PHYSIOLOGICAL RESPONSES OF GRAFTED TEA (*CAMELLIA SINENSIS* L.) TO WATER STRESS

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

Signature-------------------------------- Date-------------------------

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This thesis has been submitted for examination, with our approval as University supervisors.

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DEDICATION

To my Parents and Family

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ABBREVIATIONS

AHP	African Highland Produce
BBK	Brooke bond Kenya
EPK	Eastern Produce Kenya
ETc	Crop transpiration (mm d^{-1})
ETo	Reference crop evapotranspiration (mm d^{-1})
G_s	Stomatal conductance
HI	Harvest Index
IRGA	Infra Red Gas Analyser
JFK	James Finlay Kenya
K_c	Crop factor
Kg mt/ha	Kilograms made tea per hectare
K_y	Relative change in yield per unit drought stress
N	Nitrogen
NPKS	Nitrogen, Phosphorous, Potassium, and Sulphur
SPAD	Special Products Analysis Division
SRC	Shoot Replacement Cycle (days)
SVPD	Saturation Vapour Pressure Deficit
SWD	Soil Water Deficit
TRFCA	Tea Research Foundation of Central Africa
TRFK	Tea Research Foundation of Kenya

ABSTRACT

Currently, tea planting is expanding towards areas that are marginally dry for economic tea production and accompanied by reduced tea quality. Use of grafted materials in such areas may offer certain potentials depending on the drought tolerance of the rootstocks used. The rootstocks used in the experiments were tolerant to water stress, while the scions were high yielders and good quality. An assessment of physiological responses of composite tea to water stress and critical soil moisture level offers an identification of traits that could be used as criteria for selection of drought tolerant rootstock-scion combination.

A field experiment was set up at Kipkebe Limited, Sotik and two others inside a rain out shelter (a semi-controlled environment) at the Tea Research Foundation of Kenya, Kericho, to evaluate the physiological responses and yields of grafted tea to water stress.

Results showed that water stress significantly ($P<0.05$) reduced stomatal conductance (G_s), net CO_2 assimilation (A) and transpiration (E). High G_s by scion clones on rootstocks EPK TN 14-3, TRFK 57/15 and TRFK 303/577 suggested high tolerance to water stress. The water stress significantly ($P<0.05$) shifted the dry matter (dm) partitioning, with more dm being allocated to roots in stressed treatments. There was a significant ($P<0.05$) reduction in shoot: root ratio, leaf area and stem diameter ($P<0.03$) with water stress. However, water stress increased root length per unit volume of soil density. Reducing soil moisture contents led to higher concentration of chlorophyll contents possibly due to reduced leaf expansion, but decreased leaf water potential and relative water content. Diurnal variation was also evident in the leaf water potential, with lower values recorded at midday.

There were significant differences in air temperatures, saturation vapour pressure deficit and soil water deficit within and between the year(s) in the field site. These variables affected the yields obtained. Yields decreased (R^2=0.74***) with increase in potential soil water deficit and clonal variation was evident with scion AHP S15/10 having a decrease of 0.97 kg mt/ha/week (mm SWD)$^{-1}$. Grafting significantly ($P<0.05$) depressed the yield of the tested scion varieties, which decreased with increase in rootstock ploidy. There was a small margin in yield increases on scions grafted on diploids rootstocks. The triploids and tetraploid rootstocks depressed the yield of most scions. The water use efficiency (WUE) significantly (R^2=0.86***, 0.72***) varied and was in the ascending order of diploids>triploids>tetraploids rootstocks, for 2003, and 2004, respectively. Shoot density, development and compositions were varied between seasons, rootstock ploidy and scions. The shoot parameters contributed to varying yield levels as influenced by soil water deficits, air temperatures and saturation vapour pressure deficit.

The total chlorophyll content varied between clones and decreased significantly ($P<0.05$) with increase in soil moisture content. Rootstocks did not affect the chlorophyll content. The differences in chlorophyll content between clones significantly reduced the photosynthetic capacity. The reduced soil moisture content reduced the leaf water potential in all the clones. The diurnal differences in leaf water potential were more pronounced at the 12.5% v/v soil moisture content. Generally, the mean leaf water potential increased with a decline in soil moisture content. Based on this study, the minimum moisture level below which composite tea plants might suffer water stress is below 30% v/v, and the exposure period should be short. Rootstocks identified as being potential for commercialisation are TRFK 57/15, TRFK 8/112, TRFCA SFS 150 and EPK TN 14-3. Among the scions, AHP S15/10 was found to be more susceptible to water stress. Relative water content declined with decrease in soil moisture content ($R^2 = 0.9915$, $P<0.001$) in all the scions. Both relative water content and leaf water potential could effectively be used as water stress indicators in composite tea.

These results and effects were varietal dependent and are discussed in detail. The results point out the implications of the studies to the Kenyan tea industry bearing in mind that there may be increased frequencies of dry seasons resulting in high soil water deficits and hence decline in tea yields. The magnitude of the decrease depends on the range of soil water deficit and duration.

CHAPTER ONE

INTRODUCTION

Background information

Tea originated from Assam/Burma, and it has been introduced to many countries of the world where it is grown in a wide range of climates ranging from Mediterranean type to hot humid tropics. Tea yields are influenced by weather, and it requires a well distributed rainfall ranging from 1200-1400 mm, to over 2000 mm per annum; temperatures of 18-30^{o}C and well drained Nitosols having a pH of 4.5- 5.6 (Anonymous, 2002). Tea growth extends from Georgia (42° N) in the USSR to Argentina (27° S), and from sea level in Bangladesh (Barua, 1992) to 2700 m a.s.l. in Kenya (Anonymous, 2002).

Tea was introduced to Kenya in the early 1900's from India and China and first planted in Limuru in 1903, and was planted in other suitable areas of the country. Tea producers worldwide are currently operating in situations that face numerous problems such as high costs of production, stagnant or even declining international prices of tea and tea products. These two factors mainly reduce the profitability of tea. To keep the Industry at profitable levels requires innovative technologies and strategies that maximizes on the yield and use of better, high quality plant materials.

The tea introduced for commercial cultivation in Kenya only comprised of plants derived from seed that were low yielding, and its cultivation was hampered by lack of planting material (Wilson and Smithett, 1962). By then, the cheap and effective alternative methods of vegetative propagation from cuttings were not fully developed. Tea seeds obtained from North India were used to set up the first plantations during the first quarter of the 20th Century (Cannel *et al*., 1977). These seeds comprised mainly, random open-pollinated natural hybrids between the Assam and China varieties from Assam region (Cannel *et al*., 1977). Therefore, the genetic base and genetic variation of these progenitors were unknown, and their seed provenances were not selected for yield and quality (Banerjee, 1992). Consequently, the seedling populations were low yielding, and non-uniform (Cannel *et al*., 1977). These disadvantages led to the mass selection within the seedling populations.

The Indian hybrid seed provenance's, from which the seedling populations were established in Kenya, were polyclonal mixtures having been selected from open populated popular races (Cannel *et al*., 1977). Therefore, early planters in Kenya began to visually select jats that were closely related to the *Assamica* variety within their seedling populations, based on certain characteristics such as; their vigour, plucking point density, and shoot size, and these were used to select jats to develop seed bearers.

The establishment of the Tea Research Institute of East Africa in 1951 (Anonymous, 1966), that later became the Tea Research Foundation of Kenya from 1980, marked the beginning of major tea improvement in East Africa. Several jat stocks from the original Assam hybrid seed provenance’s, such as *Tingamira* and *Betjan,* adapted to the local growing environments in East Africa, formed a basis of the local selection of improved assamica clones from seedling tea (Wilson and Smithett, 1962; Anonymous, 1966; Cannel *et al*., 1977).

Shoot number primarily determines the selection criterion for yield potential. Shoot mass, rate of shoot regeneration and extension, which, though genetically controlled, are subject to environmental influences. These phenotypic traits have been used extensively in clonal selection. Other indicators of high yield potential include dry matter production and partitioning (Magambo and Cannel, 1981) and harvest index (Hay, 1995). The harvest index for tea ranges from 7% to 37% (Magambo and Cannel, 1981; Ng'etich, 1995). Use of dry matter production, and harvest index in clonal selection of tea is not routinely practised, because it is destructive, laborious, tedious and time consuming.

In selection for quality potential, fermentability is an important characteristic and is easy to assess even in individual bushes of progeny trials (Sanderson, 1963). The chemical parameters which determine plain tea quality comprise of theaflavins and thearubigins, whose precursors (the green leaf flavanols) are genotype dependent and consists of (+)-gallocatechin, (+)-catechin, (-)-epicatechin, (-)-epigallocatechin, (-)-epigallocatechin gallate and (-)-epicatechin gallate (Robertson, 1992). The total green leaf polyphenol content is positively correlated with brightness, thearubigin content, total colour and sensory evaluation of plain tea quality (Obanda *et al*., 1992).

Various flavour indices for predicting black tea aroma have been developed based on volatile flavour compounds. This aroma is complex and consists of 600 different compounds (Robinson and Owuor, 1992). In the past, four aroma indices were evaluated and they included those of Wickremasinghe-Yamanishi (Wickremasinghe *et al.*, 1973), Yamanishi-Botheju (Yamanishi *et al.*, 1978), Owuor's (Owuor *et al.*, 1986), and Mahanta (Mahanta *et al.*, 1988). Both Owuor and Wickremasinghe-Yamanishi indices exhibited significant relationships with sensory evaluation of black tea and, thus, can reliably be used in clonal selection (Owuor, 1992).

