THE INFLUENCE OF SOIL WATER CONTENT AND NITROGEN SUPPLY ON GROWTH, YIELD AND POLYPHENOL CONTENT OF SELECTED TEA [*Camellia sinensis* (L.) O. Kuntze] CLONES IN KENYA

Erick Kimutai Cheruiyot

A thesis submitted to the Graduate School in fulfillment for the requirements of the degree of Doctor of Philosophy in Agronomy (crop physiology) of Egerton University.

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DECLARATION AND RECOMMENDATION

DECLARATION

This thesis is my original work and it has not been previously presented in this or any other University for any degree.

Signature ..... Date 10.6.2008

Erick K. Cheruiyot

RECOMMENDATION

This Thesis has been submitted with our approval as the candidate's supervisors

Signed ..... Date 23/06.2008

Prof. Louis M. Mumera

Signed ..... Date 11th June 2008

Dr. Wilson K. Ng'etich

Signed ..... Date 16th June 2008

Prof. Ahmed Hassanali

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DEDICATION

This work is dedicated to Philip, my late father.

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ABSRACT

Tea is a major foreign exchange earner in Kenya and it accounts for about 24% of the total value of the domestic exports for the last 10 years. The sector employs 10% of Kenya's population directly and indirectly, and contributes to infrastructural development which includes construction of schools and health facilities, rural access roads and rural industries. However, tea suffers frequent droughts with significant yield decline, often accompanied by plant deaths, a condition which seems to be worsened by fertilizer input. The objectives of this work were to determine: i) the critical minimum soil water requirements in selected tea clones and show how it varies with nitrogen (N) supply ii) the effect of N supply and progressive decline of soil water content on tea shoot growth, leaf yield and black tea quality iii) the levels of tea polyphenols and define their association with water stress in tea and their suitability as indicators for drought tolerance. Two experiments were set up in a rain-out shelter at Tea Research Foundation of Kenya (TRFK) and a field experiment conducted at three different field sites in tea growing areas. The first experiment consisted of six different tea clones which were subjected to 4 levels of soil water content (SWC) (38, 30, 22 and 14% v/v) for 12 weeks. The second experiment consisted of clone BBK 35 treated with 5 different rates of N (0, 75, 150, 225 and 300 kg/ha) using NPKS (25-5-5-5) fertilizer material, and 5 levels of SWC (38, 34, 30, 26, 22, 18% v/v). Shoot and leaf growth, dry matter partitioning, photosynthesis, stomatal conductance, transpiration, leaf anatomy and shoot polyphenol content were determined. The field experiment consisted of clone BBK 35 with five rates of N, similar to the rain-out shelter and where shoot population density and yield were determined during dry months. SWC limit for tea was shown to be about 20% v/v and that fertilizer supply increased the susceptibility of tea to drought. Contrary to earlier speculations that fertilizer raises the optimal SWC for tea, it emerged that fertilizer rates above 200 kg N ha^{-1} influenced assimilate partitioning to shoot and foliage, making tea more vulnerable to drought effect. Clones with high total polyphenol content and whose levels varied less with changes in SWC were more tolerant to drought. Two catechin variants, epicatechin and epigallocatechin correlated with SWC, water stress index and shoot growth in tea, which suggests they are potential indicators for drought stress in tea.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABBREVIATIONS

CRD	**Complete randomized design**
E	**Transpiration**
EC	**Epicatechin**
ECG	**Epicatechin gallate**
EGC	**Epigallocatechin**
EGCG	**Epigallocatechin gallate**
g_s	**Stomata conductance**
LMR	**Leaf-to-mass ratio**
LRR	**Leaf-to-root ratio**
N	**Nitrogen**
RWC	**Relative water content**
SWC	**Soil water content**
SWD	**Soil water deficit**
TDR	**Time domain reflectometry**
TRFK	**Tea research foundation of Kenya**
Pn	**Net photosynthesis**
WSI	**Water stress index**

CHAPTER ONE

INTRODUCTION

1.1 Background

Tea [*Camellia sinensis* (L.) O. Kuntze] is among Kenya's leading foreign exchange earner (Republic of Kenya, 2000). It is produced on approximately 141,315 hectares, of which 70% is managed under smallholder units and 30% under large estates (Republic of Kenya, 2001; Tea Board of Kenya, 2005). The crop is principally processed to produce black tea. Annual production is currently estimated at 328,000 tonnes of processed tea, which is valued at about US$ 500 million (Republic of Kenya, 2006). Tea is consumed for its stimulant effect, which has made it an important beverage worldwide. Besides, recent studies have shown that some of the large groups of polyphenols in tea are effective in chemoprevention of various cancers and cardiovascular ailments (Schulz *et al*., 1999).

The global weather change has become unpredictable, creating uncertainty in agricultural production. Though tea crop requires a well-distributed annual rainfall of between 1150-1400 mm and a temperature regime of 18-30^{o}C (Carr, 1971; TRF, 1986), most of the tea growing areas in Kenya experience less than adequate rainfall, which at times is poorly distributed (Jaetzold and Schmidt, 1983). In addition, tea cultivation has expanded rapidly at an annual rate of 3% increase in hectarage over the last ten years (Tea Board of Kenya, 2002). A larger proportion of this expansion arises from the opening up of non-traditional tea growing areas whose major limitation is soil moisture. Besides, most of the tea growing areas experience regular 3-months drought period between November and March, during which potential soil water deficit close to 400 mm has been recorded (Ng'etich and Bore, 1998; Ng'etich, 1999). The tea yield loss

attributable to this drought period is estimated at 14-20%. However, during the more pronounced droughts such as those encountered in Kenya in 1994, 1997 and 2000, yield losses of up to 30% of annual output were reported (Ng'etich 1994, 1997). Therefore soil water deficit is, and will continue to be a major limiting factor in tea production in Kenya. Leaf yield and plant losses during such droughts could be exacerbated by addition of fertilizers (Ng'etich, 1999). Indeed, from casual observation, it has been speculated that drought effects in tea may be coupled to and aggravated by nitrogen nutrition of the plants (Ng'etich, 1999).

1.2 Statement of the problem

There is significant yield loss in tea which range from 14% to 20%, during the annual 3-months dry period and from observations, drought effects seem coupled to and aggravated by nitrogen supply to the plants. The recommended N supply for tea is 150 – 200 kg ha^{-1}, but most framers apply more than the recommended rate, and this raises the fear that this could be contributing to yield reduction and plant death during drought. The soil moisture deficit limit for tea clones is not known and that makes it difficult to synchronize N supply rate with impending soil moisture deficit period to ameliorate drought effects. Besides, specific plant organic compounds associated with drought stress and which could potentially be used to identify cultivars for drought-prone environment are not known.

1.3 Objectives

1.3.1 Broad objective

The broad objective in this study was to determine the influence of N supply regimes and soil water deficit, on the performance of selected tea clones in Kenya.

1.3.2 Specific objectives

Specific objectives were:

a) To determine the critical minimum soil water requirements in selected tea clones and show how it varies with N supply.

b) To determine the effect of N supply and progressive decline of soil water content on tea shoot growth, leaf yield and black tea quality.

c) To quantify the levels of tea polyphenols and define their association with water stress in tea and their suitability as indicators for drought tolerance.

1.4 Hypotheses

The following hypotheses were tested in the study:

i) Tea plants exposed to increasing soil water deficits show decline in shoot growth and the severity of this response is associated with tea polyphenols and clonal variation.

ii) High rates of N fertilizer supplied to tea raise the optimal soil water content for tea and subsequently decrease leaf yield and black tea quality during drought.

1.5 Justification of the study

The tea industry in Kenya employs about 3 million Kenyans, directly or indirectly, which is 10% of the population (Tea board of Kenya, 2005). The crop has also contributed to infrastructural development such as rural industrialization, rural access

roads, construction of schools and health facilities, and generation of electricity by some large tea companies to supplement their power requirement. This has partly curbed rural-urban migration and facilitates the distribution of income to rural areas. Besides, Kenya's economy is agriculture-based and economic growth is significantly influenced by the performance of the agricultural sector. The tea sub-sector is economy's main income earner, contributing 19 – 29% (average, 24%) of the total value of the domestic exports for the last 10 years (Republic of Kenya, 2000). This translates to an annual income of about US$ 500 million to the country. A significant reduction in tea yield and quality therefore adversely affects the livelihood of many Kenyans and offsets the country's economic performance.

1.6 The scope and Limitation

This study determined the response of tea to varying SWC which ranged from near soil field capacity, down to a deficit of about 60% of field capacity. Further investigations focused on tea response to different rates of fertilizer supply under declining SWC and the contribution of fertilizer to drought susceptibility of tea. Shoot polyphenols, leaf anatomy and morphology of some selected tea clones were determined and their suitability as indicators for drought tolerance evaluated. Leaf yield of field grown tea supplied with five different rates of fertilizer was determined during dry months at three different locations in tea growing areas of Kenya.

Though total polyphenols were determined in the selected clones grown under predetermined SWC, quantification of specific flavonoids was limited to catechins because it is considered the major polyphenol in tea. The effect of SWC on polyphenol oxidase (PPO) could not be done after special equipment for determination of the enzyme activity malfunctioned.

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CHAPTER TWO

A REVIEW ON SOIL WATER DEFICIT AND NITROGEN SUPPLY IN TEA [*Camellia sinensis* (L.) O. Kuntze]

Abstract

Tea is an important beverage worldwide. It is second to water as the most consumed liquid, and is rapidly assuming a significant role in human health. Though world tea [*Camellia sinensis* (L.) O. Kuntze] output has increased by about 30% during the last 10 years, its production experiences a great challenge from weather changes, particularly recurring droughts, which significantly reduce leaf yield. Severe drought also causes extensive plant deaths. Field observations indicate that despite the importance of N fertilizer on leaf yield and quality of processed tea, its supply beyond a certain level exacerbates the drought effect. The contribution of fertilizer to drought susceptibility in tea is not well understood. As a measure to minimize drought effect on tea, this chapter reviews reported works on the crop's responses to drought and fertilizer supply and discusses the need to establish the relationship between soil water deficit and fertilizer application. It also explores the role of secondary plant metabolites as indicators for water stress and hypothesizes the potential of catechins as a tool in breeding and selection of clones for drought prone environments.

2.1 Introduction

Tea [*Camellia sinensis* (L.) O. Kuntze] is ranked second after water as the most consumed drink worldwide (Wheeler and Wheeler, 2004). It is produced in over 20 countries with major producers being India, China, Sri Lanka, Kenya, Indonesia and Turkey. World production now stands at 3.2 million tonnes, up from 2.1 million tonnes in 1993 while plantation coverage has risen to some 140 million hectares from 105 during the same period (International Tea Committee, 2003; FAO, 2006).

Tea is produced for its stimulant effect which has made it an important beverage worldwide. It provides an astringent taste that is relaxing with a soothing effect. Besides being a traditional beverage, tea has assumed a more significant role as a health drink. This has been triggered partly by some positive epidemiological studies that have associated consumption of tea with either reduced occurrence or absence of certain illnesses. This positive attribute to tea stems from the fact that tea leaves contain large group of compounds, including polysaccharides, volatile oils, vitamins, minerals, purines, alkaloids and polyphenols. Perhaps the most significant of them are the polyphenols, particularly the catechins. Catechins are secondary plant metabolites which belong to the flavan-3-ol class of flavonoids which make up to 18-30% of the dry weight in green tea (Han *et al*., 2003; Fujii *et al*., 2003; F'guyer *et al*., 2003). The five major variants of catechins identified in tea are gallocatechin (GC), epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) (Fig. 2.1). These catechins have been demonstrated to contribute to the

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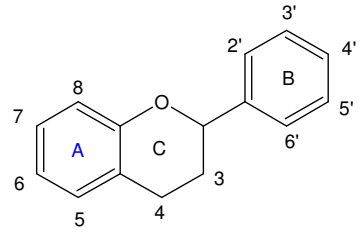
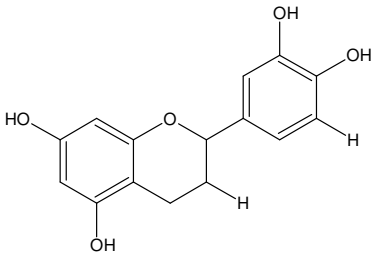


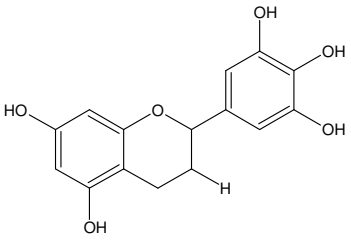
Basic structure of flavonoids

b)



(-)-Epicatechin

c)



(-)-Epigallocatechin

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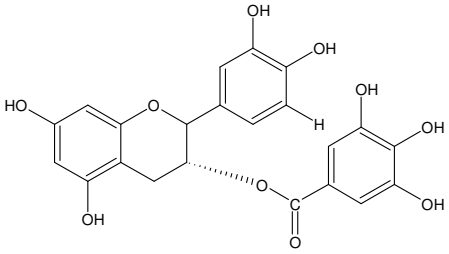
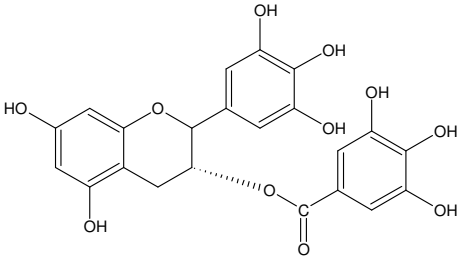


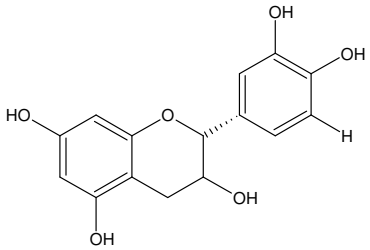
(-)-Epicatechin gallate

e)



(-)-Epigallocatechin gallate

f)



(+)-Catechin

Fig. 2.1 Catechin variants in tea.

antioxidant activities ascribed to tea, which include scavenging for reactive oxygen species such as superoxides, hydroxyl and peroxyl radicals, and inhibition of lipid peroxidation among others (Han *et al*., 2003; Mandel *et al*., 2004; Mahaboob, 2006). EGCG is the most studied and is thought to be a greater antioxidant and more potent than ascorbate (Schijlen *et al*., 2004). Besides, it has been associated with anticancer properties as shown by inhibition of tumor cells (Jung and Lee, 2001; Sato and Matsushima, 2003; Chen *et al*., 2004), treatment of periodontal disease (Yun *et al*., 2004) and decubitus ulcers (Fujii *et al*., 2003). However, though EGCG is thought to be the principal compound with healing effect, some studies seem to suggest a synergistic advantage when used along with EC, EGC and/or ECG (Morre *et al.*, 2003). Tea also contains theanine, an amino acid which is rarely found in other plants, and which has been demonstrated to induce relaxation, lower blood pressure and decrease anxiety, among other benefits (Juneja *et al*., 1999). In addition to healing effect, some study has revealed that one to two cups of tea provides about 200 mg of vitamin C which is equivalent to three glasses of orange juice (Toit *et al*., 2001). Besides, tea seed contains 40-50% oil whose quality is comparable to olive oil and is on demand as cooking oil particularly in China's southern provinces (Ruter, 2002). Other benefits of tea include antibacterial, antiseptic and detoxifying properties which help reduce digestive complaints, and guard against tooth decay due to rich fluoride content in green tea. However, there are also possible health risks associated with tea consumption, including anaemia caused by reduced ability to utilize dietary iron as a result of the antioxidant action of tea polyphenols (Toit *et al*., 2001; Nelson and Poulter, 2004).

2.2 Tea responses to water stress

Tea is a rain-fed perennial crop whose yield is greatly influenced by weather, and particularly drought. Drought effects on tea have been reported in Kenya, Tanzania and Sri lanka (Stephens and Carr, 1989; Wijeratne, 1996; Ng'etich, 1999) though all tea growing areas world wide could also be vulnerable. Soil water deficits not only limit tea yield production but may also lead to plant deaths. Training of young tea in the early stages of plant development facilitates a wide spread of leafy canopy which is required for shoot generation. The canopy so formed is associated with high water loss equated to that from an open surface (Cooper *et al*., 1979), which progressively depletes soil water in the absence of recharge from rain or through irrigation. Plant water balance is further aggravated by high environmental temperatures that usually accompany drought. Growth and obviously shoot production is limited by inability of tea plants to satisfy their internal assimilate demand as occasioned by inadequate water supply during drought. Worse still, plant deaths may occur with intensity and persistence of drought which seems accelerated by opportunistic fungal infection (Burgess and Carr, 1993) and/or large fertilizer rates (Ng'etich, 1999).

2.2.1 Processes that may limit water uptake by plants

It has been hypothesized that drought effects on tea yield and plant survival may be coupled to and aggravated by nitrogen nutrition (Ng'etich, 1999). The decline in yields in tea supplied with large amounts of N fertilizer compared to tea receiving less or no fertilizer under moisture stress suggests difficulty in water uptake by plants as N supply rate increases (Ng'etich, 1999). The effect has also been reported in tomato (*Lycopersicon esculentum* Mill.cv.UC82B) plants (Stirzaker and Passioura, 1996). The effect may be attributed to differences in solute concentration in tea plant rhizosphere. Solute concentration in the rhizosphere creates soil-root interfacial resistance to water movement into the root due to high osmotic pressure. However, osmotic pressure of soil solution between well-fertilized and moderately fertilized tomato was comparatively too small to account for a large difference in root hydraulic resistance in the two systems. Besides, cell walls of roots are a network of cellulose, hemicellulose and glycoproteins and contain about 5.0 nm pores which would offer less resistance to water flow. The diameters of some hydrated ions are 0.66 nm for K^+ and 0.82 nm for Ca^{2+}. The movement of ions into the apoplast of the root is a passive process. It is also known that the continuum of cell wall has carboxylic groups ($RCOO^-$), which act as cation exchangers (Marschner, 1995). Therefore, cations from root rhizosphere can accumulate in a non-metabolic step in root apoplast thereby favouring a rise in osmotic pressure inside rather than outside the root. As observed in the tomato plant, soil osmotic pressure in a fertilizer-supplied system does not seem to justify the discrepancy in the inhibition of water uptake by the plant. The indication that well fertilized plants experience difficulty in uptake of water compared to moderately or less fertilized ones suggests involvement of

other processes, particularly physico-chemical peculiarities of soil-root interface (Stirzaker and Passioura, 1996) or biochemical processes related to N metabolism (Stewart, 1991; Smith and Woods, 1992; Vance, 1997) and water uptake by plants. The resistance to water movement from soil to the root can also be caused by root shrinkage under dry conditions. It has been shown that root diameter may decrease by 50% or more under water stress conditions thereby creating an air gap between root and soil (Ridge, 1991). However, root hairs are known to exude sticky mucilage that ensures intimate contact with soil particles and, therefore, an air gap following root shrinkage is unlikely.

In addition to the soil-root interfacial influence on water uptake, nitrogen metabolism can indirectly influence plant physiological and biochemical processes that facilitate water uptake. Nitrogen is taken up by plants as NO_3^- and NH_4^+ and reduced to NH_3 before being incorporated into amino acids to form amides and proteins. Because NH_3 is highly toxic to the plant, it is coupled to glutamate, which is the primary acceptor molecule facilitating conversion to glutamine. The incorporation of NH_3 and glutamate to form glutamine is catalyzed by glutamine synthetase. This enzyme has high affinity for NH_3 and is usually activated by high pH, high concentration of magnesium and is ATP-dependant. Glutamate is crucial in the detoxification of ammonia, without which, ammonia will accumulate and may lead to uncoupling of photophosphorylation and loss of cell membrane integrity (Stewart, 1991; Smith and Wood, 1992; Marschner, 1995; Vance, 1997). Nitrogen being supplied to tea in Kenya is in NH_4^+ and NO_3^- forms. There are only three N fertilizer formulations recommended for tea in Kenya, which are: NPKS, 25-5-5-5; NPK, 25-5-5; and NPK, 20-10-10 (Owuor *et al*., 1994b). Their respective N analysis is given as 14% NH_4^+ -N + 11% NO_3^- -N for the first two and 12% NH_4^+ -N +

8% NO_3^- -N for the NPK (20-10-10) and these compares closely to 21% NH_4^+ in Sulphate of Ammonia (TRF, 2000). Calcium ammonium nitrate (CAN) is not used because of the tendency of calcium to raise soil pH beyond the optimum range of 4.5 – 5.6 (Owuor, 1997). Ammonia toxicity was earlier speculated to be a factor for tea growing under shade when compared with unshaded tea plants, both supplied with sulphate of ammonia (21% NH_4^+) (Othieno, 1981). Under high input of N fertilizer, there are chances of ammonia toxicity, and such processes as transpiration, photosynthesis and plant biochemical processes that sustain water uptake by plants may significantly reduce therefore leading to the apparent difficulty in water uptake by the plants. Ammonia toxicity may therefore be one of the likely causes of the observed difficulty in water uptake in tea supplied with large amounts of N fertilizer. However, this needs to be confirmed.

2.2.2 Potential use of secondary metabolites to indicate drought tolerance in tea

Plants under stress synthesize and accumulate certain organic compounds, among them sugars and sugar alcohols, betaines, proline and its analogues (Naidu, 1998) which serve to minimize stress conditions in the plants. Under water stress, abscisic acid (ABA) level is widely known to increase in both roots and leaves (Westgate *et al*., 1996; Jia and Zhang, 1999). Declining soil moisture triggers ABA and its presence in the leaves is linked to stomatal closure, which serves to minimize further water loss in the plant. In addition to ABA, other compounds associated with water stress include proline, ethylene, glycinebetaine and sucrose. Proline has been found to be the predominant osmolyte under water stress whereas glycinebetaine and sucrose are mainly associated with salt-stressed plants (Sabry *et al*., 1995). These stress related compounds are present in low

concentration but their levels usually rise to some extent during stress conditions as plant response mechanism to alleviate such conditions. Such chemicals may be useful indicators of the extent to which plants can tolerate water stress and thereby aid in selection for drought tolerance. Some of the secondary metabolites, proteins and genes have been used as markers in defining plant defense and response to biotic and abiotic stresses (Hare *et al*., 1998; Winkel-Shirley, 2001; Jwa *et al*., 2006).

Flavonoids are a group of secondary metabolites with low molecular weight in the large family of plant phenols. They are widespread in the plant kingdom and are found in leaves, bark, flower, fruit and seed. Significant roles of flavonoids in the plant system include protection against the deleterious ultraviolet radiation; provision of flower; fruit and seed pigmentation to attract pollinators and seed dispersers; defence against pathogenic micro-organisms; protection from herbivores; mediation of signal transduction in plant-microbe interaction, and facilitation of plant fertility and germination of pollen (Heim *et al*., 2002; Schijlen *et al*., 2004). Perhaps a greater attention has been focused on the contribution of the flavonoid polyphenols to humans at the expense of their natural role in plant survival and productivity. It is not surprising that the number of identified derivatives of flavonoids rose to 6000 in 2004 compared to 4000 in 2002 (Heim *et al*., 2002; Ren *et al*., 2003; Schijlen *et al*., 2004), for it partly demonstrates a growing interest in plant flavonoids and their polymeric derivatives in matters related to human nutrition and health.

The precursors of most flavonoids are malonyl-CoA, derived from carbohydrate metabolism and *p*-coumaroyl-CoA, derived from the phenylpropanoid pathway (Fig. 2.2) (Magoma *et al.*, 2000; Schijlen *et al.*, 2004; Wheeler and Wheeler, 2004). They are

primary plant metabolites whose syntheses are dependent on CO_2 and sugars produced by plants in the presence of light and soil water (Stewart *et al*., 2001). Flavonoid biosynthesis is dependent on two distinct but functionally interrelated classes of genes broadly referred to as structural and regulatory genes (Quattrocchio *et al*., 1993; Winkel-Shirley, 2001; Park *et al*., 2004; Schijlen *et al*., 2004). The structural genes encode enzymes responsible for synthesis of different derivatives of flavonoids while regulatory genes control expression of the structural genes (Schijlen *et al*., 2004). It therefore implies that availability and quantity of certain flavonoid derivatives in plant tissue is not accidental but an indication of plant response to stimuli prompted by either an internal or external factor.

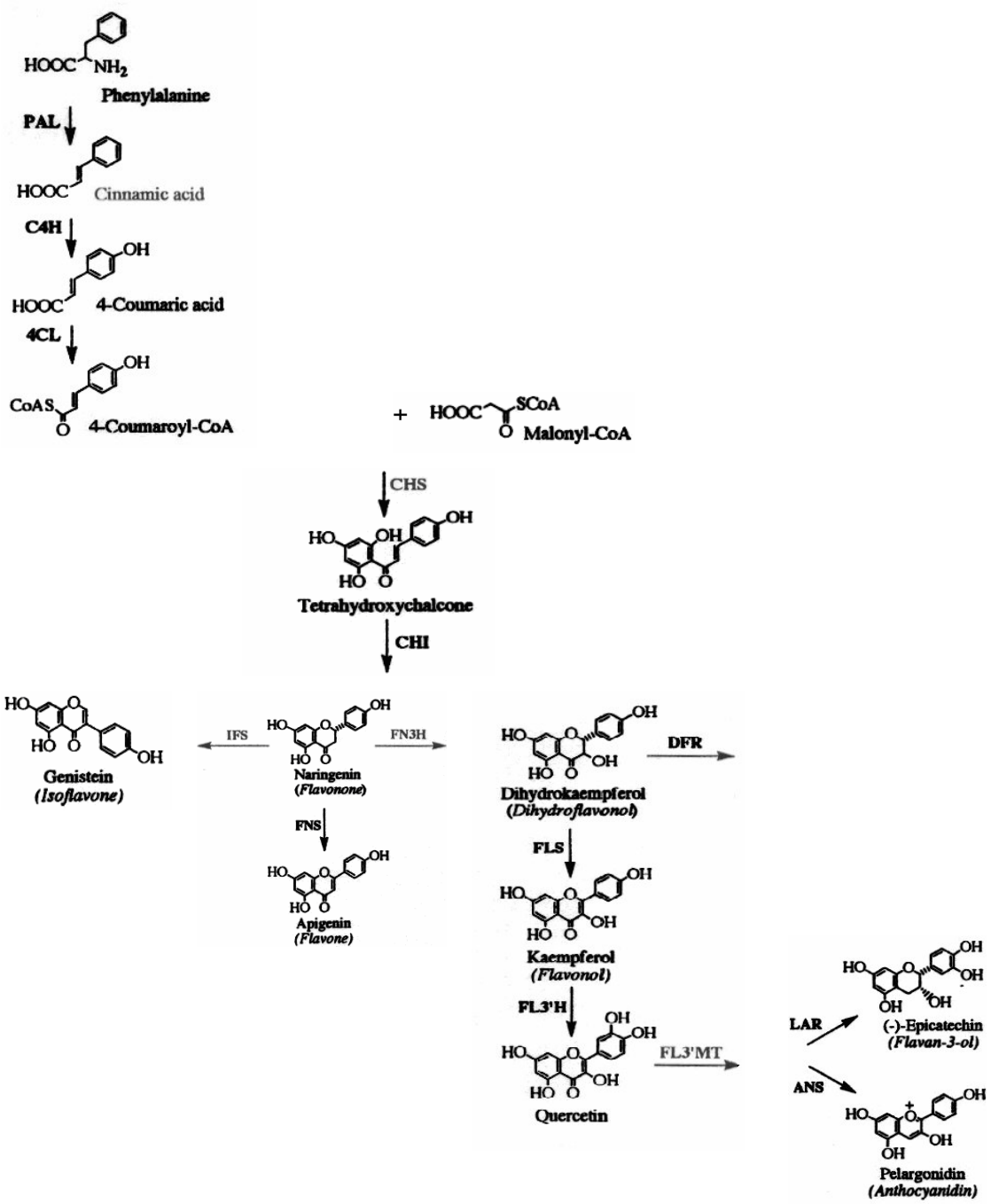


Fig. 2.2 Flavonoid biosynthesis pathway. Enzyme abbreviation: PAL, phenylalanine ammonia-lyase; C4H, cinnamate-4-hydroxylase; 4CL, 4-coumarate:CoA ligase; CHS, chalcone synthase; CHI, chalcone isomerase; IFS, 2-hydroxyisoflavanone synthase; FNS, flavone synthase; FN3H, flavanone 3-hydroxylase; DFR, dihydroflavonol reductase; LAR, leucoanthocyanidin-4-reductase; ANS, anthocyanin synthase; FLS, flavonol synthase; FL3'H, flavonol-3'-hydroxylase; FL3'MT, flavonol-3'-methylase.

The benefits of *Camellia sinensis* as discussed are associated with its rich organic compounds, most of which are secondary metabolites. Plant biosynthates have essential function in plant organisms and their basic role is plant survival. Some are produced in response to abiotic and biotic stress factors (Wan *et al*., 2002; Dixon *et al*., 2002; Fritz *et al*., 2006), to ameliorate the overall effect of stress on the plant. As has been observed in some plants, productions of certain secondary metabolites are themselves signals of external stimuli at molecular level, before manifestations are visible. For instance, increased biosynthesis of phytoalexin medicarpin in alfalfa has been observed in response to fungal attack (He and Dixon, 2000). Phenypropanoid products which include flavonoids, isoflavonoids and stilbenes are derived from deamination of phenyalanine by phenylalanine ammonia-lyase (PAL). Specific enzymes are involved in production of individual derivatives in the pathway which are encoded by specific genes (Winkel-Shirley, 1999; Wan *et al*., 2002). Production of stress-related metabolites is therefore modulated by genes whose function may include transcription of specific enzymes or encoding of specific receptors that regulate other effector genes. This stress-related biosynthesis response in plants could provide leads to plant improvement in agriculture. An understanding of flavonoid dynamics in the plant system could provide information for plant genetic manipulation and crop management strategies that would improve crop value. Isolation of the source may provide an opportunity to increase the by-product by either enhanced expression of biosynthesis enzyme activity or through introduction of accumulation mechanisms via metabolic engineering (Yazaki, 2005).

In the case of drought tolerance, elucidation of secondary metabolites associated with drought could be a key to developing crop cultivars for dry environments. It has

been observed that though drought-tolerance genes are present in plants, they are often poorly expressed during stress (Moffat, 2002). These therefore suggest the need to identify and possibly manipulate the biosynthesis signaling pathway to trigger the potentially protective genes into action. However, against the above background, there are no known attempts to use the rich tea polyphenols to address the challenges facing the crop, one of which is recurring drought. Similarly, no known attempt has been reported on use of flavonoid as a tool for plant selection or crop management. There is need to screen the various polyphenols in tea clones and evaluate their correlation to quantifiable traits such as leaf yield, response to fertilizers, but more importantly, tolerance to moisture stress or other stress inducing factors. An understanding of flavonoid dynamics in the plant system could provide information for plant genetic manipulation and crop management strategies that would improve crop value. *Camellia sinensis* is rich in catechins, the flavon-3-ol class of flavonoids (Heim *et al*., 2002), most of which have been used as parameters for tea quality, yet none of which has been linked to plant responses to diverse environmental growing conditions among them soil moisture regime and nutritional management.

2.3 Nitrogen nutrition in tea

Nitrogen (N) nutrition enhances yield in tea given favourable conditions of temperature, relative humidity, rainfall and evaporation. The crop responds to and favourably withstands large amounts of N supply. Surprisingly, favourable yields ranging from 5800 to 6400 kg made tea (mt.) ha^{-1} per year in high yielding clones have been obtained with increasing N supply of upto 600 kg ha^{-1} per year without adverse effects to the plant (Owuor, 1992; Owuor *et al*., 1994b; Owuor and Wanyoko, 1996; Owuor 1997). The incredible response of the tea plant to heavy application of nitrogen fertilizers is probably stimulated by frequent shoot harvest, which is estimated to contain 4% N (Willson, 1975). Nitrogen plays an indirect role in photosynthesis. Besides being a component of chlorophyll, N is also a major constituent of phosphoenolpyruvate carboxylase (PEPCase) and ribulose-1,5-bisphosphate carboxylase (Rubisco), both of which are catalytic enzymes in CO_2 fixation (Hall & Rao, 1995; Ranjith *et al*., 1995). In addition to the crucial role in photosynthesis, N is a substrate in amino acid and protein synthesis.

Given the response of tea to high rates of N, there has been an attempt to apply the fertilizer in splits to avert any detrimental effects of such high doses to the plant. However, split application of N fertilizer has not shown any improvement in yield and in quality of black tea, and neither has single dose application shown any severity to the plants (Owuor, 1992). It therefore makes economic sense to apply N as single dose. The implication of the heavy fertilizer input on soil chemical characteristics and ecosystem status as well as water quality in tea zones is, however, yet to be established for Kenyan soils.

Besides leaf yield, tea quality is significantly influenced by nitrogen supply. The quality of green tea progressively increases with N supply and producers of green tea for instance in Japan, are known to apply up to 1,200 kg N ha^{-1} per year to maximize on quality (Owuor, 1997). That is because increasing N supply in tea raises the levels of amino acids and fatty acids (Owuor, 1997) which are desirable for the flavour component of green tea quality. However, black tea quality significantly declines with increase in N supply in some aspects including flavour quality. Though the optimum N supply for maximum tea yield in Kenya have been shown to be 560 kg N ha^{-1} yr^{-1}, this rate produces poor quality of black tea (Owuor *et al*., 1994b). Increased N supply is thought to reduce catechin levels in tea shoots, leading to low formation of theaflavins and thearubigins which are critical parameters of black tea quality (Owuor *et al*., 1994a). The effect of N application on the activity of polyphenol oxidase enzyme which catalyzes the oxidation of catechins to theaflavins and thearubigins is, however, not known. High N has also been demonstrated to increase the levels of unsaturated fatty acids and also to reduce the ratio of the Group II: Group I volatile favour compounds (VFC) (Owuor, 1989; Watenabe, 1995), all of which depress black tea quality. The recommended optimum N rate which compromises for better yield and quality of black tea is therefore within the range of 100 – 200 kg N ha^{-1} $year^{-1}$ (Owuor, 1997).

2.4 Nitrogen and moisture stress on yield and quality of black tea

Tea yield reduction and, in severe cases, deaths of plants have been reported in plants supplied with nitrogen, against those without or with less nitrogen fertilizer (Ng'etich, 1999). Preliminary work on N effect in tea yield indicates considerable yield loss ranging

from 14-40% in tea receiving in excess of 200 N ha^{-1} during the 3-months dry period, normally between November and March in tea growing areas of Kenya (Jaetzold and Schmidt, 1983). The decline in yield during this period was highest in tea supplied with large amounts of N (Ng'etich, 1999). This suggests that nitrogen supply may accentuate moisture stress through inhibition of water uptake by plants as indicated by Stirzaker and Passoura (1996).

The minimum soil water requirement for different tea clones has not yet been established. If the data were available and with the knowledge of annual weather changes, it would be possible to project the soil water deficits for the subsequent seasons and adjust N supply for optimum production of tea during the dry season. Moreover, the response of different tea clones to the soil water deficits may provide basis for plant selection for drought tolerance. In this context, it is expected that different tea clones and cultivars express distinct responses to soil water deficit that can be linked to identifiable physiological and morphological plant characteristics, which if established, can serve as indicators for drought tolerance.

Extensive studies have been conducted to define factors influencing quality of black tea (Orchard and Owuor, 1989; Owuor, 1989; Owuor, 1992; Owuor and Obanda, 1993). Though the studies have mainly concentrated on N as a major factor in the quality of black tea, other factors have also been highlighted. These include the influence of altitude of the growing zone, plucking standards, plucking interval, pruning, and manufacturing processes. For instance, tea grown at high altitudes are of higher quality than teas grown at lower altitude (Owuor, 1989); plucking 2 leaves and a bud gives better black tea quality than plucking 3 leaves or more; shorter plucking intervals give good quality black

tea (Owuor, 1989); and shoots from recently pruned bushes produce black tea of low quality though this improves as the next pruning cycle approaches (Owuor and Langat, 1988). Very little attention has been given to the likely interaction between N and soil moisture on black tea quality and tea yields. For instance, it is debatable whether high N supply and decreased soil water content can reduce tea leaf catechin levels and/or polyphenol oxidase (PPO) activity and whether that change can explain the decline in black tea quality. Similarly, it is not clear whether the reduced water uptake as a result of high N application would also influence catechin formation and PPO activity levels.

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CHAPTER THREE

EFFECT OF SOIL WATER CONTENT ON GROWTH AND POLYPHENOL CONTENT IN TEA CULTIVARS

Abstract

An experiment was conducted to determine the soil water content (SWC) limit for tea, the association of leaf anatomical characteristics and tea polyphenols with water stress and their suitability as indicators for drought tolerance. The experiment was conducted in a rain-out shelter at Tea Research Foundation of Kenya and consisted of six tea clones (BBK 35, TRFK 6/8, TRFK 76/1, TRFK 395/2, TRFK 31/30 and TRFK 311/287) and four levels of soil water contents (38, 30, 22 and 14% v/v) which were maintained for a period of 12 weeks. The treatments were arranged in a complete randomized design and replicated three times. Shoot and leaf growth were monitored weekly for six weeks, and other measurements included relative leaf water content (RWC), shoot water potential, photosynthesis, dry matter partitioning, leaf anatomy and total polyphenols. Plant growth was monitored over six weeks and a water stress index calculated to determine water stress tolerant clones. Results indicate that declining SWC and hence water stress level reduced growth and limited physiological responses in tea. Shoot growth, photosynthesis and transpiration showed a break-point at 28% v/v while leaf expansion, RWC and shoot water potential was limited at 20-18% v/v SWC. It emerged that the SWC limit for tea is 20% v/v. Clones TRFK 6/8, TRFK 31/30 and TRFK 311/287 were more tolerant to soil water stress than the clones TRFK 76/1, TRFK 395/2 and BBK 35. Tolerant clones maintained high polyphenol content at low SWC and also showed less fluctuation in phenolics when subjected to changes in SWC. There was

significant (P<0.001) correlation between phenolic content and shoot growth of tea. The results demonstrate the potential use of polyphenols as indicators for selection of drought tolerant tea cultivars.

3.1 Introduction

Tea (*Camellia sinensis* L.) is grown as a rain fed perennial crop in Kenya. The crop requires a minimum of 1150 mm of rainfall annually, though an evenly distributed rainfall range of 1150 – 1400 mm is usually considered adequate (Carr, 1972). Although most tea growing areas achieve the minimum rainfall amount, they experience a 3-4 months of dry spell between November and February annually. Besides, there has been a progressive expansion of about 3% annually in the acreage of tea production over the last 10 years (Tea Board of Kenya 2002 and 2004). Most of this expansion has involved the introduction of tea in non-traditional tea growing areas which receive less than optimal rainfall amounts. In addition, frequent droughts have become a common occurrence, for instance, there were 4 major droughts between 1995 and 2004 during which tea yields substantially declined and many unquantified plant deaths occurred. Besides the direct contribution of drought to plant deaths, declining soil water content enhances susceptibility of tea to *Phomopsis theae* which is favoured by reduced tissue water in the bark (Burgess and Carr, 1993).

