tea should be included in the study to establish whether there is any relationship.

REFERENCES

Abrams, M.D., Kubiske, M.E. and Steiner, K.C. (1990). Drought adaptations and responses in five genotypes of *Fraxinus Pennsylvanica* Marsh: Photosynthesis, water relations and leaf morphology, *Tree Physiology.* **6**: 305-315.

Alexieva, V., Sergiev, I., Mapelli, S. and Karanov, E. (2001). The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant Cell Environment* **24**: 1337–1344.

Anandacoomaraswamy, A., De Costa, W.A.J.M., Shyamalie, H.W. and Campbell, G.S. (2000). Factors controlling transpiration of mature field-grown tea and its relationship with yield. *Agricultural Meteorolology.* **103**: 375-386.

Anesini, C.F and Filip, R. (2008). Total polyphenol content and antioxidant capacity available in Tea in Argentina. Journal of Agricultural and food chemistry **56**:9225-9229.

Bakker, J., Bridle, P., Timberlake, C.F. and Arnold, G.M. (1986). The colours, pigment and phenol contents of young port wines: Effects of cultivar, season and site. *Vitis* **25**: 40-52.

Barman, T.S, Baruah, U. and Sarma, S.K. (1993). Effect of light and shade on diurnal variation of photosynthesis, stomatal conductance and transpiration rate in tea. In: *Tea science and human health*. Proceedings of the International Symposium, Tea Tech. 1993. Calcutta, India: *Tea Research Association*, pp. 208–218.

Banerjee, B. (1992). Botanical classification of tea: In: Wilson, K.C., Clifford, M.N. (Eds), Tea. Cultivation to consumption. Chapman and Hall, London, pp. 22-51.

Bonheure, D. (1990). *Tea*. The Tropical Agricultural series. CTA, Macmillan, Education Limited pp. 102.

Bore, J.K. (2010). Plant physiology and agronomy investigation. *Annual Report*, *Tea Research Foundation of Kenya*, pp. 80-93.

Borland, A.M., Griffiths, H., Hartwell, J. and Smith, J.A.C. (2009). Exploiting the potential of plants with crassulacean acid metabolism for bioenergy production on marginal lands. *Journal Experimental Botany*, **60**: 2879–2896.

Bota, J., Stasyk, O., Flexas, J. and Medrano. H. (2004). Effect of water stress on partitioning of ^{14}C- labeled photosynthates in *Vitis vinifera*, *Functional Plant Biology* **31**: 697-708.

Braun, P., Aspinall, D. and Lenz, F. (1989). The use of physiological indicators of water status in irrigation scheduling in citrus. *Acta Horticulture*. **240**: 267-270.

Burt, C.C. (2007). Extreme weather. A guide and record book (Revised and updated). New York, USA.

Caidwell, M.M. and Flint, S.D. (1994). Internal filters prospects for UV acclimation in higher plants. *Plant Cell Environment*, **17**: 267 – 276.

Callander, B.A. and Woodhead, T. (1981). Canopy conductance of estate tea in Kenya. *Agricultural Meteorology* **23**: 151-167.

Carr, M.K.V. (2000). Shoot growth plus plucking equals profit. *TRIT Occasional Publication* No. 1, April 2000, Tea Research Institute of Tanzania, pp.1-7.

Carr, M.K.V. and Stephens, W. (1992). Climate, weather and the yield of tea. In: Willson KC, Clifford MN (eds), *Tea: Cultivation to Consumption*, pp. 87-135.Chapman & Hall, London.

Carr, M.K.V. (1977). Responses of seedling tea bushes and their clones to water stress. *Experimental Agriculture* **13**: 317-324.

Carr, M.K.V. (1974). Irrigating seedling tea in Southern Tanzania: effects on total yields, distribution of yield and water use. *Journal of Agricultural Science* (Cambridge) **83**: 363-378.

Carr, M.K.V. (1972). The climatic requirements of the tea plant: A review. *Experimental Agriculture*, **8**:1-14.

Carr, M.K.V. (1971). An assessment of some of the results of tea/soil/water studies in Southern Tanzania. In: Carr M.K.V., Carr S (eds), Water and the Tea Plant, pp.21-47. Tea Research Institute of East Africa, Kericho, Kenya.

Carr, M.K.V. (1970). The role of water in the growth of the tea crop. In: Lukwill LC, Cutting CV (eds) *Physiology of Tree Crops*, pp.287-384. Academic Press, London.

Chaves, M.M., Pereira, J.S., Maroco, J., Rodrigues, M.L., Ricardo, C.P.P., Osório, M.L., Carvalho, I., Faria, T. and Pinheiro, C. (2002). How plants cope with water stress in the field Photosynthesis and growth. *Annal of Botany* **89**: 907–916.

Cherotich, L., Kamunya, S., Alakonya, A., Msomba, S., Uwimana, M., Wanyoko, J. and Owuor, P. (2013). Variation in Catechin Composition of Popularly Cultivated Tea Clones in East Africa. *American Journal of Plant Sciences*, **4**: 628-640.

Cheruiyot, E.K., Mumera, M., Ng'etich, W.K., Hassanali, A., Wachira, F. and Wanyoko, J.K. (2008). Shoot Epicatechin and Epigallocatechin Contents Respond to Water Stress in Tea (*Camellia sinensis*). *Bioscience Biotechnology Biochememistry* **72**: 1219–1226.

Cheserek, B.C. (2011). Weather and yields. *Tea*, **32**(1): 1-2.

Christie, P.J., Alfenito, M.D., and Walbot, V. (1994). Low temperature effects on anthocyanin biosynthesis. *Plantae*, **194**: 541-549.

Cloughley, J.B. (1982). Factors influencing the caffeine content of black Tea.1. Effect of field variables. Tea Research Foundation of Central Africa. Malawi. *Food Chemistry* **4**: 269-276.

Coberly, L.C. and Rausher, M.D. (2003). Analysis of a chalcone synthase mutant in Ipomea purpurea reveals a novel function for flavonoids: amelioration of heat stress, *Molecular Ecology*, **12**: 1113-1124.

Cortell, J.M., Halbeib, M., Gallagher, A.V., Righetti, T.L. and Kennedy, J.A. (2005). Influence of vine vigor on grape (*Vitis vinifera* L. cv. Pinot noir) and wine proanthocyanidins. *Journal of Agricultural Food Chemistry* **53**: 5789-5797.

Cramer, G.R., Ergül A., Grimple J., Tillett R.L., Tattersall E.A.R., Bohlman M.C., Vincent D., Sonderegger J., Evans J., Osborne C., Quilici D., Schlauch K.A., Schooley D.A. and Cushman, J.C. (2007). Water and salinity stress in grapevines: early and late changes in transcript and metabolite profiles. *Functional Integrated Genomics*, **7**: 111-134.

Dai, J. and Mumper, R. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, **15**: 7313-7352.

DaMatta F.M., Chaves A.R.M., Pinheiro H.A., Ducatti C. and Loureiro, M.E. (2003). Drought tolerance of two field-grown clones of *Coffea canephora*. *Plant Science* **164**: 111–117.

Damayanthi, M.M.N., Mohotti, A.J. and Nissanka, S.P. (2010). Comparison of Tolerant Ability of Mature Field Grown Tea (*Camellia sinensis* L.) Cultivars Exposed to a Drought Stress in Passara Area. *Tropical Agricultural Research* **22** (1): 66 – 75.

Dan, D.H. and Lee, C.H. (2004). The effects of GA_3, CPPU and ABA applications on the quality of Kyoho (*Vitis vinifera* L. x *Labrusca* L.) Grape. *Acta Horticulture* (ISHS) **653**: 193-197.

Das, A. and Mondal, T.K. (2012). Identification of differentially expressed gene profiles in young roots of tea [Camellia sinensis (L.) O. Kuntze] subjected to drought stress using suppression subtractive hybridization. Plant Molecular Biology, 30: 1088–1101.