The tea industry in Kenya is structured into four main sectors, which are 1) the small-holder, under the Kenya Tea Development Agency, which is a private organization, contributing over 60% of Kenya's tea, 2) private large estate sector under the Kenya Tea Growers Association (KTGA), 3) government sector, under the Nyayo Tea Zone Development Corporation (NTZDC), and 4) propriety growers, who are private medium sized tea growers.

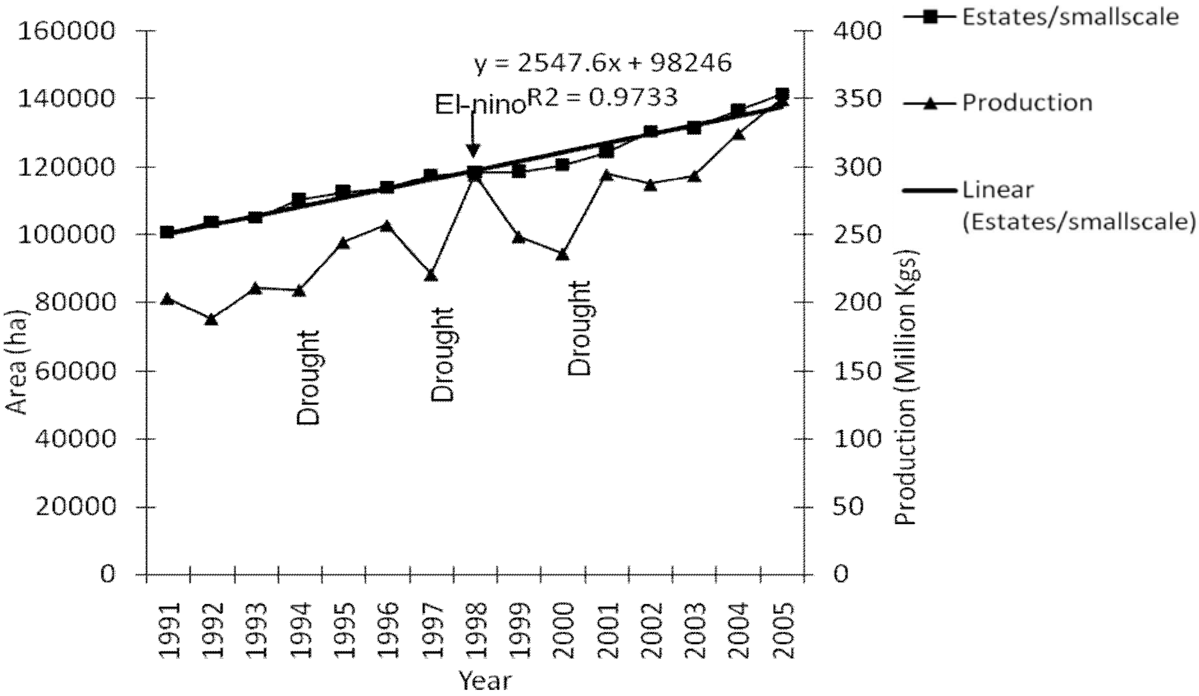

Figure 1.1. Area under tea and yield trends in Kenya

Through the years, and especially after independence, there has been a lot of progress mainly in tea production by the small-scale farmers (Figure 1). Total tea area had reached 141,315 ha, yielding 328,497,624 kg by 2005 (Anonymous, 2006). Expansion among large-scale farmers has risen from 17,921 ha in 1963 to 48,633 in 2005 yielding 130,776,195 kg, and among small-scale farmers from 3,527 ha in 1963 to 92,682 ha, yielding 197,721,429 kg in 2005. Overall production has also increased from 18,000 tons of processed tea in 1963 to over 328,467,624 kg by 2005 (Anonymous, 2006). Small-scale and large-scale growers account for 61% and 39%, respectively, of the national output. Tea is the leading agricultural export crop and foreign exchange earner for Kenya. It employs about three million people, each with more than four dependants, and supports more than 450,000 small-scale farmers. However, the numbers of farmers who are still planting tea especially at the small-scale level are numerous and this shows that the production levels will definitely increase in the near future. The foreign cash earnings by the tea farmers in 2006 amounted to Kshs. 47,297,354,324 that contributed tremendously to income for the growers (Anonymous, 2006).

Due to the increasing cost of farm inputs, continued economic production of tea will be achieved through intensive management of established tea and planting of better and improved clones. This requires new effective and efficient methods of propagation, plucking, pruning, and bringing tea into bearing and production, amongst other management aspects (Magambo, 1983). Grafting has been done to improve production in many plantation fruit crops such as woody perennials as it allows improved varieties to be used (Tubbs, 1973). It also allows use of pest, disease and drought tolerant rootstocks. However, grafting of tea has not been fully utilized to boost both yields and quality and also to improve tolerance to water stress. Screening and recommendation of specific rootstocks of tea would make tea growing possible outside the current tea planting / growing zones, and in what is currently considered to be marginal areas.

1.1.1. Grafted tea

A composite or grafted tea plant consists of a scion that is grafted onto a rootstock. The scion develops into the stems, branches and shoots whereas the rootstock forms the roots and the lower part of the trunk. The scion and the rootstock should have close botanical relationship for grafting to succeed. Some chemical compounds also play a role in influencing the success or failure of graft unions. Sreedhar and Satyanarayana (1996) reported a variation between graft partners, which was associated with levels of polyphenols, mainly orthodihydroxy phenols and catechins in the grafted plants. The content of these chemicals were significantly similar in the graft partners that had high success.

Grafting has been done in many horticultural crops mainly fruit tree species, and this has resulted in improved productivity. Similarly, grafting has exerted several other influences on the scions including improved growth, reduced tree size, precocity in flowering, fruitfulness, fruit quality, and disease resistance (Tubbs, 1973). The influence, which the rootstock has on the scion and the benefits that accrue are as a result of rootstock and scion interaction. Production of tea composites is not likely to be much more costly than conventional cuttings (Kayange and Scarborough, 1978). Producing composites by chip budding on un-rooted cuttings is easy, with a strong union of the budded plant and also no suckers grow after the rootstock has been cut back (Kayange, 1982). Apart from grafting to improve yields and retaining quality, other uses of grafting in tea include converting jat seed baries into polyclonal or biclonal baries where selection of desired plant materials can be undertaken (Barua, 1968; Grice, 1968).

Tea cultivation in Kenya depends on rainfall to maintain its soil moisture level. Generally, 1200 mm of rainfall annually is considered essential, yet the most important factor is the distribution. Inadequate rain imparts critical soil moisture stress on plants. Carr *et al.* (1987) reported that seasonal yield distribution is attributed to adverse effects of large saturation deficits on plant water status and rate of shoot extension. Clonal differences in response to drought have been demonstrated by Carr (1977) and Othieno (1978a) in Kenya, and by Nyirenda (1988) in Malawi. However, these responses have not been quantified for composite tea in relation to soil water deficits.

Water deficits reduces stomatal conductance, thus diminishing photosynthetic rates. Stomatal closure is assumed to be the main cause of decreased photosynthetic rate because it decreases CO_2 availability in the mesophyll (Cornic, 2000). An assessment of the physiological responses of tea plant to water stress is of importance in order to identify traits that could be used in selection of drought tolerant clones. Some of the physiological responses include; gas exchange, water potential, growth patterns and yields.

This work was aimed at attempting to use composite tea plants in Kenya, and exploit their full performance potential in dry matter partitioning and water use. New areas of polyploid composites and drought tolerance are addressed and the main objective of the study was to investigate the effect of grafting on tea physiological responses to water stress.

1.2. Statement of the problem

Grafting is one way in which tea production can be improved. The proportional changes of production are caused by the physiological and morphological improvements that the rootstocks impose on the scion materials. Certain improvements on the scion materials, as caused by rootstocks, confer advantages due to alleviation of stress. Water stress is alleviated when resistant and tolerant rootstocks are used. The advantage of certain rootstock attributes has only been confined to other horticultural crops such as citrus, mangoes and grapes. However, this attribute has not been exploited for tea production, thus it is an area which requires investigations.

1.3. Significance and justification of the study

In Kenya, about 8% of the total tea produced is locally consumed (Anonymous, 2006). Other Kenyans earn income through the sale of green tea leaves, which is processed into made tea. Tea is currently the leading foreign exchange earner in Kenya, and it contributes to 26% GDP of the national economy (Anonymous, 2006). The smallholder growers make a significant contribution in the industry producing approximately 61% of the total national tea production, with 66% of the tea acreage (Anonymous, 2004a). The tea sector in Kenya is globally one of the most successful (Lamb and Muller, 1982), with about 500,000 growers and 62 small holders owned processing factories managed by the KTDA and 39 private estate companies. The sector contributes to livelihood of a large proportion of population estimated at over 3 million (Anonymous, 2006). Tea farming has remained relatively more profitable compared to other agricultural enterprises (Anonymous 2004b), thus leading to its expansion to the marginal areas (Jaetzold and Schmidt, 1983). This expansion to the marginal areas has lead to increased quantity of tea production, with a potential of compromised quality.