One of the strategies to reduce crop losses and minimize plant death due to drought conditions is to develop tea cultivars that can withstand relatively low soil water content with adequate productivity. It is therefore important to first define the threshold soil water content (SWC) limit for the existing tea clones and identify individual clones that can be productive at close to the threshold limit. This should be followed by identifying key

plant indicators for desiccation tolerance in selection of tea clones among the existing pool and development of new drought resistant tea clones for drought-prone environments.

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) in response to stress factors. Though these organic compounds are species-specific, their role is not clearly defined but it is generally accepted that they contribute to ameliorating stress in plants (Hare *et al*., 1998; Sarker *et al*., 2004; Slama *et al*., 2006). Most of the stress related organic compounds are secondary plant metabolites and incidentally, tea contains large amounts of polyphenols particularly of the flavonol class. The derivatives of flavonol play a key role in quality determination in black tea (Singh *et al*., 1999) and in fruits (Stewart *et al*., 2001). However, the role of these phenolics in drought stress and their suitability as indicators of desiccation tolerance in tea has not been explored.

Beside secondary metabolites, plant morphology and anatomy have shown adaptive advantage to environmental conditions (Chartzoulakis *et al*., 1999; Aasamaa *et al*., 2001; DaMatta, 2004). Recent studies on tea leaf morphology have shown reasonable variation in stomata counts (number per unit area) and specific leaf mass in clones within and between genotypes of different ploidy levels (Ng'etich and Wachira, 2003). Leaf morphology and anatomy could play a key role in regulating plant tissue water balance and synthesis of photo assimilates during periods of soil water deficits. Linking these characteristics to plant water relations and performance in the tea plant could present a tool for clonal selection and management in water deficit environments.

The objective of this study was to i) determine the critical minimum soil water requirements in selected tea clones and show how it varies with N supply ii) determine the effect of N supply and progressive decline of soil water content on tea shoot growth, leaf yield and black tea quality iii) quantify the levels of tea polyphenols and define their association with water stress in tea and their suitability as indicators for drought tolerance. It was hypothesized that tea plants exposed to increasing soil water deficits show decline in shoot growth and the severity of this response is associated with tea polyphenols and clonal variation.

3.2 Materials and Methods

3.2.1 Set-up of rain-out shelter

A rain-out shelter was erected on 6th November 2003. The structure measured 17 m by 6.5 m ground space with a height of 2.5 m on an East-West orientation. The roof was raised and curved to give a dome-shape with a radius of 0.5m above the 2.5m height and an extended eve of 0.3 m all round (Plate 3.1). The roof design was to facilitate flow of rain water out of the structure and enhance uniform distribution of solar radiation inside. The roof was covered with an ultra violet-treated 200-micron film clear polythene sheet (Sunselector AD – IR 504) designed to transmit 82% of photo synthetically active radiation (PAR), 65% of diffused light and with 88% thermicity. The sides were covered with the same polythene but to 1 m height from ground level on the length sides and to 2.5 m on the widths. The 1.5 m uncovered space along the lengths was to allow free air flow in and out of the structure. A door measuring 1 m wide and 2 m high was made in

the middle of the westwards-facing width using wooden frames and covered with chicken wire and no polythene.

Plate 3.1 Complete structure of the rainout shelter.

Plate 3.2 Inside of the completed rain-out shelter.

3.2.2 Study materials and treatment application

i) Plant materials

Six tea clones of diverse ploidy levels were used in this study. They were clones; BBK 35 (2n), TRFK 6/8 (2n), TRFK 76/1 (3n), TRFK 395/2 (3n), TRFK 31/30 (4n) and TRFK 311/287 (4n). These clones were selected as representative of tea germplasm in Kenya. The selected diploids are commercially grown while the triploids and the tetraploids have potential traits for tea improvement purposes. These clones were raised from stem cuttings in October 2003 in seedling nursery as normally done for vegetative propagated tea (TRFK, 1986) and the plants were ready for transplanting in May 2004. The seedlings were transplanted into 1000 gauge black polythene tubes measuring 0.3 m in diameter and 0.3 m depth. Each clone had 24 potted seedlings giving a total of 144 which were later arranged into 72 experimental units.

ii) Soil medium and soil water measurement

Soil medium for raising seedlings from stem cuttings in the nursery was obtained from a forest area. The same soil medium was used for raising transplanted seedlings in the polythene tubes. Analytical status of the soil was as follows; 3.5% N, 0.16% P, 169 ppm K, 255 ppm Ca, pH 4.3, organic matter 9.3%. Its textural class was clay with 25% sand, 67% clay and 8% silt.

Soil water content was determined and maintained using a time-domain reflectometry (TDR) soil moisture meter (Trime FM-2, Eijkelkamp Agrisearch Equipment) with the 50 mm 2-rod probe. Soil water content (% v/v) was maintained within the ± 2% of the

prescribed level. Measurement was done daily at 9.00 and 14.00 hours during the 12-week period of the study.

Plate 3.3 (a) Trime FM2 soil moisture meter.

Plate 3.3 (b) Soil moisture meter probe used in the study.

iii) Arrangement and application of treatment

This study consisted of two factors in factorial combination: varying soil water content and six tea clones as follows;

W1C1	W2C1	W3C1	W4C1
W1C2	W2C2	W3C2	W4C2
W1C3	W2C3	W3C3	W4C3
W1C4	W2C4	W3C4	W4C4
W1C5	W2C5	W3C5	W4C5
W1C6	W2C6	W3C6	W4C6

Where;

W1, W2, W3, and W4 were soil water content levels: 14%, 22%, 30%, 34% and 38% v/v, respectively.
C1, C2, C3, C4, C5, and C6 were tea clones: BBK 35, TRFK 6/8, TRFK 76/1, TRFK 395/2, TRFK 31/30, and TRFK 311/287, respectively.

The six clones listed above were subjected to four soil water levels (38, 30, 22 and 14%) on a per volume basis, giving a total of 24 treatments which were arranged in a completely randomized design and replicated three times. Each experimental unit had two potted plants and this gave a total of 72 experimental units. Potted tea plants were allowed to establish for 2 months in the tea nursery prior to start of the study. The plants

were watered uniformly to field capacity twice a day during the first two weeks after which watering was progressively reduced on weekly basis. Each experimental unit was maintained at pre-determined soil water content and stabilized for a week before study commenced.

Plate 3.4 Plant arrangement inside the rain-out shelter in the experiment.
The larger tags indicate plot/ experimental unit numbers while the small tags marked the shoots and leaf for periodic measurements

iv) Growing conditions in the rain-out shelter

Growing conditions of tea in the rain-out shelter was monitored using an automatic weather station. The components used in the weather determination set up were a pyranometer (LiCor, USA), an anemometer, copper thermocouples which were connected to CR10 micro-logger (Campell Scientific) for measurements of wet and dry bulb, and soil temperature. All data were stored in data logger which was programmed to take data on 30 minutes interval but stored hourly averages. The data was off-loaded every 2 weeks using a portable computer.

3.2.3 Growth measurements

Tea response to soil water content was largely determined through shoot growth and leaf expansion. Clonal assimilate partitioning as influenced by soil water content was broadly determined by dry matter partition to stem, leaves and root sinks. Growth measurement was initiated after establishment of the plants in the rain out shelter. Leaf and shoot growth were monitored periodically for 4 and 8 weeks, respectively while destructive sampling was done on the 12^{th} week.

i) Shoot growth

Growth in tea was monitored by shoot extension. Two shoots per experimental unit were marked for subsequent weekly measurements that lasted six weeks. One shoot was selected at random in each of the two plants at each experimental unit. The selected shoots were tagged on the stem below the first opened leaf. Initial shoot length was measured from node below the tag to the upper most visible node. Subsequent

measurements were done on the same day every week for six consecutive weeks. Shoot growth was calculated on the basis of increase in shoot length (*l*) over time (*t*).

ii) Leaf expansion

Leaf expansion was determined based on increase in leaf area over time. First opened leaf was randomly selected from one shoot in each of the two plants in each experimental unit. The selected leaves were loosely tagged at the petiole and labelled as L1 and L2 respectively for every experimental unit. The area of each leaf was determined by measuring leaf length from the base of the leaf to the apex (*l*) and broadest part (*w*) and product of these two was multiplied by a factor (*k*) given as 0.62 in tea (Ng'etich and Wachira, 1992). Leaf area measurement on marked leaves was repeated the same day every week for four consecutive weeks. Incremental leaf area which was a measure of leaf growth and which was influenced by SWC and tea cultivar, was obtained by determining the difference between two successive weekly interval measurements (Causton and Venus, 1981).

iii) Dry matter partitioning

Destructive sampling to determine dry matter partitioned to different plant separates was done during the 12th week of water treatment, a time-range similar to the three months of drought commonly experienced in the tea growing zones in Kenya. One of the two potted plants in each experimental unit was used. Each potted plant was soaked by immersing the root section in a bucket of water for 10 minutes. The soaked plant was removed and placed on a polythene mat which was spread outside on grass. The shoot was cut at the crown level and all leaves were removed leaving stem and branches only. The leaves were placed in labelled paper bags while stem and branches were cut into small pieces of 8-10 cm length and placed in another labelled paper bag. The polythene tube carrying the soaked root section was cut open and water let-in through a hose pipe to run over it slowly to wash out soil. When most of the soil was removed, the roots were placed in a 2 mm mesh grid and water pressure increased to remove soil particles attached to roots and give clean roots. Care was taken to retain all roots including tiny feeder roots. After washing, the clean roots were left briefly in the sun to drain and dry off most of the water before putting them in a third labelled paper bag. The three different parts of each plant in 3 separate paper bags were dried at 70^{o}C in a drying oven to constant weight and the dry weight taken. Shoot to root ratio, leaf to root and leaf to total mass were calculated using dry weights of the different separates (Poorter and Nagel, 2000).

3.2.4 Plant water relation

i) Shoot water potential

Shoot water potential was determined six weeks after application of water treatment. It was determined using a portable plant water console (Model 3005, Soil Moisture Equipment Corp. Santa Barbara, California, USA) between 13.00 and 15.00 hours. Shoots with 3 leaves and a bud were randomly sampled in each experimental unit. A clean slanted cut was made and the cut end inserted through the hole in the leaf chamber lid. The lid was fitted and clumped in place. Pressurized nitrogen gas was released from the cylinder by turning on the inlet valve. The release valve was switched on to allow pressure build-up in the leaf chamber which pressurized leaf tissue and tissue water released through the cut surface. As soon as a water bubble was noted on the cut surface, the release valve was switched off and pressure reading on the pressure gauge was taken in bars. Each reading was an indication of the amount of pressure required by the plant to release water in its tissue (Liu and Stutzel, 2002).

ii) Leaf relative water content

The third leaf was randomly picked among shoots in each experimental unit. The leaves were detached from the stem and their fresh weight ($fr._{wt}$) taken immediately using an electric balance with accuracy of 2 decimal points of a gram. The weighed leaves were each put in a labeled polythene sleeve where distilled water was added to immerse the leaf. They were stacked in a plastic tray and placed in a fridge at $4^{o}C$ for 24 hours. After 24 hours, the leaves were removed from the fridge and each leaf blotted dry with a tissue paper to remove water on the surface and their turgid weight taken ($t._{wt}$). Each of the

weighed leaf was placed in a labeled paper bag and oven dried at 70^{o}C to constant weight (which was attained in less than 24 hours) and thereafter, dry weight was taken (d_{wt}). The leaf RWC was then calculated using the formula;

RWC = ($fr._{wt} – d_{wt}$/($t_{wt} – d_{wt}$) (Milnes *et al*., 1998).

3.2.5 Leaf morphology and anatomy

i) Leaf stomata density

Leaf sampling was done two months after end of experiment. A total of five leaves picked from 2nd leaf were sampled in each of the six clones. Sampling was done at 9 am and the leaves soaked in distilled water in 50 ml sampling bottles and taken to the laboratory. Each leaf was wiped dry and colourless nail polish applied on the lower surface then left for about 10 minutes. A cell layer on the underside of leaf was then peeled off and mounted on a microscope slide. This was viewed under a microscope (x25) and stomata counted within a calibrated graticule in the eye piece (Ng'etich and Wachira, 2003).

ii) Leaf area and specific leaf area

The third mature leaf was used in this measurement. Two leaves were randomly picked and measured in two directions; from leaf base to apex (*l*) and the broadest width (*w*) of the leaf blade. The product (*l*x*w*) was multiplied by a known constant (*k*) which is 0.62 (Ng'etich and Wachira, 1992) to give leaf area. The measured leaf was dried at 70^{o}C in a drying oven and dry weight taken. The specific leaf area (SLA) or the area to mass

ratio was obtained by dividing leaf area by dry mass of the leave (Younis *et al*., 2000; Ng'etich and Wachira, 2003; Liu and Stutzel, 2004).

3.2.6 Photosynthetic measurements

CO_2 assimilation, stomata conductance and transpiration

Measurements on photosynthesis (Pn), stomata conductance (g_s) and transpiration (E) were determined on a 2nd fully opened tea leaf using an LCA 3 portable infrared gas analyser (Analytical Development Company Bioscientific, Hoddesdon UK). The measurements were taken between 11.00 and 14.00 hours (Hunt, 2003).

3.2.7 Water stress index

The water stress index (WSI) of the clones was calculated based on shoot growth. The ratio of mean sum of growth rate at the soil water content level to the optimum growth attained by the clone in each of the SWC treatments was determined, and the following formular used:$WSI = (Y_{actual} / Y_{max})$. WSI, is calculated water stress index; Y_{actual}, is shoot growth at a given soil water content (shoot growth under stress); Y_{max}, is maximum shoot growth attained by the clone within the SWC treatments (assumption: optimum growth attained in the absence of soil water stress).

The formular was used by Younis *et al* (2000) to describe drought susceptibility index (DSI) for shoot dry weight and total green leaf area, was also used here (with slight modifications) to describe water stress level of tea in response to soil water content. Growth measurement was taken every 7 days for a period of 42 days. The WSI ranges between 0 and 1 with a value close to 0 being rated as low tolerance to water stress and a value approaching 1 being rated as tolerance to water stress.

3.3 Determination of total polyphenols

3.3.1 Sampling and sample preparation

Plant tissue for determination of total polyphenols was sampled during the 6th week of water treatment. About 500 g of two leaves-and-a bud shoots were plucked in each of the experimental units and immediately steamed for two minutes. The samples were then placed in labelled paper bags and dried in an oven at 70^{o}C for 24 hours. The dry samples were ground using a blender, sealed in paper bags and safely stored in dark and dry environment awaiting laboratory analysis (Magoma, 2001).

3.3.2 Laboratory analysis of tea shoot polyphenols

Analysis of total polyphenols followed the ISO procedure (ISO, 2003). 0.2 g of ground shoot samples were weighed into 10 ml extraction tubes, 5.0 mls of hot methanol/water extraction mixture added into each extraction tube, stoppered and mixed on the vortex mixer. The extraction tube was heated in water bath for 10 minutes while mixing on the vortex mixer after 5 and 10 minutes. The extraction tube was removed from the water bath and allowed to cool to room temperature after which the stopper was removed and the extraction tube placed in a centrifuge at 3500 revolutions per minute for 10 minutes. The supernatant was carefully decanted into 10 ml graduated tube and cold ethanol/water mixture added to make up to the 10 ml mark. The sample extract was further diluted by transferring 1.0 ml into 100 ml volumetric flask and water added to the mark. 1.0 ml of gallic acid standard solutions corresponding to 10, 20, 30, 40, and 50 μg of anhydrous gallic acid was transferred in duplicate into separate graduated tubes and a similar quantity of water for reagent blanks. 1.0 ml of diluted sample extract was

transferred into separate tubes and 5.0 ml of Folin-Ciocalteu phenol reagent was added into each tube and mixed. Within 5 minutes after the addition of Folin-Ciocalteu phenol reagent, 4.0 ml of sodium carbonate solution was added into each tube and allowed to stand for 60 minutes at room temperature. Their optical densities were measured in 10 mm cell on spectrophotometer set at 765 nm. The mass of anhydrous gallic acid in the 1.0 ml aliquots of the standard solutions was calculated using the formula:

$$(m \times V \times DM_{std} \times 10{,}000)/ (100 \times 100)$$

where,

m is the mass of gallic acid monohydrate (g) used to prepare the stock standard solution

V is the volume of gallic acid stock standard solution (ml) used to prepare the standard solution

DM_{std} is the dry matter content, expressed as a mass fraction (%) of the gallic acid

A best fit linear calibration graph from the mass of anhydrous gallic acid standards was constructed against the gallic acid standard optical densities. The total polyphenol

content, expressed as per cent by mass on a sample dry matter basis was calculated using the following formula:

$$[(OD_{sample} - OD_{intercept}) \times V \times d \times 100]/ (Slope_{std} \times m \times 10{,}000 \times DM)$$

where,

OD_{sample} is the optical density obtained for the sample test solution

$OD_{intercept}$ is the optical density at the point the best-fit linear calibration line intercepts the y-axis

$Slope_{std}$ is the slope obtained from the best-fit linear calibration

m is the mass(g) of the sample

V is the sample extraction volume (the 10 ml)

d is the dilution factor used prior to the colorimetric determination

DM is the dry matter content (expressed as a mass fraction in percent) of the test sample

3.3.3 Laboratory analysis of tea shoot catechins

The tea catechins were quantitatively analyzed using a HPLC system (Shimadzu LC 20AT) with a Gemini 5µ c6-phenyl Phenomenex column. 0.2 g of ground samples extracted with 5 ml of warm (70^{o}C) 70% methanol. The mixture was allowed to stand in warm water bath (70^{o}C) for 10 min then cooled. The mixture was then centrifuged at 3500 rpm for 10 min and extract decanted and topped up to 10 ml. 1 ml of the extract was diluted 5-fold, passed through a 0.5 µm pore filter, before injection into the HPLC column. Two mobile phases: phase A consisted of 9% (volume fraction) acetic acid with 20µl/ml EDTA, and phase B had 80% (volume fraction) acetonitrile and 2% acetic acid

with 20µg/ml EDTA. The flow rate was 1.0 ml/min and the column was operated at 40^{o}C. The sample injection volume was 20 µl. The UV spectra peaks were detected at 278 nm. The chromatographic peaks in the samples were identified by comparing their retention times and with those of caffeine-established factor with the respective chemical standards (Wang and Helliwell, 2001; ISO, 2005).

3.3.4 Data analysis

Regression analysis was done using a Gompertz exponential function (GenStat 5 release 4.2). The functional Gompertz model provided the best fit curve with relatively low residual and higher R^2 value compared to other growth models and was therefore used in this work.

3.4 Results

3.4.1 The influence of soil water content on growth of tea

i) Shoot growth of tea

The average shoot growth rate of the six tea clones was 2.22 cm/week. Soil water content (SWC) showed significant influence on shoot growth with varied response being observed between ploidy levels and individual clones. Though growth declined with progressive reduction in SWC, notable shoot growth declined sharply at about 28% SWC, which represented a 33% soil water deficit (SWD) based on the soil field capacity of 42%. A further 10% decline in SWC from 28 to 18% resulted in a large reduction in shoot growth from 2.0 to 0.5 cm which corresponded to 75% reduction compared to a reduction of only 18% with SWC decline from 38 to 28% (Fig. 3.1 a and b).

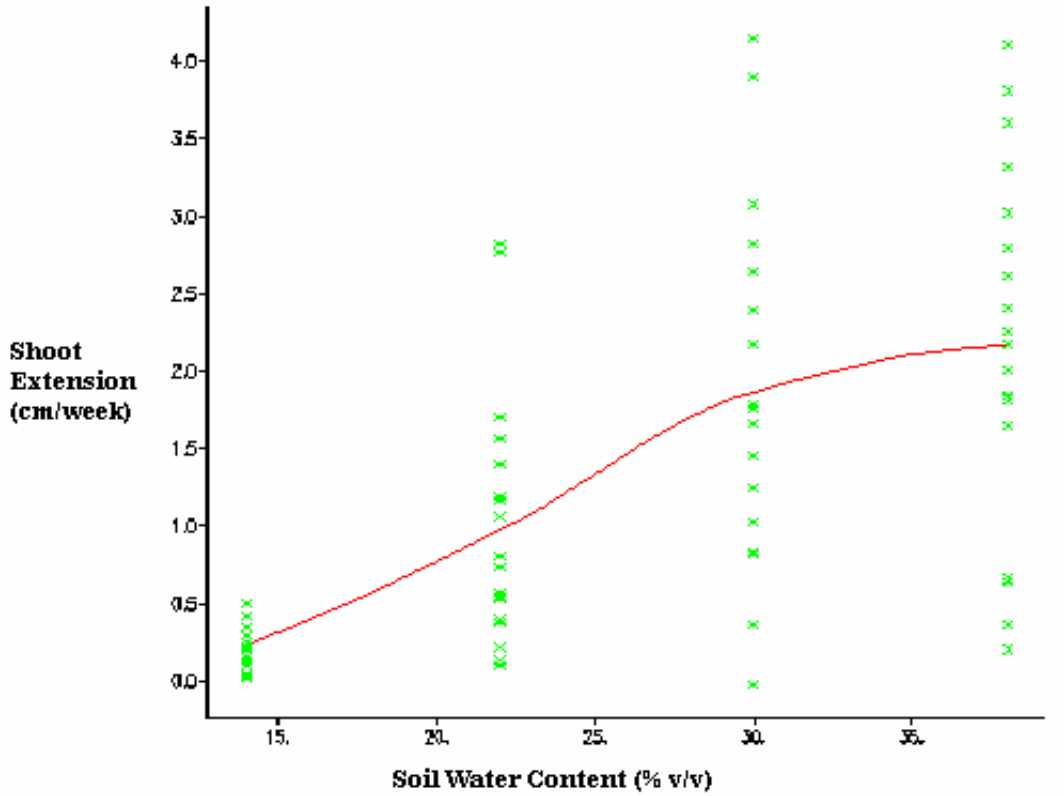


Fig. 3.1 (a) Influence of soil water content on shoot growth, mean of six tea clones during six weeks of treatment.
(P<0.001, SE = 0.91, n = 72, adjusted r^2 = 73%).

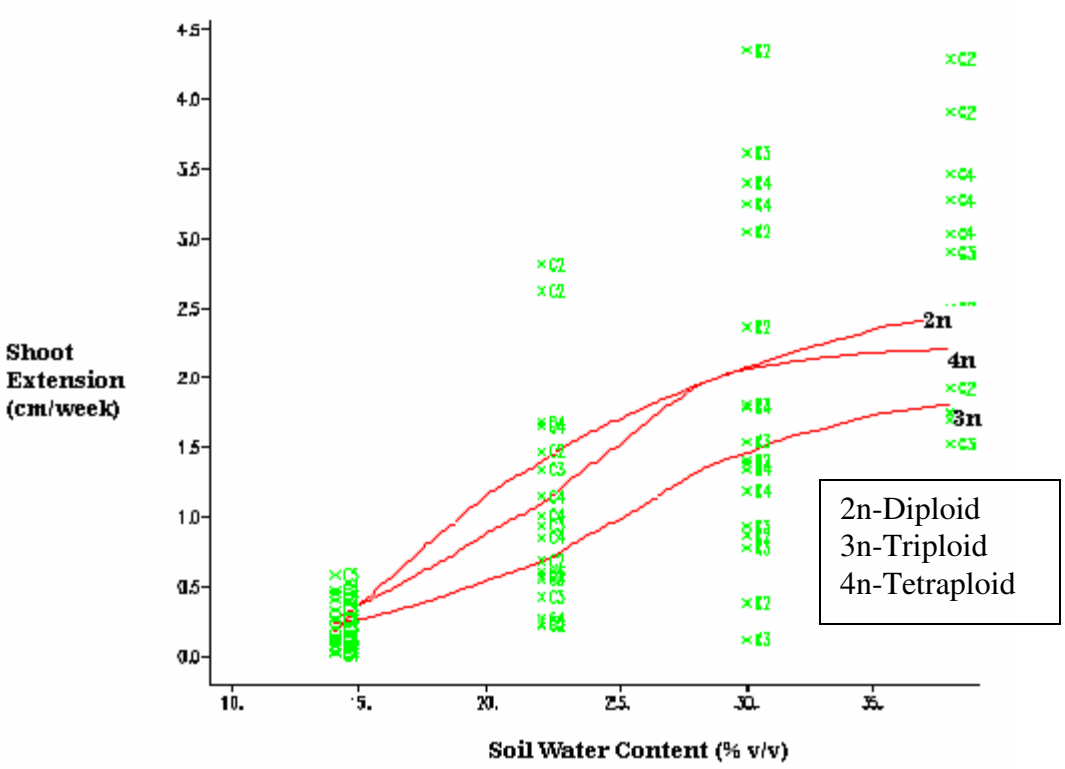


Fig. 3.1 (b) Influence of soil water content on shoot growth of diploid, triploid and tetraploid teas. (P< 0.001, SE = 0.93, n = 72, adjusted r^2 = 38%).

In reference to ploidy levels, diploids, triploids and tetraploids in the study, all shared 20% SWC as the point when half of their plants attained maximum shoot growth. However, the maximum growth attained was distinct for each ploidy group. The *2n* had higher growth rate of 2.37 cm/week, followed by 4n with 2.18 cm/week and 1.70 cm/week for *3n* teas (Table 3.1).

Table 3.1 Pooled shoot growth regression estimates for tea of different polyploidy levels as influenced by soil water content (P<0.001).

Tea ploidy level	Maximum growth attained (cm/week)	Others
2n	2.37	*B = 0.18 (0.131)
3n	1.70	M = 20.80 (2.47)
4n	2.18	SE in brackets

**Parameter estimates:* M *is soil water content when half of the population in the treatment attain maximum growth, and* B *is the gradient of the curve at point M in Fig.3.1*

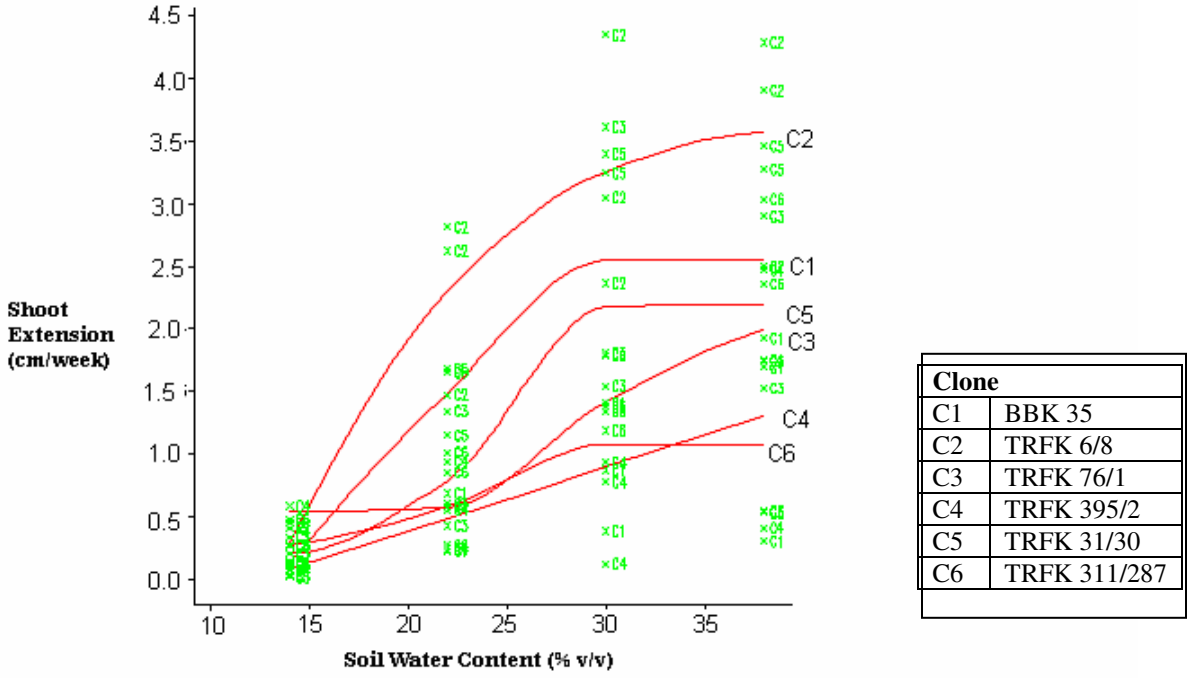


Fig. 3.1 (c) Influence of soil water content on shoot growth of the individual six clones of tea during six weeks of treatment. (P<0.001, SE = 0.76, n = 72, adjusted r^2 = 64%).

Regression analysis for individual clones showed distinct differences in response to SWC. Clones BBK 35 and TRFK 6/8 achieved a relatively higher maximum growth rate of 6.94 and 7.32 cm/week compared to 2.42, 2.0, 1.48 and 0.81 cm/week for clones TRFK 31/30, TRFK 76/1, TRFK 311/287 and TRFK 395/2, respectively. The SWC at

which maximum growth rate was achieved also differed. Clone TRFK 6/8 required as low as 13% SWC for half of the plants to achieve maximum growth rate while BBK 35 and clone TRFK 311/287 required 27.76 and 29.23%, respectively, which was more than twice that of TRFK 6/8. Clones TRFK 395/2 and TRFK 76/1 required between 21 and 23% SWC. Despite the differences, shoot growth in all clones declined at between 25% SWC and 28% SWC, an indication that they share similar break-point on SWC. The results show clone TRFK 6/8 requires less soil water to achieve large shoot growth while BBK 35 needed twice as much water for about similar growth rate (Table 3.2 a).

Table 3.2 (a) Shoot growth regression analysis estimates for six clones as influenced by soil water content ($P<0.001$)

Clone	Maximum growth attained cm/week	SWC when half the plants attain maximum growth	Difference between the maximum and minimum value	Gradient of graph when 50% attain maximum growth
BBK 35	6.94	27.76	2.69	0.03
TRFK 6/8	7.32	13.13	3.63	0.16
TRFK 76/1	2.00	23.36	0.20	0.65
TRFK 395/2	0.81	22.40	0.27	1.22
TRFK 31/30	2.42	21.87	0.13	2.00
TRFK 311/287	1.48	29.23	0.54	0.50

ii) Leaf growth

Unlike shoot growth, leaf expansion which was about 3 cm^2 per week for the six clones, was checked at much lower SWC of 18-20% (Fig. 3.2 a). Differences in leaf growth among clones also emerged. Leaf growth was generally larger for triploids with an average of 6.35 cm^2 per week and lower for tetraploids with 1.37 cm^2 per week while diploids were intermediate with 3.62 cm^2 per week (Table 3.2 b). Though triploid and

diploid teas showed sharp decline in leaf expansion at 18% SWC, tetraploid leaf growth seemed to decline at 28% SWC (Fig. 3.2 b).

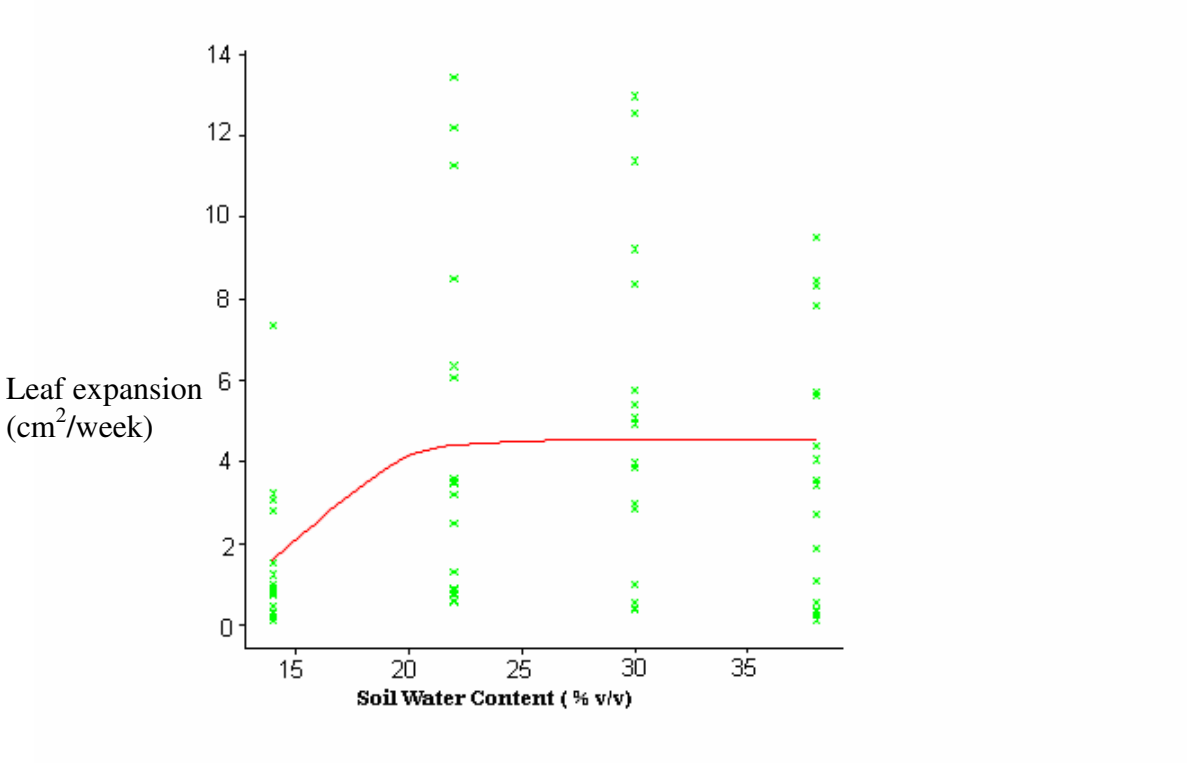


Fig. 3.2 (a) Influence of soil water content on leaf area expansion in six tea clones. (P<0.001, SE = 2.9, n = 72, adjusted r^2 = 36.5%).

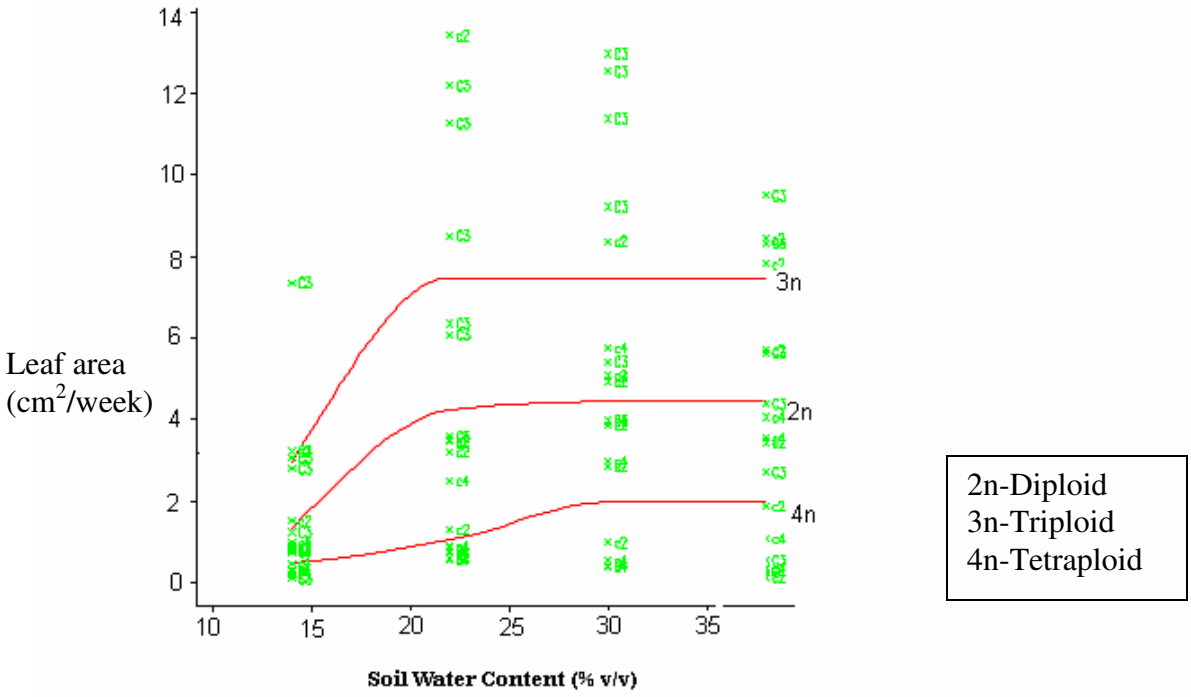


Fig. 3.2 (b) Influence of soil water content on leaf area expansion in diploid, triploid and tetraploid teas. (P<0.001, SE = 2.8, n = 72, adjusted r^2 = 40%).

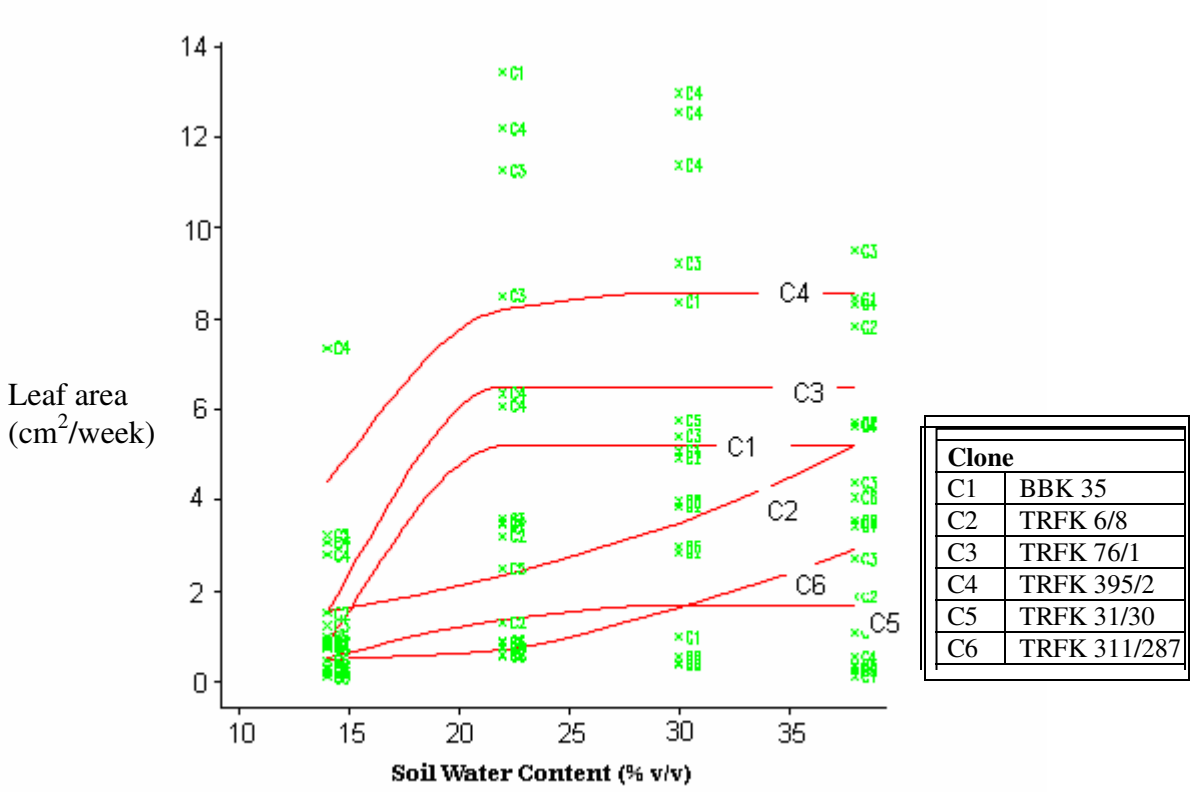


Fig. 3.2 (c) Influence of soil water content on leaf expansion (cm^2) in six individual clones. ($P<0.001$, SE = 3.05, n = 72, adjusted r^2 = 31%).