Davies, W.J. and Zhang, J. (2007). Root signals and the regulation of growth and development of plants in drying soil. *Annu. Rev. Plant Physiology Plant Molecular Biology* **102**: 55-76.

De Costa, W.A., Mohotti, A.J. and Wijeratne, M. A. (2007). Ecophysiology of tea. *Brazilian Journal of Plant Physiology* **21**(4): 299-332.

De Freitas, V.A.P., and Glories, Y. (1999). Concentration and compositional changes of procyanidins in grape seeds and skin of white *Vitis vinifera* varieties. *Journal of Science Food Agriculture* **79**: 1601-1606.

Deikman, J., and Hammer, P.E. (1995). Induction of anthocyanin accumulation by cytokinins in *Arabidopsis thaliana*. *Plant Physiology* **108**: 47-57.

Delgado, R. (2009). Changes in phenolic composition of grape berries during ripening in relation to vineyard nitrogen and potassium fertilisation rates. *Journal of Science Food Agriculture* **84**: 623-630.

Dixon, R.A., Xie, D.Y. and Sharma S.B. (2005). Proanthocyanidins: a final frontier in flavonoid research? *New Phytologist*, **165**: 9-28.

Do, C.B. and Cormier, F. (1991). Accumulation of peonidin 3-glucoside enhanced by osmotic stress in grape (*Vitis vinifera* L.) cell suspension. *Plant Cell Tissue Organ Culture* **24**: 49-54.

Dry, P.R., Loveys, B.R., Iland, P.G., Botting, D.G., McCarthy, M.G. and Stoll, M. (1998). Vine manipulation to meet fruit specifications. *In* Proceedings of the 10th Australian Wine Industry Technical Conference. R.J. Blair et al. (Eds.), pp. 208-214. AWITC, Adelaide.

Duan, L. (1992). Investigations on the physiology of tolerance in plantation crops. Effects of water stress on the growth of tea plants. *Tea Science Technical Bulletin* **1**: 12-15.

Edreva, A., Velikova, V., Tsonev, T., Dagnon, S., Gurel, A., Aktas, L. and Gesheva, E. (2008). Stress-protective role of secondary metabolites: diversity of functions and mechanisms, *Plant physiology*, **34**: 67-78.

Esteban, M.A., Villanueva, M.J. and Lissarrague, J.R. (2001). Effect of irrigation on changes in the anthocyanin composition of the skin of cv Tempranillo (*Vitis vinifera* L.) grape berries during ripening. *Journal of Science Food Agricuture*, **81**: 409-420.

Fitter, A.H. and Hay, R.K. (1981). Environmental physiology of plants. *Academic press Inc.* New York, 31- 42.

Flexas, J. and Medrano, H. (2002). Effects of drought on photosynthesis in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. *Functional Plant Biology,* **29**: 461-471.

Friedman, M., Henika, P.R., Levin, C. E., Mandrell, R.E. and Kozukue, N. (2006). Antimicrobial activities of tea catechins and theaflavins and tea extracts against *Bacillus cereus*. *Journal of Food Protein*, **69** (2): 354–361.

Fordham, R. (1969). The physiological responses of tea to moisture stress and irrigation. *Quarterly Newsletter*, Tea Research Foundation, Central Africa, **13**: 13-15.

Gill, S.S. and Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants, *Plant Physiology and Biochemistry*, **48**: 909-930.

Girona, J., Marsal, J., Mata, M., Del Campo, J. and Basile, B. (2009). Phenological sensitivity of berry growth and composition of Tempranillo grapevines (*Vitis vinifera* L.) to water stress. *Australia Journal of Grape Wine Research*, **15**: 268-277.

Gonzalez-San Jose, M.L., Santa-Maria, G. and Diez, C. (1990). Anthocyanins as parameters for differentiating wines by grape variety, wine-growing region, and wine-making methods. *Journal of Food Composition Analysis* **3**: 54-66.

Guidoni, S., Allara, P. and Schubert, A. (2008). Effect of cluster thinning on berry skin anthocyanin composition of *Vitis vinifera* cv. Nebbiolo. *American Journal of Enology and Viticulture* **53**: 224-226.

Handique, A.C. and Manivel, L. (1986). Shoot water potential in tea, Two and a Bud, **33** (1 and 2): 39-42.

Harborne, J.B. (1976). Functions of Flavonoids in Plants. In Chemistry and Biochemistry of Plant Pigments, ed. T.W. Goodwin, New York: Academic press, pp. 736-778.

Hare, P.D., Cress, W.A. and Van Staden, J. (1998). Dissecting the roles of osmolytes accumulation during stress. *Plant Cell Environment*, **21**: 535-553.

Heim, E.K., Tangliaferro, A.R. and Bobilya, D.J. (2002). Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. *Journal of Nutritional Biochemistry.* **13**: 572-584.

Hsiao, T.C. and Acevedo, E. (1974). Plant responses to water deficits, water-use efficiency, and drought resistance. *Agricultural Meteorology* **14**: 59-84.

Huang, S. (1989). Meteorology of the tea plant in China. A review. *Agricultural and Forest Meteorology*, **47**: 19-30.

Huang, X., Huang, H. and Gao, F. (2000). The growth potential generated in citrus fruit under water stress and its relevant mechanisms. *Horticultural Science* **83**: 227-240.

Jackson, D.I. and Lombard, P.B. (1993). Environmental and management practices affecting grape composition and wine quality – A review. *American Journal of Enology and Viticulture* **44**: 409-430.

Jaetzold, R. and Schmidt, H. (1983). Farm management handbook of Kenya Vol. II. Natural conditions and farm management information. Ministry of Agriculture, Kenya in cooperation with the Germany Agriculture Team (GAT) of the Germany Agency for Technical Cooperation (GTZ), Nairobi, Kenya.

Jeong, S.T., Goto-Yamamoto, N.M., Kobayashi, N. and Esaka, M. (2004). Effects of plant hormones and shading on the accumulation of anthocyanins and the expression of anthocyanin biosynthetic genes in grape berry skins. *Plant Science* **167**: 247-252.

Kamunya, S.M. (2010). Genetic Parameters and Quantitative Trait Loci Mapping in Tea (*Camellia sinensis* L). PhD Thesis, Egerton University, Njoro, Kenya.

Karunarathne, P.M.A.S., Wijeratne, M.A. and Sangakkara, U.R. (2004). Osmotic adjustment and associated water relations of clonal tea (*Camellia sinensis* L.) Sabaragamuwa, *University Journal* **2**(1): 77-85.

Keller, M. and Hrazdina, G. (1998). Interaction of nitrogen availability during bloom and light intensity during veraison. II. Effects on anthocyanins and phenolic development during grape ripening. *American Journal of Enology and Viticulture* **49**: 341-349.

Keller, M., Arnink, K.J. and Hrazdina, G. (1998). Interaction of nitrogen availability during bloom and light intensity during veraison. I. Effects on grapevine growth, fruit development and ripening. *American Journal of Enology and Viticulture* **49**: 333-340.

Kim, H., Quon, M.J. and Kim, J. (2014). New insights into the mechanisms of polyphenols beyond antioxidant properties; lessons from the green tea polyphenol, epigallocatechin 3-gallate. *Redox Biology*, 2: 185-195.

Khan, N. and Mukhtar, H. (2007). Tea polyphenols for health promotion. *Life Sciences.* **81** (7): 519-533.

Kliewer, W.M. (1977). Influence of temperature, solar radiation and nitrogen on coloration and composition of Emperor grapes. *American Journal of Enology and Viticulture* **28**: 96-103.

Kramer, P.J. (1983). "Water Relations of Plants." Academic Press, New York.

Koes, R.E., Quattrocchio, F. and Mol, J.N.M. (1994). The flavonoid biosynthetic pathway in plants: *Function and evolution*, **16**: 123-132.