Sometimes drought occurs and tea production declines, therefore causing green leaf loss and a severe reduction in income to farmers who depend on tea production (Figure 1.1). It is therefore important that an understanding of a rootstock's influence on scion material and its related response to plant water status is undertaken. Realizing the actual potential of composite use in all tea areas such as, dry areas, is important and it may give an opportunity for planting tea slightly outside the current gazetted tea planting areas, provided that yield is sustainable in water stressed environments.

1.4. Objectives

The broad objective of this study was to determine the influence of grafting tea on its physiological responses.

The specific objectives for this study were:

1) To determine the effects of grafting on performance of tea under water stress.
2) To determine the effects of rootstocks and scions on tea yield components, and dry matter partitioning.
3) To determine the critical water deficit of composite plants.

1.5. Hypotheses

This study was done to test the following hypotheses

1) Grafting tea affects growth and performance of tea.

2) Grafting improves tea plant water status and its response to water stress.

Grafting affects Carbohydrate reserves and influences other physiological parameters.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. Rootstocks and effects on yield and quality

Work previously done on tea grafting has mainly been to determine the effect of rootstocks on yield and yield components. Since a composite tea plant is a combination of two plants of varied characteristics and performance, its overall productivity is thus dependant on the genetic potential of each of these two plant materials, i.e. scion and rootstock. In various studies in India, Malawi and Kenya, use of vigorous rootstocks, on low yielding scions (Barua and Saikia, 1973; Kayange *et al.,* 1981) improved their yields without any significant effect on quality (Kayange *et al.,* 1981; Pool and Nyirenda, 1981; Bore *et al.,* 1995). In one study conducted at Malawi using differenr scions and rootstocks it was observed that after three seasons, use of TRFCA MFS 87 as a rootstock had significantly increased the yield of scions TRFCA PC 1 and TRFCA SFS 204 without significant effect on quality (Kayange *et al.,* 1981; Pool and Nyirenda, 1981).

Yield increases as a result of the increase in the number of shoots was also found in grafted grapes (Zelleke and Kliewer, 1979). Since the main objective of using composites is to improve yields without loss of quality or detrimental effect on plant health and plant longevity (Harvey, 1989), the choice of grafting material is very crucial. Grafting very vigorous scions onto weak rootstocks will result in depressed yield. In Malawi, use of tea clone TRFCA

SFS 150 as scion on weak rootstocks depressed the yield of scion clones in experimental production (Nyirenda and Ridpath, 1983). Weak rootstocks exerted negative effect on plant growth (Nyirenda and Kayange, 1984). Bezbaruah (1971) reported that little success occurred when scions from vigorous clones were grafted on stocks of poor vigour. Depressed yields of vigorous scion clones on weak rootstocks have also been reported in Kenya (Bore *et al.*, 1995). Similarly, use of young clonal scions on old seedling rootstocks results in the same phenomenon, thus making grafting on old established tea unsuccessful (Singh, 1980). Seedlings are mixed genotypes and often within the mixture there may be vigorous high yielding, and weak low yielding, drought resistant and susceptible bushes which potentially can be useful rootstocks (Bezbaruah, 1971). Success is thus, unlikely to be uniform on different seedling bushes because of their genetic differences. High yielding scion clones on very vigorous rootstocks produced plants with thicker stems than ungrafted plants (Nyirenda and Kayange, 1984).

With proper selection and use of rootstocks Kayange *et al*. (1981) reported yield increases in Malawi, where TRFCA SFS 150 and TRFCA MFS 87 increased yield of scions TRFCA PC 1 and TRFCA SFS 204 by 42%, and TRFCA MFS 87 and TRFCA SFS 42 increased by 38%. These yield increases were due to increase in shoot numbers that had increased by almost the same percentage (Pool and Nyirenda, 1981).

In Sri-Lanka, grafting has been used to increase yields of tea by about 35% in a pruning cycle. However, at high altitude areas in Kenya, grafting failed to realize yield increase though few marginal yield increases have been reported (Njuguna, 1992; Bore *et al.*, 1995). The failure of composites to increase yields in Kenya could be due to environmental factors of the test sites and poor selection of rootstocks and scions combinations. The test site, Tea Research Foundation of Kenya [Latitude 0° 22’S, Longitude 35° 22’E] is situated at an altitude of 2180 m a.m.s.l. It has a long-term mean rainfall of 2120 mm per year (54 years), which is well distributed with no distinct seasons that are extremely dry except in some years when December-March period is dry and soil water deficits of 300mm is common. This rainfall distribution favours tea growth, and does not offer a moisture deficit that creates a differential necessary for different rootstocks to show their marked potential in water and nutrient uptake.

Failure of these composites may also have been due to the previous selection criteria, where unsuitable rootstock materials might have been tested without establishing their vigour and rooting depth. A field survey conducted at Kericho and Nandi Hills in Kenya identified a few rootstocks that had been observed to show good potential in yield increase. These included BBK 207, AHP SC 12/28, EPK TN 14-3 and EPK C 12 (Njuguna, 1992). Clones TRFK 11/26 and TRFK 6/8 had their yields improved by grafting on rootstock AHP SC-12/28 by 26% and 7%, respectively while grafting on BBK 207 depressed yields of both clones by 1% and 21%, respectively (Bore, 2000). Shoot replacement cycle has also been improved by grafting. Their influences seem to vary and are clonally dependent. For instance, EPK TN 14-3 increased annual yields of BBK 35, TRFK 31/8, AHP S15/10, BBK 152 by 11%, 13%, 20% and 37%, respectively.

Mean yield of AHP S15/10 on its own rootstock, on TRFCA SFS 150, TRFK 6/8, and TRFCA PC 81, decreased linearly with the maximum soil water deficit at a mean rate of 5.3 Kg $(\text{ha mm})^{-1}$. Yields of AHP S15/10 were increased differently by different rootstocks. Clone TRFK 6/8 as a rootstock increased the yield by up to 32% and TRFCA PC 81 by only 11% (Mizambwa, 2000).

Successes in use of composite plants have been recorded mainly in environments that suffer considerable lengths of drought spells (Pool and Nyirenda, 1981; Kayange *et al.*, 1981; Harvey, 1989; Pallemulla *et al.*, 1992). Composite plants therefore require to be tested, evaluated and screened in moisture stressful environments. Preliminary results of a grafting experiment in a low altitude at Kipkebe, in Kenya indicated that, yield increase of up to 20% and an improvement in shoot growth rate were achievable (Wachira and Bore, 2000). The response however, varied with clones and seasons, similar to observations in Malawi (Pool and Nyirenda, 1981).

Studies on the influence of the rootstock on the scion in tea revealed that success of grafting was directly related to vigour of the rootstock and inversely related to the vigour of the scion (Barua and Saikia, 1973; Harvey, 1989). Similar observations were made on woody perennial tree crops such as rubber (*Havea brasiliensis*) (Ho, 1981); apples (Mosse, 1951; Viser and Kehl, 1958) and guava (*Psidium guajava*) (Teaotia and Phogat, 1971). These yield improvements in composites is achievable where the amounts of polyphenols and catechins in the two graft partners are similar (Sreedhar and Satyanarayana, 1996). Either rootstock or scion with a high level of polyphenols will cause delayed incompatibility hence leading to depressed yields and graft failure.

2. 2. Composites and water stress

Drought normally restricts the potential size and the general crop yield. The physiological linkage between drought and sink size is rather complex and probably has direct and indirect effects.

In rootstocks selection where grafting is done to create drought tolerance, an assessment should be made on the vigour and tolerance to water stress conditions (Harvey, 1988). Visual assessment in the preliminary stages of clonal selection particularly in comparing the performance of source (mother) bushes with that of their clonal derivatives gave inconsistent results (Green, 1971) and attempts to modify the method were also unsuccessful (Magambo, 1982; Njuguna, 1984). However, Viser and Kehl (1958), and Barua (1964; 1965) recommended visual assessment for vigour expressed by bush area, canopy and shoot population density. It is now a confirmed method for use in identifying high yielding clones from a large base population of seedling tea (Nyirenda, 1989). This method can therefore be used effectively to select potential rootstocks for evaluations.

One method of plant adaptation is their root capability to extend to deeper horizons and is thus able to use water efficiently. These plants tend to have a high root-shoot ratios (Koslowski, 1976). Scion clones grafted to deep rooting rootstocks are able to yield substantially higher under stressful environments such as drought. For example, TRFCA SFS 150 is a deep rooter and has big root system (Harvey, 1989) and are, more successful rootstocks. Composite plants with deep rooted rootstocks are more drought tolerant than ungrafted plants (Kayange, 1988). Visual assessment of composite plants at Tea Research Foundation of Kenya during the 1996-1997 drought revealed that scion clones TRFK 6/8, TRFK 11/26 and TRFK 57/15 grafted on TRFK 8/112, TRFK 39/10 and TRFK 6/10 were able to resist drought and were even flushing after about 50 days with no rain (Bore, 1997). This showed that, with proper selection, good rootstocks could improve yields and avoid death of scion materials in the event of prolonged drought.