Table 3.2 (b) Leaf expansion parameter estimates from regression analysis of the six tea clones

Clone	Leaf expansion (cm^2/week)
BBK 35	4.10
TRFK 6/8	3.14
TRFK 76/1	5.26
TRFK 395/2	7.43
TRFK 31/30	1.31
TRFK 311/287	1.43

3.4.2 Plant water status

i) Leaf relative water content (RWC)

Leaf relative water content (RWC) significantly declined at 20% soil water content which represented about 53% soil water deficit (Fig.3.3 a, b & c). On average, RWC of tea leaf was 93% at soil field capacity and upto 20% SWC but a soil water deficit of just 5% below 20% SWC (Fig. 3.3 a)resulted in a sharp reduction in RWC from 93% to 70% (Fig. 3.3 a). However, regardless of tea ploidy level, the critical soil water content for leaf RWC was 20%. Variations were evident at SWC above 20% where tetraploid tea had higher RWC, followed by triploids and relatively lower for the diploid tea (Fig. 3.3 b).

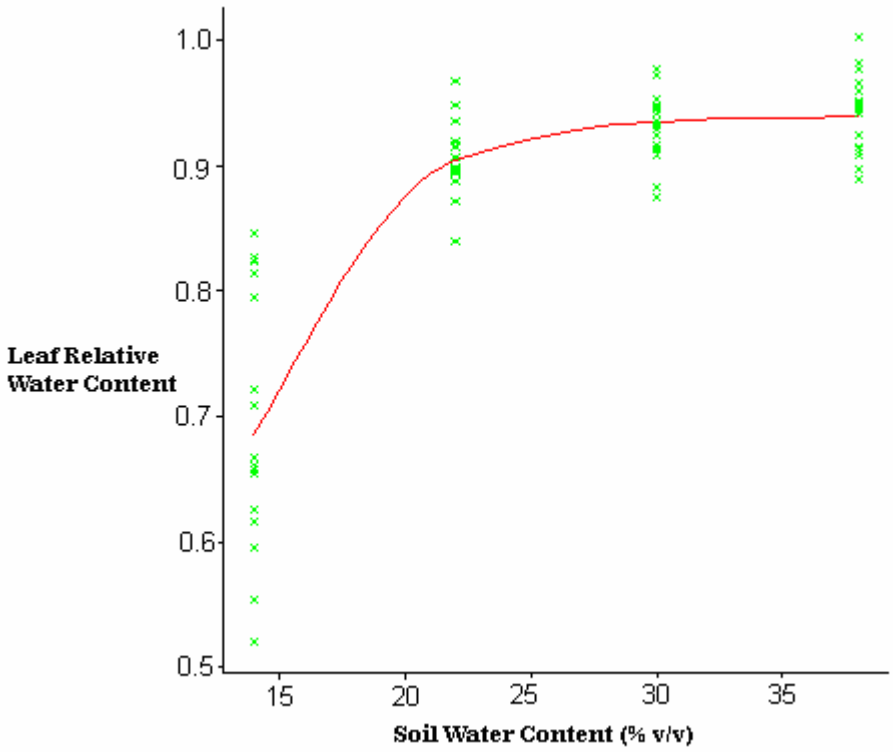


Fig. 3.3 (a) Influence of soil water content on relative water content in tea leaf. (P<0.001, SE = 0.05, n = 72, adjusted r^2 = 79%).

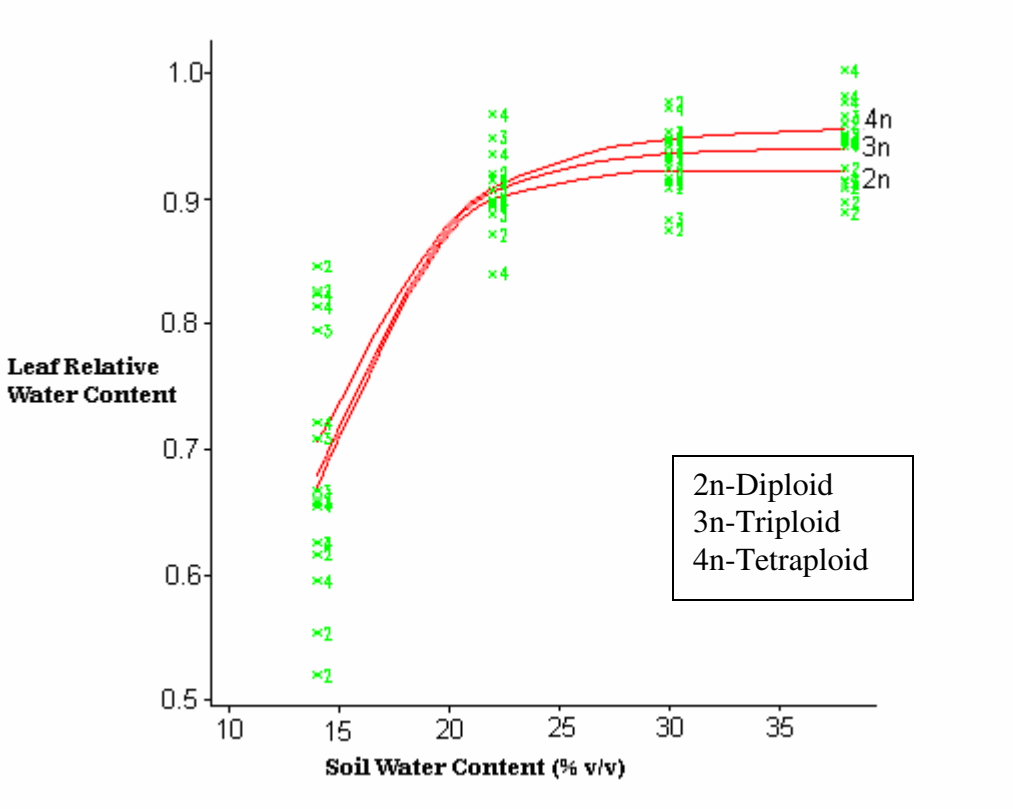


Fig. 3.3 (b) Influence of soil water content on relative water content in tea of different ploidy level**.** (*P*<0.001, SE = 0.05, n = 72, adjusted r^2 = 78.6%).

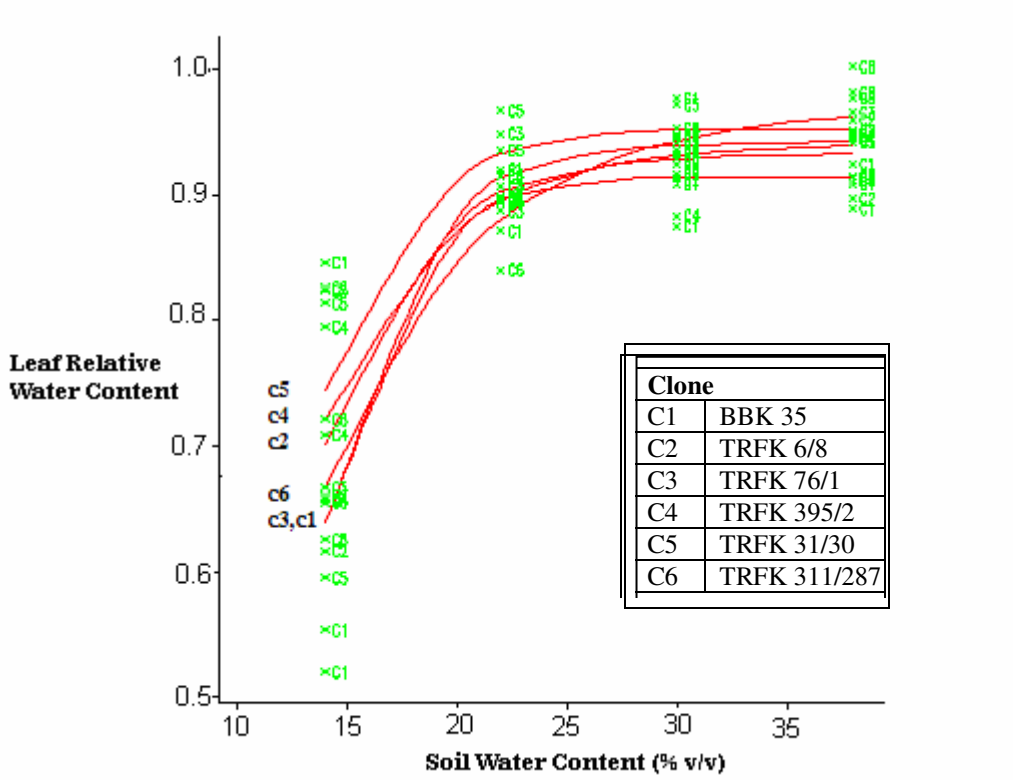


Fig. 3.3 (c) Influence of soil water content on relative water content of six tea clones. (P<0.001, SE = 0.05, n = 72, adjusted r^2 = 78.4%).

Table 3.3 Leaf RWC regression estimates for six tea clones

Clone	Maximum RWC attained	Soil water content when 50% of tea attained maximum RWC	Gradient of the curve when 50% of the plants are at maximum RWC	Variation accounted for (adjusted r^2 %)	SE
BBK 35	0.91	11.82	0.388	62.5	0.089
TRFK 6/8	0.93	10.13	0.286	75.5	0.056
TRFK 76/1	0.94	11.82	0.341	97.0	0.023
TRFK 395/2	0.94	8.80	0.229	85.5	0.038
TRFK 31/30	0.95	11.93	0.626	63.5	0.066
TRFK 311/287	0.96	9.80	0.194	91.2	0.038

The leaf RWC estimates show that clones TRFK 31/30 and TRFK 311/287 attained higher RWC of 95 and 96%, respectively, followed by clone TRFK 76/1 and TRFK 395/2 with 94% each while clone BBK 35 and TRFK 6/8 registered lower RWC of 91 and 93% respectively. The maximum RWC was attained at different soil water contents.

Generally, the maximum RWC was attained at SWC above 10.31, 10.86 and 10.97 for the triploids, tetraploids and diploids respectively. Individually, clones TRFK 395/2, TRFK 311/287 and TRFK 6/8 attained maximum RWC at low soil water content of 8.80, 9.80 and 10.13%, respectively, while clones BBK 35, TRFK 76/1 and TRFK 31/30 required relatively higher amounts of 11.82, 11.82 and 11.93% soil water to attain maximum leaf tissue water, respectively (Table 3.3).

ii) Shoot water potential

Shoot water potential reduced in tea shoots with decreasing SWC indicating increasing water deficit in plant tissue. However, there were variations in shoot water potential trends in different clones (Fig. 3.4 a & b), with clones TRFK 31/30 and TRFK 311/287 showing less deficit (-7 bars) compared to clones TRFK 76/1, TRFK 395/2, BBK 35 and TRFK 6/8 whose pressure ranged from 8.8 to 9.8 (Fig. 3.4 c and Table 3.4). Again, clones TRFK 31/30 and TRFK 311/287 attained the maximum pressure at much higher SWC of 23 and 29%, respectively, which was equivalent to about 45-30% soil water deficits. In addition to the large force to which clones TRFK 76/1, TRFK 395/2, BBK 35 and TRFK 6/8 can withhold tissue water, their maximum capacity was overstretched at much lower soil water content of 14-21%. In terms of elasticity of shoot water potential to changes in SWC, clones TRFK 76/1 and TRFK 395/2 showed large capacity to adjust which stretched to 4.17 and 2.98 bars, respectively, with clones BBK 35 and TRFK 6/8 being restricted to 2.72 and 1.22 bars, while TRFK 31/30 and TRFK 311/287 had much lower elasticity of 0.6 and 1.9, respectively (Table 3.4).

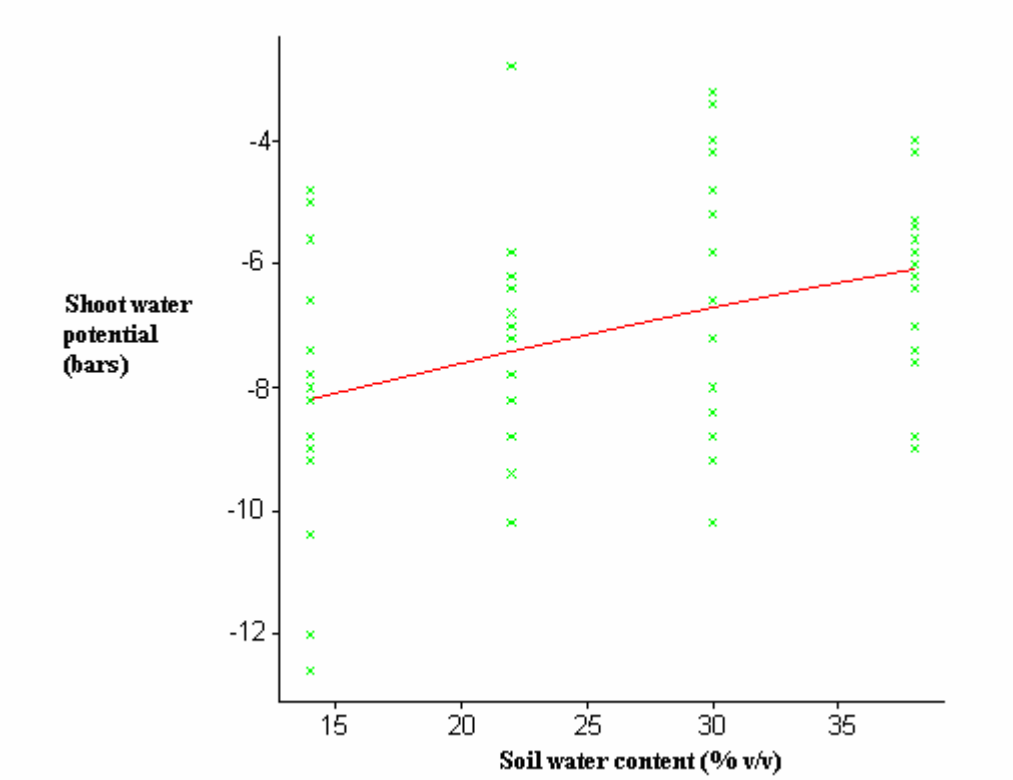


Fig. 3.4 (a) Effect of soil water content on shoot water potential of tea clones. (P<0.001, SE = 1.96, n = 72, adjusted r^2 = 13%).

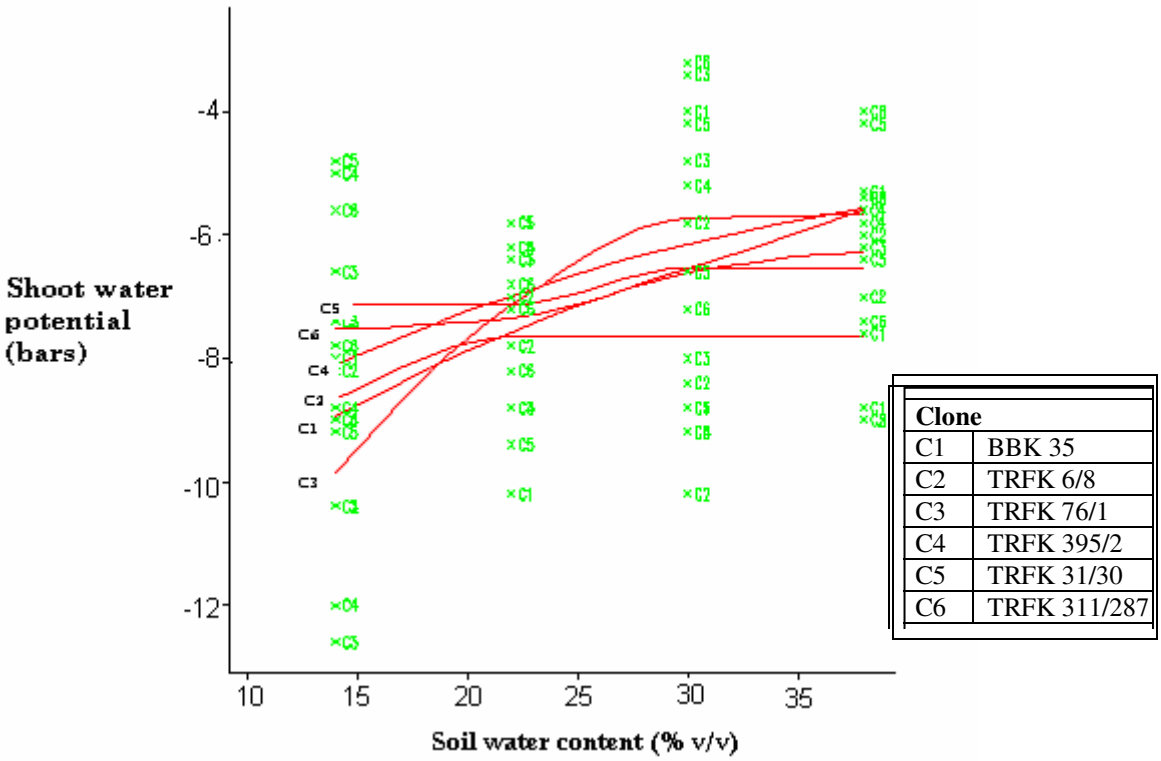


Fig. 3.4 (b) Effect of soil water content on shoot water potential.
(P<0.05, SE = 1.9, n = 72, adjusted r^2 = 10.2%).

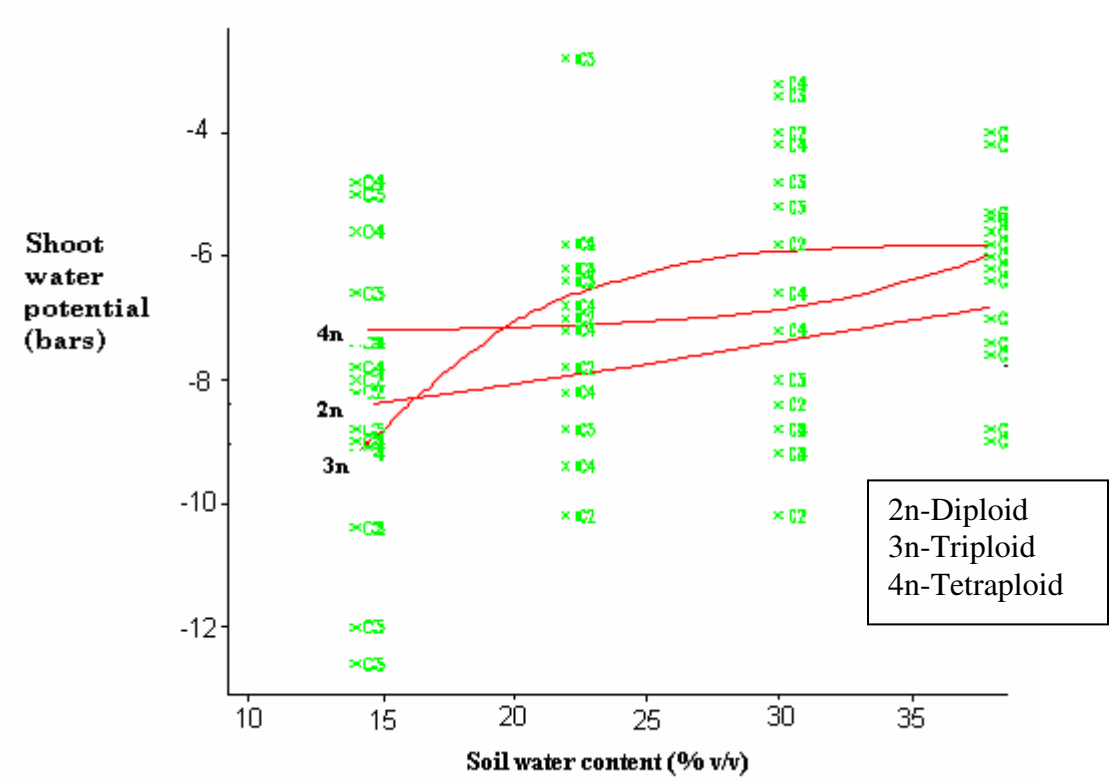


Fig. 3.4 (c) Effect of soil water content on shoot water potential of diploid and polyploid tea. (P<0.05, SE = 1.96, n = 72, adjusted r^2 = 13.4%).

Table 3.4 Parameter estimates for regression analysis (P<0.05) of shoot water potential for six clones of tea.

Clone	Gradient of the graph when 50% of shoots attain maximum pressure	Soil water content when 50% of the shoots attain maximum pressure (% v/v)	Pressure range between minimum and maximum (bars)	Pressure required to release water in tea shoot (bars)
BBK 35	2.01	21.64	-2.72	-9.133
TRFK 6/8	0.86	14.69	-1.22	-8.865
TRFK 76/1	1.99	21.57	-4.17	-9.867
TRFK 395/2	0.89	14.72	-2.98	-9.047
TRFK 31/30	1.54	23.53	-0.60	-7.133
TRFK 311/287	0.38	29.24	-1.90	-7.433

3.4.3 Effect of soil water content on dry matter partitioning

i) Shoot-to-root ratio

Shoot-to-root ratio (SRR) of the tea clones as influenced by SWC and clonal variation ranged from 1.9 – 2.9. Clones TRFK 6/8, TRFK 395/2 and TRFK 31/30 had large ratios, which were; 2.95, 2.71 and 2.87, respectively, while clones BBK 35, TRFK 76/1 and TRFK 311/287 gave low ratios of 1.95, 2.20 and 2.38, respectively (Table 3.5 and Fig. 3.5 b). Though the response pattern was nearly similar, the SRR in clone TRFK 31/30 was almost linear with changes in soil water whereas that of TRFK 395/2, TRFK 76/1 and TRFK 311/287 reached their lowest value as SWC approached 20%. Clone TRFK 6/8 and BBK 35 had their break point at about 30% SWC (Fig.3.6 b). Elasticity in adjustment of SRR under water deficits was large in clone TRFK 31/30 suggesting that though it attained maximum SRR of 2.87, it could reduce to as low as 0.83 when SWC declined. Clone BBK 35 had a narrow range of 0.47 giving a lower SRR of 1.90 from 1.95. The SWC above which SRR appreciated varied with clone though it generally ranged from 22-30% SWC. Clone TRFK 76/1 and TRFK 395/2 had their SRR rising at much lower SWC of 22% while BBK 35, TRFK 6/8 and TRFK 311/287 experienced SRR increase at 30% SWC.

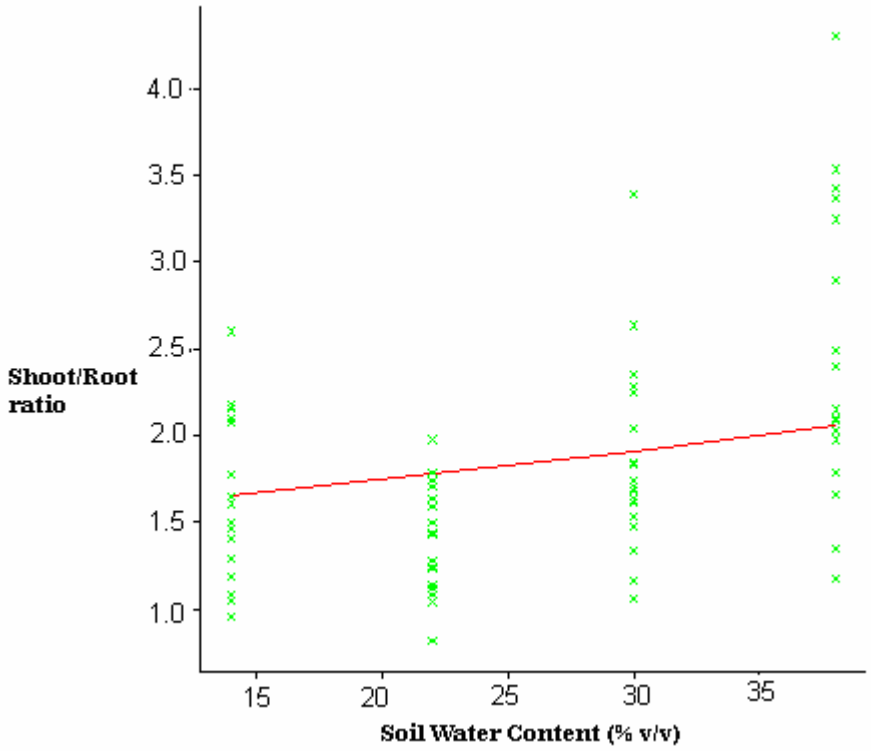
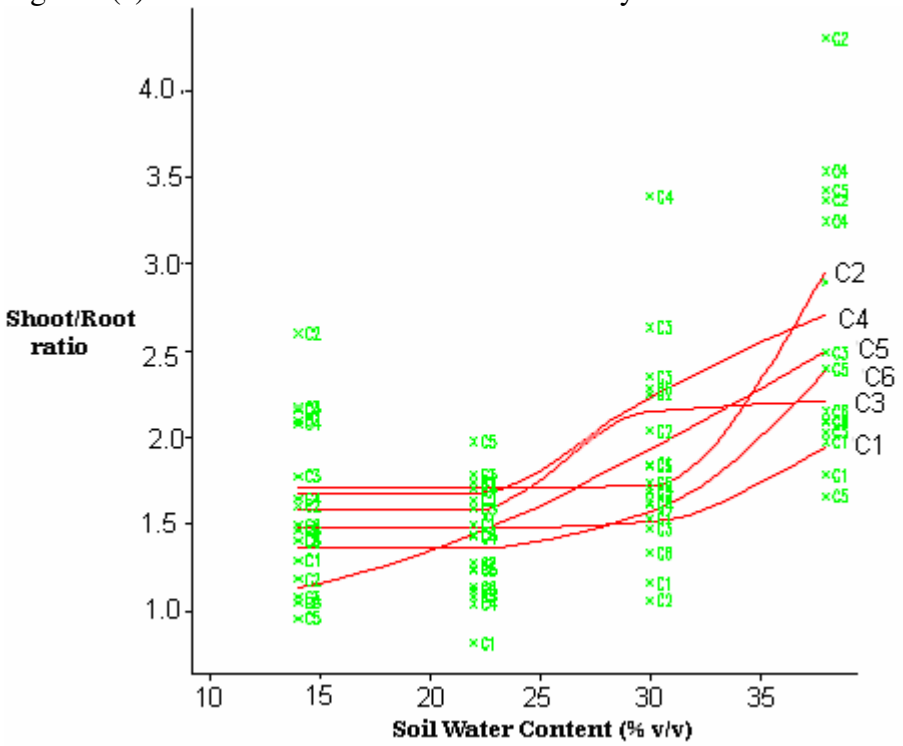


Fig. 3.5 (a) Influence of soil water content on dry matter in shoot-to-root ratios of tea



Clone	
C1	BBK 35
C2	TRFK 6/8
C3	TRFK 76/1
C4	TRFK 395/2
C5	TRFK 31/30
C6	TRFK 311/287

Fig. 3.5 (b) Influence of soil water on dry matter partitioning as determined in shoot-to-root ratios of six tea clones. ($P < 0.001$, SE = 0.57, n = 72, adjusted $r^2 = 30$).

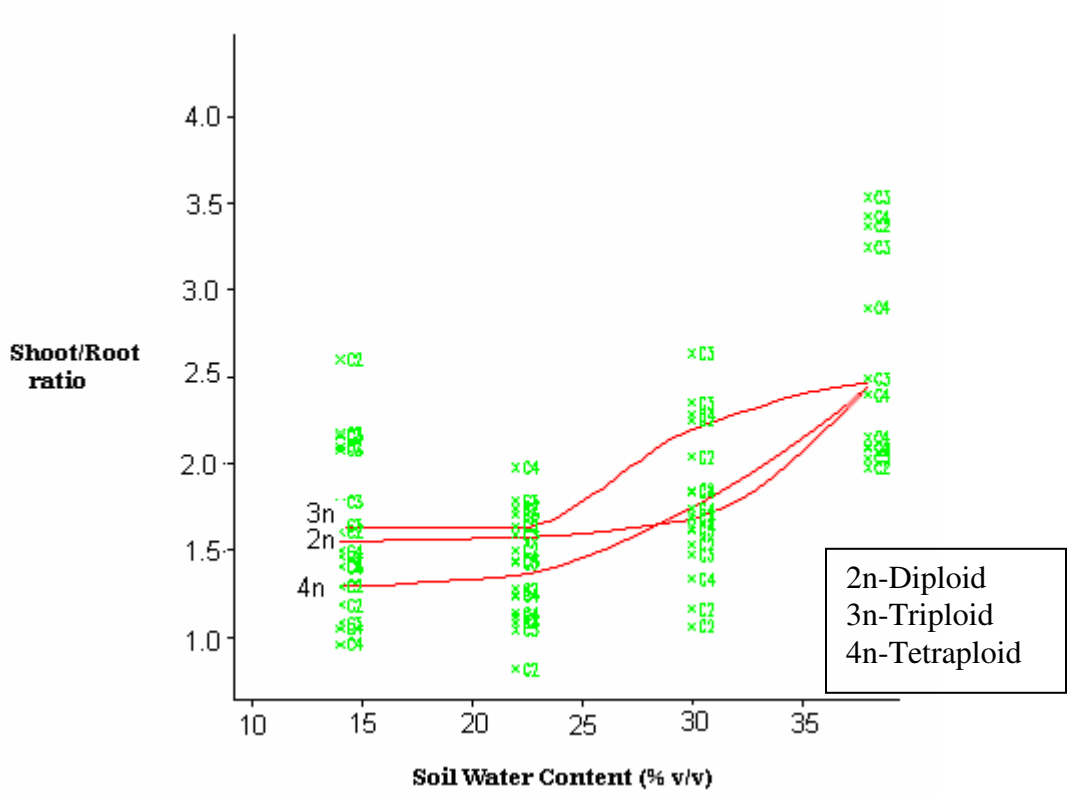


Fig. 3.5 (c) Influence of soil water content on dry matter partitioning as determined in shoot-to-root ratios of two clones each from diploid, triploid and tetraploid tea. (P<0.001, SE = 0.58, n = 72, adjusted r^2 = 27.7%).

Table 3.5 Shoot-to-root ratio (SRR) regression estimates of six tea clones as influenced by SWC.

Clone	Shoot/root ratio	SWC (%) when 50% are at maximum SRR	Elasticity	Gradient of the graph when 50% are at maximum SRR
BBK 35	1.95	31.36	-0.470	-1.867
TRFK 6/8	2.95	34.97	-1.240	-0.939
TRFK 76/1	2.20	29.49	-0.62	-1.835
TRFK 395/2	2.71	30.14	-1.033	-1.890
TRFK 31/30	2.87	32.60	-2.049	-0.096
TRFK 311/287	2.38	30.78	-1.016	-1.925

3.4.4 Stomatal conductance, photosynthesis and transpiration

Response of stomatal conductance to soil water content followed the same pattern as shoot growth where there was drastic decline at 28% SWC (Fig 3.6 a). The response to stomatal conductance varied with ploidy level in tea and of significance was the SWC range within which each group restricted their stomatal (Fig. 3.6 b). Under unlimited SWC, diploid tea had higher stomatal conductance followed by tetraploids and triploids. When tea was subjected to declining soil water content, the diploids and tetraploids restricted their stomata at 28% SWC, while triploids restricted theirs at 20% SWC. In the individual clones, BBK 35 had generally higher stomatal conductance with insignificant response to SWC. Clone TRFK 6/8 restricted its stomata at about 30% SWC and maintained it at about 0.3 mol m^{-2} s^{-1} with further soil water deficit. Clone TRFK 311/287 maintained the lowest stomatal conductance up to 23% SWC and increased to a maximum of 0.5 mol m^{-2} s^{-1} at 28% SWC (Fig 3.6 c). The response of CO_2 assimilation to changes in SWC was similar to that of leaf growth (Fig 3.7 a and b) with a break point occurring at 20% SWC. However, leaf transpiration trend followed that of the shoot growth (Fig. 3.8).

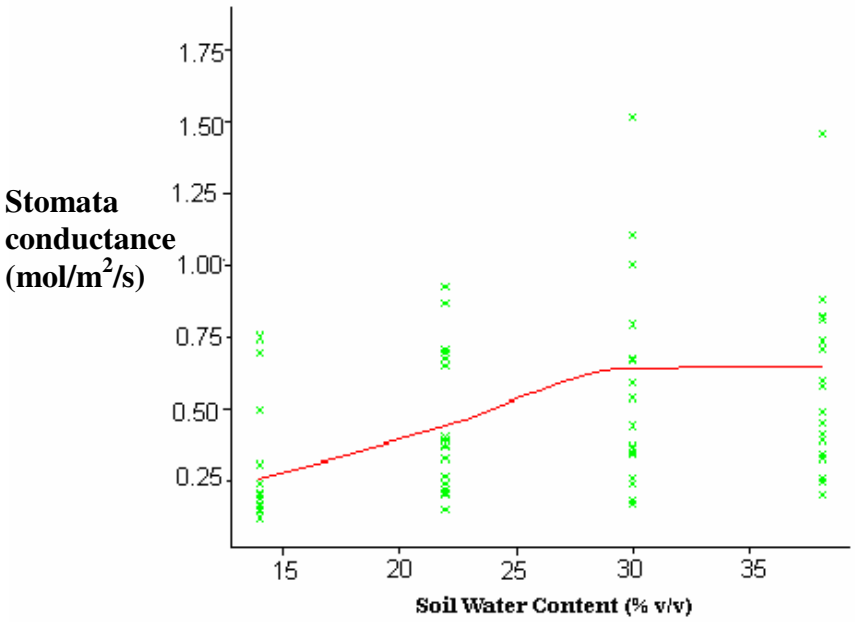


Fig. 3.6 (a) Changes in stomata conductance in tea grown at different soil water content. (P<0.01, SE = 0.3, n = 72, adjusted r^2 = 43.7%).

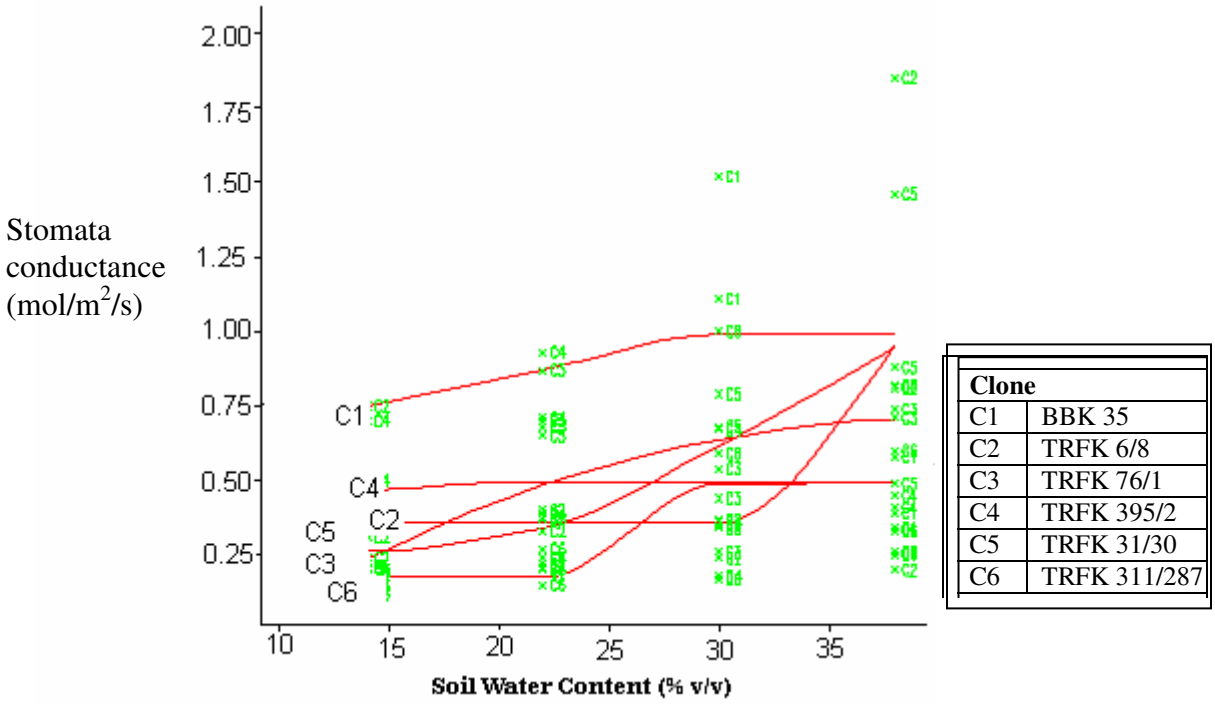


Fig.3.6 (b) Response of stomata conductance to soil water deficit in selected tea clones. (P<0.01, SE = 0.36, n = 72, adjusted r^2 = 16.7%).