Kottur, G., Venkatesan, S., Senthil Kumar, R.S. and Murugesan, S. (2010). Influence of season on biochemical parameters of green shoots and quality parameters of made tea under south Indian conditions. *Journal of Biosciences and Research* **1** (2): 74-82.

Kozlowski, T.T., and Pallardy, S.G. (2007). Physiology of woody plants. San Diego: Academic Press. **21**(5): 287-298.

Lakmini, W.G.D., Nainayake, N.P.A.D. and De Costa, W.A.J.M. (2006). Biochemical changes of four different coconut (*Cocos nucifera* L.) forms under moisture stress conditions. *Journal of Agricultural Science* **2** (3): 1-7.

Lawlor, D.W. and Tezara, W. (2008). Causes of decreased photosynthetic rate and metabolic capacity in water-deficient leaf cells: A critical evaluation of mechanisms and integration of processes, *Annals of Botany*, 1-19.

Levy, Y. (1980). Effect of evaporative demand on water relations of *Citrus limon*. *Annal of Botany* **46**: 695-700.

Liyanapatabendi, N.K.L.P., Mohotti, A.J. and Sangakkara, U.R. (2007). Rooting behaviour of field grown seedling and vegetatively propagated tea (*Camellia sinensis* (L) O. Kuntze). In: Proceedings of the 27th Annual Sessions of the Institute of Biology of Sri Lanka, Sri Lanka, pp.33.

Ludlow, M. M., Santamaria, J. M. and Fukai, S. (1990). Contribution of osmotic adjustment to grain yields in *Sorghum bicolor* (L.) Moench under water-limited conditions. II. Water stress after anthesis. *Australian Journal of Agricultural Research*, **41**: 67-78.

Magoma, G.N., Wachira, F.N., Obanda, M., Imbuga, M. and Agong, S.G. (2000). The use of catechins as biological markers in diversity studies of tea (*Camellia sinensis*), *Genetic Resource and Crop Evolution* **47**: 107-114.

Magambo, M.J.S. and Othieno, C.O. (1983). Influence of soil temperature variation on early flowering of tea plants. *Tea* **4**: 21-27.

Magambo, M.J.S. (1983). Dry matter production and partioning in clonal tea (*Camellia sinensis* L.) in Kenya. PhD. Thesis, University of Nairobi.

Malec, L.S. (2006). Seasonal variations in theaflavin, thearubigin and caffeine contents of Argentinean black teas. *Journal of the Science of Food and Agriculture* **45** (2): 185-190.

Marschner, H. (1995). Mineral Nutrition of Higher Plants. Academic Press, London.

Mayer, A. (2006). Polyphenol oxidases in plants and fungi: going places? A review. *Phytochemistry*, **67**: 2318-2331.

McDonald, M.S., Hughes, M., Burns, J., Lean, M.E.J., Matthews, D., and Crozier, A. (1998). Survey of the free and conjugated myricetin and quercetin content of red wines of different geographical origins. *Journal of Agricultural Food Chemistry* **46**: 368- 375.

Medrano, H., Chaves, M.M., Porqueddu, C. and Caredda, S. (1998). Improving forage crops for semi-arid areas. *American Journal of Enology and Viticulture* **27**: 89-94.

Mohotti, A.J. and Lawlor, D.W. (2008). Diurnal variation of photosynthesis and photoinhibition in tea: effects of irradiance and nitrogen supply during growth in the field. *Journal of Experimental Botany* **53**: 313-322**.**

Molla, M.M. (1981). Study on the nature of polyphenols in BTRI clones. *Tea Journal of Bangladesh* **17**: 20-25.

Mondal, T.K., Bhattacharya, A., Laxmikumaran, M. and Ahuja, P.S. (2004). Recent advances of tea (*Camellia sinensis*) biotechnology. *Plant Cell, Tissue and Organ Culture* **76:** 195-254.

Morgan, J.M. (1984). Osmoregulation and water stress in higher plants. *Annual Review of a Plant Physiology*, Agricultural Research Center, Australia. **35**: 299-319.

Morris, J.R., Sims, C.A. and Cawthon, D.L. (1983). Effects of excessive potassium levels on pH, acidity and color of fresh and stored grape juice. *American Journal of Enology and Viticulture* **34**: 35-39.

Mullet, J.E. and Whitsitt, M.S. (1997). Plant cellular responses to water deficit. In: Belhassen E (ed), Drought Tolerance in Higher Plants: *Genetical, Physiological and Molecular Biological Analysis*, pp. 41-46. Kluwer Academic Publishers, Dordrecht, the Netherlands.

Nadal, M. and Arola, L. (1995). Effects of limited irrigation on the composition of must and wine of Cabernet Sauvignon under semiarid conditions. *Vitis* **34**: 151-154.

Nagarajah, S. (1989). Physiological responses of grapevines to water stress. *Acta Horticulture* **240**: 249-256.

Nainanayake, A.D. (2004). Impact of drought on coconut (*Cocos nucifera* L.): Screening germplasm for photosynthesis tolerance in the field, PhD thesis, University of Essex, UK.

Nakamura, N., Nakamae, H. and Maekawa, L. (1980). Effects of light and kinetin on anthocyanin accumulation in the petals of *Rosa hybrida*, Hort. Cv. *Ehigasa*. *Z. Pflanzenphysiology* **98**: 263-270.

Navaratne, D.K. (1992). A survey of drought damage to tea, in the Ratnapura region *Tea Bulletin* **2**: 34-42.

Ng'etich, W.K. (1995). An evaluation of response of some tea clones to environments in Kenya. PhD. Thesis, Cranfield University, Silsoe, U.K.

Ngetich, W.K. (1997). Weather and tea. *Tea* **18**: 58-59.

Ngetich, W.K. and Bore, J.K. (1998). Perfomance of clonal tea in rehabilitation and replanted fields. II. Effects of soil water deficits. *Tea* **19**: 77-83.

Nissanka, S.P. (2007). Report of the Plant Physiology, Plant Propagation and Breeding Division, *Annual Report , The Tea Research Institute of Sri Lanka*, Talawakelle.

Nogués, S., Allen, D.J., Morison, J.I.L. and Baker, N.R. (1998). Ultraviolet-B radiation effects on water relations, leaf development, and photosynthesis in droughted pea plants. *Plant Physiology* **117**: 173–181.

Noori, M., Amini, F. and Foroghi, M. (2010). Zn effects on flavonoid compounds of Coronilla varia L., 16th National and 4th International Conference of Biology, Ferdowsi University of Mashhad, Mashhad, Iran, 14-16 September 2010, pp. 1380.

Northcote, K.H. (1992). Soils and Australian viticulture. *In* Viticulture. Vol. 1. Resources. B.G. Coombe and P.R. Dry (Eds.), pp. 61-90. Winetitles, Adelaide.

Obaga, S.M.O., Squire, G.R. and Langat, J.K. (1988). Altitude, temperature and the growth rate of tea shoots. *Tea* **9**: 30-35.

Odhiambo, H.O., Nyabundi, J.O. and Chweya, J. (1993). Effect of soil moisture and vapour pressure deficits on shoot growth and the yield of tea in the Kenya Highlands. *Experimental Agriculture* **29**: 341-350.

Ogren, E. and Oquist, G. (1985). Effects of drought on photosynthesis, chlorophyll fluorescence and photoinhibition susceptibility in intact willow leaves. *Plantae* **166**(3): 380-388.

Ohashi, Y., Nakayama, N., Saneoka, H. and Fujita, K. (2006). Effects of drought stress on photosynthetic gas exchange, chlorophyll fluorescence and stem diameter of soybean plants. *Biology of Plants* **50:** 138–141**.**

Ojeda, H., Andary, C., Kraeva, E., Carbonneau, A. and Deloire, A. (2002). Influence of pre- and postvéraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* cv. Shiraz. *American Journal of Enology and Viticulture.*, **53**: 261-267.