In Tanzania, composite trials showed that some rootstocks improved yields and tolerance to drought during different water regimes. However, Clone AHP S15/10 as a scion had its yields decreased significantly with increasing soil water deficits (Mizambwa, 2000).

With the current tea expansion even into the marginal zones experiencing long drought spells, use of composite plants may be inevitable if yield/production has to be sustained, provided the rootstocks are selected for drought and/or adverse environment tolerance and for their yield under such adverse conditions.

Clones vary in their shoot water potential and it is this variation that indicates a differential in drought tolerance (Handique and Manivel, 1986). Yield response seems to be a function of water availability and scion clone, for example, response of ungrafted AHP S15/10 was 5.9 Kg $(ha\ mm)^{-1}$, and for BBK35 the range was 3.0-4.5 Kg $(ha\ mm)^{-1}$ but had a high response when grafted to rootstock TRFCA PC81 and AHP S15/10. However, the response of ungrafted BBK 35 was 3.6 Kg $(ha\ mm)^{-1}$ (Kimambo, 2000).

In the study, there was a significant yield difference between ungrafted clones where TRFCA PC 81 performed best and TRFCA PC 105 and EPK TN 14-3 performed poorly under full irrigation and drought treatment. The potential soil water deficit (SWD) of 240 mm reduced yields by about 750 Kg ha^{-1}, which is equivalent to a yield response to irrigation (200 mm) of 4 Kg $(ha\ mm)^{-1}$.

Sepaskhah and Kasherfipour (1994) studied the relationship between leaf water potential, and Crop Water Soil Index (CWSI) on grafted lime. Results showed that, the lower baseline for CWSI in terms of the air Vapour Pressure Deficit (VPD) and the temperature differential between the canopy and the air $(T_C\text{-}T_a)$=3.61-1.74 (VPD) and the upper limit for CWSI was 50^{o}C. The maximum fruit yield was obtained at pan Evaporation fraction of 0.75, corresponding to CWSI of 0.103 and leaf water potential of –2.03 MPa with minimum water use efficiency of 26.8 Kg mm^{-1}. The relationship between leaf water potential and CWSI was dependent on VPD. However, Carr (1977) and Sandanam *et al.* (1981) evaluated some clones and seedling tea and concluded that shoot water potential in mature tea under moisture stress is an index of the water status and hence, their ability to withstand drought. This method of identifying clones with such characters may be useful in selecting drought tolerant rootstocks.

Similarly, the variety of the clone/stock has an influence on the degree of drought tolerance in tea. China varieties seemed to be more tolerant to drought than Cambods whereas Assam was the most susceptible (Handique and Manivel, 1986). However, Assam-China hybrids with dominating Chinary features are both high yielders and drought tolerant. This combination may be exploited when these hybrids are considered for their potential as rootstocks.

2. 3. Ploidy of rootstocks

In evaluating tea clones it has been observed that ploidy influences performance. The general performance of polyploids is far better than that of other non-polyploids. Growth characteristics of polyploid teas have been indicated to be of better stability, higher and prolific growth rate than diploids (Kulasegaram, 1980; Wachira, 1990). Polyploids have larger leaves, shoots, guard cells, stomates (Ng'etich and Wachira, 1992; Wachira, 1997) and flowers (Chaudri and Bezbaruah, 1985), substantial amounts of alkaloids (Singh, 1980) and faster recovery from pruning than diploids (Wachira, *personal communication*). Some of these polyploids have been noted to withstand cold conditions better than diploids (Kulasegaram, 1980).

The general vigour that polyploids exhibit may be useful if polyploid rootstocks are evaluated for yields, drought tolerance or their quick recovery from prune. Yields could be increased and their adaptability to cold stress may allow tea growing in certain stressful environments for instance the very high altitude areas with prevailing low temperatures. Ng'etich and Wachira (2003) reported that there was significant variation in different attributes depending on their ploidy levels. Specific leaf mass were higher in tetraploids but had lower net carbon dioxide assimilation and stomatal conductance than either diploids or triploids. Also the effect of increased ploidy of rootstocks (2x, 3x, 4x) on selected scion clones did not shown clear ploidy effects (Wachira and Bore, 2000).

An evaluation on the effect of polyploid rootstocks on the photosynthetic efficiency of the scion clones was quantified to assess their influence, in terms of improved photosynthetic activities, and in relation to plant water status.

2.4. Physiological responses

2.4.1. General responses of plants to drought

Generally plants that are actively growing in a well-watered environment exert little control over water use and transpires at rates that are determined by the evaporative demand (Black and Ong, 2000). Terrestrial plants are exposed to varying degrees of water stress throughout their ontogeny, ranging from small fluctuations in atmospheric humidity to extreme water deficits and low humidity in arid environments. Plants in semi-arid environment invariably experience some degree of drought stress (Morgan, 1984). In these water limited environments there is a need in managed systems to maximize growth using available water (Hall *et al*., 1993). At whole plant level, limited water supplies may cause major effects on the development, activity and duration of various source and sink organs by reducing the rates of leaf and shoot expansion, inducing changes in root to shoot ratio and growth rate in woody plants (Hall *et al*., 1993). General production of total dry matter, leaf area ratio, lateral branching and leaf production may be reduced (Fernandez *et al*., 2002).

According to Xiongwen (2002), water availability affects trees in various ways and responses to drought are complex. Different plant species adopt a range of strategies to reduce water stress, including escape, avoidance and tolerance (Freitas, 1996), and have evolved some mechanisms to respond to and survive drought (Levitt, 1980; Laffray and Louguet, 1990). Plants capable of escaping drought have a capacity to develop faster and complete their life cycle before severe water deficits develops (Chaves *et al.,* 2003). Examples of stress avoidance mechanisms include rapid completion of ontogeny, low stomatal conductance (Freitas, 1996), reduction in canopy area caused by reductions in leaf growth and premature abscission of leaves, accumulation of resources in organs such as stems or roots prior to drought (Chaves *et al.,* 2003) and leaf rolling (Tanimoto and Itoh, 2000) to reduce light interception and consequent water use. Levit (1980) and Larcher (1995) reported that plants enhance drought tolerance by evolving mechanisms including extensive and deep root systems to increase length of absorbing root per unit area of transpiring leaves and osmotic adjustment.

Plants may also undergo a series of morphological, phenological, anatomical, physiological, biochemical and molecular changes which include increases in the production and relocation of abscisic acid (ABA), stomatal closure, decreased in photosynthesis and changes in membrane properties

(Freitas, 1996; Biswal and Biswal, 1999). ABA and possibly other chemical signals (Hartung *et al.*, 1998; Stoll *et al.*, 2000) are thought to be generated in the roots that are exposed to the drying soil and then transported in the xylem to stomata guard cells where the water loss and carbon dioxide is assimilation is regulated.

Recently, it has been confirmed that total polyphenols in tea varies with clones. However, the clones with high polyphenol content under low soil water content exhibited drought tolerance (Cheruiyot *et al*., 2007). What these findings imply is that total polyphenols and catechins in tea could be used to screen for clones that are drought tolerant.

2.4.2. Transpiration

Photosynthetic carbon fixation occurs in the leaves. These leaves consume most of the plant's water and they are linked to the soil moisture reserves by the absorptive capacity of the roots and the xylem transport system. The roots and the xylem transport system extract and transport enough water to the shoots to maintain hydration despite rapid transpiration (Coomstock, 2002). Pereira and Chaves (1993), reported that under water stress conditions, the changes in assimilate partitioning favour the structures involved in water uptake and transport to enhance plants survival. Extensive root system offers a continued maintenance of turgor and transpiration by continuously taking sufficient water at the root-soil interface (Hall *et al*., 1993). However, when the soil dries, both the soil and roots shrink hence decreasing the contact between roots and the surrounding soil. The decline in soil water content reduces water uptake, stomatal closure, photosynthesis and biomass production (Hall *et al.*, 1993). Studies of various tree species such as deciduous (Liang and Murayama, 1995), and savannah tree species (Eamus *et al.*, 1999), have demonstrated reductions in stomatal conductance and transpiration in response to water stress.

Plants have various adaptations in response to water stress for example foliar modifications (Pugnaire *et al.*, 1999). Changes in diffusive resistance i.e. the boundary layer and stomata have greater influence on transpirational loss of water than CO_2 uptake. Xerophytes and mesophytic species have evolved adaptations to reduce transpiration during photosynthesis by increasing diffusive resistance of the stomata to water loss and locating them in depression in the epidermis. Other adaptations are deposition of epicuticular wax to limit water loss through epidermis (Chaves *et al.*, 2003), together with formation of thick leaves that are adapted to high light and temperature environments (Chaves *et al.*, 2002). These adaptations reduce water loss by decreasing leaf area and increasing the length of the diffusion pathway.

At low water potentials, ABA accumulates to high concentrations and is a prime candidate regulating growth under low moisture content (Sestak and Catsky, 1997; Coomstock, 2002). The studies suggest that ABA is a signal in root-to-shoot signalling since it is synthesized in the drying roots and transported via the transpiration stream to the shoots where it induces stomatal closure and influence leaf growth.