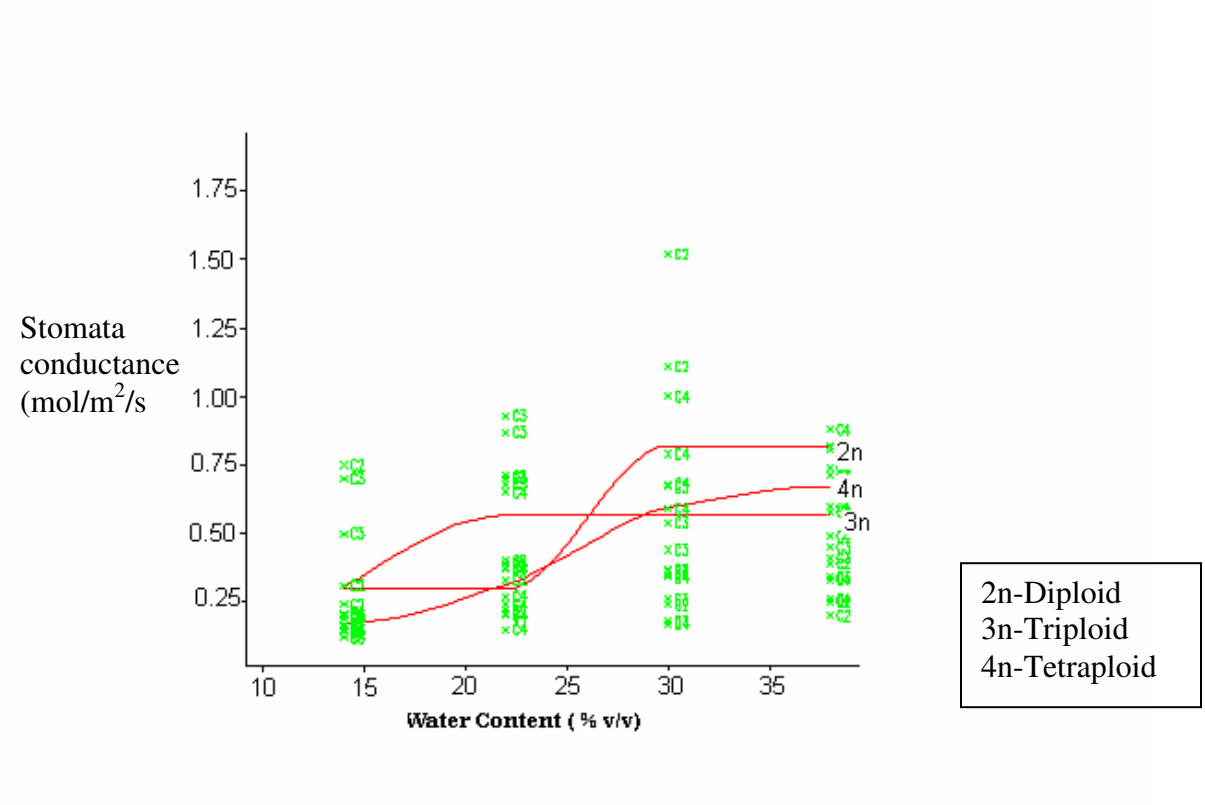


Fig. 3.6 (c) Influence of soil water content on stomata conductance in selected teas of different polyploidy level. (P<0.01, SE = 0.3, n=72, adjusted r^2 = 14.4%).

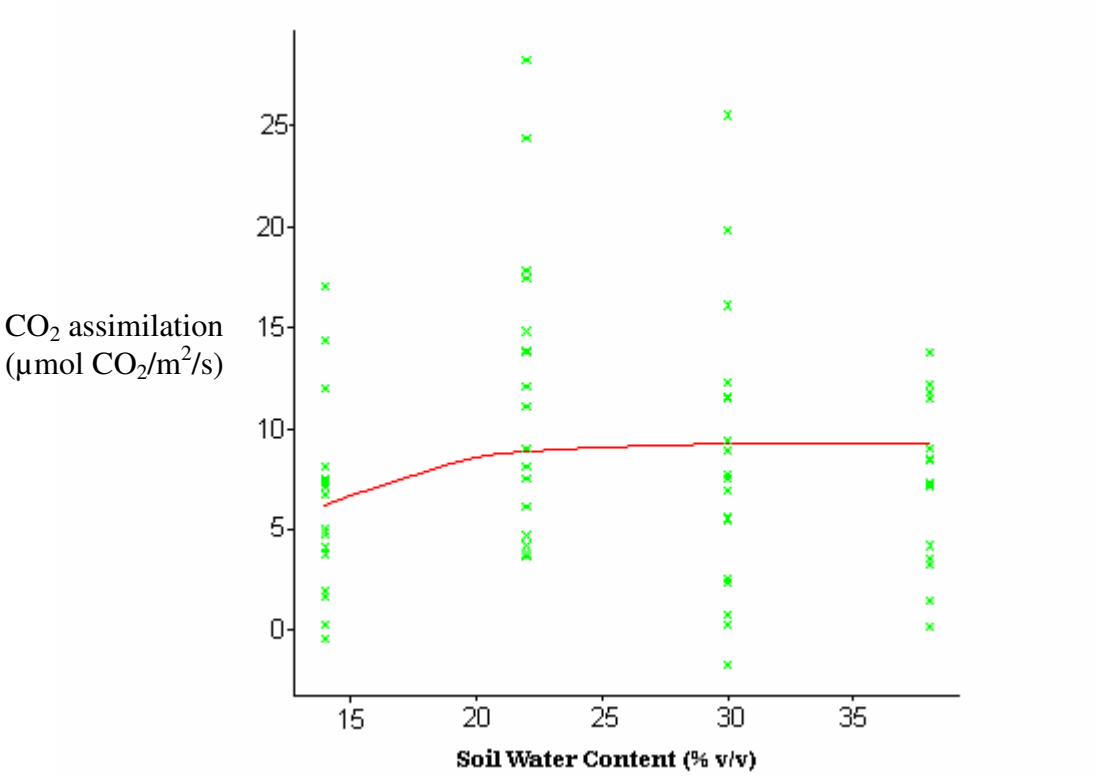


Fig. 3.7 (a) Influence of soil water content on CO_2 assimilation in tea during the 9th week of soil water treatment.

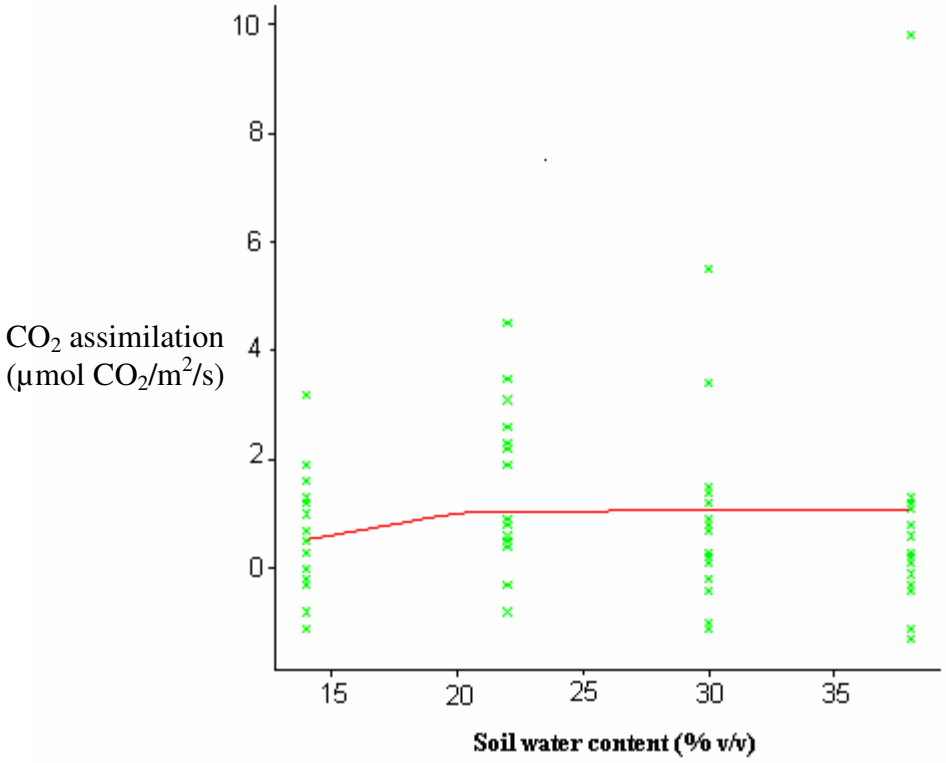


Fig. 3.7 (b) Influence of soil water content on CO_2 assimilation in tea during the 10th week of soil water treatment. ($P < 0.001$, SE = 1.7, n = 72, adjusted r^2 = 33.9%)

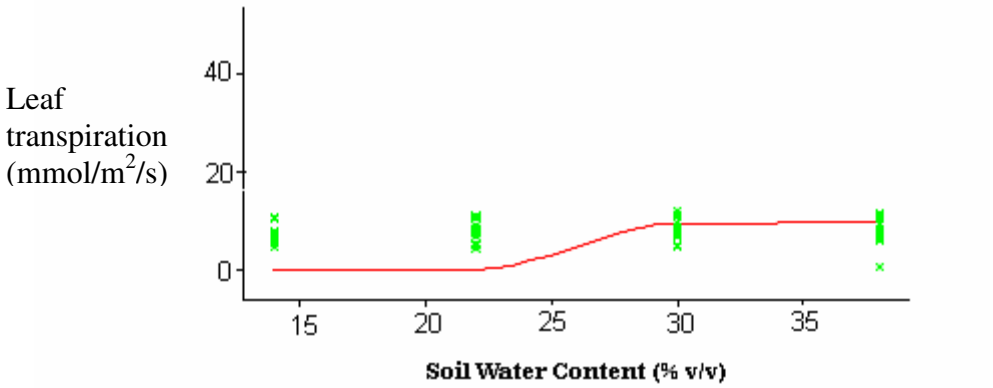


Fig. 3.8 Influence of soil water content on leaf transpiration in tea (P<0.001, SE = 5, n = 72, adjusted r^2 = 46)

3.4.5 Leaf area and specific leaf area

Leaf area varied with clones but notable leaf size variation was observed in triploids which had larger leaves (Fig 3.9 a). In response of leaf size to SWC leaf area was improved with increase in SWC (Fig. 3.9 a and b). Though specific leaf area almost followed similar trend to that of leaf size, individual clones showed notable differences. For instance, clone TRFK 6/8 had higher specific leaf area which was almost unresponsive to changes in SWC (Fig. 3.10 a, b and c).

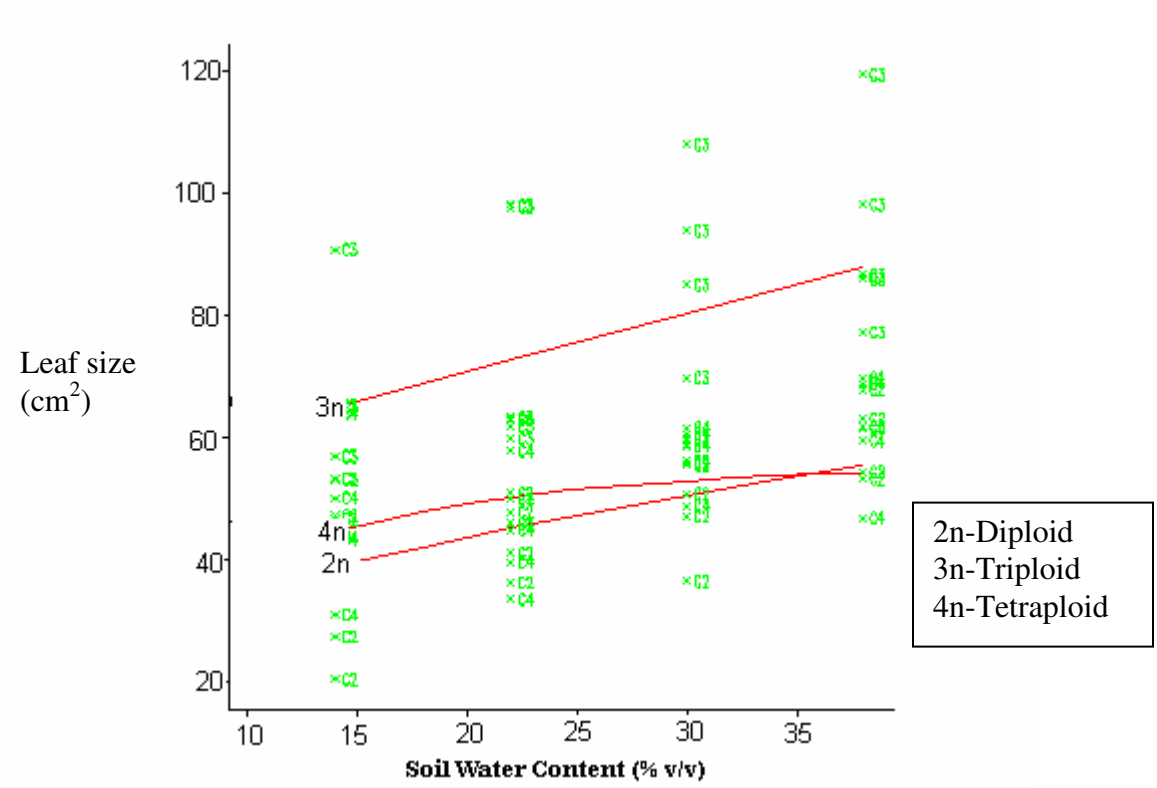


Fig. 3.9 (a) Effect of soil water content on leaf size in tea of different ploidy level. (P<0.01, SE = 13.5, n=72, adjusted r^2 = 51.5%).

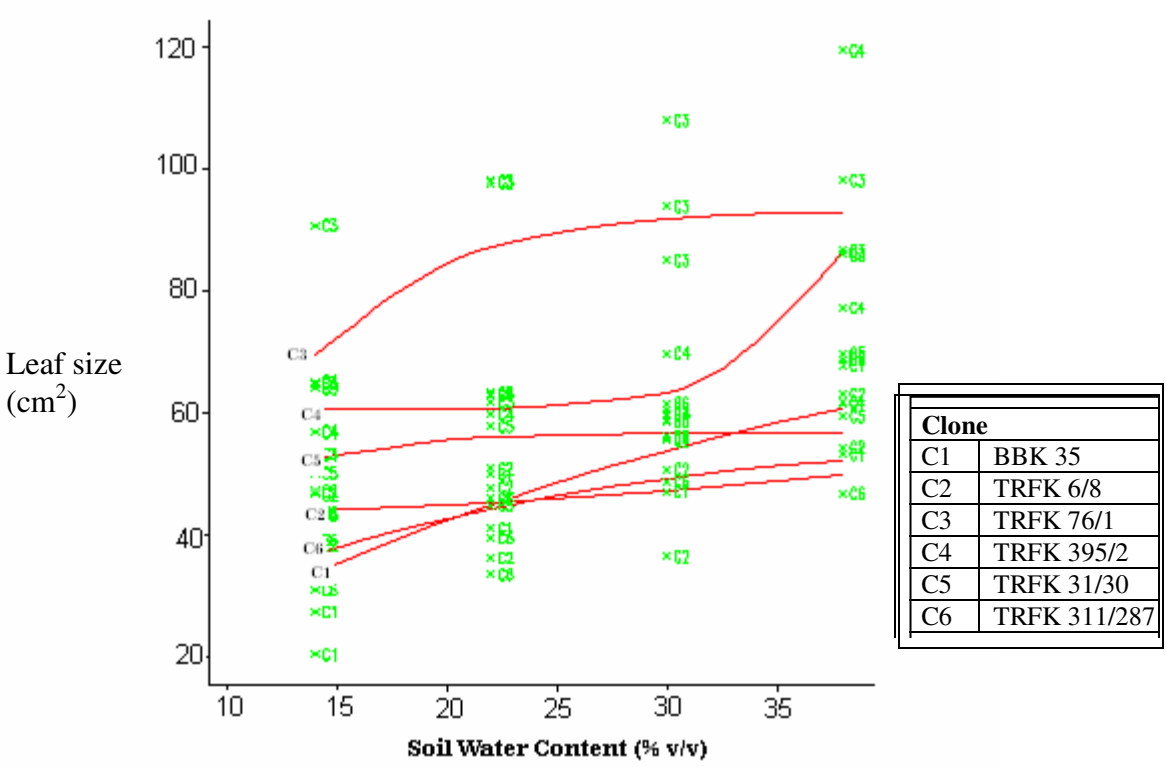


Fig. 3.9 (b) Effect of soil water content on leaf size in selected tea clones.
(P<0.001, SE = 12.1, n=72, adjusted r^2 = 61.3%).

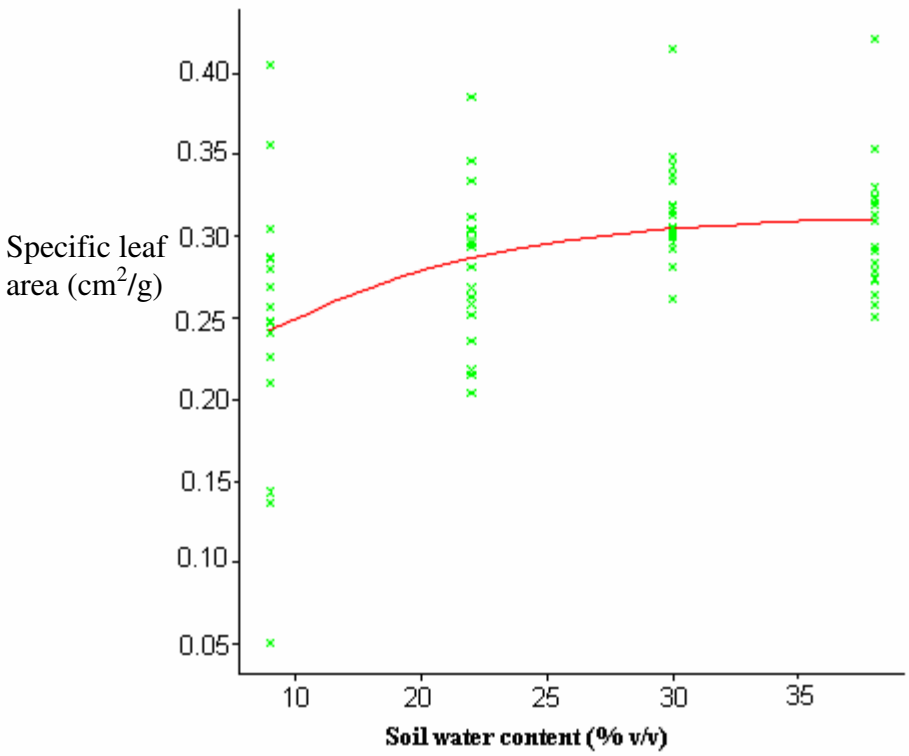


Fig.3.10 (a) Effect of the soil water content on specific leaf area in tea. (P<0.001, SE = 0.05, n=72, adjusted r^2 = 16.8%).

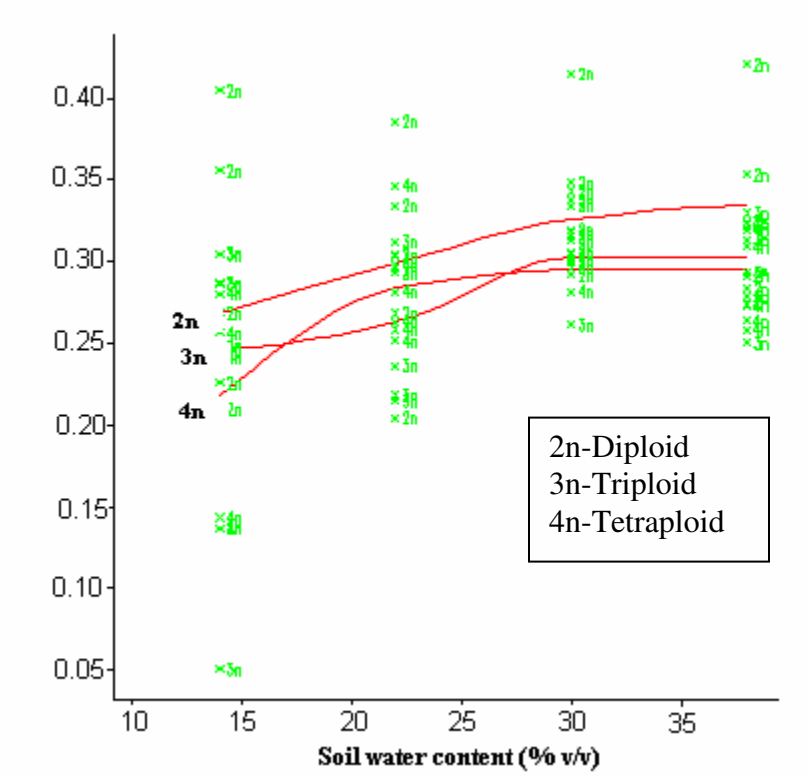


Fig. 3.10 (b) Effect of soil water content on specific leaf area in selected teas of different ploidy level. (P<0.01, SE = 0.06, n = 72, adjusted r^2 = 18.2%).

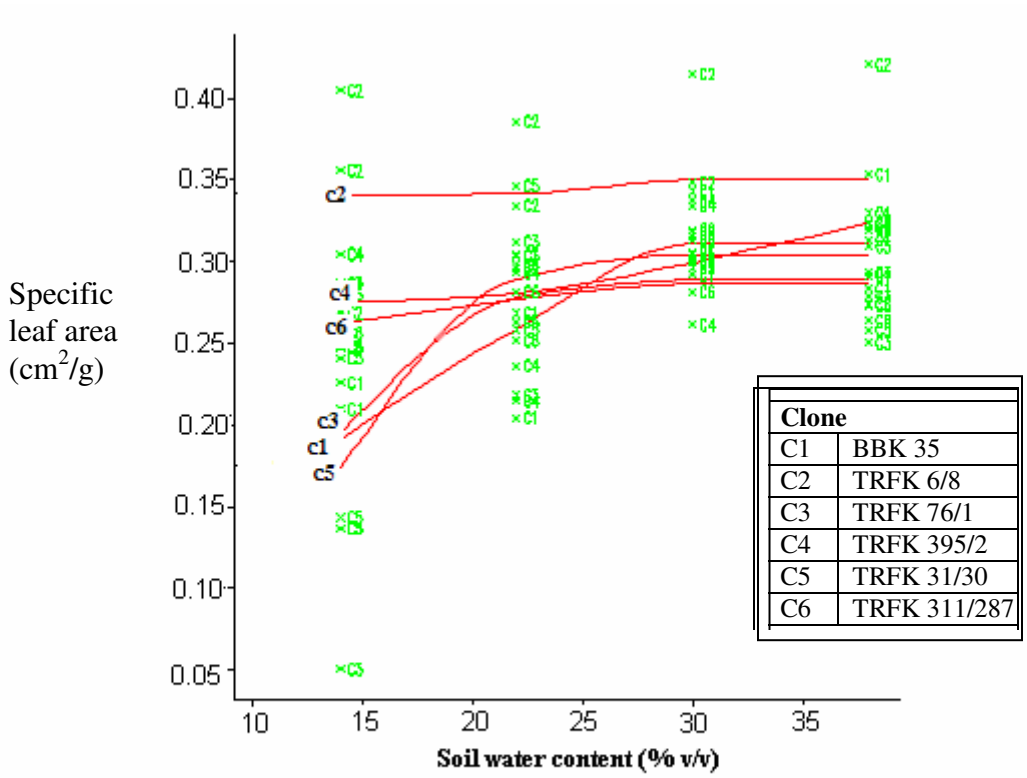
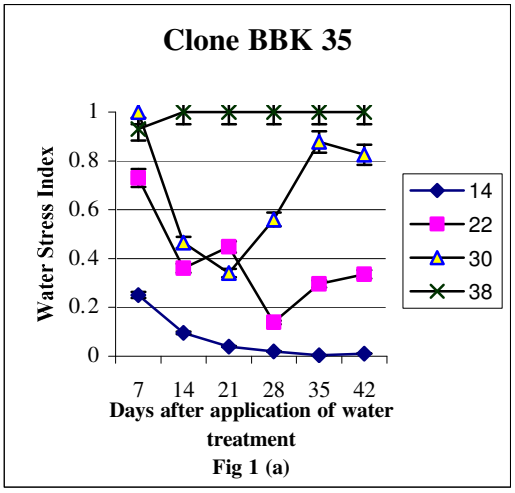
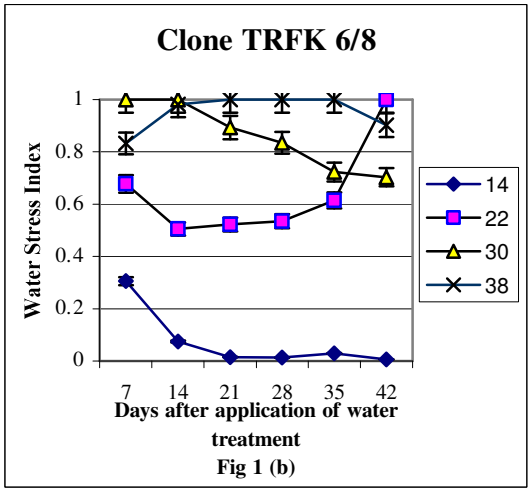


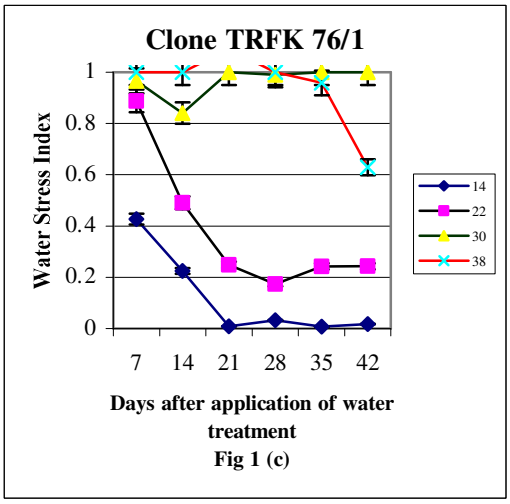
Fig. 3.10 (c) Effect of soil water content on specific leaf area in selected tea clones. (P<0.001, SE = 0.05, n = 72, Adjusted r^2 = 35.5%).

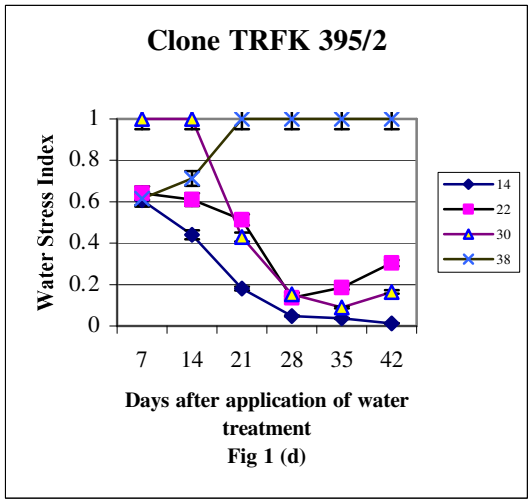
3.4.6 Water stress tolerance

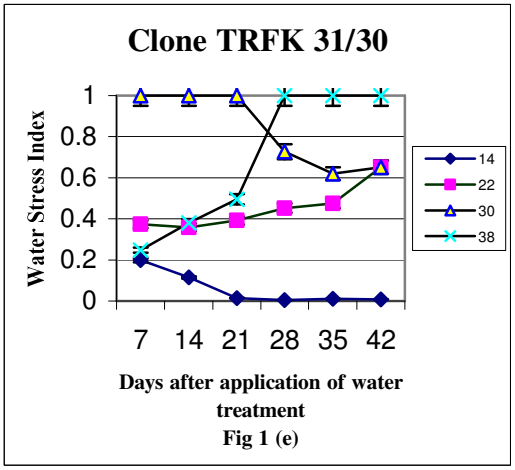
Results indicate that all clones succumbed to water stress at 14% SWC though after varied exposure time. Water stress index (WSI) for clones TRFK 311/287 and TRFK 395/2 approached zero on the 28th day at 14% SWC while the others approached zero earlier. Based on SWC above 22% and given the observed threshold SWC of 20%, clones TRFK 6/8 and TRFK 31/30 had WSI $\geq$ 0.4 suggesting relatively higher tolerance to water stress compared to clones TRFK 76/1, BBK 35, TRFK 311/287 with $\geq$ 0.2 and TRFK 395/2 with $\geq$ 0.1 (Figs. 3.11 a-f).











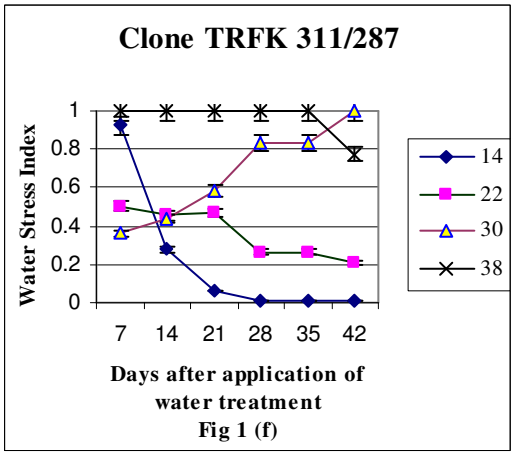


Fig. 3.11 Water stress index for the six tea clones (a)-(f) at 14, 22, 30, and 38% soil water content (SWC).

3.5 Effect of soil water content on polyphenols content in tea shoots

The total polyphenol content in shoots of the six tea clones varied but it ranged between 12 and 25% on dry weight basis. Results in this study showed that total polyphenol content was influenced by soil water content. Declining SWC progressively reduced shoot polyphenol content and the degree of that response varied with clone (Fig 3.12 a and b). The total polyphenol content in clones TRFK 6/8 and TRFK 31/30 showed almost linear response to SWC changes and were relatively higher at low SWC compared to other clones (Fig. 3.12 b). Regression analysis also indicates the range to which shoot polyphenols fluctuated in each clone when subjected to varying SWC. Clones TRFK 6/8 and TRFK 31/30 had 2.68 and 2.74% ranges, respectively which were low compared to clone TRFK 76/1 with a high range of 11.20% (Table 3.6). This response was similar to the calculated water stress tolerance index (Fig. 3.11) where clones TRFK 6/8, TRFK 31/30 and TRFK 311/287 had higher indices. The total polyphenols positively correlated with shoot extension ($P < 0.001$) and the calculated WSI while there was no significant correlation with leaf expansion (Table 3.7). There was also linear relationship between shoot growth, WSI and polyphenols content (Figs. 3.13 and 3.14).

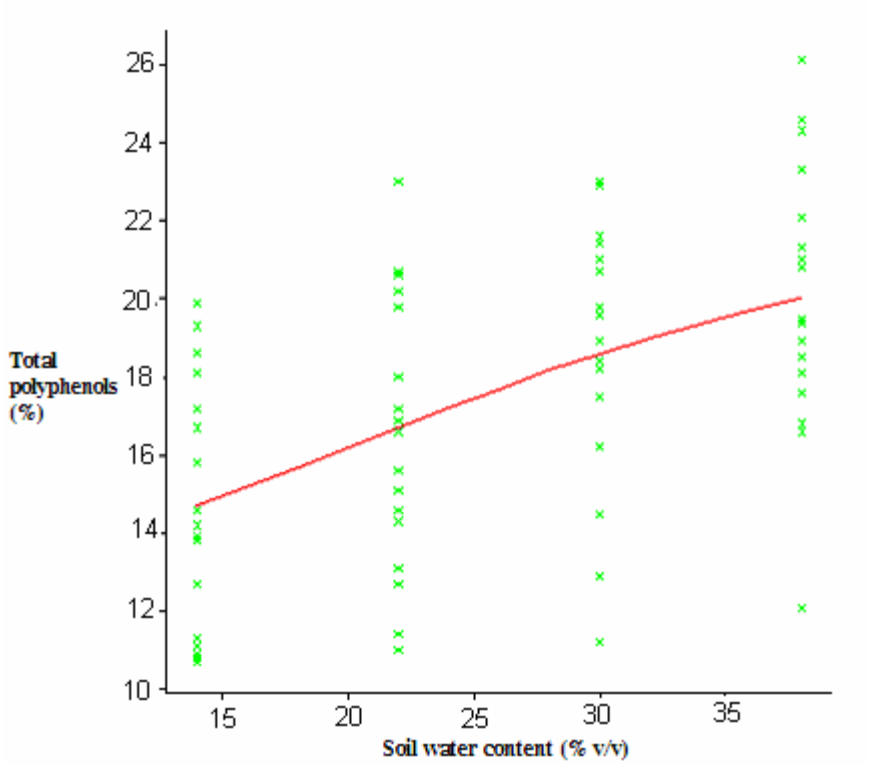


Fig. 3.12 (a) Influence of soil water content on total polyphenols in tea shoots.

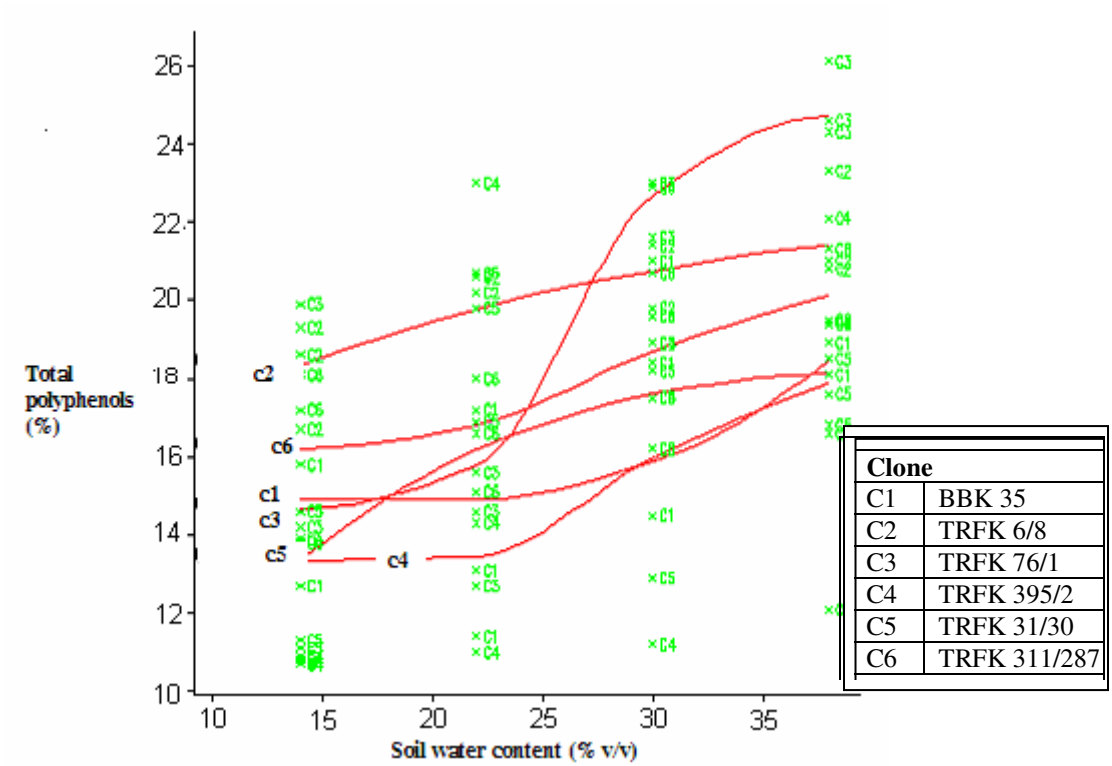


Fig. 3.12 (b) Influence of soil water content on total polyphenols in shoots of six clones.

Table 3.6 Total polyphenol content estimates, fluctuation ranges (%) obtained from regression analysis (P<0.001).

Clone	**Fluctuation range (%)**	**Lowest limit attained (%)**
BBK 35	4.71	13.62
TRFK 6/8	2.68	18.70
TRFK76/1	11.20	13.73
TRFK 395/2	5.49	12.36
TRFK 31/30	2.74	14.93
TRFK 311/287	4.07	15.88

Table 3.7 Correlation analysis for soil water content, total polyphenol content, leaf expansion, shoot growth and water stress index in tea.

		Soil water content	Polyphenols	Leaf expansion	Shoot extension	Water Stress Index
Soil water content		1.000				
Polyphenols		0.63 (0.0009)*	1.000			
Leaf expansion		0.28 (0.1803)	0.02 (0.9260)	1.000		
Shoot extension		0.76 (<0.0001)	0.74 (<0.0001)	0.03 (0.8728)	1.000	
Water Stress Index	at 5th week	0.91 (<0.0001)	0.72 (<0.0001)	0.04 (0.8491)	0.83 (<0.0001)	1.000
	at 6th week (of stress)	0.82 (<0.0001)	0.67 (<0.0004)	0.02 (0.9368)	0.82 (<0.0001)	

**P values in bracket*

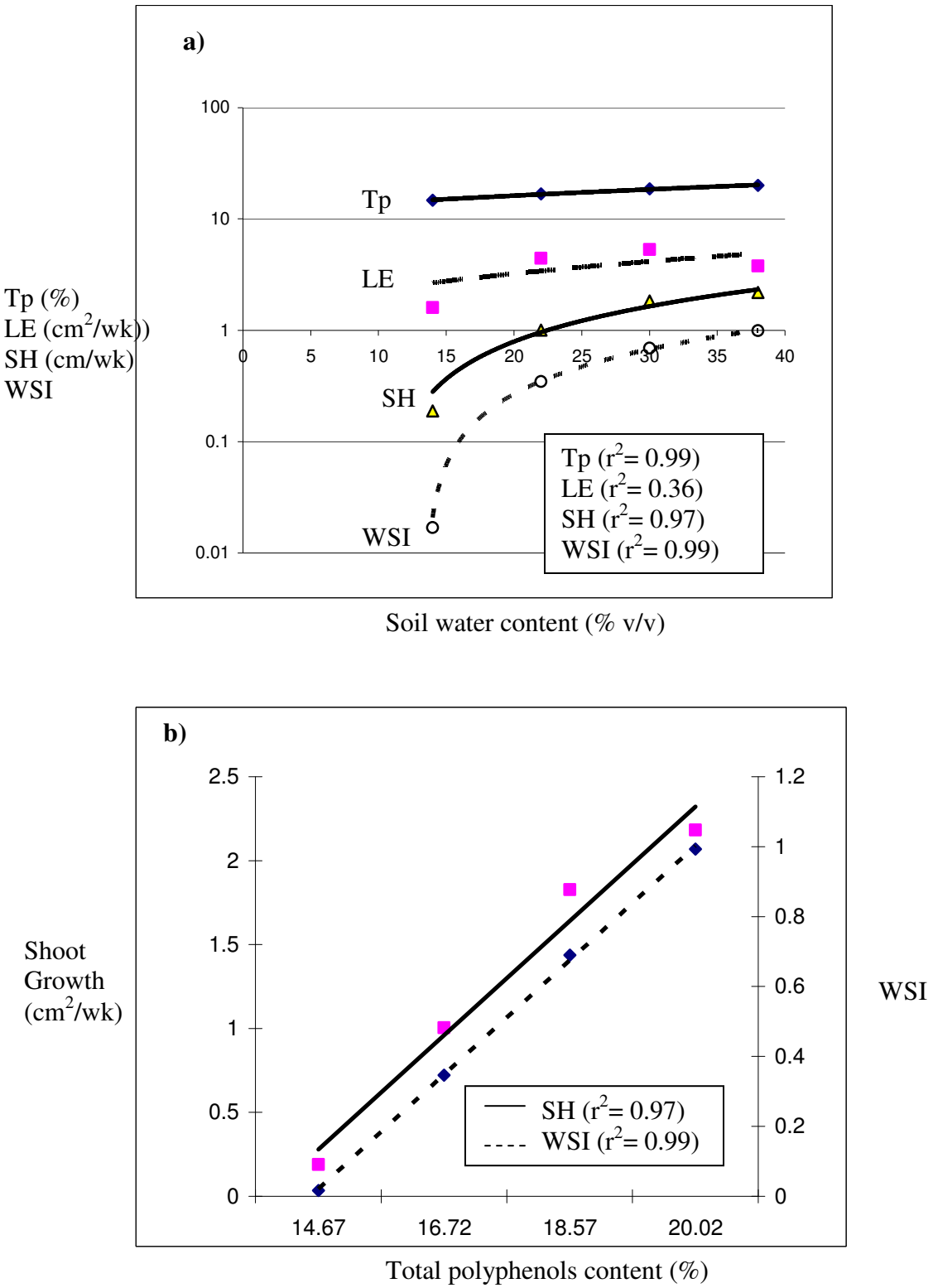


Fig. 3.13 (a) Influence of soil water content on total polyphenol (Tp), leaf expansion (LE), shoot growth (SH) and WSI in tea and b) linear relation between shoot polyphenol, shoot growth and WSI in tea.