Olsen, P.E., Castro, A., Joern, M.M., Duteau, N.A.H., Pilon-smits, E.F. and Reardon, K. (2007). Comparison of Plant families in a greenhouse phytoremediation study on an aged polycyclic aromatic hydrocarbon-contaminated soil, *Journal of Environmental Chemistry* **36**: 1461-1469.

Othieno, C.O., Stephens, W., and Carr, M.K.V. (1992). Yield variability at the Tea Research Foundation of Kenya. *Agriculture and Meteorology* **61**: 237-252.

Othieno, C.O. (1982). Diurnal variations in soil temperature under tea plants. *Experimental Agriculture* **18**: 195-202.

Othieno, C. O. (1978). Supplementary irrigation of young clonal tea in Kenya. Internal water status. *Experimental Agriculture* **14**: 309-316.

Othieno, C.O. (1973). Physical characterisation of soils, In: *Annual Report, Tea Research Institute of East Africa, Kericho*, Kenya, pp. 38-41.

Ozeki, Y., and Komamine, A. (1981). Induction of anthocyanin synthesis in relation to embryogenesis in a carrot suspension culture: Correlation of metabolic differentiation with morphological differentiation. *Physiology of Plants* **53**: 570-577.

Peterson, J., Dwyer, J., Jacques, P., Rand,W., Prior, R., Chiu, K. (2004). Tea variety and brewing techniques influence flavonoid content of black tea. *Journal of Food Composition and Analysis.* **17** (3-4): 397-405.

Phene, C.J., Reginato, R.J., Itier, B. and Tanner, B.R. (1990). Sensing irrigation needs. p. 208-261. In: G. J. Hoffman, T. A. Howell, and K. H. Solomon (eds.). Management of farm irrigation systems. ASAE., Joseph, Mich.

Pinheiro, H.A. (2004). Physiological and morphological adaptations as associated with drought tolerance in robusta coffee (*Coffea canephora* Pierre var. *kouillou*). Viçosa, Universidade Federal de Viçosa, PhD Thesis.

Pinheiro, H.A., DaMatta, F.M., Chaves, A.R.M., Fontes, E.P.B. and Loureiro, M.E. (2004). Drought tolerance as related to protection against oxidative stress in clones of *Coffea canephora* subjected to long-term drought. *Plant Science*. **24**: 5-11.

Plaut, Z. and Meiri, A. (1994). Crop Irrigation. pp. 47-63. In: K.K. Tanji and B.Yaron (eds.). Management of water use in agriculture. Springer-Verlag, New York, N.Y.

Rajagopal, V. and Balasimha, D. (2004). Drought tolerance in plantation crops. *Advance Horticulture* **21**: 1200.

Raj Kumar, R. and Mohan Kumar, P. (2010). Contributing factors of crop productivity in tea. A case study in the Anamallais. *Planters' Chronicle*, **106** (1): 4-5.

Reddy, A.R., Chaitanya, K.V. and Vivekanandan, M. (2010). Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *Journal of Plant Physiology.* **174**: 1189–1202.

Reynolds, A.G., Lowrey, W.D., Tomek, L., Hakimi J. and de Savigny, C. (2007). Influence of irrigation on vine performance, fruit composition, and wine quality of Chardonnay in a cool, humid climate. *American Journal of Enology and Viticulture*, **58**: 217-228.

Ripullone, F., Guerrieri, M.R., Nole, A., Magnani, F. and Borghetti, M. (2007). Stomatal conductance and leaf water potential responses to hydraulic conductance variation in *Pinus pinaster* seedlings. *Trees*, **21**: 371-378.

Roberts, E.H., Summerfield, R.J., Ellis, R.H., Craufurd, P.Q. and Wheeler, T.R. (1997). The induction of flowering. In: Wien HC (ed), The Physiology of Vegetable Crops, pp. 69-100. CAB International, Wallingford.

Robredo, A., Pérez-López, U., Sainz de la Maza, H., González- Moro, B., Lacuesta, M. and Mena-Petite, A. (2007). Elevated CO_2 alleviates the impact of the drought on barley improving water status by lowering stomatal conductance and delaying its effects on photosynthesis. *Environmental and Experimental Botany*, **59**: 252-263.

Roby, G., Harbertson, J.F., Adams, D.O. and Matthews, M.A. (2004). Berry size and vine water deficits as factors in winegrape composition: Anthocyanins and tannins. *Australian Journal of Grape Wine Research*, **10**: 100-107.

Russell, E.W. (1961). Soil Conditions and Plant Growth. Longmans, Green and Co., London.

Sabry, S.R., Smith, L.T. and Smith, G.M. (1995). Osmoregulation in spring wheat under drought and salinity stress. *Journal of Genetics and Breeding,* **49**: 55-60.

Salleo, S., Nardini A., Pitt, F. and Lo Gullo, M.A. (2000). Xylem cavitation and hydraulic control of stomatal conductance in Laurel (*Laurus nobilis* L.). *Plant Cell Environment*, **23**: 71-79.

Salón, J.L., Chirivella, C. and Castel, J.R. (2005). Response of cv. Bobal to timing of deficit irrigation in Requena, Spain: water relations, yield, and wine quality. *American Journal of Enology and Viticulture*, **56**: 1-8.

Sanaravan, M., Maria, J., Raj, K., Pius, P.K. and Sai Kumar, R. (2005). Genetic diversity of UPASI tea clones on the basis of total catechins and their fractions. *Phytochemistry* **66**: 561 – 565.

Seigler, D.S. (1998). Plant Secondary Metabolism. Dordrecht: Kuwar Academic Press.

Sivilotti, P., Bonetto, C., Paladin, M. and Peterlunger, E. (2005). Effect of soil moisture availability on Merlot: from leaf water potential to grape composition. *American Journal of Enology and Viticulture*, **56**: 9-18.

Slama, I., Messedi, D., Ghnaya, T., Savoure, A. and Abdelly, C. (2006). Effects of water deficit on growth and proline metabolism in *Sesuvium portulacastrum*. *Environment and Experimental Bot*any, **56**: 231-238.

Sperry, J.S., Hacke, U.G., Oren, R. and Comstock, J.P. (2002). Water deficits and hydraulic limits to leaf water supply. *Plant Cell Environment*, **25**: 251-263.

Stephens, W. and Carr, M.K.V. (1990). Seasonal and clonal differences in shoot extension rates and numbers in tea (*Camellia sinensis*). *Experimental Agriculture*, **26**: 83-98.

Stephens, W. and Carr, M.K.V. (1991). Responses of tea (*Camellia sinensis*) to irrigation and fertilizer. II. Water use. *Experimental Agriculture.* **27**: 193-210

Stephens, W. and Carr, M.K.V. (1989). A water stress index for tea (*Camellia sinensis*). *Experimental Agriculture*, **25**: 545-548.

Squire, G.R., Obaga, S.M.O. and Othieno, C.O. (1993). Altitude, temperature and shoot production of tea in the Kenyan Highlands. *Experimental Agriculture*, **29**: 107-120.

Squire, G.R. (1990). The Physiology of Tropical Crop Production. CAB International, Wallingford.

Squire, G.R. and Callander, B.A. (1981). Tea plantations. In: Kozlowski, T.T (ed), Water Deficits and Plant Growth, Vol. VI, pp. 471-510. Academic Press, New York.

Squire, G.R. (1979). Weather, physiology and seasonality of tea (*Camellia sinensis*) yields in Malawi. *Experimental Agriculture* **15**: 321-330.

Squire, G.R. (1978). Stomatal behavior of tea in relation to environment. *Journal of Applied Ecology.* **15**: 287-301.

Squire, G.R. (1977). Seasonal changes in photosynthesis of tea (*Camellia sinensis*). *J. Applied Ecology*, **14**: 303-316.

Squire, G.R. (1976). Xylem water potential and yield of tea (*Camellia sinensis*) clones in Malawi. *Experimental Agriculture*, **12**: 289-297.