Other stress-induced changes include accumulation of proline in the cytosol and mitochondria (Chaves *et al.*, 2003). In tea, high transpiration rates from extensive canopies cause significant soil water deficits, which are responsible for decreased leaf expansion rates (Stephen and Carr, 1993). When the soil is wet, the excessive transpiration rates resulting from high irradiance and saturation deficits around mid-day could cause transient water deficits within the plant (Smith *et al.*, 1994). Transpiration is linked to photosynthesis which is the primary process responsible for growth of young leaves which form the economic yield of tea. Anandacosmaraswamy *et al.* (2000) reported that when soil moisture content was near field capacity, maximum transpiration rates occurred between 10.00 am and 15.00hrs. However, transpiration rates were found to decline rapidly at 15% soil moisture content.

2.4.3. Water Use Efficiency

Water use efficiency (WUE) is defined either as the ratio of dry matter accumulated per unit of water consumed during the entire season (Zhang *et al.*, 1997) or the ratio of photosynthetic (A) to transpiration (E) rates over a short period (Hall *et al.*, 1993). The ratios are expressed as:

$$WUE_t = \frac{\text{Accumulated dry matter production or crop yield}}{\text{Water transpired}} \qquad \text{Equation 2.1}$$

$$WUE_i = A/E \qquad \text{Equation 2.2}$$

Where WUE_t and WUE_i have units of gKg^{-1} and $\mu mol\ mmol^{-1}$ (Li Chunyang, 2000).

Zhang *et al*. (1997) reported that plants may achieve high WUE values through high net photosynthetic rates or low transpiration rates or a combination of both. Generally, the WUE of plants is affected by the photosynthetic pathways. In hot, high radiation and water stressed environments, C4 plants have higher WUE than C3 plants (Wahid *et al*., 1997). The difference in stomatal conductance and photosynthetic capacities contributes to variations in WUE. These differences are mainly due to differing leaf morphologies (Zhang *et al*., 1997).

Moderate to severe water stress conditions affects leaf metabolism and have considerable influence on WUE (Chaves *et al*., 2002). Though WUE is reported to increase with decrease in soil moisture, there are variations between species and the studies have not provided evidence that drought tolerance is linked to high WUE (Zhang *et al*., 1997; Muthuri, 2004). It is the possible that high WUE may be considered as an adaptive mechanism in plants that are exposed to extended periods of water stress (Pugnaire *et al*., 1999). In plants, there is a linear relationship between dry matter production and transpiration, and this is based on the fact that the stomata which determines diffusion of CO_2 in to the leaf also determines the rate of diffusion of water out of the leaf (Bierhuizen and Slatyer, 1965). WUE of tea has been found to reduce with nutrient and drought stress (Stephens and Carr, 1991a).

2. 4.4. Photosynthesis of tea

Photosynthesis is one of major processes within higher plants, and factors that influence it include chlorophyll content, surface area, variation in stomatal size and aperture, irradiance and potential to utilize CO_2 (Da Matta *et al.*, 1997; Wahid *et al.,* 1997). Photosynthetic rate has been used as an index of growth potential and it generally increases until the leaves are fully expanded, before declining, and varies between leaves of differing longevity (Sestak and Catsky, 1997).

According to Parsons *et al.* (1997), the changes in photosynthetic capacity with leaf age are related to anatomical and physiological factors that include leaf expansion, development of internal tissues and stomata, synthesis of chlorophyll and changes in stomatal conductance. Chaves *et al.* (2002) reported that water is essential for photosynthesis and drought is an environmental factor that decreases photosynthesis. The decrease is mainly attributed to decreases in soil moisture content and a reduction in leaf water potential. The response of photosynthesis to drought is however, complicated by the fact that it is governed by stomatal and non-stomatal factors (Shanguan *et al.,* 1999).

The decrease in photosynthesis is a consequence of limitation of CO_2 diffusion into the leaf (Chaves *et al.*, 2002) resulting from the decreased stomatal resistance to gaseous diffusion associated with stomatal closure to conserve water (Sestak and Catsky, 1997). Under mild water stress the reduced photosynthesis is by decreased CO_2 uptake as a result of stomatal closure (Tezara *et al.*, 1998). On the other hand, when relative water content is reduced to 70% by leaf water deficits, photosynthetic CO_2 fixation is again impaired and stomatal conductance is decreased by stomatal closure (Cornic, 1994). Under prolonged drought, relative water content falls below 70% there are additional reductions in the capacity for photosynthetic O_2 evolution and electron transport in photo system II (PSII) energy conversions (Yordanov *et al.,* 2002) and biochemical reactions involved in photosynthetic process observed (Tezara *et al.,* 1999). These observations support the view that reductions in photosynthesis during severe water stress result from a combination of stomatal and non-stomatal response (Yordanov *et al.,* 2000).

Light, carbon dioxide and temperature are the main factors, which directly affect photosynthesis. Many attempts have been made to correlate photosynthesis or factors related to photosynthesis with crop yield (Devanathan, 1975; Hadfield, 1975) but the interpretation of results in terms of photosynthesis, or yield is complicated because climatic conditions favourable to photosynthesis are also favourable to shoot growth in tea. The conclusions from such attempts were that, semi-erect type of tea produced six-fold number of shoots per unit area than Assam leaf type, hence more active sink. Light saturation point for photosynthesis in mature tea canopy exceeds 700 Wm^{-2} (Sakai, 1975) and in single leaves it is about 350 Wm^{-2} (Squire, 1977).

In tea dry matter partitioning, Tanton (1979) reported that tea harvests is only about 2.5 ton ha^{-1} $year^{-1}$ indicating that yield is limited by lack of assimilates due to partitioning to wood and roots or by limiting factors other than supply of carbohydrates. During selection, a lot of emphasis is put on quantity and whereas quality is secondary, it is a factor that has to be maintained for future tea sales and marketability. Use of rootstocks is aimed at improving yields of low yielding scion clones and retaining their quality. This possibility is a strategy for increasing yields from grafted clones, as their shoots will be larger and more than those grown on their own roots.

Grafting in horticultural crops has boosted the scions performance and especially in matters related to photosynthesis. Medina *et al.* (1998) reported that under well watered conditions, CO_2 assimilation rate of limes (*Citrus limona*) was lower in plants grafted to Troyer and Cleopatra than plants grafted on Rangpur lime. The greatest CO_2 assimilation rate was high at 9.00 h (8 μ mol m^{-2} s^{-1}) followed by a decrease possibly due to the increase in water vapour deficit; from 11.00 h to 14.00 h. Decreasing leaf water potential was observed in all plants when water was withheld. CO_2 assimilation rate decreased rapidly after withholding water. However, there was a rootstock differential response to water deficit. Hydraulic conductivity was highest in Rangpur lime.

In same study, Medina *et al.* (1998), observed that there was no decrease in CO_2 assimilation rate, transpiration rate, water use efficiency, relative water content when the substrate potential was above -0.04 MPa. However, when substrate water potential was below - 0.05 MPa, plants grafted to Rangpur lime and *Poncitrus trifoliata* rootstocks showed different responses. Plants on Rangpur lime had their CO_2 assimilation rate, water use efficiency and substrate water potential decreasing one day earlier than those on *P. trifoliata*. This suggested that maintenance of high values of CO_2 assimilation rate; stomatal conductance; water use efficiency and substrate water potential indicated that plants on *P. trifoliata* showed a higher tolerance to water stress, hence, could survive drought conditions better. From the foregoing, it is evident that rootstock response to water deficit can affect carbon dioxide assimilation, hence, affecting photosynthesis.

Although controlled experiments have provided details on CO_2 assimilation, on many other crops, still there is little information on CO_2 assimilation at the crop community level e.g. plantation crop such as tea composites. This has arisen due to influences from seasonal changes in environmental factors that are constantly changing and differential plant responses of cropping environments.

2. 5. Status of composites in plantations

Currently, use of composite tea commercially is still very low in Kenya and a lot of experimental work is being done to generate the necessary vital information. In Malawi, only about 10% of the plants in new plantations are composites and the planting materials are not available due to large inputs of time and skill required (Harvey, 1989). Composite plants for specific environments are under test in Tanzania (Nixon and Sanga, 1995). Expansions of plantation of composites will increase in the near future provided a proper criterion of selecting rootstocks is done and only if benefits of use of composites can be demonstrated and adopted. The composite trials ought to have clear objectives that demonstrate clear advantages relative to use of ungrafted clones.

Problems caused by stress e.g. pest/disease, drought and/or cold, should be addressed. The choice of composite components ought to have certain characters for example, rootstock clones should be deep rooting, fast and vigorously growing in the field and even if they have poor quality or posses undesirable morphological characters such as small shoots, erect growth habits or tough to pluck. Such clones are best used as rootstocks to boost yields of high quality low yielding scions (Nyirenda and Ridpath, 1983).

Little research on dry matter partitioning, plant water relations of composite tea plants have been done in tea. Wachira and Bore (2000) reported that root biomass was high in rootstock clone TRFCA SFS 150, followed by TRFK 371/1, TRFK 311/287 and EPK TN14-3. Rooting depth and spread were high in EPK TN14-3 and there were clonal variation in their partitioning either to roots, frames and leaves. As soon as more information becomes available, use of composites will be widely adopted, and provided confidence grows in the use of composites, their impact on the industry is likely to be more than that of vegetatively propagated cuttings. However, physiological studies on composite plants in relation to water deficits should be done to create an understanding of the mechanism between tissues of distinct genotypes and their ability to influence each other’s growth and performance.