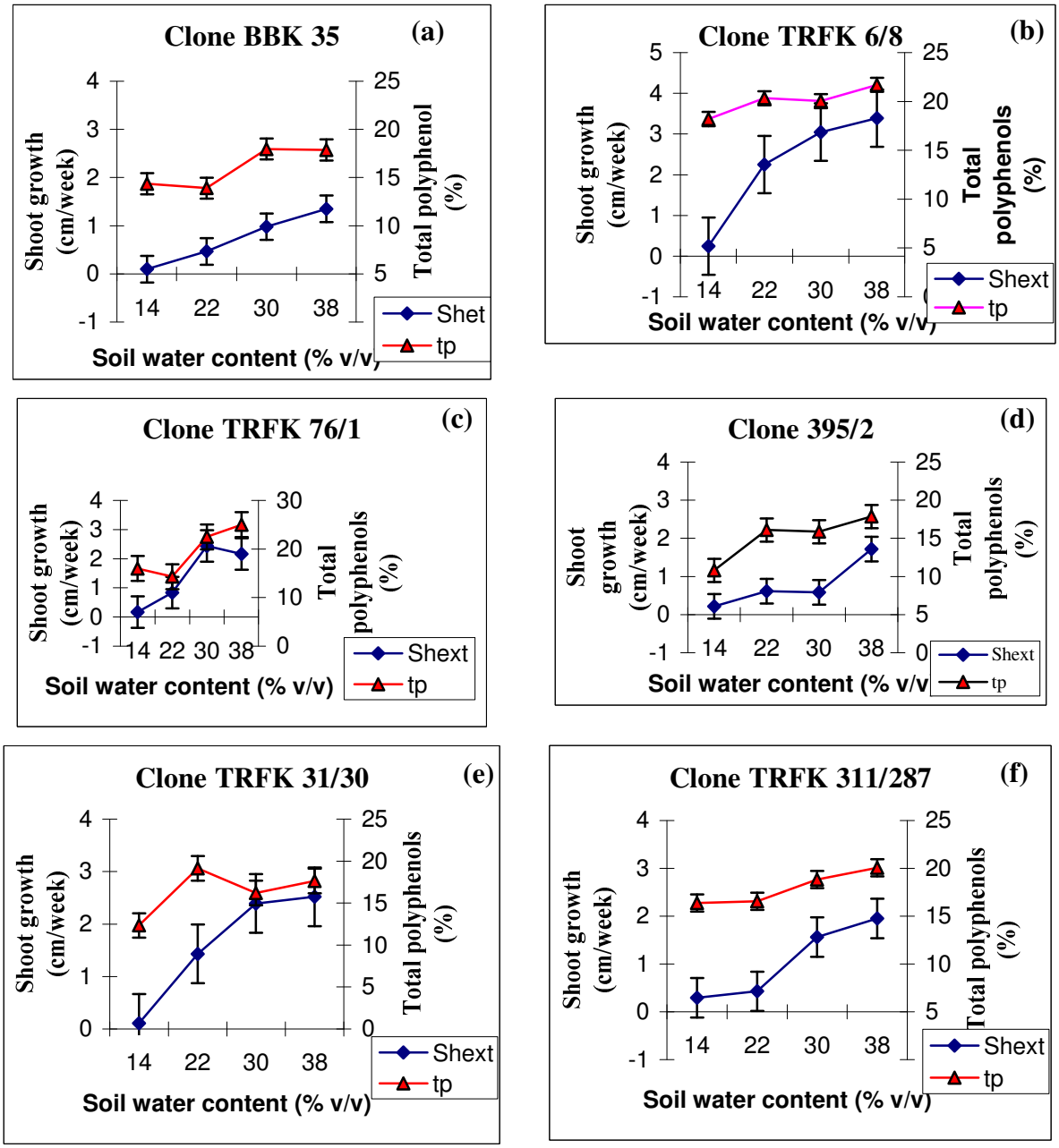


Fig. 3.14 Response of shoot growth (Shext) and shoot polyphenol (tp) content to soil water content in tea.

3.6 Effect of soil water content on catechins content in tea shoots

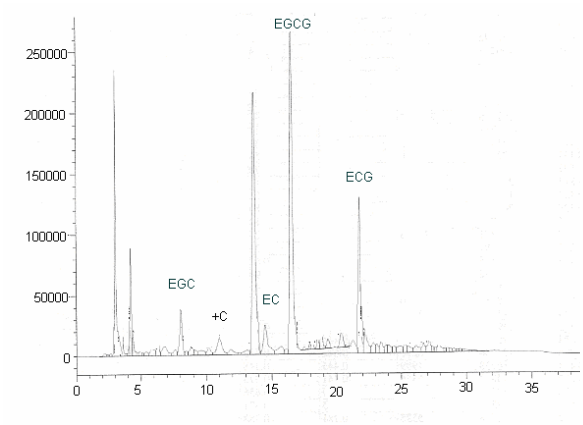
Total catechins (TC) were influenced by shoot growth (65%) and their response correlated with WSI (67%) (Table 3.8). The catechin variants determined with the HPLC were (+) catechin, epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG), of which EGCG and ECG were relatively higher in amounts (Fig. 3.15 a-l). However, not all catechin variants responded similarly when tea was subjected to varying SWC. Both (EGC) and (EC) correlated to SH (65% and 58%, respectively), WSI (65% and 63%, respectively) and SWC (50% and 62%, respectively). EGC shoot content decreased in TRFK 6/8, TRFK 76/1, TRFK 395/2 and TRFK 31/30 with decline in SWC but increased in TRFK 311/287 and BBK 35 ($P< 0.001$). The decline was gradual in TRFK 31/30, TRFK 395/2 and TRFK 6/8 with gradients of their regression curves being 0.00117, 0.00118 and 0.00118, respectively (Fig.3.16). EC content in tea shoots of the six clones declined with SWC. However, the decline rate, as indicated by the gradient of the curve varied with tea clone. The gradient of regression curve for the six clones were; 0.049, 0.001, 0.116, 0.001, 0.001 and 0.295 for clones BBK 35, TRFK 6/8, 76/1, 395/2, 31/30 and 311/287, respectively, a further indication that the decline was gradual for clones TRFK 6/8, TRFK 395/2 and TRFK 31/30 (Fig. 3.17).

Table 3.8 Correlation analysis between total polyphenols (TP), shoot growth (SH), leaf expansion (LE), leaf relative water content (RWC), water stress index (WSI), total catechins (TC), and some flavanoid derivatives in tea.

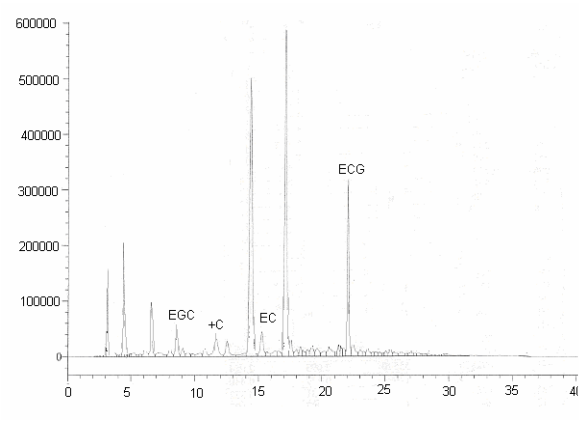
	TP	**SH**	**LE**	**RWC**	**WSI**	**SWC**
TP	1.000					
SH	0.74 *(<0.0001)	1.000				
LE	0.02 (0.9260)	0.03 (0.8728)	1.000			
RWC	0.52 (0.0092)	0.70 (0.0001)	0.40 (0.0513)	1.000		
WSI	0.72 (<0.0001)	0.83 (<0.0001)	0.04 (0.8491)	0.77 (<0.0001)	1.000	
SWC	0.63 (0.0009)	0.76 (<0.0001)	0.28 (0.1803)	0.82 (<0.0001)	0.91 (<0.0001)	1.000
TC	0.90 (<0.0001)	0.65 (0.0006)	-0.09 (0.6718)	0.44 (0.0330)	0.67 (0.0004)	0.54 (0.0066)
GC	0.20 (0.3493)	-0.06 (0.7867)	0.41 (0.0476)	0.23 (0.2677)	0.05 (0.8032)	-0.04 (0.8550)
GA	0.04 (0.8640)	-0.13 (0.5468)	0.14 (0.5029)	0.15 (0.4742)	-0.14 (0.5086)	-0.10 (0.6524)
EGCG	0.39 (0.0579)	0.28 (0.1907)	-0.26 (0.2242)	0.26 (0.2230)	0.27 (0.1976)	0.14 (0.5018)
EGC	0.81 (<0.0001)	0.65 (0.0006)	-0.07 (0.7445)	0.31 (0.1375)	0.60 (0.0021)	0.50 (0.0133)
ECG	0.67 (0.0004)	0.38 (0.0656)	0.10 (0.6379)	0.45 (0.0258)	0.55 (0.0049)	0.48 (0.0164)
EC	0.83 (<0.0001)	0.58 (0.0032)	0.20 (0.3444)	0.36 (0.0803)	0.63 (0.001)	0.62 (0.0014)
CAFF	0.24 (0.2591)	-0.09 (0.6643)	0.21 (0.3245)	0.01 (0.9632)	-0.12 (0.5771)	-0.23 (0.2849)

**P values in bracket*

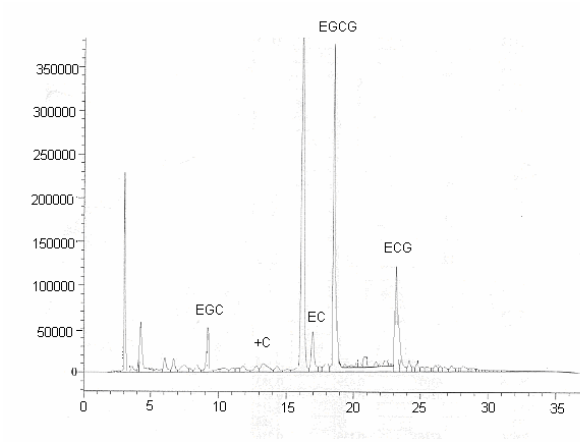
a BBK35 (14% v/v SWC)



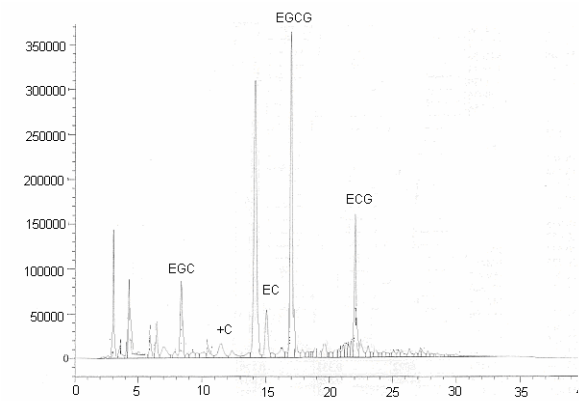
b BBK35 (30% v/v SWC)



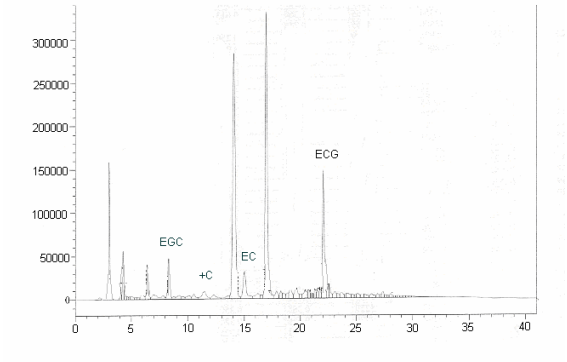
c TRFK 6/8 (14% v/v SWC)



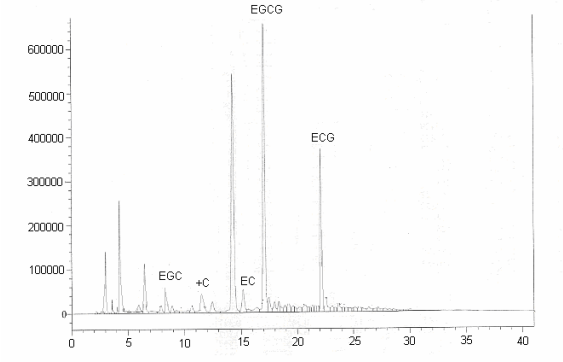
d TRFK 6/8 (30% v/v SWC)



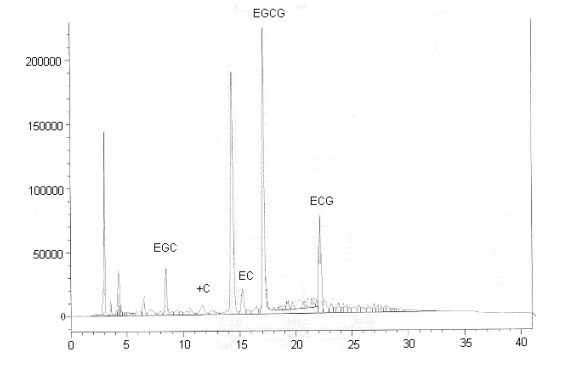
e TRFK 76/1 (14% v/v SWC)



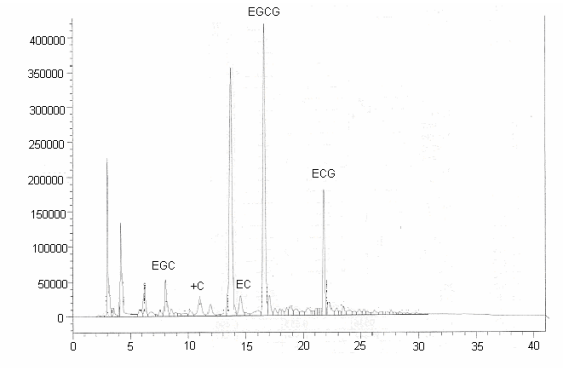
f TRFK 76/1 (30% v/v SWC)



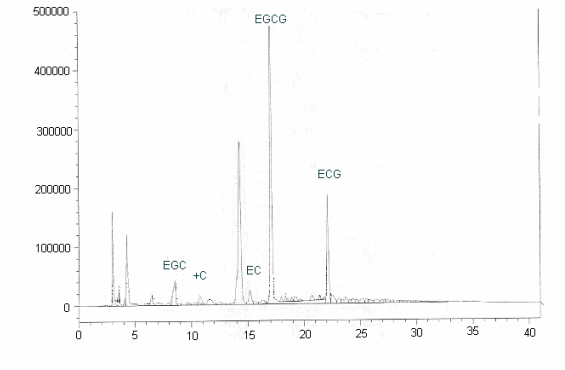
g TRFK 395/2 (14% v/v SWC)



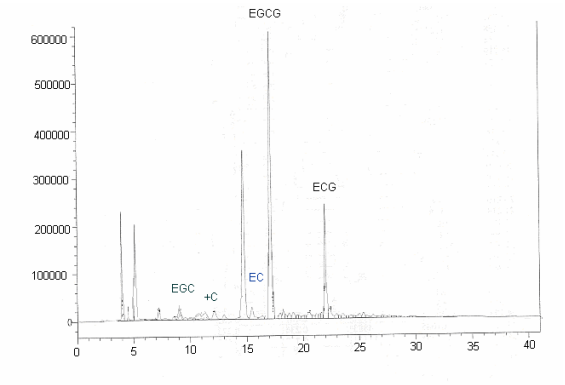
h TRFK 395/2 (30% v/v SWC)



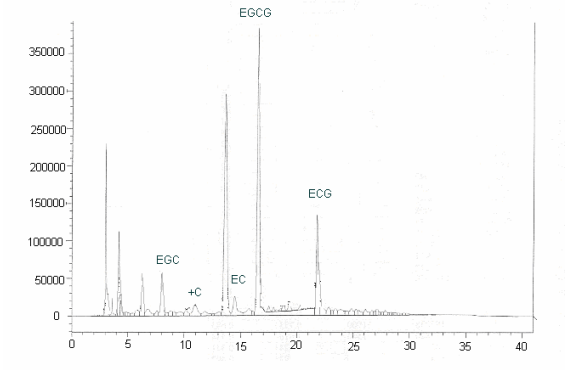
i TRFK 31/30 (14% v/v SWC)



i TRFK 31/30 (30% v/v SWC)



k TRFK 311/287 (14% v/v SWC)



l TRFK 311/287 (30% v/v SWC)

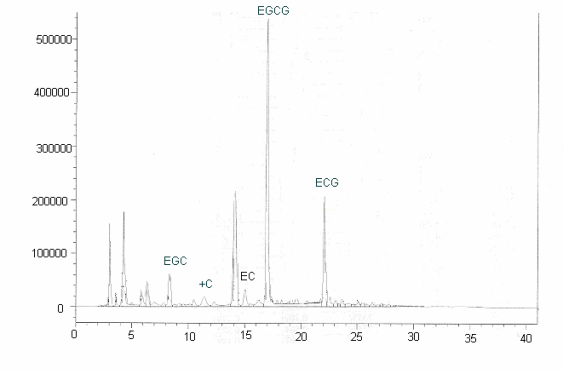
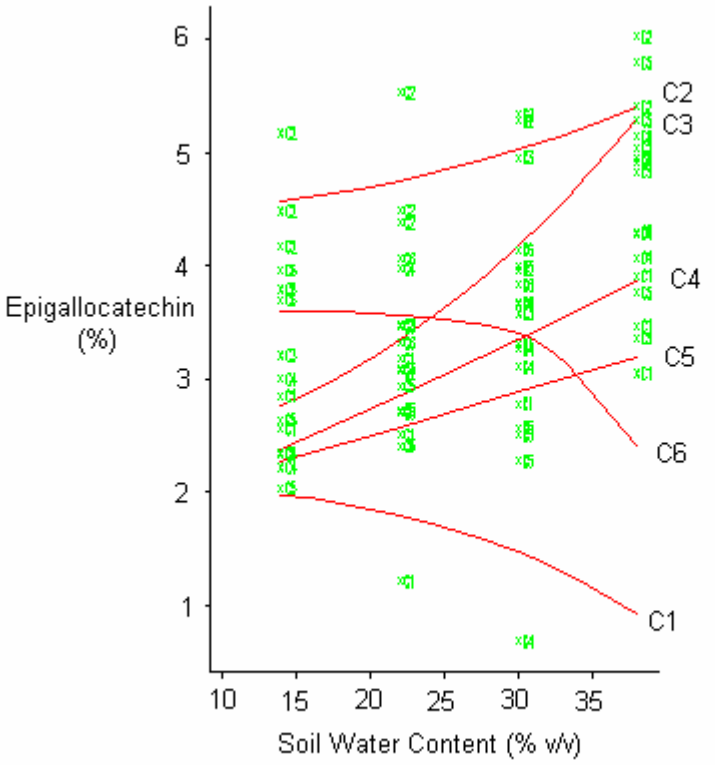
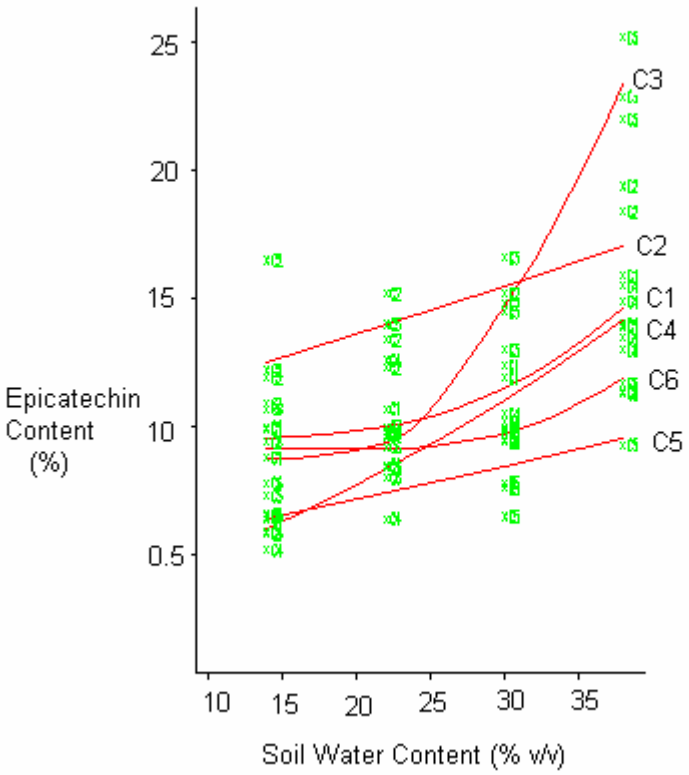


Fig. 3.15 HPLC elution profiles of the catechin variants in the six tea clones at two of the four soil water contents treatments.



Clone	
C1	BBK 35
C2	TRFK 6/8
C3	TRFK 76/1
C4	TRFK 395/2
C5	TRFK 31/30
C6	TRFK 311/287

Fig. 3.16 Effect of SWC on epigallocatechin in shoots of the six tea clones. ($P < 0.001$, SE = 0.69, n = 72, adjusted r^2 = 61%).



Clone	
C1	BBK 35
C2	TRFK 6/8
C3	TRFK 76/1
C4	TRFK 395/2
C5	TRFK 31/30
C6	TRFK 311/287

Fig. 3.17 Effect of SWC on epicatechin in shoots of the six tea clones.
($P < 0.001$, SE = 0.22, n = 72, Adjusted r^2 = 73.4%).

3.7 Discussion:

Results from this study indicate that declining soil water content limits growth of tea and that there is a SWC threshold below which normal physiological function is checked with resultant limitation to tea growth. Soil water deficit (SWD) elicited responses in tea which influenced shoot growth and leaf expansion, changes in leaf RWC, stomata conductance, net photosynthesis and dry matter partitioning. Shoot growth was limited at 28% SWC along with stomatal conductance, transpiration and photosynthesis while leaf expansion, RWC and shoot water potential declined at 18% SWC, indicative of shoot sensitivity to drought conditions compared to leaf expansion (Otieno *et al*., 2001). An impending soil water deficit is perceived by plant roots and a signal which could be mediated by ABA, and possibly other organic compounds (Cruz *et al*., 1998; Liu *et al*., 2005), is transmitted to leaves for appropriate response the function of which is mostly to curtail tissue water loss. However, though efflux of water was greatly reduced by restriction of stomata (Fig.3.6 and 3.8), a progressive reduction in photosynthesis occurred and this could be attributed to restricted influx of CO_2 and declining tissue water (Fig.3.7).

Dry matter (DM) partitioning in tea is estimated at 25-40% in the leaves, 40-52% in woody tissue and 13-32% in the roots (Wachira and Ng'etich, 1999). Soil water deficit reduces dry matter yield in plants, causing a major reduction in shoot leading to a reduced shoot-to-root ratio (Steinberg *et al.,* 1990), which was the case in this study. The limited photosynthates caused by the effect of soil water deficits may have led to the observed DM partitioning. It has been shown that root development in tea is influenced by soil water status. For instance, rooting depth in tea has been found to increase in dry

environments compared to wet ones and mulching as a cultural practice encourages shallow roots in tea due to conserved soil water in the upper soil layer (Prematilake *et al.*, 2004). It has been argued under the theory of functional balance put forth by Thornley (1972) that plants will react to a limited water availability with a relative increase in the flow of assimilates to the roots leading to reduced shoot-to-root ratio. Therefore, increased DM partitioned to roots in tea suggests a plant strategy to increase root volume for water harvesting (Gindaba *et al*., 2005; Slama *et al*., 2006) while reducing transpiration surface by significant reduction in shoot growth (Liu *et al*., 2005). Secondly, it may be a survival strategy whereby after sensing drought, perennial plants invest in storage organs, mostly stem and roots. Results on dry matter partitioning in this study implies a potential beneficial effect of mild drought in tea in the long term. A short dry spell that may reduce SWC to 28-20% can stimulate an increase in root volume which could ensure better survival in future recurrence of soil water deficit.

Plant water status affected tea performance. Leaf RWC was positively correlated with SWC (r^2= 76%, P<0.001) and shoot extension (r^2= 56%, P<0.001). However, shoot water potential was weakly correlated to SWC (r^2= 37%, P<0.001) and shoot growth (r^2= -38%, P<0.001) which suggests that leaf water status and growth depended on the soil water reservoir to a great extent and less on plant suction force. Photosynthesis was less sensitive to declining SWC unlike stomatal conductance, which corroborates work done elsewhere (Liu *et al*., 2005). This observation explains why the break-point for photosynthesis was later than that of stomatal conductance as SWC declined (Fig 3.6 and 3.7). Restriction of stomata as a response to declining leaf tissue water is a plant strategy to reduce water loss (Cruz *et al*., 1998; DaMatta, 2004; Pezeshki and Goodwin 2004;

Romero and Botia, 2006) and is aimed at improving photosynthetic water use efficiency (A/gs) at initial stages of water stress (Liu and Stutzel, 2002 and 2004). However, further decline in soil water leads to a reduction in leaf RWC which, in addition to limiting photosynthesis and cellular metabolism, can disrupt enzyme function, interfere with transport channels, disorganize intercellular organelles and result in shrinkage of cell wall (Franca *et al*., 2002; Gindaba *et al*., 2005), a process likely to have occurred when SWC declined below 20%. Cell turgidity fell at 18-20% SWC as indicated by leaf RWC with a concurrent further drop in photosynthesis. Given that cell functions are dependent on cell integrity, which seemed curtailed at 20% SWC, it therefore appears that the soil water content limit for tea is 20% v/v. This study clearly indicates that though significant growth reduction occurred at 28% SWC, which could lead to yield reduction under field conditions, the threshold SWC below which disruption of crucial biochemical processes occur in tea is or close to 20%. Therefore leaf productivity in tea can progressively appreciate at SWC above 28% and is expected to decline with varying degree depending on individual clone response when SWC fall below 28%.

Shoot water potential (Ψ_w) indicates the relative pressure a plant needs to exert in order to draw water through its xylem vessel. Thus, clones with higher (Ψ_w) would be expected to maintain high RWC. Clones TRFK 31/30, TRFK 311/287 (4n) and clone TRFK 6/8 with higher (Ψ_w) at low SWC subsequently showed higher leaf RWC compared to the other clones in the study (Fig. 3.4 and Table 3.3). The same clones attained lower SRR at decreasing SWC which could be a complementary effect to maintaining adequate tissue water by minimizing transpiration surface through restricting

shoot over root growth (Fig 3.5 b) and imposing greater stomata restriction (Fig. 3.6 b). By restricting stomata it was possible for these clones to conserve tissue water and hence the high RWC unlike the other clones. Though SRR declined with SWC, shoot growth was not severely affected even though leaf expansion reduced.

In addition, elasticity in shoot water potential with the SWC spectrum seems to indicate water stress tolerance. Clones which could stretch for less than 2 bars have equally high WSI while those with ≥ 2 showed varied responses (Table 3.4 and Fig. 3.10). In that respect therefore, clone TRFK 6/8 and the two tetraploids (TRFK 31/30 and TRFK 311/287) are more tolerant to soil water stress among the clones in the study.

With regards to tea polyphenols content, water stress lowered total polyphenols content in tea shoots (Figs. 3.12, 3.13 and 3.14) and there was significant correlation between SWC and phenolic content, WSI and shoot growth in tea (Table 3.7). This was expected because water is one of the raw materials in photosynthesis and directly impacts on organic synthesis for both growth and secondary metabolites. Though a significant correlation between polyphenol content and shoot growth was observed, there was none with leaf expansion. Leaf is the source of photo assimilates which yields the precursors for secondary metabolites such as malonyl-CoA and *p*-coumaroyl-CoA (Magoma *et al.*, 2000; Schijlen *et al.*, 2004; Wheeler and Wheeler, 2004) which are dependent on light and SWC (Stewart *et al*., 2001). In resource-limited environment, the theory of functional balance explains why shoot growth and not leaf growth correlates with phenolic content (Thornley, 1972). The organ that has close proximity to a limiting resource is less affected by unavailability of that resource compared to a far-off sink within the plant.

Of significance was the potential amount and extent of variation of total polyphenols content in each clone which could provide a basis for clonal selection, improvement and/or management of tea for better yield and quality. Clones TRFK 6/8, TRFK 31/30 and TRFK 311/287 had relatively higher polyphenols content compared to the other clones in the study. Similarly, the same clones had higher WSI, indicating that they were more tolerant to water stress. Given the close correlation of shoot growth (as influenced by SWC) and WSI to total polyphenols, these results suggest an association of plant polyphenols with water stress in tea. This observation agrees with results of Yaginuma *et al*. (2002) who noted increased polyphenols in light and water stress resistant Safflower and Cucumber seedlings compared to those which responded weakly to the stresses. Further investigation on flavonoid contents in Safflower revealed that a strong antioxidant was responsive to both light and water stress while a weak antioxidant remained unchanged (Yaginuma *et al*., 2003). It therefore provides pointers that though total tea polyphenols content may be related to water stress tolerance in tea, flavonoid derivatives particularly the rich tea catechins could be more useful and that should be subject of further investigation. Besides absolute amount of polyphenols, results in this study showed varied fluctuation of polyphenols content with changes in SWC and seem to suggest that clones with more stable polyphenols are more tolerant to water stress (Table 3.6). It implies that tea cultivars which have more stable phenolic content are less affected by changes in soil water and photo assimilates and this therefore reflects tolerance to drought. However, owing to the large groups of polyphenols, there are limitations in use of total polyphenols as reliable response indicator to stress and therefore there is need to go into specific compounds.

In this study, the response of tea catechins (in the flavan-3-ol) to SWC was determined in which only EC (epicatechin) and EGC (epigallocatechin), among the other catechin variants, correlated with shoot growth, WSI and SWC in tea (Table 3.9). The observed differences could be due to the chemical structures of the catechin variants. Both EC and EGC have 3', 4'-dihydroxy group in the B-ring, and an H^+ and OH^- at position 5' in the C-ring for EC and EGC, respectively (Fig 2.1 b and c). Though ECG (epicatechin gallate) and EGCG (epigallocatechin gallate) also have the 3', 4'-dihydroxy group in B-ring, the notable difference is a keto-group attached to position 3 in the C-ring (Fig. 2.1 d and e). It has been shown that the molecular structure and particularly the hydroxyl groups influence the radical-scavenging activity of flavonoids (Amic *et al*., 2003). Increase in antiradical activity has been associated with substitution of 3', 4'-dihydroxy on the B-ring while low antiradical scavenging activity was observed where both the 3-hydroxyl group in C-ring and the 3', 4'-dihydroxy in the B-ring were missing (Amic *et al*., 2003). Plants under water stress produce oxygen derived free radicals, the reactive oxygen species (ROS), which are associated with tissue damage (Pirker *et al*., 2002 and 2003). The ROS include superoxide anions (O_2^-•), hydroxyl radicals (•OH) and hydrogen peroxide (H_2O_2). The ROS usually occur within tolerable balance in plant tissue, but that balance may be exceeded under stress condition, with a likely damage to the tissue. In order to counter the likely damage caused by the free radicals, plants produce antioxidants such as ascorbate, glutathione, carotenoid and flavanoids among others (Tűrkan *et al*., 2005; Wu *et al*., 2005). In the current study, though total polyphenols correlated with drought stress in tea, the response of catechin variants, notably EC and EGC, positively correlated with water stress in tea clones (Table 3.8).

The response of EC and EGC, unlike ECG and EGCG to water stress in tea can be attributed to structural differences, particularly the absence of the carboxyl-group in EC and EGC (Fig. 2.1 d and e).

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CHAPTER FOUR

EFFECT OF NITROGEN SUPPLY AND SOIL WATER CONTENT ON GROWTH, SHOOT DENSITY, AND YIELD OF TEA [*Camellia sinensis* (L.) O. *Kuntze*]

Abstract

In experiments to determine the association of fertilizer with soil water deficit in field-grown and potted tea plants of clone BBK 35, five rates of fertilizer (0, 75, 150, 225 and 300 kg/ha) and six levels of soil water content (38, 34, 30, 26, 22 and 18% v/v) in potted plants were applied in complete randomized design and replicated three times. The soil water treatment in potted plants was maintained for a period of 12 weeks during which shoot growth, plant water relation and dry matter partitioning in tea was monitored. Response of field grown tea to fertilizer and soil water deficit was determined during dry months between November and March at three locations in major tea growing areas of Kenya. Field data included shoot population density and yield, and soil water content. Fertilizer supply exacerbated drought effect on tea through disproportionate partitioning which consequently weakened tea tolerance to desiccation. It increased leaf-to-root and leaf-to-mass ratios ($P<0.001$), reduced shoot growth, shoot water potential and specific leaf area ($P<0.001$). These results are discussed in the light of tea plant's requirements for N nutrition and a possible new dimension on fertilizer management for tea during recurring drought periods in tea zones.

4.1 Introduction

Fertilizer nutrition is among the regular field management practices with significant bearing on both yield and quality of tea [*Camellia sinensis* (L.) O. Kuntze]. Tea leaf yield responds favourably to large amounts of N fertilizer under suitable growing conditions of adequate rainfall amounts (1400-1500 mm), fairly distributed throughout the year and mean air temperature of 22^{o}C (Carr, 1971). The incredibly high response of tea to N supply could be due to frequent loss of N through harvest of shoots whose N content is about 4%. Though N supply improves yield of tea, it reduces the quality of black tea (Owuor, 1997). Quality of tea is a priority for sustenance of market demands particularly for Kenyan tea where 95% of the produce is destined for export market (International Tea Committee, 2003). Though positive yield response of tea to N supply rate of upto 500 kg N ha^{-1} has been reported (Owuor *et al*., 1994), N supply rarely exceeds 300 kg N ha^{-1} due to its negative effect on black tea quality. Consequently, the recommended N rate that gives best compromise for both yield and quality in Kenya is 150-200 kg N ha^{-1} (Owuor, 1997) supplied as NPKS (25-5-5-5), NPK (25-5-5) or NPK (20-10-10) fertilizer material sources.

Tea production in Kenya and in the region experiences frequent droughts and this remains the single most recurring challenge. Kenya's tea growing areas experience a regular dry spell between November and March, usually ranging from 3 to 4 months during which yield losses occur and sometimes plant deaths in severe cases. It is estimated that the annual 3-4 months dry spell result in 1.5-2% plant deaths (Kenya Tea Growers Association, personal communication)

A worrying situation has emerged following observation that fertilizer could be compounding drought effect, and thus negating potential gains of prudent management practice in tea. Ng'etich (1999) has reported plant deaths during drought in tea supplied with higher fertilizer rates which suggests an association between fertilizer supply and plant susceptibility to water stress. In related instances cotton plants supplied with low N were also found to be tolerant to dehydration compared to plants supplied with higher rate of N (Radin and Parker, 1979). Though N improves productivity in most plants, it seems to compromise plant tolerance to desiccation. It is not clear whether lack of tolerance to water stress as a result of high rate of N could be due to interference of N in plant biosynthesis, soil-plant hydraulic conductivity and/or assimilate partitioning.

This study was conducted to investigate the relationship between N nutrition and drought susceptibility of tea. It was hypothesized that high rates of N fertilizer in tea raises the optimal soil water content required for tea and subsequently lowers tea productivity and survival during drought.

4. 2 Materials and Methods

4.2.1 Rain-out shelter experiment

Set-up of a rain-out shelter

This study was conducted in a rain-out shelter with conditions similar to those in a green house, and whose dimensions were 17 x 6.5 m ground space and was raised to a height of 2.5 m. A dome-shaped roof above the 2.5 m height was erected whose diameter was 0.5 m with a 0.3 m extended eve all round. The roof was covered with an ultra violet-treated 200-micron film clear polythene sheet (Sunselector AD – IR 504) designed to transmit 82% of photosynthetically active radiation (PAR), 65% of diffused light and with 88% thermicity (Pate 3.1 and 3.2). The sides were covered with the same polythene but to 1 m height from ground level on the length sides and to full height on the widths. A free space of 1.5 m along the lengths allowed free air flow in and out of the structure. An entrance measuring 1 m wide by 2 m high was made in the middle of the westwards-facing width using wooden frames and covered by chicken wire with no polythene.

Study materials and treatment application

a) Plant materials

Camellia sinensis clone BBK 35, was used in this study because it was the same one available for field study (in another but related experiment) in three diverse sites (section 4.2.2). The plant materials were developed from vegetative leaf cuttings and raised in seedling nursery as recommended (TRFK, 1986). The seedlings were transplanted into 1000 gauge black polythene sleeve with a diameter of 0.22 m and a depth of 0.40 m. A total of 270 seedlings were transplanted in the polythene sleeves and given three months

to establish after which nipping of the shoots was done to encourage regeneration of more shoots. The plants were transferred to the rain-out shelter two months later.

Soil medium for raising seedling from stem cutting in nursery was obtained from forest area. The same soil medium was used for raising transplanted seedling in this experiment. Result of chemical analysis was as follows: 3.5% N, 0.16% P, 169 ppm K, 255 ppm Ca, pH 4.3, organic matter 9.3% and its textural class was clay with 25% sand, 67% clay and 8% silt.

b) Application of treatments

Each tea plant was subjected to 6 different levels of soil water content and 5 rates of NPKS fertilizer. The soil water content levels were 38, 34, 30, 26, 22, and 18% v/v while NPKS rates were, 33.91, 25.43, 16.96, 8.48 and 0 gms per pot, which when calculated on the basis of amount hectare, taking consideration of a 30 cm depth plough layer was equivalent to 300, 225, 150, 75 and 0 kg N ha^{-1}, respectively. The thirty factorial treatment combinations were as follows;

N0W1	N1W1	N2W1	N3W1	N4W1
N0W2	N1W2	N2W2	N3W2	N4W2
N0W3	N1W3	N2W3	N3W3	N4W3
N0W4	N1W4	N2W4	N3W4	N4W4
N0W5	N1W5	N2W5	N3W5	N4W5
N0W6	N1W6	N2W6	N3W6	N4W6

Where;

W1, W2, W3, W4, W5, and W6 are soil water contents levels: 18%, 22%, 26%, 30%, 34%, and 38% v/v, respectively.