Stephens, W. and Carr, M.K.V. (1993). Responses of tea (*Camellia sinensis*) to irrigation and fertilizer. III. Shoot extension and development. *Experimental Agriculture*, **29**:323-339.

Subair, M.A. (2010). Response of tea plants to irrigation in Wayanada. *Newsletter of UPASI Tea Research Foundation* **20**: 2-3.

Suzuki, M. (1995). Enhancement of anthocyanin accumulation by high osmotic stress and low pH in grape cells (*Vitis* hybrids). *Journal of Plant Physiol*ogy **147**: 152-155.

Taiz, L. and Zeiger, E. (2006). Plant Physiology, 4th ed. Sinauer, Sunderland, M.A.

Tanton, T.W. (1992). Tea crop physiology. In: Willson K.C. and Clifford M.N. (eds), Tea: Cultivation to Consumption, pp. 173-199.

Tanton, T.W. (1982). Environmental factors influencing the yield of tea (*Camellia sinensis*). I. Effects of air temperature. *Experimental Agriculture*. **18**: 47-52.

Tanton, T.W. (1979). Some factors limiting yields of tea (*Camellia sinensis*). *Experimental Agriculture* **15**: 187-192.

Tardieu, F. (2005). Plant tolerance to water deficit: physical limits and possibilities for progress. *Geoscience*, **337**: 57-67.

Tea Research Foundation of Kenya, (2012). Tea Cultivation Manual for Good Agricultural Practices Inkit, Nairobi, p. 70.

Tea Board of Kenya, (2011). In: Kenya tea industry performance highlights for 2010 and outlook for 2011. *Annual report*, Tea Board of Kenya.

Tea Board of Kenya, (2008). In: Kenya tea industry performance highlights for 2007. *Annual report*, Tea Board of Kenya.

Tea Board of Kenya, (2004). Smallholder tea growing enterprise. *Productivity and profitability survey report.* Tea Board of Kenya.

Tezara, W., Mitchell, V., Driscoll, S.P. and Lawlor, D.W. (2002). Effect of water deficit and its interaction with CO2 supply on the biochemistry and physiology of photosynthesis in sunflower. *Journal of Experimental Botany*, **53** (375): 1781-1791.

Treutter, D. (2006). Significance of flavonoids in plant resistance. Springer-Verlag, *Environmental Chemistry Letters*, **4**: 147-157

Turner, N.C. (1981). Techniques and experimental approaches for the measurement of plant water status. *Plant and Soil*, **58**: 339-366.

Upadhyaya, H. and Panda, S.K. (2013). Abiotic stress responses in tea [Camellia sinensis L. (O) Kuntze]: An overview. *Reviews in Agricultural Science*, **1**: 1-10

Van der Vossen, H.A.M. and Wessel, M. (2000). Plant resources of South-East Asia (PROSEA) No. 16 Stimulants. Backhuys publishers, Leiden, The Netherlands, pp 55-63

Warren, C.R. (2007). Stand aside stomata, another actor deserves centre stage: the forgotten role of the internal conductance to CO2 transfer. *Journal of Experimental Botany* **59**: 1475 - 1487.

Warren, J. and Mackenzie, S. (2001). Why are all color combinations not equally represented in as flower-colour polymorphism? *New Phytologist*, **151**: 237-41

Watson, M. (1986). Soil and climatic requirements. In: Sivapalan P, Kulasegaram S, Kathiravetpillai A. (eds), Handbook on Tea, pp. 3-5. Tea Research Institute, Talawakelle, Sri Lanka.

Williams, M., Law, B.E., Anthoni, P.M. and Unworth, M.H. (2001). Use of simulation model and ecosystem flux data to examine carbon water interactions in ponderosa pine. *Tree Physiology* **21**(5): 287-298.

Williams, E.N.D. (1971). Investigations into certain aspects of water stress in tea. In: Carr, M.K.V, Carr, S. (eds). Water and the Tea Plant, pp. 79-87. Tea Research Institute of East Africa, Kericho, Kenya.

Wink, M. (2010). Biochemistry, physiology and ecological functions of secondary metabolites. In Biochemistry of Plant Secondary Metabolism (second edition), ed. M. Wink, pp. 1-17. New York: Wiley–Blackwell Publishing.

Winkel, B.S. (2001). Flavonoid Biosynthesis. A Colourful Model for genetics, biochemistry, cell biology and biotechnology. *Plant Physiology*, **126**: 485-493.

Wijeratne, M.A., Anandacoomaraswamy, A., Amarathunga. M.K.S., Ratnasiri, J., Basnayake, B.R.S. and Kalra, N. (2007). Assessment of impact of climate change on productivity of tea (*Camellia sinensis* L.) plantations in Sri Lanka. *Journal of Natural Science* Sri Lanka **35**: 119-126.

Wijeratne, M.A. (2006). Some adaptations of the tea plant to dry environment, Adapting to climate change: *Assessments and issues*, Springer, New York, NY. pp. 174-187.

Wijeratne, M.A. (2004). Effect of soil moisture deficit and temperature on dry matter accumulation in tea shoots. Sri Lanka. *Journal of Tea Science* **69**: 40-50.

Wijeratne, M.A., Fordham, R. and Anandacoomaraswamy, A. (1998). Water relations of clonal tea (*Camellia sinensis* L.*)* with reference to drought resistance: II. Effect of water stress. *Tropical Agricultural Research and Extension* **1**: 74-80.

Wijeratne, M.A., and Fordham, R. (1996). Effect of environmental factors on growth and yield of tea (*Camellia sinensis* L.) in the low country wet zone of Sri Lanka. Sri Lanka. *Journal of Tea Science* **46**: 21-34.

Wijeratne, M.A. (1996). Vulnerability of Sri Lanka tea production to global climate change. *Water, Air and Soil Pollution* **92**: 87-94.

Wijeratne, M.A. (1994). Effect of climatic factors on the growth of tea (*Camellia sinensis* L.) in the low country wet zone of Sri Lanka. London, Wye College, University of London, PhD thesis.

Wood, D.J., Bhatia, I.S., Chakraborty, S., Choudhury, M.N.D., Deb, S.B., Roberts, E.A.H. and Ullah, M.R. (2006). The chemical basis of quality in tea:1. Analyses of freshly plucked shoots. *Journal of the Science of Food and Agriculture* **15**(1): 8-14.

Zaveri, N.T. (2006). Green tea and its polyphenolic catechins: medicinal uses in cancer and non cancer applications. *Life Sci.*, **78** (18): 2073-2080.

Appendix I (a)

Cool and wet season

Data File : PROP

Title : CATSMC

Case Range : 96 - 105

Variable 4 : CATECHINS

Function : RANGE

Error Mean Square = 0.005000

Error Degrees of Freedom = 29

No. of observations to calculate a mean = 3

Least Significant Difference Test

LSD value = 0.1181 at alpha = 0.050

Original Order				Ranked Order			
Mean	1 =	0.3833	ab	Mean	5 =	0.4700	a
Mean	2 =	0.1867	d	Mean	3 =	0.4267	ab
Mean	3 =	0.4267	ab	Mean	1 =	0.3833	ab
Mean	4 =	0.2300	cd	Mean	6 =	0.3233	bc
Mean	5 =	0.4700	a	Mean	10 =	0.3200	bc
Mean	6 =	0.3233	bc	Mean	7 =	0.3100	bc
Mean	7 =	0.3100	bc	Mean	4 =	0.2300	cd
Mean	8 =	0.1800	d	Mean	9 =	0.2067	cd
Mean	9 =	0.2067	cd	Mean	2 =	0.1867	d
Mean	10 =	0.3200	bc	Mean	8 =	0.1800	d

Appendix I (b)

Warm and wet season

Data File :