CHAPTER THREE

PLANT GROWTH, GASEOUS EXCHANGE AND WATER RELATIONS OF YOUNG GRAFTED TEA IN RESPONSE TO WATER STRESS

3.1. Introduction

The proportion of harvestable dry matter for tea is affected by several environmental factors and clonal material. The conversion efficiency of intercepted solar radiation to effective dry matter is low, and the ultimate crop production/ performance is limited to the economic yield (Squire, 1985). The economic yield of tea consists of tender shoots bearing one mature leaf and a bud up to three fully expanded leaves and an immature bud. According to Clowes (1989), these shoots comprise of 25% green stems and 75% leaf, respectively.

There is a relationship between dry matter (dm) produced and transpiration. Transpiration efficiency remains constant where production is limited by water (DeWit, 1958). The physiological basis of this is that, influx of carbondioxide in to the leaf needed for photosynthesis is controlled by the same stomata that regulate transpiration (Bierhuizen and Slatyer, 1965). In plants, high temperatures, radiation, and leaf area, not only increase total photosynthesis, but also increase transpiration. According to Bierhuizen and Slatyer, (1965), the factors that increases the ratio of CO_2 uptake to transpiration are higher partial gradients of CO_2 and lower vapour pressure deficit. These two factors are related to carboxylation pathway and are high in C4 species such as sorghum (*Sorghum-bicolor*) than for C3 species such as tea (Lodlow and Muchow, 1991).

Squire (1990) reported that a decrease of vapour pressure deficit increases transpiration efficiency of groundnut (*Arachis hypogaea*) from 15 Kg ha^{-1} mm^{-1} to 52 Kg ha^{-1} $mm^{-1.}$ This therefore shows that transpiration efficiency is directly influenced by VPD. The field determination of dry matter of tea is generally through destructive harvesting of the whole plant and root excavation. However, a non-destructive method that can be used is the stem diameter. Linear relationships between total dm and stem diameter in young tea were reported by Fordham (1971). This method is also used in forestry management to determine total dry matter in forest trees (Causton, 1985).

High yielding scion clones grafted on vigorous rootstocks produce plants with thicker stems (Bezbaruah, 1971). Good rootstocks enable the scion clones to tolerate drought, more than the ungrafted plants (Bore, 1997). In Malawi, rootstocks with good roots have been used with success (Kayange, 1988; Harvey, 1989). Tea clones are often propagated from seedling tea bushes after having been observed to withstand a period of stress in the hope that they will be drought resistant (Carr, 1977). To see whether this practise is justified, measurements of internal water status are necessary. Clonal rootstocks are widely used to control vegetative vigour and to improve yields and quality. The mechanisms for these commercial useful rootstocks effects are complex and poorly understood. Low root conductivity decrease stomatal conductance, photosynthesis and shoot growth for a given investment in root biomass (Clearwater *et al.*, 2004).

Changes in rainfall pattern directly impact soil and foliage water status, resulting in physiological modification in trees that can affect carbon assimilation rates (Briggs *et al.,* 1986). A decrease of water potentials in the root and/or foliage can affect the carbon assimilation by adjusting stomatal conductance (Lowenstein and Pallardy, 1998), or possibly by directly impacting the biochemical potential for carbon assimilation in the leaf (Escalona *et al.*, 1999).

Tree species differ in their morphology and use of physiologic adaptations to avoid the negative impacts of drought on carbon assimilation (Briggs *et al.*, 1986). Species-specific differences in gas exchange response to drought depend on characteristics such as rooting depth, stomatal sensitivity, osmotic adjustment, cavitation avoidance, and increased tolerance of desiccation (Lowenstein and Pallardy, 1998).

In Kenya, no information is available on composite tea water relations, stomatal conductance and dry matter partitioning with respect to soil water stress. The proportional partitioning of dm composites to their respective plant part portions need to be quantified with respect to soil water deficits. Therefore, this study aimed at evaluating the effects of water stress and rootstocks on growth, stomatal conductance and water relations of young grafted tea.

3.2. MATERIALS AND METHODS

3.2.1. Establishment of plant materials

This study was carried out at Tea Research Foundation of Kenya, Kericho. Composite (grafted) tea plants were prepared in 2002 by leaving selected materials to grow freely for five months according to procedures of Tea Research Foundation of Kenya (Anonymous, 2002). The rootstocks and scions were grafted using chip budding, as out lined by Bore *et al.* (1995). Briefly; from the selected unrooted cuttings of the rootstocks, a cut was made at an angle about one quarter through the stock. A second cut was made 2.5cm above the first cut, going downwards till it met with the first cut. The cuts that were made to remove the bud from the stick were similar to that of the stock. When both scion and stock were ready, they were placed together. To obtain a good fit, both cuts were of the same size and shape. The cambium layer of the bud piece had to coincide with that of the rootstock. The union was wrapped with adhesive tape, and the stock was not cut till the union had healed after hardening.

Clones used as scions were TRFK 6/8, TRFK 31/8, while the rootstocks were TRFK 303/577, TRFK 57/15, EPK TN14-3, and TRFCA SFS150. Clone TRFK 6/8 has excellent quality, is a moderate yielder but it is drought susceptible; clone TRFK 31/8 and TRFK 303/577 are high yielders and have good quality. TRFK 57/15 is drought tolerant, moderate yielder and good quality; EPK TN14-3 is drought tolerant, low yielder and good quality; and TRFCA SFS150 is drought tolerant and high yielder with good quality.

The scion clones were also grafted on their own rootstocks and potted in 4.8 litre (30 x 15.3 cm) polyethylene containers. The potting medium used contained 25% sub-soil and 75% top soil (Anonymous, 2002). 18 months after grafting, the plants which were between 30 to 40cm tall, and had 23 leaves on average were transferred to a propagation shelter (covered with a transparent polyethylene sheet) and arranged on a raised weld mesh table at the Tea Research Foundation, Kericho; (altitude 2178 m a.m.s.l; Latitude 0^0 22' S, longitude 35^0 $21^{'}$ E.; mean annual rainfall of 2120 mm (54 years), mean annual maximum and minimum temperature of 24.5^oC, and $9.2\ ^oC$, respectively.

3.2.2. Treatments

The main treatments consisted of two watering regimes (well watered and stressed) and 6 rootstocks as sub-treatments laid down in a randomized complete block design, replicated three times. All the plants were allowed to acclimatize for one month with constant watering after which they were put in two groups for watering regimes treatments. Stress treatments were watered weekly with 35mm of water (10%$\underline{+}$2 %v/v). This amount of water gives the annual minimum rainfall for tea (Anonymous, 2002). This amount of water resulted in a moderate stress level. The control plants were subjected to adequate watering to 30%v/v (as measured by Time Domain Reflectometer), which was maintained constant throughout the experimentation time. Watering of the control plants was done when the soil moisture content declined below 30%v/v. 30 plants were allocated to each treatment. The treatments were continued for one year.

3. 2.3. Measurements

Plant growth measurements

The measurements were taken as: a) Gas exchange, b) water potential, c) plant height, d) stem diameter, e) trunk cross sectional area, f) leaf area, g) root lengths, h) root length density. Dry matter partitioning was determined by cutting the plants at the collar region and separating the roots, stems and leaves (fallen leaves excluded). These were oven dried at 85-90°C till a constant weight was achieved. Roots were washed and dried.

Gas exchange

Carbon dioxide assimilation rate (A), internal CO_2 concentration (C_i), stomatal conductance (G_s) and transpiration rate (E) and leaf temperature were measured once every three months to represent different seasons. These measurements were done on fully expanded leaves attached to the shoots with a portable Infra Red Gas Analyser (LCA 2 -Analytical Development Co., Limited, Hodderdon, Herts, U.K.) every two hours from 9.00 – 11.00am, 12.00 – 01.00pm and 2.00 – 4.00pm. The gas exchange rates were calculated using equations developed by Von Caemmere and Farquhar (1981).

Water use efficiency (WUE_i) was computed as the ratio of photosynthetic (A) to transpiration (E) rates over a short period (Hall *et al*., 1993). The ratios are expressed as:

$$WUE_i = A/E \qquad \text{Equation 3.0}$$

Where WUE_i have units of gKg^{-1} and $\mu mol\ mmol^{-1}$ (Li Chunyang, 2000).

Water potential

Xylem water potential of tea shoots measuring 10-12cm were measured using pressure chamber technique developed by Scholander *et al.* (1965). Three shoots were taken from each treatment on the same date as stomatal conductance and gas exchange measurement. Measurements were done between 09.00 - 11.00 hrs, 12.00 -13.00hrs and 14.00 - 16.00hrs. The selected shoots were cut and transferred to a site within the experiment plot, where 1cm of the shoot was cut again. The shoot was immediately inserted in to the gas chamber. Using the compressed Nitrogen gas, the key was turned on until the air bubbles were first released from the cut shoot surface.

Basal stem diameter

The basal stem diameter measurements were obtained by using vernier callipers. The base of the stem of the potted sleeved plants was measured. Three plants were used for this exercise.

Leaf area

The first normal leaf was used to determine the leaf area. Leaf area (LA) was estimated at monthly intervals during the experimental period, using the following equation:

$$LA = 0.623*(L \times B) \qquad \text{Equation 3.1}$$

where L and B are the length (cm) and breath (cm), respectively (Ng'etich and Wachira, 1992).