N0, N1, N2, N3, and N4 are nitrogen fertilizer supply rates: 0, 75, 150, 225, and 300 kg ha^{-1}, respectively.

The 6 x 5 treatments were arranged in a CRD and replicated 3 times. Each experimental unit had 3 potted plants which were maintained uniformly except for the specified treatments.

The fertilizer treatment was carried out soon after placing the plants in the rain-out shelter, about 5 months after transplanting, which was sufficient time to allow for roots to establish in the pot media. Following fertilizer treatment the plants were adequately supplied with 160-200 mm of water per pot which was given in two splits daily but avoiding drainage. This was done consistently for four weeks, a period thought adequate for one to observe N response. Thereafter, the water supply was progressively reduced to 80-100 mm for 4 days and further reduced to 50 mm for another 4 days, after which SWC level for each experimental unit was effected which took a week to stabilize. Soil water content was checked and maintained twice daily using a Trime FM-2 TDR (Eijkelkamp Agrisearch Equipment) soil moisture meter. When the pre-set SWC levels were attained (about two weeks after introducing water treatment), tagging of plants and initial shoot and leaf measurements followed, and thereafter, done every week.

Plate 4.1 Trime FM2, TDR soil moisture meter inserted in soil and soil water content indicated on read-out circled with blue.

Plate 4.2 Trime FM2 probe used for soil water content determination in the experiment.

Growth measurement

Growth as influenced by both soil water content and fertilizer treatment was determined by shoot extension, leaf expansion and dry matter partitioning.

(i) Shoot growth

Two shoots, one from each of the two plants in each experimental unit, were randomly selected and tagged. The shoots were tagged between the first and second leaf in the second week of soil water treatment, and labeled as S1 and S2 respectively in each experimental unit. Initial shoot measurement was done immediately after tagging and repeated weekly. Shoot length was measured in centimeters from the node above the tag to the uppermost visible node, and then corrected to two decimal places using veneer caliber. Increase in shoot growth was calculated by subtraction of previous reading from every successive measurement and results expressed as shoot growth (l) over time (t).

(ii) Leaf expansion and specific leaf area

Two leaves from separate shoots in two different plants in each experimental unit were randomly selected and tagged for leaf growth measurements during the second week following water treatment. In each, the first opened leaf was selected and tag tied at the petiole and labeled as L1 and L2 respectively. Leaf area of the leaves was determined by measuring the length of leaf blade (l) from the base to the apex, and width (w) on the broadest part. The product of the two (lxw) was multiplied by a correction factor k known to be 0.62 for tea (Ng'etich and Wachira, 1992). Leaf area measurements were initially done immediately after tagging and repeated weekly for four successive weeks. Increase

in leaf area on weekly basis was determined by subtracting the previous from succeeding leaf area and the magnitude of increase was an indication of tea response to soil water and fertilizer treatments.

After four weeks, the tagged leaves were picked and oven-dried at 65^{o}C to constant weight and dry weight taken. Leaf-to-mass ratio (LMR) or specific leaf area (Younis *et al.,* 2000) was calculated as leaf mass/leaf area (leaf area was determined before drying).

Plate 4.3 Tagged leaf for serial measurements on leaf expansion.

Plate 4.4 Leaf area measurement; *l* (blue arrow) and *w* (red arrow).

Plate 4.5 Tagged shoot and marked portion indicating extent measured during the weekly interval.

(iii) Dry matter partitioning

Destructive sampling to determine dry matter partitioning was done during 12th week after water treatment, which corresponds to the three month dry period in most tea zones of Kenya. One of the three potted plants in each experimental unit was randomly picked and then soaked by immersing the root section in a bucket of water for a period of 10 minutes. The soaked plant was removed and placed on a polythene mart which was spread outside on grass. The shoot was cut at the crown level and all leaves plucked leaving stems and branches only. The leaves were placed in labelled paper bags while stem and branches were cut into small pieces of 8-10 cm length and placed in a separate labelled paper bag. The polythene tube carrying the soaked root section was cut-open and roots washed slowly in running water let-in through a hose pipe. When most of the soil was removed, the roots were placed in a 2 mm mesh grid and water pressure increased to remove soil particles attached to roots to give clean roots. Care was taken to retain all roots including tiny feeders. After washing, the clean roots were left briefly in the sun to drain off water before putting them in a third labelled paper bag. The three different parts of each plant in 3 separate paper bags were dried at 65^{o}C in drying oven to constant weight and the dry weight taken. Shoot-to-root ratio (SRR), leaf-to-root (LRR) and leaf-to-total mass (LMR) were calculated using the obtained data. Each of the dry parts was subsequently milled using a blender and samples stored for laboratory analysis.

Plant water relation

Plant water status was determined by shoot water potential (ψ_w) using pressure chamber (Plant Water Console 3005, Soil Moisture Equipment Corp., U.S.A.) and leaf relative water content (RWC) calculated as follows (Milnes *et al*., 1998); RWC = (fresh leaf mass-dry mass)/(fully turgid fresh mass-dry weight). Leaf stomata conductance and transpiration rate was determined as a response to water status. The stomata conductance and transpiration was taken on the second leaf on randomly picked shoot in each experimental unit. This was done between 11.00 and 14.00 hrs using a steady state porometer (Li-Cor, Inc. Lincoln, NE, USA) model LI-1600 (Sanchez-Blanco *et al*., 2002).

Monitoring environmental conditions of rain-out shelter

Growing conditions of tea in green house were monitored using an automatic weather station. The components used in the weather determination set up were a pyranometer (LiCor, USA), an anemometer, copper thermocouples which were connected to CR10 micro-logger (Campbell scientific, USA) for measurements of wet and dry bulb, and soil temperature respectively. All data were stored in data logger which was programmed to take data on 30 minutes interval but stored in hourly averages. The data was off loaded every 2 weeks using a portable computer.

4.2.2 Field experiment

Response of fertilizer supplied tea to drought was monitored in an established field experiment. The experiment was set up in 1998 by TRFK in selected tea growing areas to

establish appropriate fertilizer rates for tea. This work picked on only three sites, which represents the diverse environmental conditions in tea growing zones of Kenya, and where the experiment was running. The sites were Timbilil ($0^{o}22'S\ 35^{o}\ 21'E$), Changoi ($0^{o}29'S\ 35^{o}14'E$) and Karirana ($01^{o}05'S\ 36^{o}42'E$).

a) Site characteristics;

Timbili estate ($0^{o}22'S\ 35^{o}\ 21'E$) is situated in Kericho District and is at 2200 m above sea level. It receives 2100 mm of rainfall annually and a monthly range of 70-270 mm which peaks in April and May and with dry months in December – February. The daily mean temperature is $15^{o}C$ (Jaetzold and Schmidt, 1983). The soils are well-drained, deep dusky red to dark reddish brown, friable clay with acid humic topsoil referred to as *humic Nitisols*. Karirana ($1^{o}10'S\ 36^{o}\ 20'E$) situated in Limuru, is a relatively cold and wet location. It is at 2300 m above sea level and receives 1900 mm of rain annually. The monthly rainfall ranges between 50-260 mm with the highest amounts being received in April and May and lowest in December to February. The mean daily temperature is $14^{o}C$ and well drained, deep, dusky red to dark reddish brown, friable clays with acid humic topsoil (*humic Nitisols*). Changoi ($0^{o}29'S\ 35^{o}14'E$) is situated in Kericho and has well drained, deep soils. It is at 1800 m above sea level and receives about 1700 mm of rainfall annually. The soils are dark to reddish brown with acid humic topsoil classified as *humic Nitisols*. (Jaetzold and Schmidt, 1983; Ng'etich, 1995).

b) Application of treatments

The experiment was conducted on clone BBK 35 which was supplied with five different rates of N fertilizer. The fertilizer rates were 0, 75, 150, 225 and 300 kg N ha^{-1} $year^{-1}$ with source being NPKS (25-5-5-5), arranged in randomized complete block design and replicated three times. Each experimental unit measured 6 x 3.6 m with at total of 4 rows of 60 established tea bushes spaced at 1.2 x 0.6 m. The fertilizer was supplied in one doze in July annually, which corresponds to the farmers' practice, a time based on low labour demand for tea plucking due to low leaf yield.

Data on tea response to drought was determined on shoot regeneration and yield between December and March in 2003/04 and 2004/05 seasons. Sampling and harvesting of shoots was done at 2 weeks interval between December and March. During sampling, a 0.5 m quadrant was randomly thrown on tea table in each plot and all shoots at 2 leaves and a bud within the quadrate harvested. The shoots were counted and placed in a labelled paper bag and oven dried at 70^{o}C to constant weight. Both the shoot count and shoot dry weights were recorded during the dry season.

c) Soil Water Content

Soil water content during the dry season was monitored at Timbilil and Changoi only during 2004/05 season using Trime FM2 TDR soil moisture meter (Eijkelkamp Agrisearch Equipment, The Netherlands). The limitation in site and season for this measurement was due to availability and transport of the soil moisture measuring equipment.

4.2.3 Data analysis

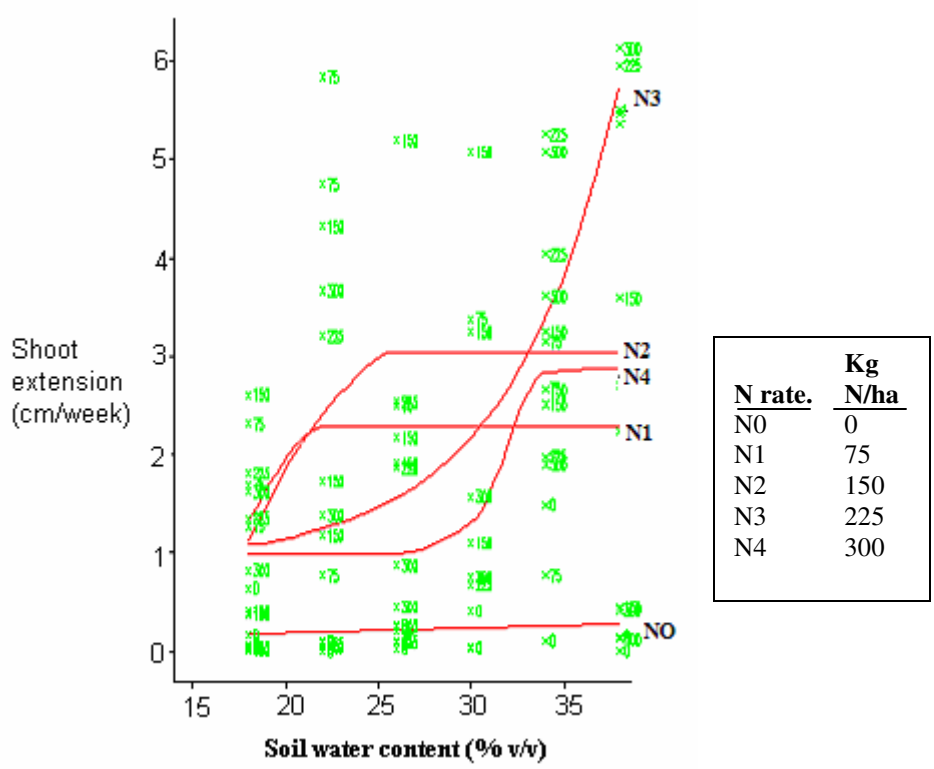
Regression analysis was done using a Gompertz exponential function (GenStat 5 release 4.2). The functional Gompertz model gave the relationship between soil water content and growth in tea, and provided the best fit curve with relatively low residual and higher R^2 value (adjusted r^2) compared to other growth models and was therefore used in this work.

4.3 Results

4.3.1 Influence of N fertilizer and SWC on shoot growth of tea.

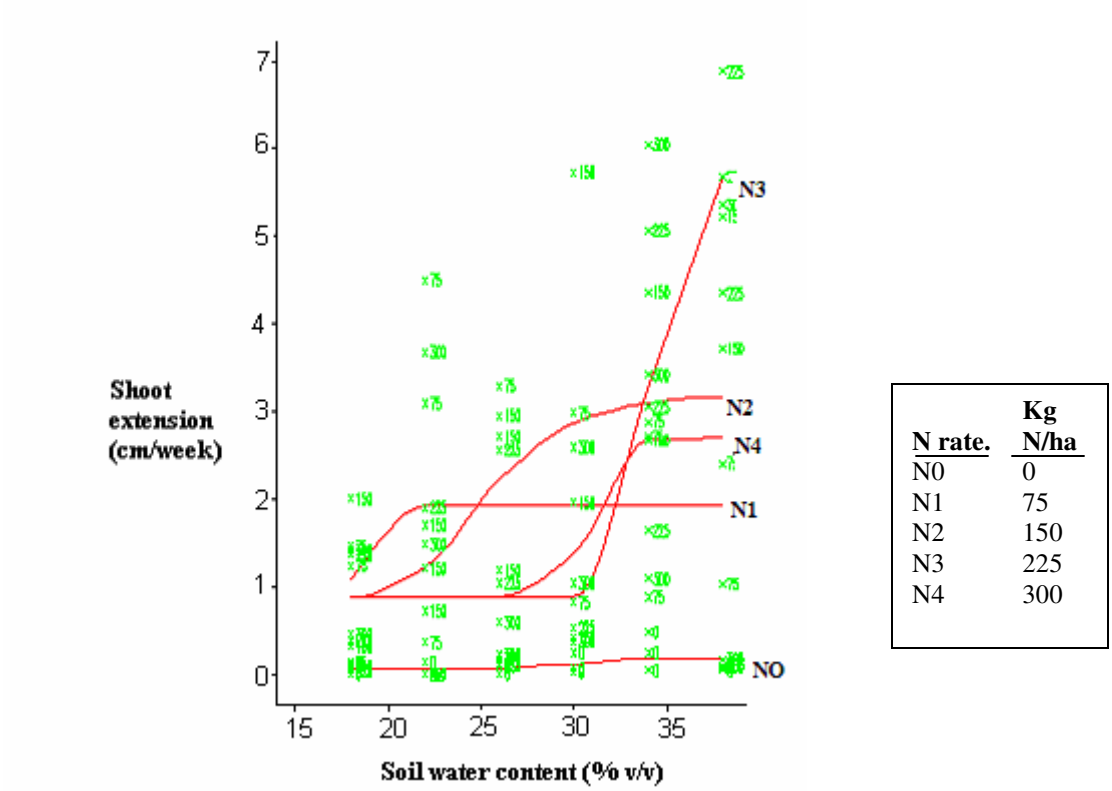
a) Shoot extension

Inorganic nitrogen supply improved shoot growth in tea while growth was noticeably constrained on unfertilized tea. However, growth response to fertilizer for each rate applied was influenced by SWC. Decline in shoot growth was observed at much lower SWD with higher rate of fertilizer; for instance break point in shoot growth occurred at >30%, 28% and at 20% SWC for 225, 150 and 75 kg N ha^{-1} respectively (Fig 4.1 a-d). Growth response at 225 Kg N rate consistently increased at SWC>30%, which could suggest favourable fertilizer and soil water content for tea.



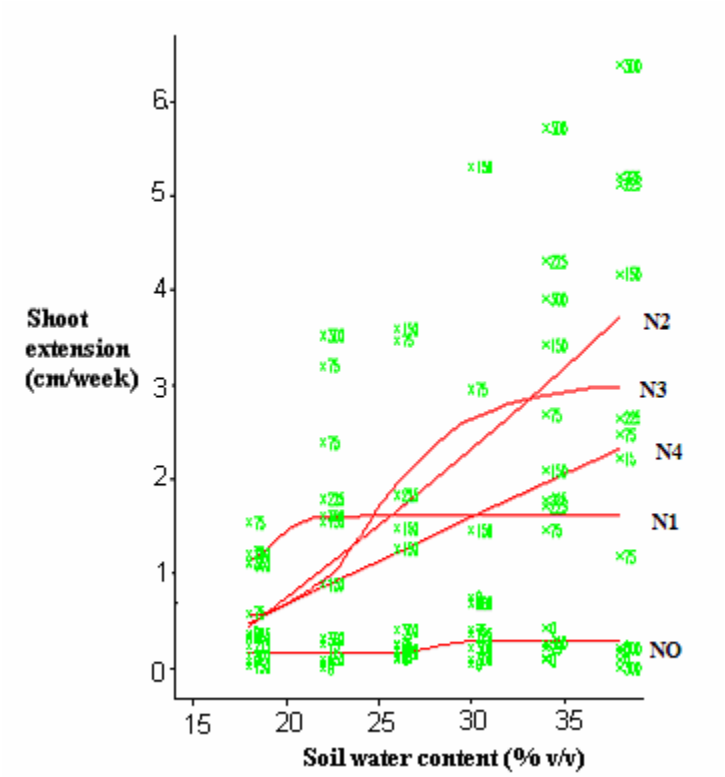
N rate.	Kg N/ha
N0	0
N1	75
N2	150
N3	225
N4	300

Fig. 4.1 (a) Shoot extension during the second week after water treatment. (P<0.01, SE = 1.48, adjusted r^2 = 34%).



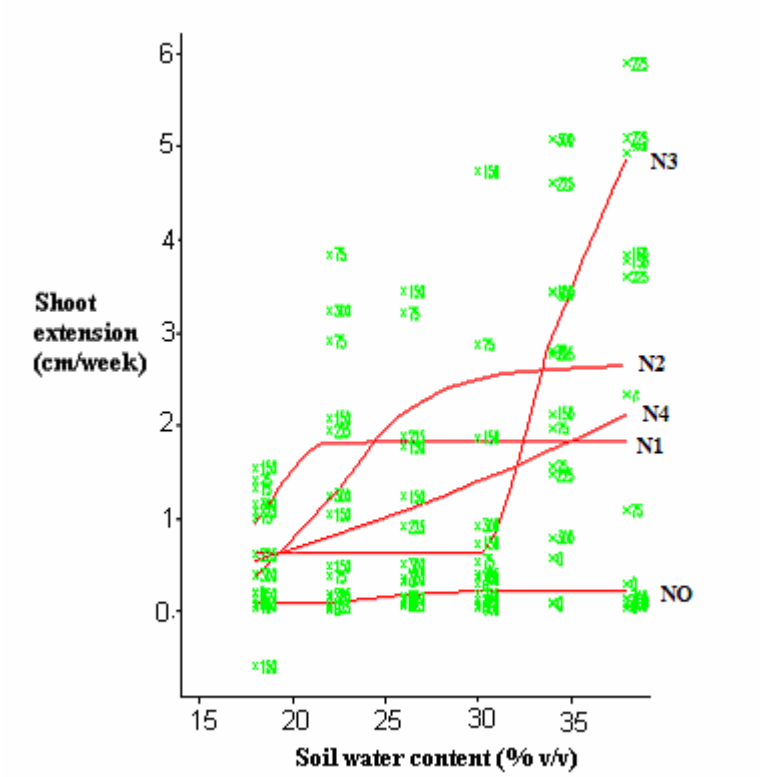
N rate.	Kg N/ha
N0	0
N1	75
N2	150
N3	225
N4	300

Fig. 4.1 (b) Shoot extension during the third week of water treatment. (P<0.001, SE = 1.4, n = 90 adjusted r^2 = 37%).



N rate.	Kg N/ha
N0	0
N1	75
N2	150
N3	225
N4	300

Fig. 4.1 (c) Shoot extension during the fourth week of water treatment. (P<0.001, SE = 1.41, n = 90, adjusted r^2 = 36%).



N rate.	Kg N/ha
N0	0
N1	75
N2	150
N3	225
N4	300

Fig. 4.1 (d) Shoot extension as mean of first 4 weeks of water treatment. (P<0.001, SE = 1.24, n = 90, adjusted r^2 = 35%).

b) Leaf growth

Leaf growth of tea significantly improved with nitrogen fertilizer supply and again, this response was influenced by SWC. Though leaf growth declined with SWD, that decline was gradual with fertilizer rates at 150 kg N ha^{-1} and above, whereas at both 0 and 75 kg N ha^{-1} gave a stable response at lower SWC (Fig 4.2).

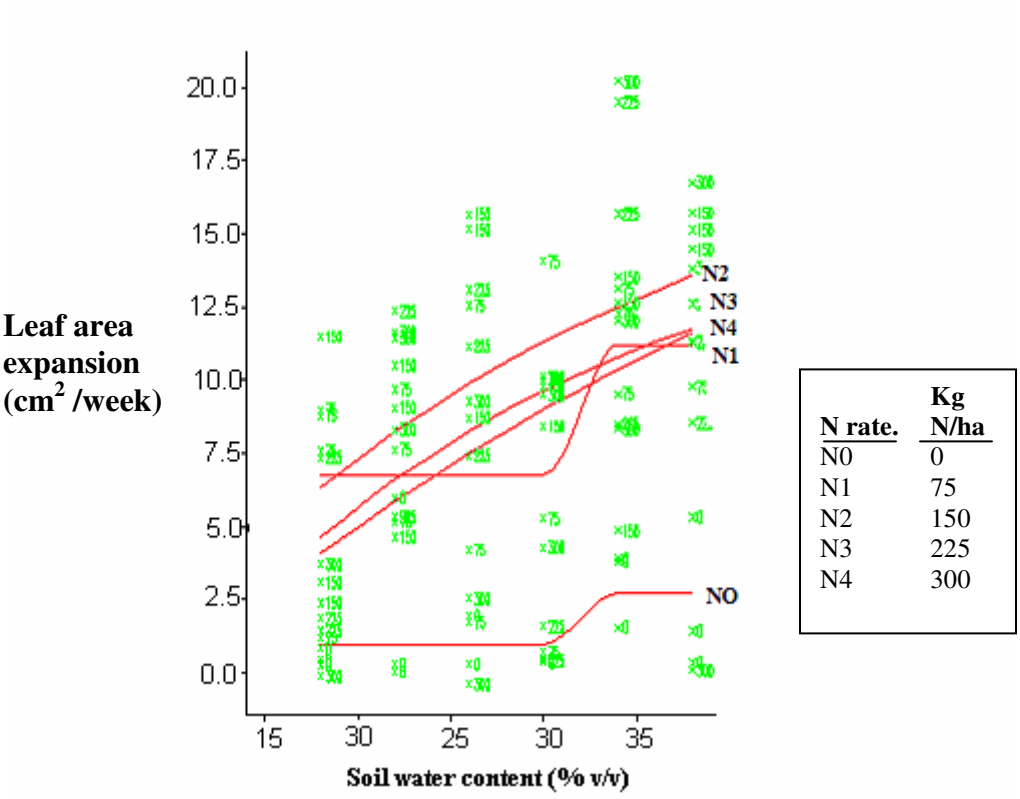


Fig. 4.2 Influence of fertilizer and SWC on leaf expansion. (P<0.001, SE = 4.36, n = 90, adjusted r^2 = 40.53%).

4.3.2 Plant water relation

Leaf relative water content and shoot water potential

The shoot water potential as influenced by fertilizer rates showed wide variations at lower SWC but such differences were minimal at higher SWC. High amounts of fertilizer lowered plant water potential and this was noticeable at lower SWC (Fig. 4.3). Shoot water potential in tea growing at different soil moisture regimes was also influenced by fertilizer rates. High amounts of fertilizer lowered plant suction pressure which is required for water uptake (Fig. 4.3 b).

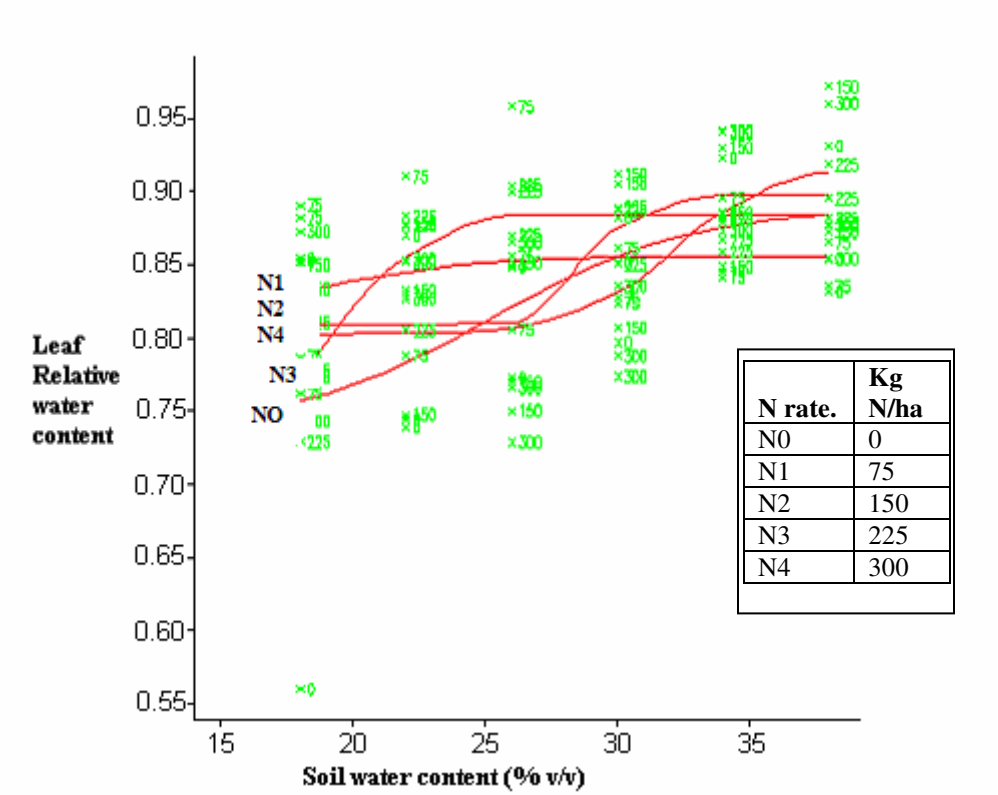
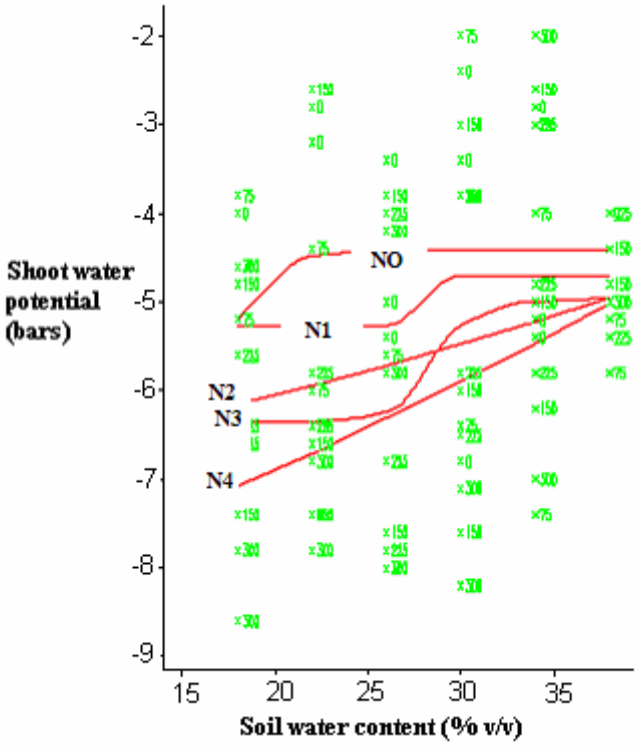


Fig. 4.3 (a) Influence of fertilizer supply and SWC on leaf relative water content in tea. (P<0.001, SE = 0.05, n = 90, adjusted r^2 = 24%).



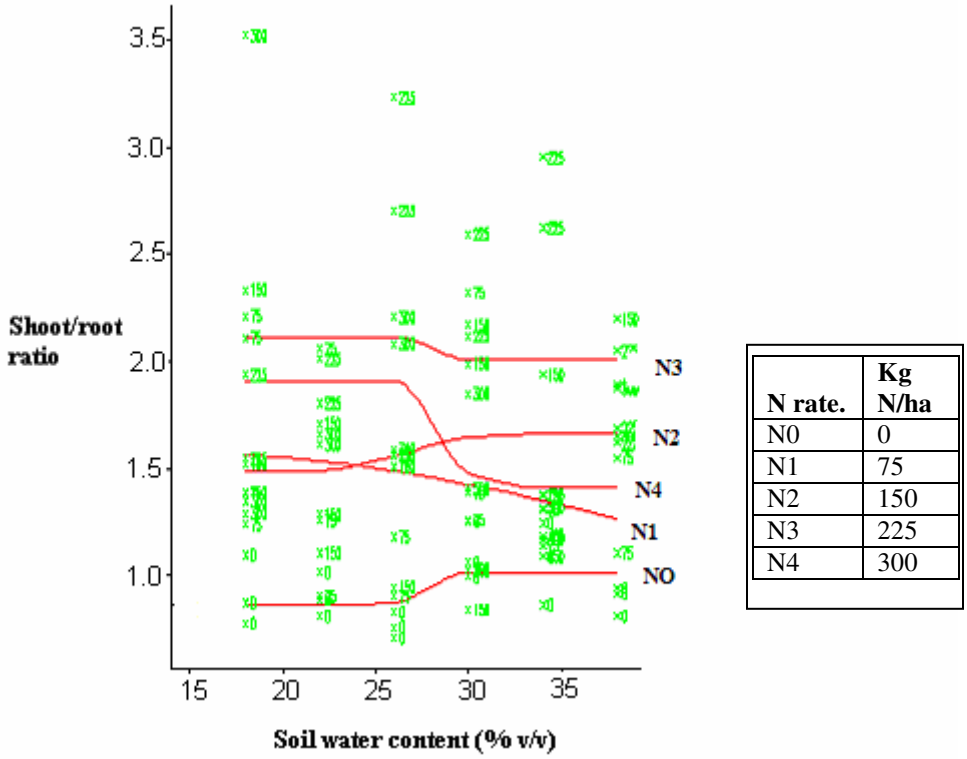
N rate.	Kg N/ha
N0	0
N1	75
N2	150
N3	225
N4	300

Fig. 4.3 (b) Shoot water potential of tea as influenced by fertilizer supply to tea and SWC. (P<0.001, SE = 1.77, n = 90, adjusted r^2 = 63.7%).

4.3.3 Dry matter partitioning

a) Shoot-to-root ratio

Shoot-to-root ratio (SRR) was much higher in tea supplied with fertilizer than in unfertilized tea. However, SRR response to fertilizer at different SWC varied with N rates above 225 kg ha^{-1} showing high SRR at SWC <26% which declined with increase in SWC. In contrast, SRR in tea with no fertilizer applied showed lower SRR at low SWC and which appreciated at 25% SWC to maximum at 30% SWC (Fig. 4.4).



N rate.	Kg N/ha
N0	0
N1	75
N2	150
N3	225
N4	300

Fig. 4.4 (a) Influence of fertilizer supply and SWC on shoot-to-root ratio of dry matter in tea. (P<0.01, SE = 0.33, n = 90, adjusted r^2 = 40.6%).

b) Leaf-to-root ratio

Fertilizer supply improved foliage development over root biomass in tea regardless of soil moisture regime (Fig. 4.4 b and c). Nonetheless, fertilizer supply influenced allocation of greater proportion of dry matter to leaves than to roots at lower SWC unlike in unfertilized tea (Fig 4.4 c).

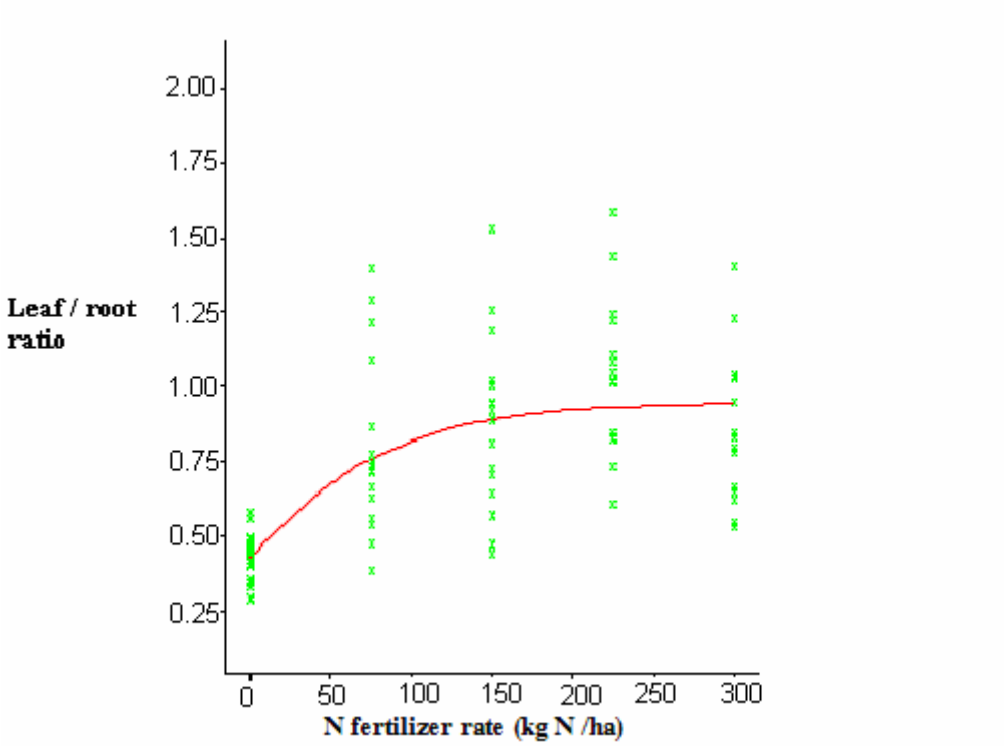
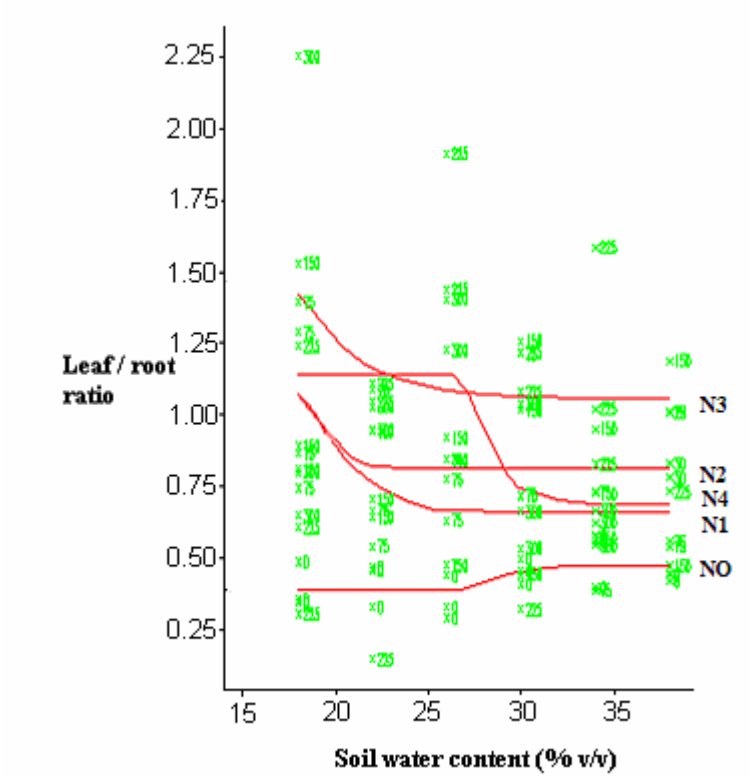


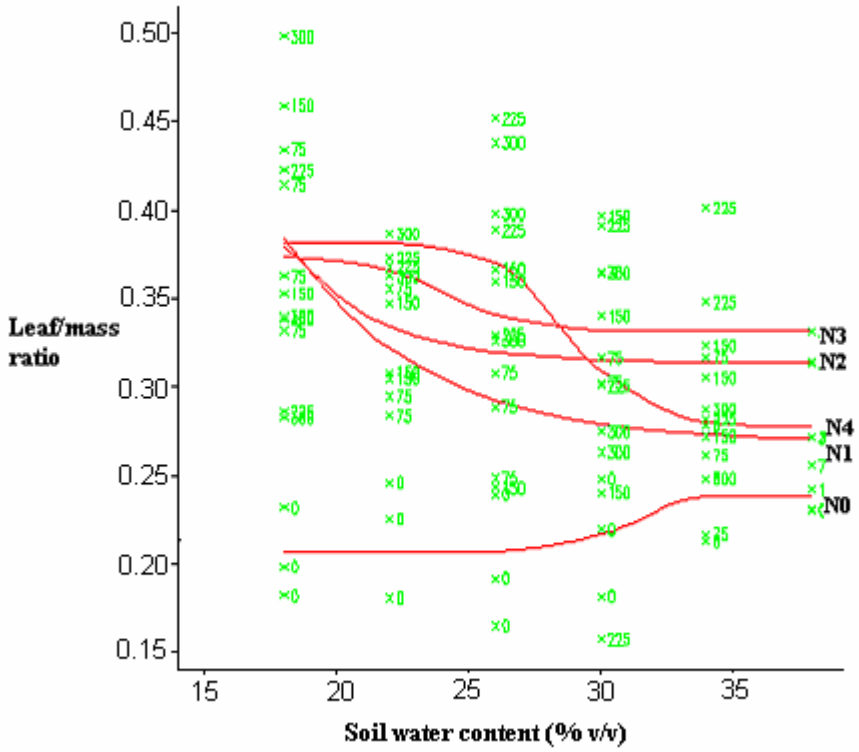
Fig. 4.4 (b) Influence of fertilizer supply on leaf-to-root ratio in tea.
(P<0.001, SE = 0.33, n = 90, adjusted r^2 = 72%).



N rate.	Kg N/ha
N0	0
N1	75
N2	150
N3	225
N4	300

Fig. 4.4 (c) Influence of fertilizer supply and SWC on leaf-to-root ratio in tea. (P<0.001, SE = 0.33, n = 90, adjusted r^2 = 46%).

Fertilizer improved leaf-to-mass ratio (LMR) in tea which was enhanced under water stress and the ratio ranged from about 0.28-0.38 with lower range at near filled capacity. In contrast, unfertilized tea had very low LMR which ranged from 0.21-0.25 with lower range under limited SWC and upper range at higher SWC. Whereas increase in SWC improved LMR in unfertilized tea, it was the reverse in fertilizer supplied tea (Fig. 4.4 d). Specific leaf area (SLA) declined with increase in fertilizer supply rate to tea (Fig. 4.5) and stomata conductance was generally higher where no fertilizer was applied but was lower in fertilizer applied tea (Fig. 4.6).