PROP

Title : CATSMC

Case Range : 96 - 105

Variable 4 : CATECHINS

Function : RANGE

Error Mean Square = 0.009000

Error Degrees of Freedom = 29

No. of observations to calculate a mean = 3

Least Significant Difference Test

LSD value = 0.1584 at alpha = 0.050

Original Order			Ranked Order		
Mean 1 =	0.4033	ab	Mean 5 =	0.4767	a
Mean 2 =	0.1300	d	Mean 1 =	0.4033	ab
Mean 3 =	0.4033	ab	Mean 3 =	0.4033	ab
Mean 4 =	0.3300	abc	Mean 4 =	0.3300	abc
Mean 5 =	0.4767	a	Mean 10 =	0.3167	bc

Mean	6 =	0.3033	bc	Mean	6 =	0.3033	bc
Mean	7 =	0.2967	bc	Mean	7 =	0.2967	bc
Mean	8 =	0.1867	cd	Mean	9 =	0.1900	cd
Mean	9 =	0.1900	cd	Mean	8 =	0.1867	cd
Mean	10 =	0.3167	bc	Mean	2 =	0.1300	d

Appendix I (c)

Dry and hot season

Data File : PROP

Title : CATSMC

Case Range : 96 - 105

Variable 4 : CATECHINS

Function : RANGE

Error Mean Square = 0.003000

Error Degrees of Freedom = 29

No. of observations to calculate a mean = 3

Least Significant Difference Test

LSD value = 0.09147 at alpha = 0.050

Original Order			Ranked Order		
Mean	1 = 0.2433	a	Mean	5 = 0.2600	a
Mean	2 = 0.09000	d	Mean	1 = 0.2433	a
Mean	3 = 0.2133	ab	Mean	3 = 0.2133	ab
Mean	4 = 0.2067	abc	Mean	4 = 0.2067	abc
Mean	5 = 0.2600	a	Mean	10 = 0.1767	abcd
Mean	6 = 0.1333	bcd	Mean	6 = 0.1333	bcd
Mean	7 = 0.1300	bcd	Mean	7 = 0.1300	bcd
Mean	8 = 0.1200	cd	Mean	8 = 0.1200	cd
Mean	9 = 0.09667	d	Mean	9 = 0.09667	d
Mean	10 = 0.1767	abcd	Mean	2 = 0.09000	d

Appendix II (a)

Analysis of Variance

Soil moisture Content [Cool and wet season]

One Factor Randomized Complete Block Design

Factorial ANOVA for the factors:

- Replication (REP) with values from 1 to 3
- Factor A (CLONE) with values from 1 to 10

Grand Mean = 54.472 Grand Sum = 1634.160 Total Count = 30

T A B L E O F M E A N S

1	2	4	Total
1	*	54.332	543.320
2	*	53.880	538.800
3	*	55.204	552.040
*	1	54.643	163.930
*	2	57.673	173.020
*	3	53.147	159.440
*	4	52.873	158.620
*	5	52.447	157.340
*	6	56.693	170.080
*	7	52.210	156.630
*	8	54.130	162.390

*	9	55.763	167.290
*	10	55.140	165.420

ANALYSIS OF VARIANCE TABLE

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability
1	Replication	2	9.059	4.529	0.5765	
2	Factor A	9	92.921	10.325	1.3140	0.2963
-3	Error	18	141.428	7.857		
	Total	29	243.408			

Coefficient of Variation: 5.15%

s_ for means group 1: 0.8864 Number of Observations: 10
y

s_ for means group 2: 1.6183 Number of Observations: 3
y

Appendix II (b)

Analysis of Variance

Soil Moisture Content [Warm and wet season]

One Factor Randomized Complete Block Design

Factorial ANOVA for the factors:
Replication (REP) with values from 1 to 3
Factor A (CLONE) with values from 1 to 10

Grand Mean = 57.319 Grand Sum = 1719.580 Total Count = 30

TABLE OF MEANS

1	2	4	Total
1	*	56.993	569.930
2	*	57.295	572.950
3	*	57.670	576.700
*	1	58.810	176.430
*	2	60.590	181.770
*	3	58.333	175.000
*	4	54.840	164.520
*	5	56.020	168.060
*	6	58.947	176.840
*	7	54.293	162.880
*	8	56.093	168.280

*	9	59.657	178.970
*	10	55.610	166.830

ANALYSIS OF VARIANCE TABLE

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability
1	Replication	2	2.301	1.150	0.1305	
2	Factor A	9	130.427	14.492	1.6440	0.1765
-3	Error	18	158.674	8.815		
	Total	29	291.402			

Coefficient of Variation: 5.18%

s_y for means group 1: 0.9389 Number of Observations: 10

s_y for means group 2: 1.7142 Number of Observations: 3

Appendix II (c)

Analysis of Variance

Soil Moisture Content [Dry and hot season]

One Factor Randomized Complete Block Design

Factorial ANOVA for the factors:

Replication (REP) with values from 1 to 3

Factor A (CLONE) with values from 1 to 10

Grand Mean = 30.572 Grand Sum = 917.160 Total Count = 30

TABLE OF MEANS

1	2	4	Total
1	*	29.881	298.810
2	*	30.386	303.860
3	*	31.449	314.490
*	1	33.610	100.830
*	2	31.430	94.290
*	3	31.380	94.140
*	4	30.960	92.880
*	5	30.940	92.820
*	6	28.253	84.760
*	7	27.760	83.280
*	8	30.370	91.110

*	9	29.927	89.780
*	10	31.090	93.270

ANALYSIS OF VARIANCE TABLE

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability
1	Replication	2	12.812	6.406	1.6949	0.2116
2	Factor A	9	74.741	8.305	2.1972	0.0742
-3	Error	18	68.033	3.780		
	Total	29	155.586			

Coefficient of Variation: 6.36%

s_ for means group 1: 0.6148 Number of Observations: 10
y

s_ for means group 2: 1.1224 Number of Observations: 3
y

Appendix II (d)

Analysis of Variance

Catechin levels [Cool and wet season]

One Factor Randomized Complete Block Design

Factorial ANOVA for the factors:
Replication (REP) with values from 1 to 3
Factor A (CLONE) with values from 1 to 10

Grand Mean = 0.304 Grand Sum = 9.110 Total Count = 30

TABLE OF MEANS

1	2	5	Total
1	*	0.290	2.900
2	*	0.302	3.020
3	*	0.319	3.190
*	1	0.383	1.150
*	2	0.187	0.560
*	3	0.427	1.280
*	4	0.230	0.690
*	5	0.470	1.410
*	6	0.323	0.970
*	7	0.310	0.930

*	8	0.180	0.540
*	9	0.207	0.620
*	10	0.320	0.960

ANALYSIS OF VARIANCE TABLE

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability
1	Replication	2	0.004	0.002	0.4419	
2	Factor A	9	0.281	0.031	6.4973	0.0004
-3	Error	18	0.086	0.005		
	Total	29	0.372			

Coefficient of Variation: 22.83%

s_ for means group 1: 0.0219 Number of Observations: 10
y

s_ for means group 2: 0.0400 Number of Observations: 3
y

Appendix II (e)

Analysis of Variance

Catechin levels [Warm and wet season]

One Factor Randomized Complete Block Design

Factorial ANOVA for the factors:
Replication (REP) with values from 1 to 3
Factor A (CLONE) with values from 1 to 10

Grand Mean = 0.304 Grand Sum = 9.110 Total Count = 30

TABLE OF MEANS

1	2	5	Total
1	*	0.295	2.950
2	*	0.310	3.100
3	*	0.306	3.060
*	1	0.403	1.210
*	2	0.130	0.390
*	3	0.403	1.210
*	4	0.330	0.990
*	5	0.477	1.430
*	6	0.303	0.910

*	7	0.297	0.890
*	8	0.187	0.560
*	9	0.190	0.570
*	10	0.317	0.950

ANALYSIS OF VARIANCE TABLE

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability
1	Replication	2	0.001	0.001	0.0671	
2	Factor A	9	0.322	0.036	3.9841	0.0061
-3	Error	18	0.162	0.009		
	Total	29	0.485			