Root length density

After cutting the stem at the soil level of the potted sleeved plants, a slice of soil containing roots measuring 1 cm^3 was taken at the plant base, the total root length per cm^3 was determined by taking a sum of all root fragments within the soil volume obtained. The root length density (RLD) at a point corresponding to a centroid of a cubic slice was defined as the total length of roots per unit volume of soil sampled (Loach, 1978).

Dry matter partitioning

Dry matter partitioning was determined at the end of the experiment after 12 months of treatments. Single plants from each clone and treatment (watered and stressed) were destructively harvested, and partitioned in to leaves, stems, and roots. The leaves were stripped off and stems cut into small pieces. The roots and soils were washed and sieved through a $10mm^2$ mesh. The leaves, stems and roots were dried in an oven at 85°C for 48 hours or till a constant weight was achieved. Their respective dry weights were then determined.

The proportional amount of total dry matter produced by each clonal composite combination and treatment were calculated. The amount of individual dry matter partitioned were calculated and expressed as a proportion of the total accumulated dry matter. The amount of dry matter allocated to roots was divided by the total allocated to both shoots and leaves for each clone and treatment, respectively in order to obtain root: shoot ratio.

3.2.4. Data analysis

For each of the parameters assessed, data were subjected to the analysis of variance (ANOVA), and where significant F-values ($P < 0.05$) resulted, mean separations were done using either the Least Significant Difference (LSD).

3.3. RESULTS AND DISCUSSION

3.3.1. Leaf temperature

The leaf temperature was significantly ($P \leq 0.05$) affected by the water treatments. Leaf temperature in the stressed plants was on average 2.0°C higher as compared to the controls. Transpiration may have moderated the leaf temperature of the watered treatments. Ungrafted TRFK 6/8 and TRFK 6/8 grafted on its own rootstock had higher leaf temperature in the stressed treatment. This clone has previously been confirmed to be drought susceptible (Njuguna, 1982). Clonal variation was evident, implying that they had different capabilities in withstanding water stress.

In the watered treatments, grafting clone TRFK 6/8 on EPK TN14-3 reduced the leaf temperatures (Table 3.1). This rootstock may be drought tolerant. These results imply that, grafting a drought susceptible clone to drought tolerant rootstock, may improve the scions capability to withstand some moderate water stress level. Schultz and Matthews (1997) reported that, water stored in leaf cells served as the initial source for transpiration upon high light exposure. This effect increased with increasing Vapour Pressure Deficit (VPD) and temperature. The pronounced decrease in leaf water content over time in high light intensity caused a rapid decrease in leaf water potential and a concomitant increase in water potential gradient between stem and leaf. It is argued that wilting substantially contributes to creating a sufficient driving force for water uptake from the soil, and reducing the VPD (through a decrease in radiation load and thus leaf temperature) to avoid excessive dehydration.

Table 3.1. Changes in leaf temperatures (^{o}C) during the day in the stressed and watered treatments at Tea Research Foundation, Kericho-Kenya on 15.07.2004.

Clones	09.00-11.000hrs		12.00-13.00 hrs		14.00- 16.00hrs	
	Stressed	Watered	Stressed	Watered	Stressed	Watered
TRFK6/8	30.60	29.60	32.13	30.57	22.80	22.40
TRFK31/8	30.33	28.53	31.80	30.60	22.87	22.40
TRFK6/8 on 6/8	33.17	29.27	31.70	29.77	22.87	22.00
TRFK31/8 on 31/8	32.87	29.13	31.63	30.57	22.73	22.17
TRFK6/8 on SFS150	30.43	30.03	31.13	30.57	22.67	22.40
TRFK31/8 on TRFCASFS150	30.70	29.00	31.20	31.23	22.67	22.47
TRFK6/8 on EPKTN14-3	30.60	28.53	31.13	30.13	22.87	22.40
TRFK31/8 on EPKTN14-3	31.27	28.87	31.13	30.13	22.07	22.60
TRFK6/8 on 303/577	30.50	29.00	31.13	30.67	22.73	22.53
TRFK31/8 on 303/577	30.97	29.87	31.13	29.90	22.87	22.60
TRFK6/8 on 57/15	30.30	29.87	31.17	31.20	22.67	22.67
TRFK31/8 on 57/15	31.67	30.37	31.20	30.30	22.67	22.80
Means	31.12	29.33	31.38	30.47	22.71	22.45
CV (%)	3.43		4.14		1.58	
LSD (P≤0.05)	Water 0.483 Clones NS Interaction NS		Water 0.598 Clones NS Interaction NS		Water 0.166 Clones NS Interaction NS	

Exposure to high VPD and high light intensity causes substantial and rapid dehydration of leaves. However, dehydration could be prevented under high humidity, irrespective of temperature (Schultz and Matthews, 1997). The increased mean leaf temperature that accompanied water stress may actually accentuate the effect of water stress such as preventing transpiration from increasing, thus was reducing the potential negative effects of water stress.

There were significant ($P=0.004$) diurnal leaf temperature differences between clones prevailing only in the morning hours. Higher leaf mean temperatures were recorded in the afternoon, and lowest in late afternoon, though not significant between water treatments. The diurnal leaf temperatures indicated a significant clonal difference, which occurred in mid-morning and was absent in mid-afternoon and late afternoon data, respectively. Although notable clonal differences occurred in mid-morning, leaf temperatures of ungrafted TRFK6/8 had a mean of 1 ^{o}C above others, and the same scenario persisted till afternoon. Interestingly, clone 6/8 on 6/8 registered the highest leaf temperature (33.17 ^{o}C) in the stressed treatment at mid-morning (Table 3.1).

The clonal leaf temperature amplitude was low at mid-morning and high in the afternoon whereas the interaction was highest in the afternoon. High leaf temperatures may have been responsible for lower assimilation rates in the afternoon (Prior *et al.*, 1997). Assimilation and stomatal conductance in *Terminalia ferdinandiana* decreased when leaf temperatures rose above 38 ^{o}C and/or the leaf-to-air vapour pressure difference exceeded 4 - 4.5 KPa. Partial wilting was observed in stressed treatments during midday and this could have substantially contributed to creating a sufficient driving force for water uptake from the soil as wad reported by Schultz and Matthews (1997) on grapes. This ultimately contributed to a cooling effect of the plants, hence the registered lower leaf temperature.

Temperatures have a distinct influence on photosynthesis. The high morning temperature could have led to high photosynthetic rates. This could be followed by decline by midday without a decrease in ambient temperature or radiation (Kumudini, 2004). The observed diurnal pattern is consistent with a midday depression, which has been reported, in a number of horticultural crops including carob trees (Demming-Adams *et al.*, 1989).

3.3.2. Stomatal conductance

The watering regimes had significant ($P<0.001$) effects on stomatal conductance (G_s) of the scion clones. Generally, low G_s were recorded in the water stressed plants, and high G_s for well watered treatments. Stomatal conductance also varied between rootstocks and scions (Table 3.2). Between 9.00- 11.00 hrs under the adequately watered regimes, clone TRFK 6/8 on TRFK 6/8; TRFK 31/8 on EPK TN 14-3 and TRFK 31/8 on TRFK 57/15 had high G_s. The high G_s of clone TRFK 6/8 had previously been identified by infiltration score measurements (Othieno, 1978b). Water stressed plants had lower stomatal conductance, irrespective of rootstock. High values of G_s under watered stressed treatments suggest that potted tea scions on TRFK 57/15; and EPK TN 14-3, showed higher tolerance to water stress. These results concur with those of Medina *et al.* (1998). Clone TRFK 31/8 was better than TRFK 6/8, and this implies that TRFK 6/8 is more susceptible to water stress as compared to TRFK 31/8. Clone TRFK 31/8 on TRFK303/577, EPK TN14-3 and TRFCA SFS 150 had similar and comparable G_s in the watered treatments. Sandanam *et al.* (1981) found differences in clonal response to water stress and attributed it to leaf age.