N rate.	Kg N/ha
N0	0
N1	75
N2	150
N3	225
N4	300

Fig. 4.4 (d) Effect of soil water content and fertilizer on leaf-to-mass ratio. (P<0.001, SE = 0.06, n = 90, adjusted r^2 = 43.5%).

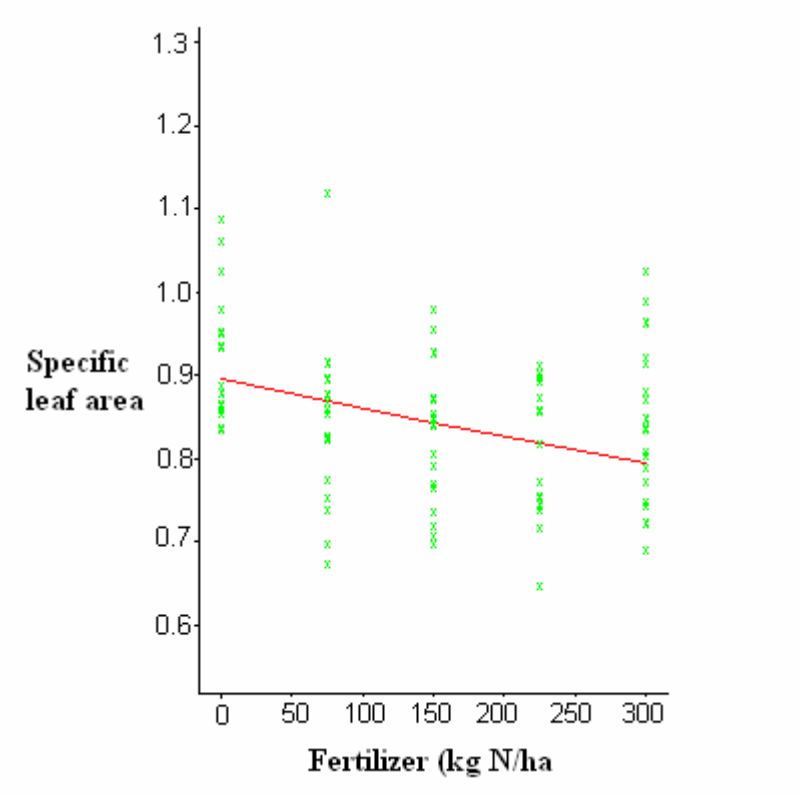
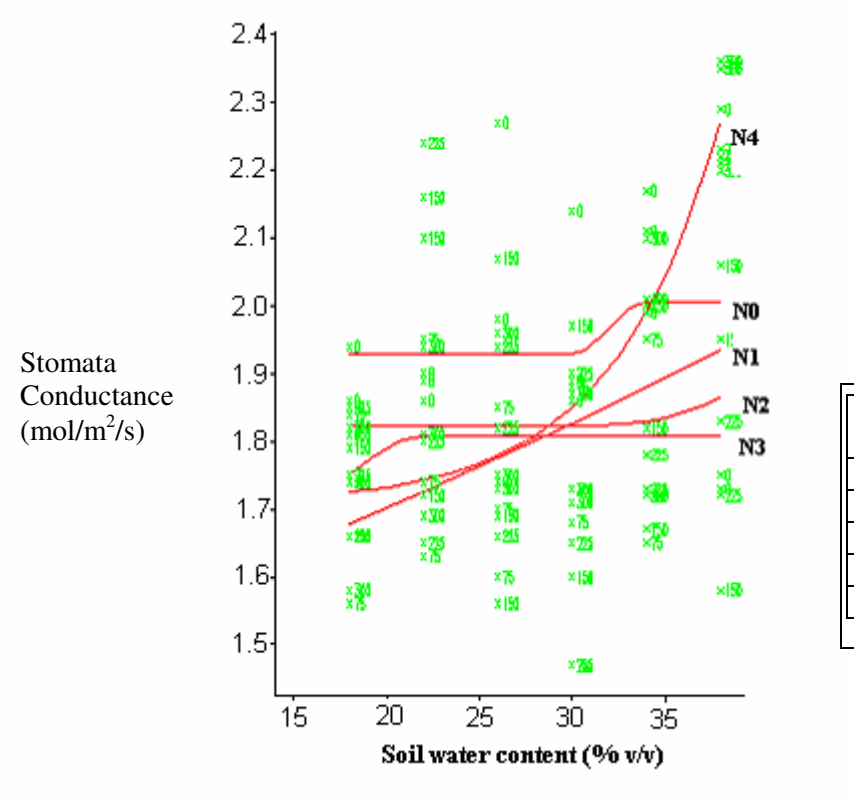


Fig. 4.5 Effect of fertilizer on specific leaf area in tea. (P<0.01, SE = 0.11, n = 90 adjusted r^2 = 89.2%).



N rate.	Kg N/ha
N0	0
N1	75
N2	150
N3	225
N4	300

Fig. 4.6 Stomata conductance in tea leaf at different fertilizer rates and SWC levels. (P<0.001, SE = 0.20, n = 90, adjusted r^2 = 52%).

Plate 4.6 Tea supplied with no fertilizer (F0) and maintained at 18% soil water content (W1).

Plate 4.7 Tea supplied with fertilizer at rate equivalent to 225 kg N /ha and maintained at 18% soil water content.

Plate 4.8 Tea supplied with fertilizer at the rate of 75 kg N /ha equivalent, and maintained at 18% soil water content.

Plate 4.9 Tea supplied with fertilizer at the rate of 225 kg N /ha equivalent, and maintained at 38% soil water content.

4.4 Influence of N fertilizer and drought on shoot density and yield of tea.

4.4.1 Shoot count and yield

Dry period in tea growing areas usually begins in November or December and its effect on tea can be observed a month later. Data in this experiment was collected between December and March in 2003/04 and 2004/05 seasons. Harvestable shoots on tea bushes following drought varied with both location and year. Shoot count and leaf yield declined during dry season with greater decline in the warm highlands (Fig. 5.1 a & b and 5.2 a & b) than in cool highlands (Fig. 5.1 c and 5.2 c). Though drought affected shoot count and yield in all fertilizer rates, greater reduction was observed in tea supplied with higher fertilizer rates. In some instances, tea with lower fertilizer rates performed better than those with higher rates (Fig. 5.1 b and 5.2 b &c).

a) Shoot count

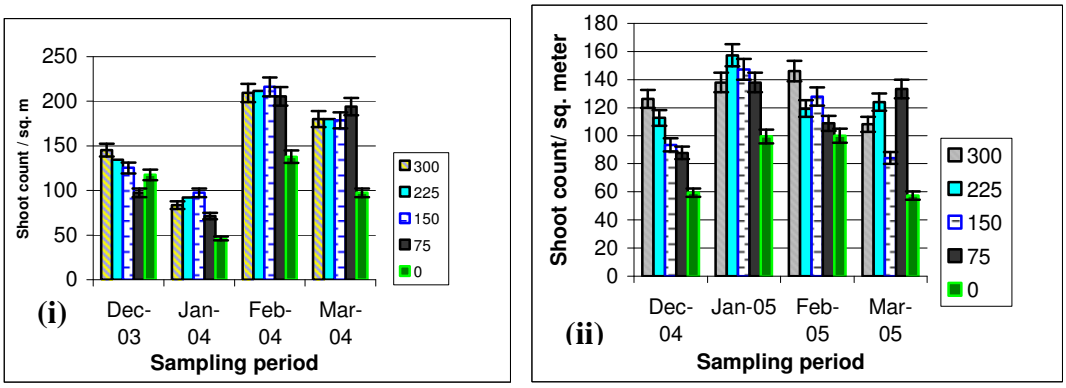


Fig. 5.1 (a) Harvestable tea shoot count obtained from Changoi in 2003/04 (i) and in 2004/05 (ii) seasons

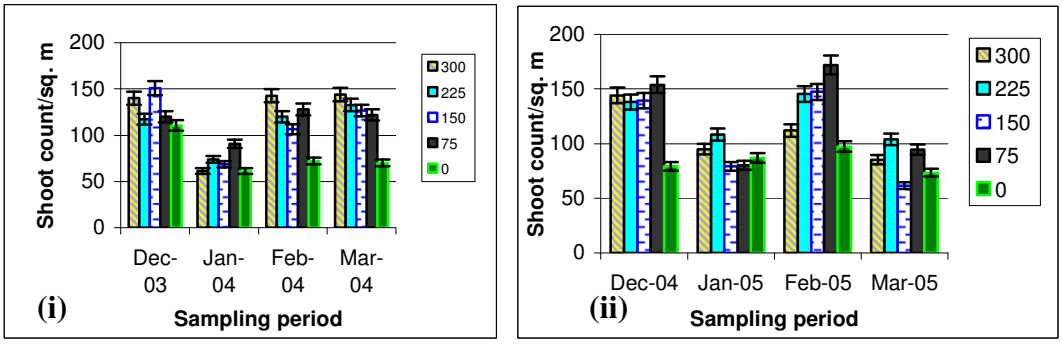


Fig. 5.1 (b) Harvestable tea shoot count obtained from Timbilil in 2003/04 (i) and in 2004/05 (ii) seasons.

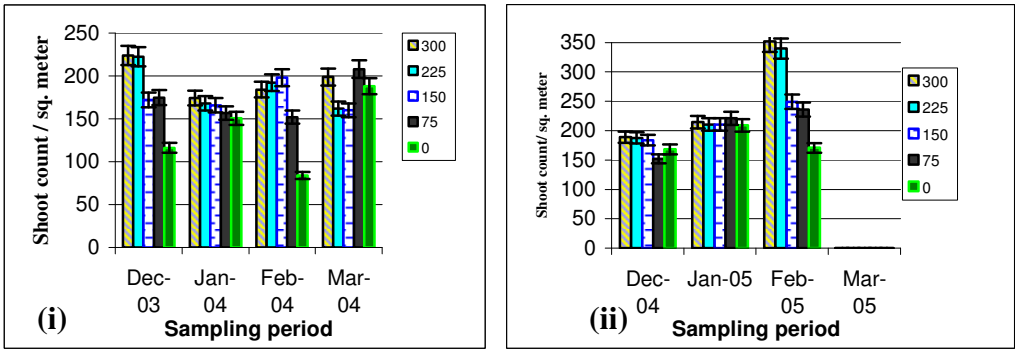


Fig. 5.1 (c) Harvestable tea shoot count obtained from Karirana in 2003/04 (i) and in 2004/05 (ii) seasons

b) Shoot Yield

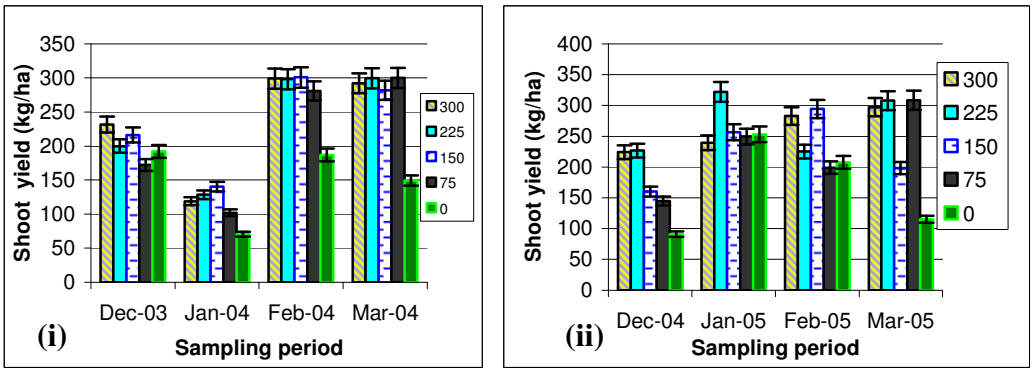


Fig. 5.2 (a) Shoot yield obtained from Changoi in (i) 2003/04 and in 2004/05 (ii) seasons

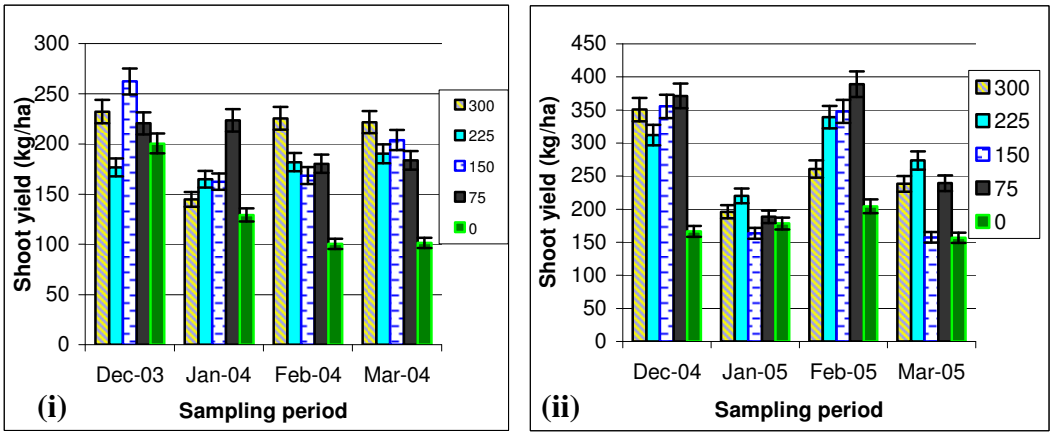


Fig. 5.2 (b) Shoot yield obtained from Timbilil in (i) 2003/04 and in 2004/05 (ii) seasons

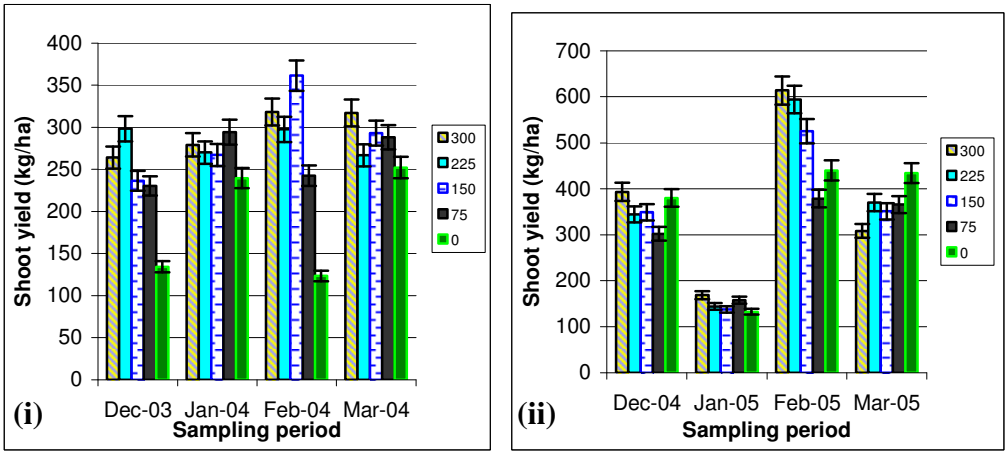
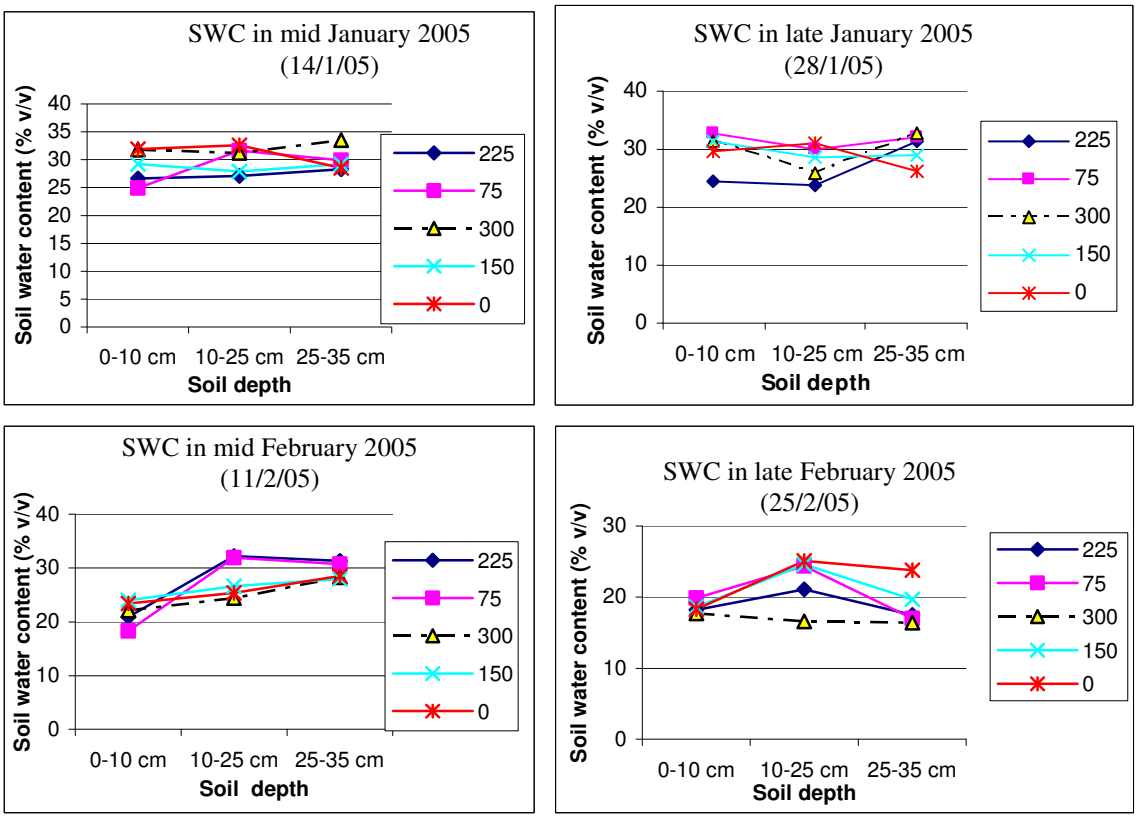


Fig. 5.2 (c) Shoot yield obtained from Karirana in (i) 2003/04 and in 2004/05 (ii) seasons

4.4.2 Soil water content in Changoi and Timbilil tea estates during the December-March season in 2004/05.

Soil water content (SWC) declined from about 30% to 20% v/v during the dry season (Fig. 5.3). Soil water content declined most at the upper 0-10 cm soil layer compared to lower horizons. However, of greater significance was the change in SWC as influenced by fertilizer rates. Higher fertilizer rates, especially 300 kg N/ha consistently showed lower SWC compared to lower or zero fertilizer rates at the three soil depth levels (Fig. 5.3 a & b). Among the two locations, Timbilil experienced greater SWC decline unlike Changoi.

a) Changoi



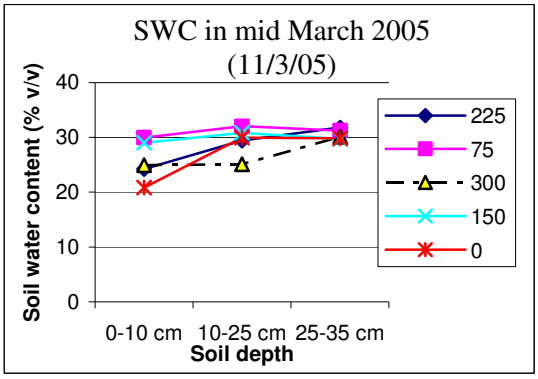
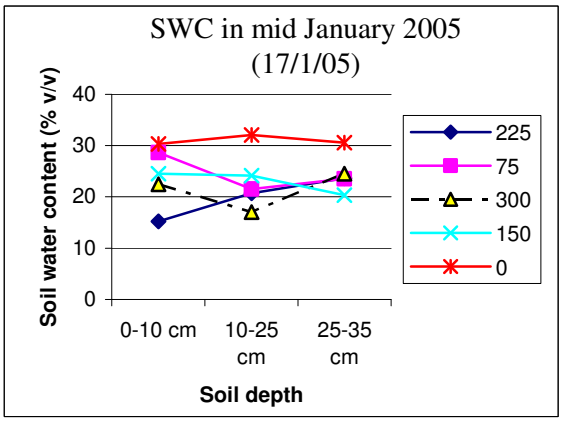
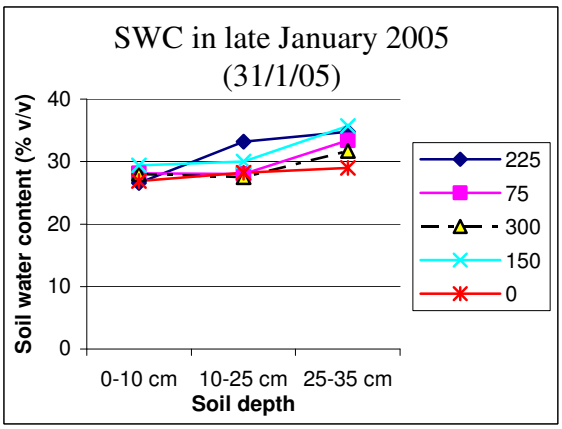
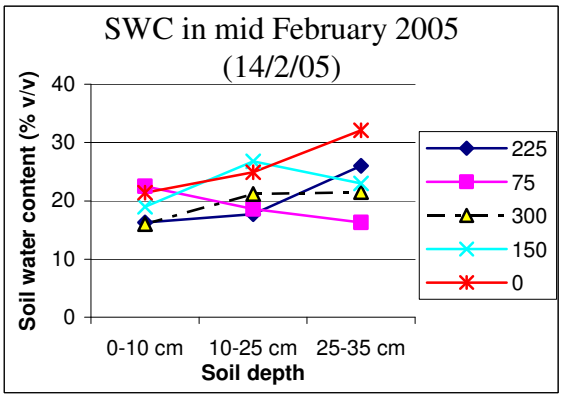


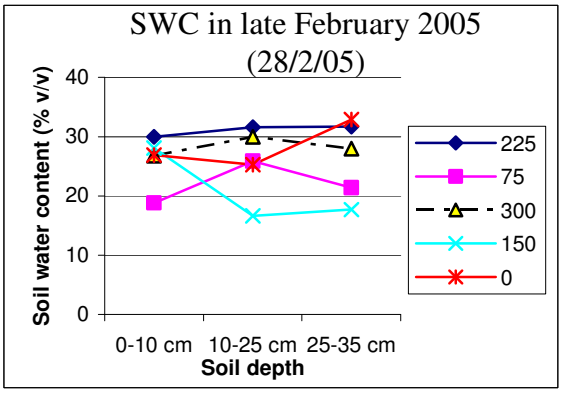
Fig. 5.3 (a) Changes in soil water content in the tea experiment in Changoi during dry months.

b) Timbilil









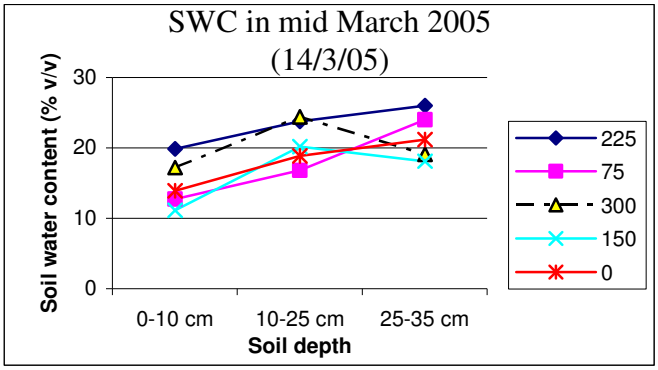


Fig. 5.3 (b) Changes in soil water content in the tea experiment in Timbilil during dry months.

4.5 Discussion

The hypothesis in this study was that high rates of N fertilizer raises the optimal soil water requirement for tea and subsequently decrease tea productivity during drought. Results obtained provide strong evidence that tea plants supplied with fertilizer have limited growth during drought. Results from the field experiment indicate a decline in tea leaf yield during drought and the extent of that decline is influenced by fertilizer supplied to tea. Higher fertilizer rates reduced tea yield (Fig 5.1 and 5.2) which corroborates the work on potted tea experiment (Fig. 4.1 and 4.2). Fertilizer in excess of 200 kg N ha^{-1} negated tea performance at low SWC compared to N rates of 75-150 kg ha^{-1} but the trend was reversed when SWC increased above 30% v/v (Fig 4.1 & 4.2). This observation conforms with leaf tissue water, which was relatively lower at 20% SWC in plants receiving high compared to low fertilizer input (Fig. 4.3 a). Fertilizer supply reduced shoot water potential (Fig 4.3 b) which could have contributed to the sustained decline in growth (Fig 4.1 and 4.2).

Results on dry matter partitioning shows increased shoot-to-root ratio (SRR) following fertilizer supply, which was enhanced under lower SWC. The leaf fraction, determined as leaf-to-total mass ratio (LMR) and leaf-to-root ratio (LRR), was notably increased under water stress. These results agree with the theory of "functional balance", which explains plant responses to the complexities of assimilate partitioning where shortage of an essential resource will necessitate the plant to invest in the structures responsible for the acquisition of that limiting resource (Davidson, 1969). Allocation of dry matter in the plant has been related to the ratio of total dry matter to shoot and roots as two major sinks within the plant. Shoot provides roots with carbohydrates while roots

provide the shoot with mineral nutrients and water which they require. Shoot-to-root ratios are thus modulated as a response variate to factors influencing carbon synthesis and nutrient acquisition by the plant (Davidson, 1969; Thornley, 1972; Hilbert, 1990; Dewar, 1993; Linker and Johnson, 2005). N uptake via roots is then assimilated and incorporated in proteins, enzymes and structural molecules such as chlorophyll. Carbon is primarily synthesized by the leaves in the presence of PAR and CO_2 before being translocated to various sinks within the plant. Plants receiving adequate supply of N with no limitation to light, CO_2 and water will lead to increase in assimilates to shoot for structural growth, therefore SRR will increase (Andrews *et al*., 1999; Grechi *et al*., 2007). This probably explains why fertilizer improved SRR and LRR of tea. However, under water stress, plants are generally known to invest more dry matter in roots over shoot and gradually shift the allocation in favour of shoot when SWC become less limiting (Munns and Cramer, 1996) which conformed to data derived from fertilizer deprived tea in this study. Contrary to the expectation, N supplied tea grown in low soil moisture regime allocated larger proportion of dry matter to the shoot against roots. The observed trend suggests strong significance of nutrient supply over water deficit as a signal to partitioning of dry matter in tea. This may reflect limitation in root development caused by N fertilizer which corroborates work by Chamuah (1988) on tea and Arora and Mohan (2001) on wheat. Relatively high investment in shoot over roots by the plant at low soil water content has, therefore, negative implications on productivity and survival of tea plants.

Though RWC declined with reducing SWC, that decline was more rapid in unfertilized tea (Fig. 4.3 a). Fertilizer increased leaf thickness in tea (Fig. 4.4 d), which could explain the high RWC of tea. Leaf thickness is often reduced in plants exposed to

drought due to leaf sensitivity to low soil water content (Fernandez *et al*., 2002; Liu and Stutzel, 2004; Zhang *et al*., 2005). A thinner leaf is an adaptation against desiccation because it improves water use efficiency and reduces transpiration in plants. Thicker leave is also associated with higher N content and more mesophyll cell density both of which enhance CO_2 assimilation (Liu and Stutzel, 2004) thereby necessitating higher demand for water. Such leaves are also associated with higher density of chlorophyll and proteins per unit leaf area and would therefore have greater capacity for photosynthesis than thinner leaves (Reich *et al*., 1998). Consequently, DM increased which partly explains the observed increase in leaf-to-mass ratio (LMR) and leaf-to-root ratio LRR in fertilizer supplied against unfertilized tea under SWD.

The preferential development of shoot over root and particularly LRR at low SWC in high N supply explains why fertilizer disadvantages tea during drought periods. A larger LRR will lead to canopy demand for water and mineral ions which cannot be sustained under water deficit conditions therefore resulting in growth decline. This explains why tea supplied with higher fertilizer rates experienced greater decline in soil water content during drought (Fig 5.3) and this could be the major cause of yield reduction and plant deaths during drought.

Besides being a major organ for plant assimilates, plant leaf is the avenue through which tissue water is lost via transpiration. It would have been expected that the plants growing in low SWC would equally invest in roots to meet the expected water demand (Fernandez *et al*., 2002). However, this was not the case in fertilized tea. The increase in leaf-to-root ratio (LRR) at lower soil water content meant an increase in water demand for both physiological functions and biochemical processes, through a relatively limited

root system. Consequently, insufficient supply of water to shoot canopy demand was expected which led to the observed decline in growth. These results agree with Nixon *et al*. (2001) who reported drought sensitivity of young tea with shoot-to-root ratio of 2:1 compared to 1:1 of mature tea, showing vulnerability of tea to drought contributed by dry matter partitioning. Secondly, fertilized tea had reduced shoot water potential, which made it difficult for the plants to access the limited soil water. The influence of N fertilizer on shoot water potential is not clear but is similar to Stirzaker and Passioura (1996) observations that plants in nutrient-flushed soil had difficulty extracting water, a condition that needs further investigation. Given the combined effect of increased leaf canopy with limited root system and reduced suction pressure of tea, N fertilizer plays a key role in growth decline and death of some plants in fertilized tea during drought, a scenario commonly observed in tea fields. These results highlight the need to optimize N fertilizer supply to tea in relation to drought occurrence in tea zones to achieve consistent favourable leaf yield and to avoid plant deaths.

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CHAPTER FIVE

SUMMARY AND CONCLUSION

This work aimed at establishing soil water content limit below which tea performance is negated. It was also intended to establish the cause of observed susceptibility to drought in fertilizer-supplied tea and to determine some suitable indicators for water stress tolerance in tea.

It has been shown that tea yield is severely compromised during drought as a result of the decline in SWC. The manifestation of drought effect is commonly observed in growth among other plant variables. Shoot growth was limited at 28% SWC while leaf growth, leaf RWC and shoot water potential had a breakpoint at 18-20% SWC. It implies that cell turgidity fell at 18-20% SWC, and therefore the 18-20% SWC becomes the critical SWC below which tea production is curtailed.

Tea response to water stress was confirmed to be exacerbated by fertilizer supply. Fertilizer nourishment indirectly contributes to drought susceptibility in tea through disproportionate assimilate partitioning to shoot over root sinks. N supply encourages proliferation of shoot and foliage which is desirable for tea production. However, the contribution of N to productivity in tea is checked during drought with a risk of plant deaths. Results in this study explain why high plant deaths often occur in high fertilizer supplied compared to less fertilized tea, a situation frequently observed under severe drought, commonly experienced between December and February. While mild drought may be important for physiological root development of tea, high fertilizer supply negates this significant structural investment of the plant. There is therefore need to judiciously limit fertilizer supply to balance both plant productivity and structural

development. Results in this study indicate that fertilizer rate above 150 kg N ha^{-1} significantly negated tea growth during drought.

According to clonal response to water stress, there exist a pool of drought tolerant and susceptible tea clones within 2n, 3n and 4n ploidy levels. Some diploids and tetraploids seem to have water stress tolerant genes which could be incorporated in the commercially cultivated diploids. Tea polyphenols are potential indicator for drought tolerance in tea. There was a correlation between growth and polyphenol content and the response of total polyphenols to SWC fitted with the determined WSI, an indication that some compounds within the large phenolic family can be useful indicator(s) for water stress. The catechin variants, EC and EGC are potential indicators for drought stress in tea, unlike ECG and EGCG. The TRFK 6/8 and TRFK 31/30 clones which were more tolerant to soil water deficit had less fluctuation of their EC and EGC content, when they were subjected to varying SWC. These results give a pointer to the fact that some flavonoid derivatives are potential indicators of drought stress in tea.

APPENDIX

Appendix 1: Experimental Layouts

Experiment 1: Effect of Soil Water Content on Growth and Polyphenol Content in Tea Cultivars

REP I		REP II		REP III	
Plot N0.	**Treatment**	**Plot N0.**	**Treatment**	**Plot N0.**	**Treatment**
1	W2C6	25	W1C3	49	W2C2
2	W3C4	26	W4C5	50	W4C4
3	W2C1	27	W3C4	51	W3C6
4	W4C3	28	W3C6	52	W3C3
5	W1C2	29	W4C1	53	W1C3
6	W3C5	30	W3C2	54	W3C5
7	W2C4	31	W1C3	55	W1C2
8	W3C3	32	W1C6	56	W3C1
9	W2C5	33	W2C3	57	W2C5
10	W4C2	34	W4C5	58	W4C3
11	W3C6	35	W3C1	59	W3C5
12	W1C1	36	W1C4	60	W4C1
13	W3C2	37	W4C2	61	W2C3
14	W1C4	38	W1C1	62	W2C4
15	W3C1	39	W2C6	63	W4C6
16	W1C5	40	W4C3	64	W4C2
17	W4C1	41	W2C5	65	W1C1
18	W1C6	42	W2C4	66	W4C5
19	W4C4	43	W2C2	67	W3C2
20	W2C2	44	W2C1	68	W2C6
21	W2C3	45	W4C6	69	W1C4
22	W4C6	46	W3C3	70	W1C5
23	W2C1	47	W1C5	71	W1C6
24	W1C2	48	W4C4	72	W3C4

Where;

W1, W2, W3, and W4 were soil water content levels: 14%, 22%, 30%, 34% and 38% v/v, respectively.

C1, C2, C3, C4, C5, and C6 were tea clones: BBK 35, TRFK 6/8, TRFK 76/1, TRFK 395/2, TRFK 31/30, and TRFK 311/287, respectively.

Experiment 2: Effect of Nitrogen Supply and Soil Water Content on Growth of Tea [*Camellia sinensis* (L.) O. *Kuntze*

REP I		REP II		REP III	
Plot N0	**Treatment**	**Plot N0**	**Treatment**	**Plot N0**	**Treatment**
1	F2W3	31	F4W4	61	F2W3
2	F3W2	32	F3W1	62	F3W6
3	F0W6	33	F4W2	63	F4W3
4	F3W3	34	F2W6	64	F0W3
5	F1W5	35	F1W2	65	F1W6
6	F3W6	36	F3W2	66	F2W1
7	F1W2	37	F3W4	67	F4W4
8	F4W1	38	F4W5	68	F3W1
9	F2W5	39	F2W2	69	F4W2
10	F0W4	40	F4W6	70	F2W6
11	F4W3	41	F2W5	71	F1W2
12	F2W6	42	F3W3	72	F3W2
13	F0W1	43	F1W1	73	F0W6
14	F4W6	44	F4W3	74	F4W1
15	F3W5	45	F2W3	75	F0W1
16	F0W3	46	F2W1	76	F2W4
17	F4W2	47	F0W3	77	F1W3
18	F2W4	48	F3W6	78	F0W5
19	F2W2	49	F1W3	79	F3W4
20	F1W1	50	F0W1	80	F4W5
21	F1W6	51	F4W1	81	F2W2
22	F3W1	52	F0W5	82	F4W6
23	F1W3	53	F2W4	83	F2W5
24	F4W5	54	F0W6	84	F3W3
25	F0W2	55	F0W2	85	F1W4
26	F2W1	56	F3W5	86	F0W2
27	F4W4	57	F1W5	87	F1W1
28	F0W5	58	F1W4	88	F3W5
29	F3W4	59	F1W1	89	F0W4
30	F1W4	60	F0W4	90	F1W5

Where;

W1, W2, W3, W4, W5, and W6 are soil water contents levels: 18%, 22%, 26%, 30%, 34%, and 38% v/v, respectively.

N0, N1, N2, N3, and N4 are nitrogen fertilizer supply rates: 0, 75, 150, 225, and 300 kg ha^{-1}, respectively.

Experiment 3: Effect of Nitrogen Supply and Soil Water Content on Shoot Density, and Yield of Tea [*Camellia sinensis* (L.) O. *Kuntze*

REP I

Plot N0	**Treatment (kg N ha^{-1})**
4	225
6	75
8	300
12	150
14	0

REP II

Plot N0	**Treatment (kg N ha^{-1})**
17	75
19	225
22	300
23	150
29	0

REP III

Plot N0	**Treatment (kg N ha^{-1})**
31	225
32	75
37	150
38	300
45	0

Appendix 2: Leaf anatomy

Leaf anatomy

Fresh second leaves were plucked and fixed with formalin acetic alcohol for 24 hours. The treated leaves were then dehydrated in stages with a series of alcohol in different concentrations for 4 days. This was followed by cleaning with xylene and impregnated in wax at 90^{o}C, then allowed to cool. The embedded material was sectioned using Rotary microtome and the sections mounted on slides, dried and stained using xylene, alcohol and safranin stain (Cutler, 1978).

Table A1 Leaf thickness of some selected tea clones.

Clone	Thickness including air spaces (µm)				Total leaf thickness (µm)
	Palisade	Spongy mesophyll	Upper epidermis	Lower epidermis	
BBK 35	49 b	184.5 b	21.0 a	17.0	271 b
TRFK 6/8	49 b	177.0 bc	17.5 b	15.5	259 b
TRFK 76/1	51 b	189.5 b	19 ab	17.5	277 b
TRFK 395/2	36 c	150.5 c	17.5 b	16.0	220 c
TRFK 31/30	65 a	229.5 a	19.5 ab	17.0	331 a
TRFK 311/287	48 b	198.5 b	21.0 a	16.5	284 b
P (0.05)	8.74	28.39	3.02 ns	2.09 ns	29.93

Means followed by the same letter in the same column are not significantly different as per LSD means separation procedure ($\propto$=0.05)

Table A2 Cell numbers and their measure in some selected tea leaves.