Coefficient of Variation: 31.23%

s_y for means group 1: 0.0300 Number of Observations: 10

s_y for means group 2: 0.0547 Number of Observations: 3

Appendix II (f)

Analysis of Variance

Catechin levels [Dry and hot season]

One Factor Randomized Complete Block Design

Factorial ANOVA for the factors:

Replication (REP) with values from 1 to 3

Factor A (CLONE) with values from 1 to 10

Grand Mean = 0.167 Grand Sum = 5.010 Total Count = 30

TABLE OF MEANS

1	2	5	Total
1	*	0.182	1.820
2	*	0.165	1.650
3	*	0.154	1.540
*	1	0.243	0.730
*	2	0.090	0.270
*	3	0.213	0.640
*	4	0.207	0.620
*	5	0.260	0.780
*	6	0.133	0.400

*	7	0.130	0.390
*	8	0.120	0.360
*	9	0.097	0.290
*	10	0.177	0.530

ANALYSIS OF VARIANCE TABLE

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability
1	Replication	2	0.004	0.002	0.6090	
2	Factor A	9	0.102	0.011	3.4556	0.0121
-3	Error	18	0.059	0.003		
	Total	29	0.164			

Coefficient of Variation: 34.23%

s_y for means group 1: 0.0181 Number of Observations: 10

s_y for means group 2: 0.0330 Number of Observations: 3

Appendix II (g)

Analysis of Variance

Shoot water potential [Cool &Wet season]

One Factor Randomized Complete Block Design

Factorial ANOVA for the factors:

Replication (REP) with values from 1 to 3

Factor A (CLONE) with values from 1 to 10

Grand Mean = 4.407 Grand Sum = 132.200 Total Count = 30

TABLE OF MEANS

1	2	3	Total
1	*	4.240	42.400
2	*	4.540	45.400
3	*	4.440	44.400

---

*	1	4.600	13.800
*	2	4.867	14.600
*	3	4.400	13.200
*	4	4.000	12.000
*	5	3.733	11.200
*	6	4.800	14.400

*	7	4.400	13.200
*	8	4.667	14.000
*	9	4.333	13.000
*	10	4.267	12.800

ANALYSIS OF VARIANCE TABLE

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability
1	Replication	2	0.467	0.233	2.1141	0.1497
2	Factor A	9	3.345	0.372	3.3678	0.0136
-3	Error	18	1.987	0.110		
	Total	29	5.799			

Coefficient of Variation: 7.54%

s_y for means group 1: 0.1051 Number of Observations: 10

s_y for means group 2: 0.1918 Number of Observations: 3

Appendix II (h)

Analysis of Variance

Shoot water potential [Warm & Wet season]

One Factor Randomized Complete Block Design

Factorial ANOVA for the factors:

Replication (REP) with values from 1 to 3

Factor A (CLONE) with values from 1 to 10

Grand Mean = 6.370 Grand Sum = 191.100 Total Count = 30

TABLE OF MEANS

1	2	4	Total
1	*	6.420	64.200
2	*	6.400	64.000
3	*	6.290	62.900

---

*	1	6.733	20.200
*	2	6.533	19.600
*	3	6.533	19.600
*	4	6.000	18.000
*	5	6.033	18.100
*	6	6.000	18.000
*	7	6.400	19.200

*	8	6.200	18.600
*	9	6.867	20.600
*	10	6.400	19.200

ANALYSIS OF VARIANCE TABLE

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability
1	Replication	2	0.098	0.049	0.5328	
2	Factor A	9	2.550	0.283	3.0805	0.0202
-3	Error	18	1.655	0.092		
	Total	29	4.303			

Coefficient of Variation: 4.76%

s_ for means group 1: 0.0959 Number of Observations: 10
y

s_ for means group 2: 0.1751 Number of Observations: 3
y

Appendix II (i)

Analysis of Variance

Shoot water potential [Dry & Hot season]

One Factor Randomized Complete Block Design

Factorial ANOVA for the factors:
Replication (REP) with values from 1 to 3
Factor A (CLONE) with values from 1 to 10

Grand Mean = 17.447 Grand Sum = 523.400 Total Count = 30

TABLE OF MEANS

1	2	5	Total
1	*	17.500	175.000
2	*	17.580	175.800
3	*	17.260	172.600
*	1	16.033	48.100
*	2	18.367	55.100
*	3	18.933	56.800
*	4	17.167	51.500
*	5	18.733	56.200
*	6	17.467	52.400
*	7	16.500	49.500

* 8	15.800	47.400
* 9	18.967	56.900
* 10	16.500	49.500

ANALYSIS OF VARIANCE TABLE

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Probability
1	Replication	2	0.555	0.277	0.9881	
2	Factor A	9	40.808	4.534	16.1552	0.0000
-3	Error	18	5.052	0.281		
	Total	29	46.415			

Coefficient of Variation: 3.04%

s_ for means group 1: 0.1675 Number of Observations: 10
y

s_ for means group 2: 0.3059 Number of Observations: 3
y

Appendix III (a)

Least Significant Difference Test

Title: Shoot water potential [Cool & Wet]

Case Range: 36 – 65

Function: RANGE

Error Mean Square = 0.1100
Error Degrees of Freedom = 18
No. of observations to calculate a mean = 3

Least Significant Difference Test
LSD value = 0.5689 at alpha = 0.050

Original Order

Mean	1 =	-4.600	ab
Mean	2 =	-4.867	a
Mean	3 =	-4.400	abc
Mean	4 =	-4.000	cd
Mean	5 =	-3.733	d
Mean	6 =	-4.800	ab
Mean	7 =	-4.400	abc
Mean	8 =	-4.667	ab
Mean	9 =	-4.333	abc
Mean	10 =	-4.267	bcd

Appendix III (b)

Least Significant Difference Test

Shoot water potential [Warm & Wet]

Case Range: 36 - 65
Function: RANGE

Error Mean Square = 0.09200
Error Degrees of Freedom = 18
No. of observations to calculate a mean = 3

Least Significant Difference Test
LSD value = 0.5203 at alpha = 0.050

Original Order

Mean	1 =	-5.733	def
Mean	2 =	-5.533	defg
Mean	3 =	-5.533	defg
Mean	4 =	-5.350	efg
Mean	5 =	-5.367	efg
Mean	6 =	-5.667	def
Mean	7 =	-5.400	efg
Mean	8 =	-5.533	defg
Mean	9 =	-5.867	cde
Mean	10 =	- 5.400	efg

Appendix III (c)

Least Significant Difference Test

Title: Shoot water potential [Dry & Hot]