Clone	Mean number of cell layers on mesophyll		Palisade (width x breath)	Spongy mesophyll (width x breath)
	Palisade	Spongy		
BBK 35	1.10	9.00 bc	900.0 b	690.8
TRFK 6/8	1.20	9.96 a	872.9 b	699.8
TRFK 76/1	1.20	9.23 ab	873.0 b	794.3
TRFK 395/2	1.00	8.10 c	555.8 c	562.5
TRFK 31/30	1.56	9.46 ab	1282.5 a	909.0
TRFK 311/287	1.00	9.20 ab	1057.5 ab	794.3
P (0.05)	ns	0.92	296.28	ns

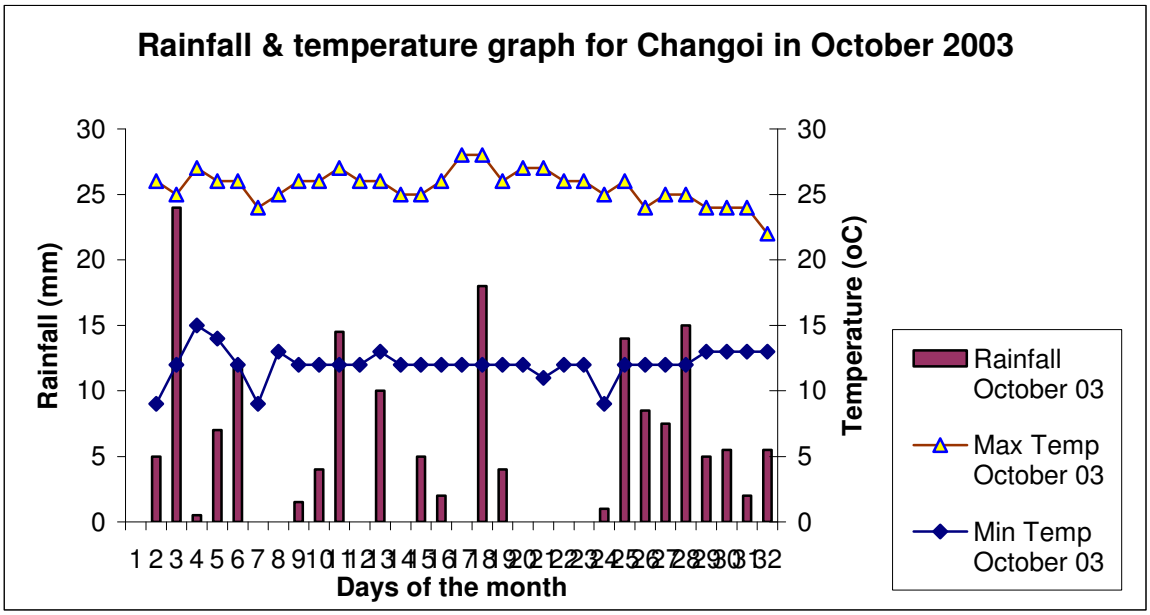
Means followed by the same letter in the same column are not significantly different as per LSD means separation procedure (∝=0.05)

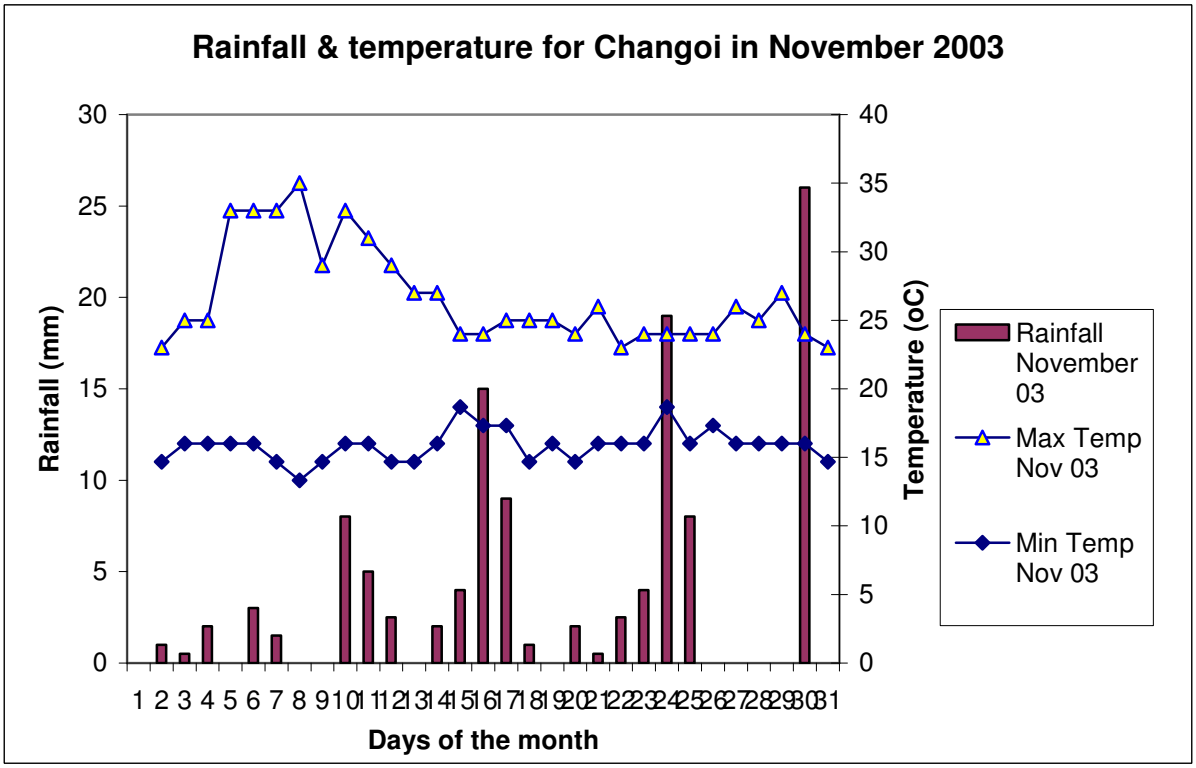
Table A3 Stomata density and size of guard cell of tea clones n the study.

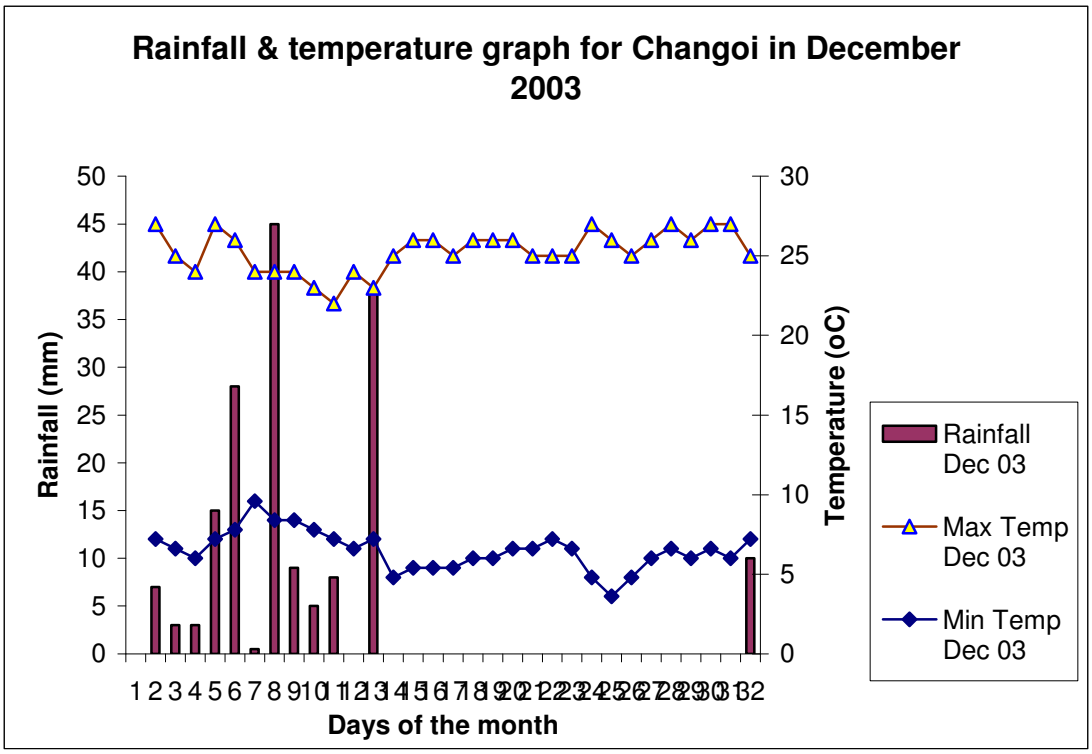
Clone	Length of the guard cell (L) (µm)	Width of the guard cell (W) (µm)	Approximate size (LxW)	Stomata Density (mm^{-2})
BBK 35	54.45 a	46.80 ab	2554 ab	236 a
TRFK 6/8	56.25 a	50.40 a	2861 a	198 b
TRFK 76/1	54.90 a	44.10 bc	2426 b	175 c
TRFK 395/2	45.00 b	40.05 c	1802 c	199 b
TRFK 31/30	46.80 b	41.85 c	1960 c	153 d
TRFK 311/287	55.80 a	47.25 ab	2647 ab	195 b
P (0.05)	4.45	4.22	364	9.9

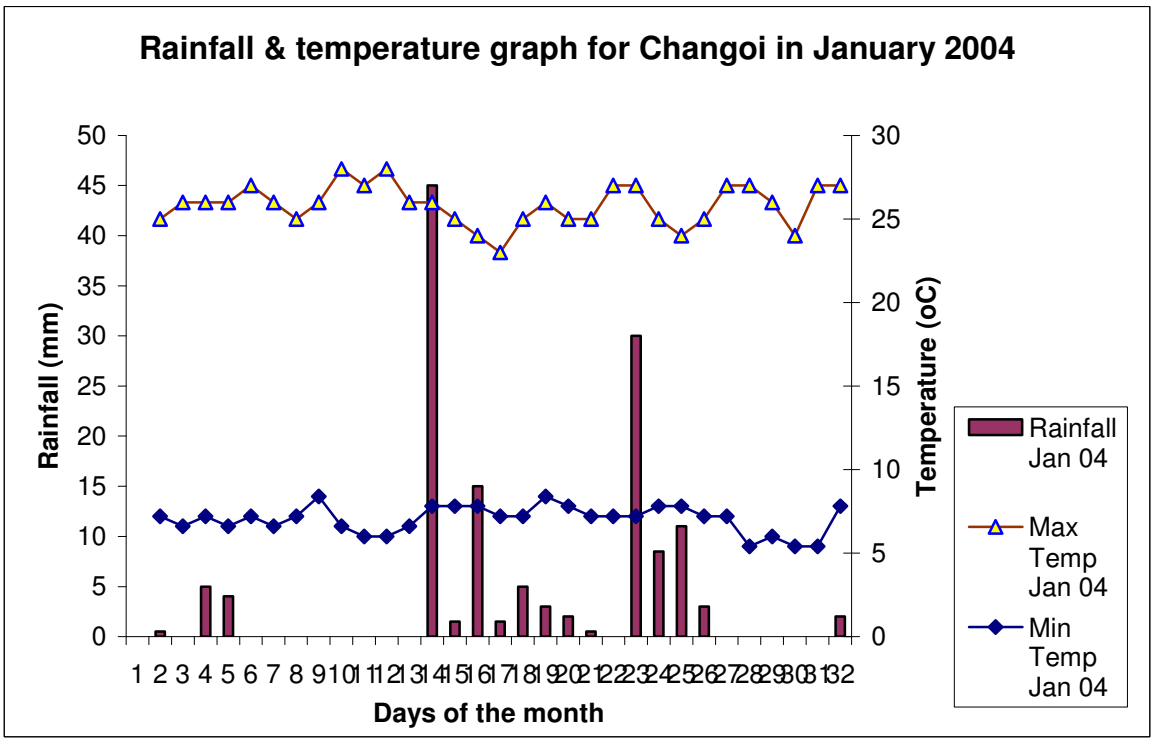
Means followed by the same letter in the same column are not significantly different as per LSD means separation procedure (∝=0.05).

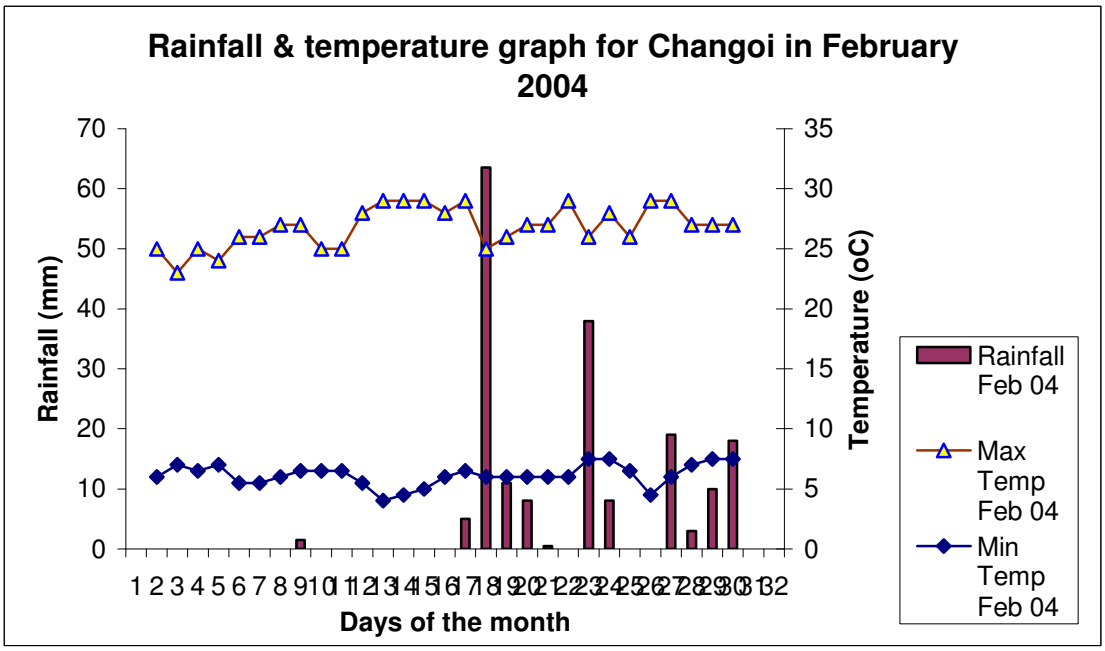
Appendix 3: Rainfall and Temperature for Changoi and Karirana field sites

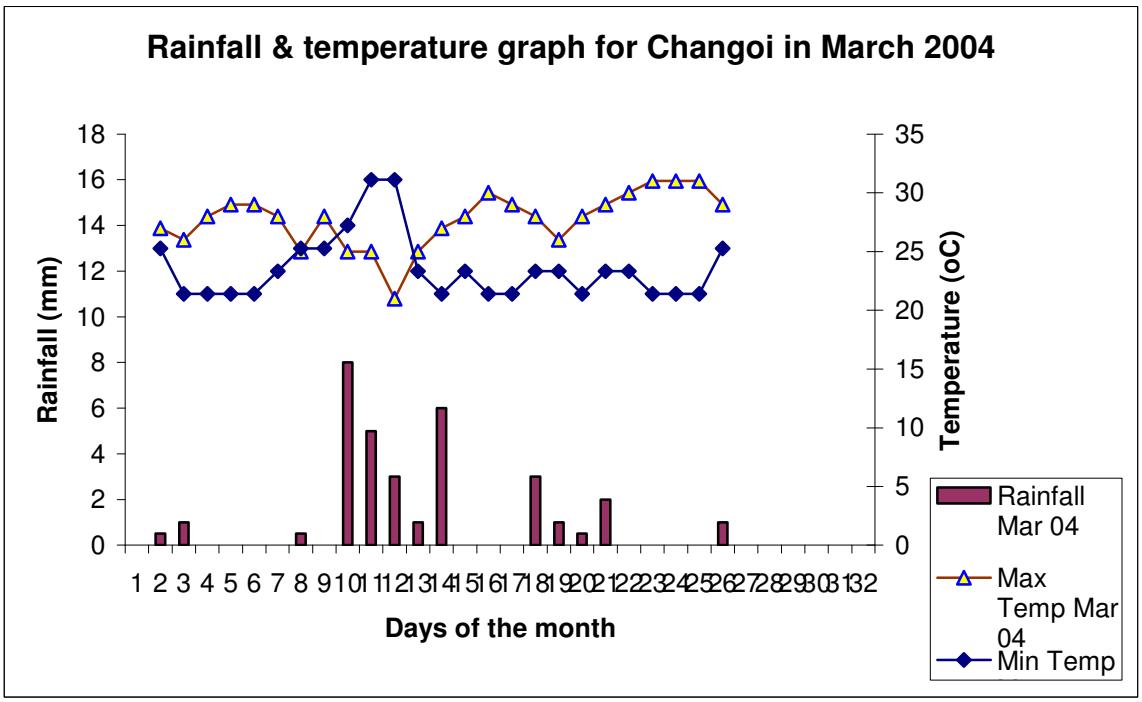




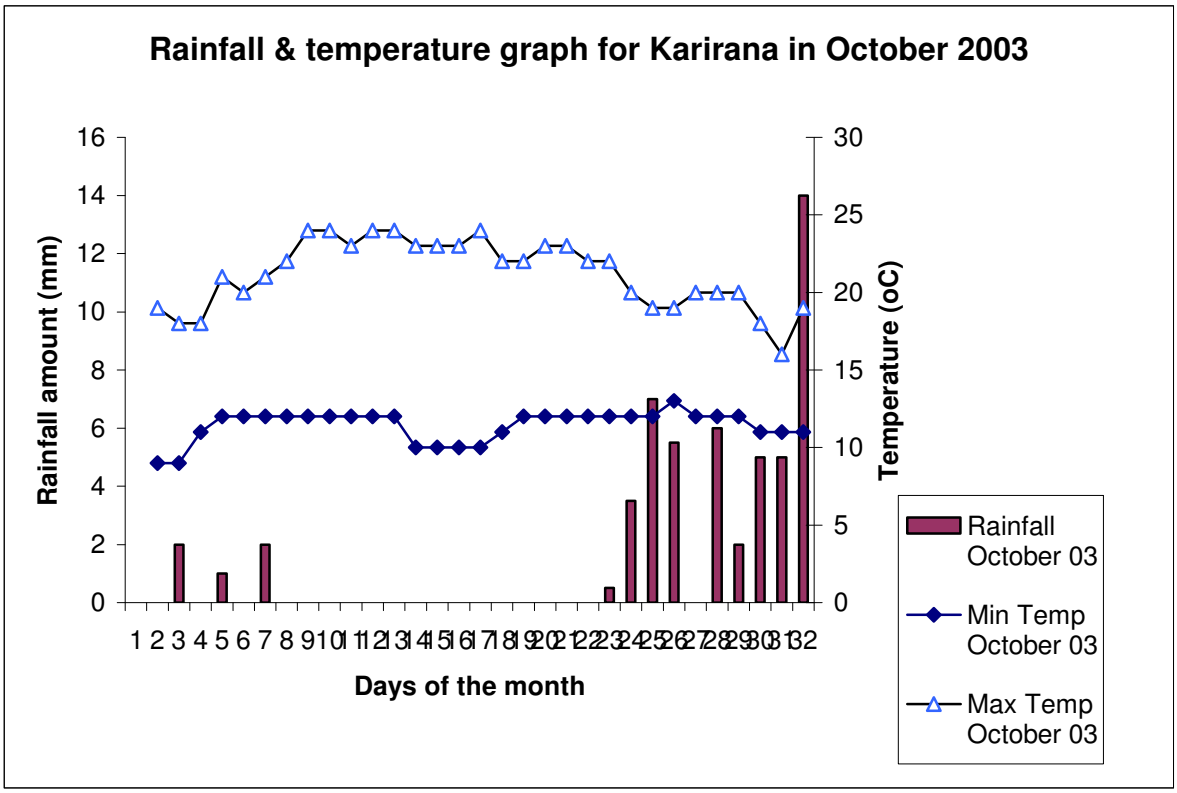

Rainfall & temperature graph for Changoi in December 2003
Rainfall (mm)
Temperature (oC)
Days of the month
Rainfall Dec 03
Max Temp Dec 03
Min Temp Dec 03

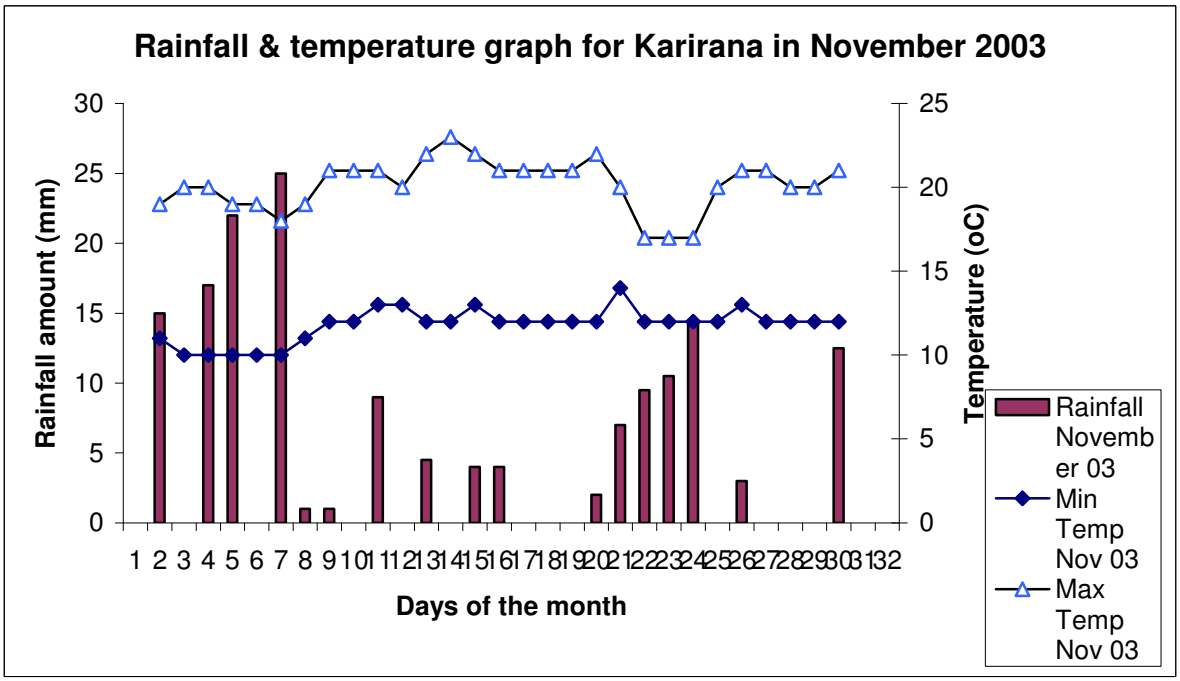

Rainfall & temperature graph for Changoi in January 2004
Rainfall (mm)
Temperature (oC)
Days of the month
Rainfall Jan 04
Max Temp Jan 04
Min Temp Jan 04

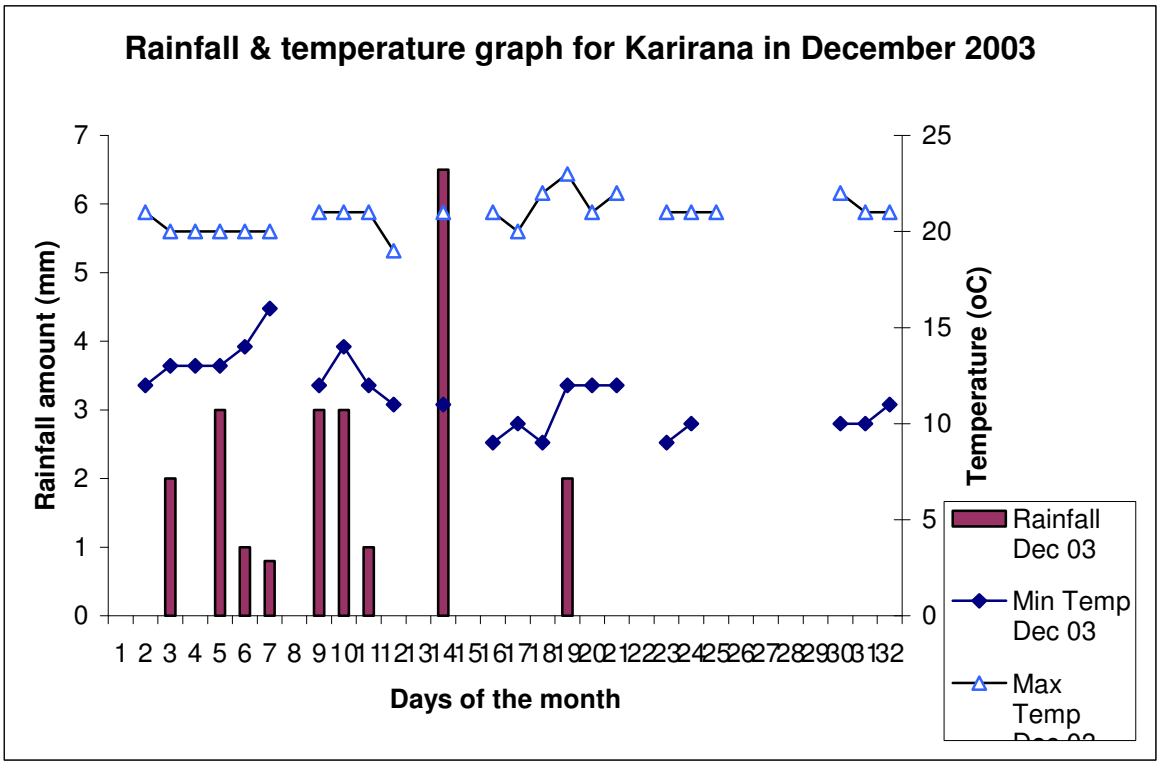

Rainfall & temperature graph for Changoi in February 2004
Rainfall (mm)
Temperature (oC)
Days of the month
Rainfall Feb 04
Max Temp Feb 04
Min Temp Feb 04


Rainfall & temperature graph for Changoi in March 2004
Rainfall (mm)
Temperature (oC)
Days of the month
Rainfall Mar 04
Max Temp Mar 04
Min Temp

Karirana


Rainfall & temperature graph for Karirana in October 2003
Rainfall amount (mm)
Temperature (oC)
Days of the month
Rainfall October 03
Min Temp October 03
Max Temp October 03


Rainfall & temperature graph for Karirana in November 2003
Rainfall amount (mm)
Temperature (oC)
Days of the month
Rainfall November 03
Min Temp Nov 03
Max Temp Nov 03


Rainfall & temperature graph for Karirana in December 2003
Rainfall amount (mm)
Temperature (oC)
Days of the month
Rainfall Dec 03
Min Temp Dec 03
Max Temp

Appendix 4: Publications

Biosci. Biotechnol. Biochem., 71 (9), 2190–2197, 2007

JSBA

Polyphenols as Potential Indicators for Drought Tolerance in Tea (*Camellia sinensis* L.)

Erick K. CHERUIYOT,[1,†] Louis M. MUMERA,[1] Wilson K. NG'ETICH,[2] Ahmed HASSANALI,[3] and Francis WACHIRA[4]

[1]*Egerton University, Department of Crop, Horticulture and Soil Sciences, P.O. Box 536, Njoro, Kenya*
[2]*Tea Research Foundation of Kenya, P.O. Box 820, Kericho, Kenya*
[3]*International Centre for Insect Physiology and Ecology, P.O. Box 30772-00100, Nairobi, Kenya*
[4]*Egerton University, Department of Biochemistry and Molecular Biology, P.O. Box 536, Njoro, Kenya*

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Plant polyphenols have gained prominence in quality of plant products and in human health. An experiment was conducted to determine the association of tea polyphenols with water stress and their suitability as indicators for drought tolerance. The experiment was conducted in a 'rain-out' shelter, and consisted of six tea clones (BBK 35, TRFK 6/8, TRFK 76/1, TRFK 395/2, TRFK 31/30, and TRFK 311/287) and four levels of soil water contents (38, 30, 22, and 14% v/v), which were maintained for a period of 12 weeks. The treatments were arranged in a completely randomized design and replicated three times. Plant growth was monitored over 6 weeks, and a water stress index was calculated to determine water-stress tolerant clones. Total polyphenols in tea shoots was analyzed and a regression analysis done. The results indicate that declining soil water content (SWC) reduced both growth and content of polyphenols in tea. Tolerant clones maintained a high polyphenol content at low SWC, and also showed less fluctuation in phenolics when subjected to changes in SWC. There was significant ($P < 0.001$) correlation of total polyphenol content with shoot growth and WSI of tea, and a linear relationship ($r^2 = 0.97$) between SWC for tea and both, water stress index and shoot polyphenol content. We report that there is a potential to use polyphenols as indicators for selection of drought-tolerant tea cultivars.

Key words: tea (*Camellia sinensis* L.); polyphenols; soil water content; drought tolerance

Tea (*Camellia sinensis* L.) is rich in polyphenol compounds which have been a subject of study as to their effects on human health.[1–8] The crop is the source of manufactured tea, which is consumed worldwide but its production is constrained by frequent recurrence of drought in major production areas.[9–11] Tea germplasm that can tolerate low soil water content (SWC) can reduce the losses occasioned by drought in production areas. Readily identifiable indicators for drought tolerance would hasten development and selection of tea germplasm for water stress environments. Plant response to stress is often manifested by its physiological and biochemical reactions, which can provide a basis for screening and selection of individual varieties and germplasm resistant to stress factors. For instance, plants are known to accumulate organic osmolytes, such as proline, glycine betaine, non-reducing sugars, and polyols[12,13] in response to stress factors. Though these organic compounds are species specific, their role is not clearly defined, but it is generally accepted that they contribute to ameliorating stress in plants.[13–15] Most of the stress-related organic compounds are secondary plant metabolites and incidentally, tea contains large amounts of polyphenols, particularly of the flavonol class. Some polyphenol derivatives have been used in quality determination in black tea[16] and in fruits,[17] but the role of polyphenols in drought stress and their suitability as indicators of desiccation tolerance in tea have not been explored.

The objective of this study was to quantify levels of green leaf polyphenols in tea and to define their association with SWC and their suitability as indicators for drought stress. It was hypothesized that tea plants exposed to increasing soil-water deficits show a decline in shoot growth and that the severity of this response can be predicted by tea polyphenols.

Materials and Methods

Set-up of a rain-out shelter. A rain-out shelter measuring 17 m by 6.5 m on the ground and a height of 2.5 m was erected. The roof was raised and curved to give a dome-shape with a radius of 0.5 m above the

† To whom correspondence should be addressed. Fax: +254-51-2217737; E-mail: cheruiyotke@yahoo.com

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Shoot Epicatechin and Epigallocatechin Contents Respond to Water Stress in Tea [*Camellia sinensis* (L.) O. Kuntze]

Erick K. CHERUIYOT,[1,†] Louis M. MUMERA,[1] Wilson K. NG'ETICH,[2] Ahmed HASSANALI,[3] Francis WACHIRA,[4] and John K. WANYOKO[2]

[1]*Egerton University, Department of Crop, Horticulture and Soil Sciences, P.O. Box 536, Njoro, Kenya*
[2]*Tea Research Foundation of Kenya, P.O. Box 820, Kericho, Kenya*
[3]*International Centre for Insect Physiology and Ecology, P.O. Box 30772-00100, Nairobi, Kenya*
[4]*Egerton University, Department of Biochemistry and Molecular Biology, P.O. Box 536, Njoro, Kenya*

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An experiment was conducted to determine the association of tea catechins to water stress in tea, with the objective of determining their suitability as indicators for predicting drought tolerance in tea (*Camellia sinensis*). The study consisted of six tea clones (BBK 35, TRFK 6/8, TRFK 76/1, TRFK 395/2, TRFK 31/30, and TRFK 311/287) and four levels of soil water content (38, 30, 22, and 14% v/v), which were arranged in a complete randomized design and replicated 3 times. The treatments were maintained for a period of 12 weeks. Tea shoots were sampled for catechin analysis during the 6th week of water treatment, in which fresh shoots with two leaves and a bud were plucked and steamed for 2 min, and dried at 70 °C to constant weight. Subsequently, the samples were ground and analyzed for catechins using an HPLC system. The total catechins showed significant correlation with shoot growth ($r = 0.65$, $P = 0.006$), soil water content ($r = 0.54$, $P = 0.0066$), and water stress index ($r = 0.67$, $P = 0.0004$). The epicatechin (EC) correlated with shoot growth ($r = 0.58$, $P = 0.0032$), soil water content ($r = 0.62$, $P = 0.0014$), and water stress index ($r = 0.63$, $P = 0.0010$). Similarly, epigallocatechin (EGC) correlated with shoot growth ($r = 0.65$, $P = 0.0006$), soil water content ($r = 0.50$, $P = 0.0133$), and water stress index ($r = 0.60$, $P = 0.0021$). However, epigallocatechin gallate (EGCg) and epicatechin gallate (ECG) showed no significant response to changes in soil water content. The shoot contents of EC and EGC in the six clones showed varied responses, with a distinct pattern in the water-stress tolerant clones (TRFK 6/8 and TRFK 31/30). The results suggest a potential use for EC and EGC as indicators in predicting drought tolerance in tea.

Key words: catechins; drought stress; flavan-3-ol; free radicals

Plants are known to accumulate organic osmolytes such as proline, glycine betaine, non-reducing sugars, and polyols[1,2] in response to stress factors. Though these organic compounds are species-specific their role is not clearly defined, but it is generally accepted that they contribute to ameliorating stress in plants.[2–4] Most stress-related organic compounds are secondary plant metabolites, and tea (*Camellia sinensis*) contains large amounts of polyphenols, mainly catechins, that belong to the flavan-3-ol class. Flavonoids play a key role in quality determination in black tea,[5] and in fruits,[6] but their role as indicators of desiccation tolerance in tea has not been explored. The precursors of most flavonoids are malonyl-CoA, derived from carbohydrate metabolism and *p*-coumaroyl-CoA, from the phenylpropanoid pathway.[7–9] Phenylpropanoids, which include flavonoids, isoflavonoids, and stilbenes, are derived from deamination of phenylalanine by phenylalanine ammonia-lyase (PAL). Flavonoid biosynthesis is dependent on structural and regulatory genes; structural genes encode enzymes catalyzing the biosynthesis, while regulatory genes control the expression of the genes.[8,10–14] This implies that the availability and quantity of certain flavonoids in plant tissues is an indication of plant response to either internal or external stimuli.

As observed in some plants, the production of certain secondary metabolites serves as a signal generated by an external factor, and manifested at the molecular level, prior to morphological symptoms. For instance, increased biosynthesis of the phytoalexin medicarpin in alfalfa has been observed in response to fungal attack.[15] Therefore, the production of stress-related metabolites is modulated by genes whose functions may include transcription of specific enzymes or encoding of specific receptors that regulate other effecter genes. The dynamics of the biosynthesis of organic compounds in response

† To whom correspondence should be addressed. Fax: +254-51-2217942; E-mail: cheruiyotke@yahoo.com

Abbreviations: EC, epicatechin; ECG, epicatechin gallate; EGC, epigallocatechin; EGCg, epigallocatechin gallate; RWC, relative water content; TF, theaflavins; TR, thearubigins

Submitted with correction
Journal of Plant Nutrition

High Fertilizer Rates Increase Susceptibility of Tea [*Camellia sinensis* (L.) O. Kuntze] to Water Stress.

Erick K. Cheruiyot,[1] Louis M. Mumera,[1] Wilson K. Ng' etich,[2] Ahmed Hassanali,[3] and Francis N. Wachira[4]

[1]Egerton University, Department of Crop, Horticulture and Soil Sciences, P.O. Box 536 - 20115, Egerton – Kenya.

[2]Moi University, Department of Soil Science, P.O. Box 1125 – 30100, Eldoret – Kenya.

[3]International Centre for Insect Physiology and Ecology, P.O. Box 30772 – 00100, Nairobi - Kenya

[4]Egerton University, Department of Biochemistry and Molecular Biology, P.O. Box 536 - 20115, Egerton – Kenya.

Address correspondence to Erick K. Cheruiyot, Egerton University, Department of Crop, Horticulture and Soil Sciences, P.O. Box 536 - 20115, Egerton – Kenya. Tel: +254 051 2217877-81 ext 229; Fax: +254 051 2217861; E-mail; cheruiyotke@yahoo.com

ABSTRACT

A study to determine the association of fertilizer with soil water deficit in tea [*Camellia sinensis* (L.) O. Kuntze] was conducted in a rain-out shelter using potted plants, in which five rates of fertilizer (0, 75, 150, 225 and 300 kg N ha^{-1}) and six levels of soil water content (38, 34, 30, 26, 22 and 18% v/v) were applied in a complete randomized design and replicated three times. The soil water treatment was maintained for a period of 12 weeks during which shoot growth, plant water relations and dry matter partitioning in tea were determined. A parallel field experiment with the above fertilizer rates was conducted at three sites in which shoot density and shoot weight were determined during the dry season. Fertilizer improved leaf-to-root and leaf-to-total mass ratios ($P<0.001$), reduced shoot growth, shoot water potential and specific leaf area ($P<0.001$). The fertilizer exacerbated drought effect on tea through disproportionate assimilate partitioning which consequently weakened the ability of tea to tolerate water stress. Results suggest an indirect contribution of fertilizer supply to drought susceptibility in tea.

Keywords: drought, dry matter partitioning, fertilizers, leaf-to- root ratio, soil water content

Submitted, and still being reviewed

Threshold soil water content and the influence of soil water deficit on growth in tea [*Camellia sinensis* (L.) O. Kuntze]

Erick K. Cheruiyot, [a,*] Louis M. Mumera, [a] Wilson K. Ng'etich, [b] Ahmed Hassanali, [c] and Francis Wachira [d]

[a] Egerton University, Department of Crop, Horticulture and Soil Sciences, P.O. Box 536, Njoro, Kenya
[b] Tea Research Foundation of Kenya, P.O. Box 820, Kericho, Kenya
[c] International Centre for Insect Physiology and Ecology, P.O. Box 30772-00100, Nairobi,-Kenya
[d]Egerton University, Department of Biochemistry and Molecular Biology, P.O. Box 536, Njoro-Kenya

[*]Corresponding author. Fax: +254-51-2217737; E-mail: cheruiyotke@yahoo.com

Abstract
The limiting soil water content (SWC) for tea (*Camellia sinensis* L.) production was determined at Tea Research Foundation of Kenya. Tea producers continue to incur huge losses due to drought and hence the need to establish the threshold SWC for tea and to pave way for mitigating measures. The study consisted of six tea clones (BBK 35, TRFK 6/8, TRFK 76/1, TRFK 395/2, TRFK 31/30 and TRFK 311/287) and four levels of SWC (38, 30, 22, and 14% v/v), which were arranged in a complete randomized design and replicated three times. The treatments were maintained for observations for 12 weeks. Leaf and shoot growth were measured weekly for four and eight weeks, respectively, while dry matter yield and partitioning was determined in the 12th week. Other measurements included leaf relative water content (RWC), CO_2 assimilation, stomata conductance and transpiration. Shoot growth, photosynthesis and transpiration had a break-point at 28% v/v SWC, while that of leaf expansion and RWC was observed at 20-18% v/v SWC. The results suggests that SWC below 28% v/v, which was equivalent to ≥33% soil water deficit (SWD), significantly lowers tea yields while SWD above ≥ 52% risk survival of tea plants. It emerged that 20% SWC is the critical limit for tea. Data may be useful in irrigation management for irrigated tea and in clonal selection if clones with better performance at SWD close to the threshold limit are picked.
Key words; soil water deficit (SWD); shoot growth; leaf expansion; drought; relative water content (RWC)

Abbreviations
A/gs, photosynthetic water use efficiency; E, leaf transpiration; gs, stomata conductance; IRGA, infra-red gas analyzer; PAR, photosynthetically active radiation; Pn, net photosynthesis; ppm, parts per million; RWC, relative water content; TDR, time-domain reflectometry; SWC, soil water content; SWD, soil water deficit.