Case Range: 36 - 65
Function: RANGE

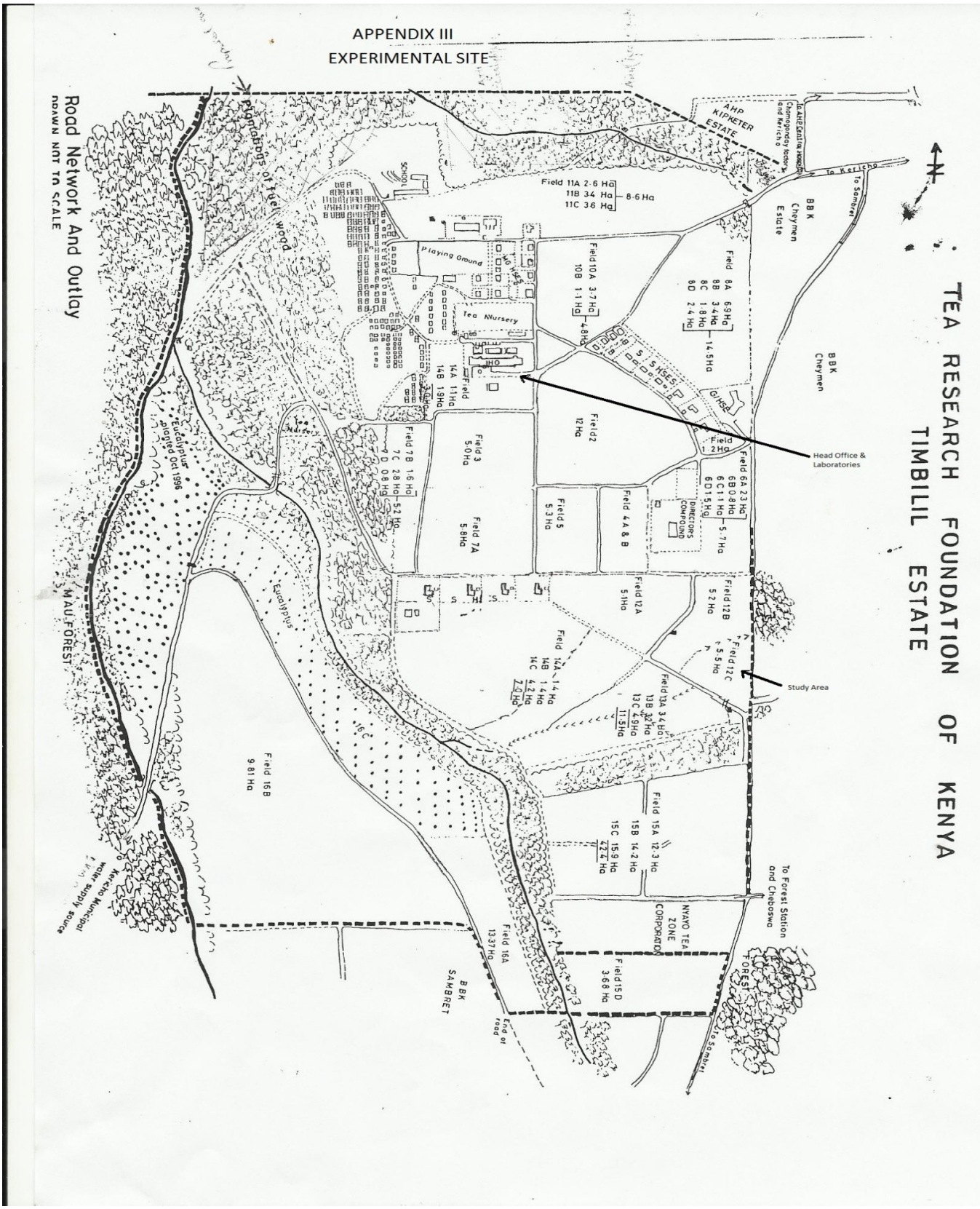
Error Mean Square = 0.2810
Error Degrees of Freedom = 18
No. of observations to calculate a mean = 3

Least Significant Difference Test
LSD value = 0.9093 at alpha = 0.050

Original Order

Mean	1 =	-16.03	e
Mean	2 =	-18.37	ab
Mean	3 =	-18.93	a
Mean	4 =	-17.17	cd
Mean	5 =	-18.73	a
Mean	6 =	-17.47	bc
Mean	7 =	-16.50	de
Mean	8 =	-15.80	e
Mean	9 =	-18.97	a
Mean	10 =	-16.50	de

Appendix IV


APPENDIX III
EXPERIMENTAL SITE
TEA RESEARCH FOUNDATION OF KENYA
TIMBILIL ESTATE
Road Network And Outlay
DRAWN NOT TO SCALE
Plantations of fuel wood
Eucalyptus planted Oct 1996
Eucalyptus
MAU FOREST
To Metric Municipal water supply source
AHP KIPKETER ESTATE
To Kericho
To Sambret
BB K Cheymen Estate
BB K Cheymen
Playing Ground
Tea Nursery
Field 10A 3.7 Ha
10B 1.1 Ha
4.8Ha
Field 8A 6.9Ha
8B 3.4Ha
8C 1.8 Ha
8D 2.4 Ha
14.5Ha
Field 11A 2.6 Ha
11B 3.4 Ha
11C 3.6 Ha
8.6 Ha
Field 14A 1.1Ha
14B 1.9Ha
Field 2 12 Ha
Field 1 2Ha
Head Office & Laboratories
Field 3 5.0Ha
Field 7A 5.8Ha
Field 7B 1.6 Ha
7C 2.8 Ha
7D 0.8 Ha
5.2 Ha
Field 5 5.3 Ha
Field 4A & B
DIRECTORS COMPOUND
Field 6A 2.3 Ha
6B 0.8 Ha
6C 1.1 Ha
6D 1.5 Ha
5.7 Ha
Field 12A 5.1Ha
Field 12B 5.2 Ha
Field 12C 5.5 Ha
Study Area
Field 14A 1.4 Ha
14B 1.4 Ha
14C 4.2 Ha
7.0 Ha
Field 13A 3.4 Ha
13B 3.2 Ha
13C 4.9Ha
11.5Ha
Field 15A 12.3 Ha
15B 14.2 Ha
15C 15.9 Ha
42.4 Ha
Field 16B 9.81 Ha
Field 16A 13.37 Ha
NYAYO TEA ZONE CORPORATION
Field 15D 3.68 Ha
To Forest Station and Cheboswa
FOREST
BBK SAMBRET
End of road
To Sambret

Appendix V

Experimental design and treatments

SEASON I

	REP		
Clone	I	II	III
31/8	13	15	27
15/10	4	29	11
7/9	25	14	3
6/8	8	21	18
12/19	2	9	16
31/11	17	20	24
301/4	6	10	26
303/577	19	1	28

SEASON II

	REP		
Clone	I	II	III
31/8	25	14	3
15/10	19	1	28
7/9	6	10	26
6/8	17	20	24
12/19	8	21	18
31/11	4	29	11
301/4	12	5	22
303/577	30	23	7

SEASON III

	REP		
Clone	I	II	III
31/8	17	20	24
15/10	2	9	16
7/9	12	5	22
6/8	30	23	7
12/19	19	1	28
31/11	25	14	3
301/4	13	15	27
303/577	8	21	18

BBK 35	30	23	7	BBK 35	13	15	27	BBK 35	4	29	11
SFS 150	12	5	22	SFS 150	2	9	16	SFS 150	6	10	26

KEY: 1- 30 (Plot numbers)

Appendix VI

Summary of meteorological observations at TRFK from June 2011 to February, 2012

	Rainfall	Temperatures			Radiation	Vapor pressure deficit	Evaporation monthly totals		Deficit
Month	Mean totals	Max	Min	Mean	Gunn Bellani [MJm-1		Penman Estimate Eo[evapo ration]	ET = 0.85 Eo [Evapot ranspira tion]	Monthly potential soil water deficit
	mm	°C	°C	°C	cm-²/day	MPa	mm	mm	Mm
June	231.1	22.7	9.2	16	18.9	5.81	151	130	0
July	165.5	22.0	8.3	15.2	23.32	7.92	139	118	0
August	169.8	22.7	9.4	16.1	15.92	4.71	130	110	0
September	261.1	23.1	9.1	16.1	17.67	5.77	137	117	0
October	204.8	23.7	9.9	16.8	21.61	7.23	153	130	0
November	503.7	22.4	10.4	16.4	17.72	5.25	121	103	0

December	103.3	23.6	10.5	17.1	25.12	9.46	157	134	30.3
Jan, 2012	0.0	25.7	7.7	16.7	37.53	19.73	179.3	164.14	164.14
Feb, 2012	0.9	27.2	9.0	18.1	31.49	20.68	193.1	155.49	128.69
TOTALS	**1640.2**	**213.1**	**83.5**	**148.5**	**209.28**	**86.56**	**1360.4**	**1161.6**	**323.13**
MEAN	**182.24**	**23.67**	**9.2**	**16.5**	**41.86**	**17.31**	**272.08**	**232.33**	**64.63**