Table 3.2. Stomatal conductance (mmolm^{-2}s^{-1}) during the day in tea plants subjected to different watering regimes between 09.00 hrs to 16.00 hrs in 2004 at Tea Research Foundation, Kericho- Kenya

Clones	09.00-11.000hrs		12.00-13.00 hrs		14.00- 16.00hrs	
	Stressed	Watered	Stressed	Watered	Stressed	Watered
TRFK6/8	213	520	180	196	288	570
TRFK31/8	188	499	180	208	220	471
TRFK6/8 on 6/8	176	860	180	172	236	480
TRFK31/8 on 31/8	188	676	164	184	244	591
TRFK6/8 on SFS150	184	716	160	176	224	540
TRFK31/8 on TRFCASFS150	198	700	168	180	260	450
TRFK6/8 on EPKTN14-3	183	712	168	176	236	482
TRFK31/8 on EPKTN14-3	224	820	156	176	248	495
TRFK6/8 on 303/577	175	528	152	168	236	573
TRFK31/8 on 303/577	192	720	156	168	220	482
TRFK6/8 on 57/15	183	672	164	209	210	408
TRFK31/8 on 57/15	220	816	164	184	232	490
Means	194	687	166	180	238	503
CV (%)	11.55		7.04		3.02	
LSD (P≤0.05)	Water 23.72 Clones 58.10 Interaction 82.18		Water 5.73 Clones 14.04 Interaction NS		Water 5.21 Clones 12.76 Interaction 18.04	

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There were diurnal differences with a consistent trend in G_s between the adequately watered and water stressed plants. In mid morning, G_s were higher in watered plants, and then progressively decreased at midday and increased again in the afternoon (Table 3.2). The higher G_s values for watered plants indicate that they were responding to prevalence of high soil moisture content, which could have influenced the stomatal activity. The results also suggested an influence of the soil water status on the diurnal stomatal activity. In contrasts, stressed plants had low G_s values in the morning, thereafter decreasing at midday and peaked again in the afternoon. Within a watering regime, the rootstocks did not significantly influence $G_{s.}$

Low differences in the water stressed plants may be attributed to the high air temperatures. Winkel and Rambal (1993) reported that diurnal changes in G_s are mainly influenced by saturation vapour pressure, which fluctuates during a given day. Stomatal conductance is related to the saturation vapour pressure deficits of the air and is reported to decrease linearly with increasing leaf-to-air vapour pressure difference (Thomas *et al.*, 2000). The high G_s in the watered treatments in the morning were attributed to high air humidity as influenced by the low leaf-to-air vapour pressure difference (Vadell *et al.*, 1995; Prior *et al.*, 1997; Kurpius *et al.*, 2003). This observation could probably be related to low air temperatures in the morning. Scion clones on rootstocks TRFK 303/577; TRFCA SFS 150; TRFK 57/15 and EPK TN 14-3 had high G_s values in the afternoon. Plants subjected to progressive reduction in soil moisture may develop morphological changes such as reduced leaf area as a way of acclimatization and have low stomatal conductance as an avoidance mechanism

(Freitas, 1996). The reduced G_s may lead to fluctuations in diurnal leaf gas exchange hence a decline in carbon assimilation during drought period. These fluctuations may improve water-use efficiency, probably by enabling the plant to survive in periods of high air humidity (Vadell *et al.*, 1995). Stomatal conductance is related to the saturation vapour pressure deficits of the air and is reported to decline linearly with increasing leaf-to-air vapour pressure difference in all species (Thomas *et al.*, 2000).

Midday depression of stomatal conductance has been observed in many plants, and is characterized by a decline in photosynthetic rates around noon. Studies on midday depressions have suggested that the decline in photosynthetic rates would depend on environmental conditions and can vary daily (Kuppers *et al.*, 1986). Consistent with this suggestion, our results indicate a significant day effect. Midday depression has been thought to be a consequence of either stomatal limitation or biochemical limitations from photo inhibition. Midday depression is attributed to stomatal closure in inhibition of photosynthesis by water stress. Demming –Adams *et al. (*1989) have suggested that increasing water stress and vapour pressure deficit in mid afternoon, feedback inhibition from carbohydrate accumulation or decreased carboxylation efficiency may contribute to midday depression. These factors can directly or indirectly affect $G_{s.}$

Although some of the values of stomatal conductance reported in this study are high in some clones, others are comparable with those reported by Ng'etich and Wachira (2003). However, the difference could have been contributed by the differences in clones and the water treatment regimes. Also, the pattern of G_s in the stressed treatments suggests that they are insensitive to shoot water potential but closely related to rate of photosynthesis (Squire, 1978).

3.3.3. Transpiration

The watering regimes significantly (P<0.05) influenced the mean rate of transpiration. Water stressed plants had low transpiration rates compared to the adequately watered plants (Table 3.3). Rootstocks did influence transpiration rates in either water stressed or adequately watered plants in early morning and at midday. Reductions in photosynthesis and transpiration were reported in Cashew nut trees and attributed to low (0.05 mol m^{-2} s^{-1}) stomatal conductance in the drying trees (Blaikie and Chacko, 1998).

Diurnal transpiration rates significantly (P<0.05) varied between watering regimes (Table 3.3). For water stressed plants, transpiration rates were low in the morning up to midday, thereafter decreasing in the afternoon. In contrast, adequately watered plants had high transpiration rates in the morning, which slightly decreased at midday and were moderately higher in the afternoon. In the afternoon, transpiration was twice higher in adequately watered plants than in the stressed plants.

Transpiration varied significantly between clones and with the time of day. The variation was only absent at midday. Benkert *et al.* (1995) observed that diurnal changes of xylem pressure in *Tetrastigma voinierianum* revealed that, early morning, tensions developed more or less rapidly with time, depending on light intensity. On sunny days, negative pressures down to -0.4 MPa were recorded around noon, whereas on rainy or cloudy days the xylem pressure was low. Towards the evening the tension decreased, i.e. the xylem pressure shifted to about atmospheric. These results strongly suggest that the xylem tension induced by transpiration is not the sole force for water ascent but other forces, such as osmotic pressure of convectional and interfacial forces, which to a remarkable extent have already been postulated for decades, seem to be equally important (Benkert *et al.*, 1995). In this study, the trends in stomatal conductance and transpiration are very similar thus indicating that xylem-induced tension could be the sole force for water ascent.

Table 3.3. Transpiration (mmolm^{-2}s^{-1}) during the day for tea plants subjected to watering and water stress between July and September 2004 at Tea Research Foundation, Kericho, Kenya.

Clones	09.00-11.000hrs		12.00-13.00 hrs		14.00- 16.00hrs	
	Stressed	Watered	Stressed	Watered	Stressed	Watered
TRFK6/8	12.977	32.917	29.307	34.630	14.823	62.217
TRFK31/8	12.247	29.400	31.197	37.397	18.283	53.510
TRFK6/8 on 6/8	17.690	32.797	31.830	41.660	17.383	39.977
TRFK31/8 on 31/8	18.400	30.720	32.320	39.710	17.007	47.180
TRFK6/8 on SFS150	18.950	32.377	33.330	39.297	17.847	60.867
TRFK31/8 on TRFCASFS150	19.843	29.530	29.507	38.800	15.900	60.643
TRFK6/8 on EPKTN14-3	19.540	30.083	33.040	38.427	17.310	60.203
TRFK31/8 on EPKTN14-3	18.443	26.703	34.203	38.697	17.173	59.027
TRFK6/8 on 303/577	10.970	32.430	34.857	39.663	17.110	60.867
TRFK31/8 on 303/577	19.610	31.823	33.633	40.473	18.227	62.433
TRFK6/8 on 57/15	19.993	33.527	30.833	38.960	18.663	60.603
TRFK31/8 on 57/15	18.900	28.970	31.983	38.613	17.533	60.840
Means	17.297	39.940	32.170	38.861	17.272	57.364
CV (%)	10.60		10.97		6.38	
LSD ($P \leq 0.05$)	Water 1.007 Clones 2.467 Interaction NS		Water 1.818 Clones NS Interaction NS		Water 1.111 Clones 2.722 Interaction 3.849	

Further research is required on tea to clearly state a cause and effect relationship between G_S and photosynthetic decline during midday.

Relationship between G_s and transpiration (E) in watered and stressed treatments (Figure 3.1) indicates that correlation coefficients between E and G_s in watered treatments were significantly linear and showed an increase in E with increasing G_s (R^2 =0.71; P<0.0001).

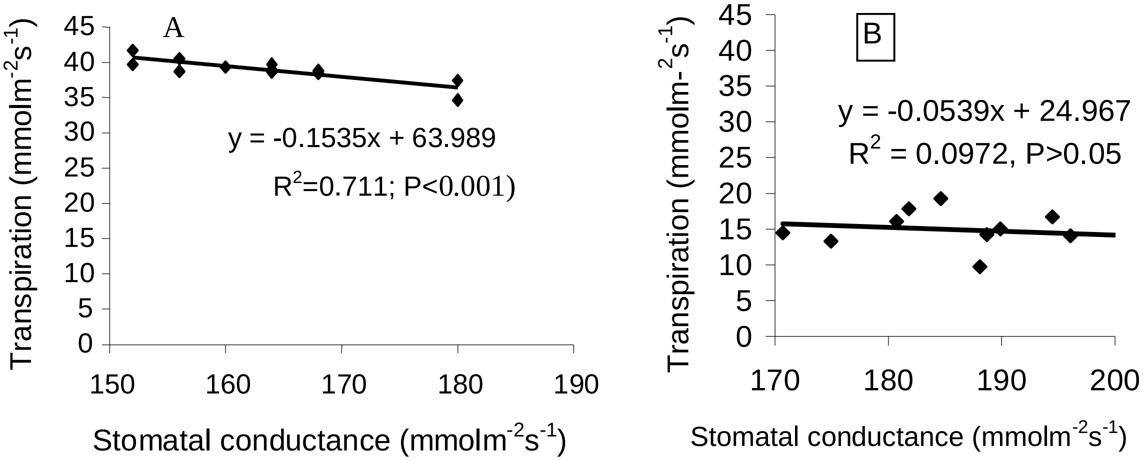

Figure 3.1. Relationship between stomatal conductance ($mmolm^{-2}s^{-1}$) and transpiration ($mmolm^{-2}s^{-1}$) of (A) adequately watered (R^2 =0.71; P<0.001) and (B) stressed grafted tea plants. Data points represent clones.

The low transpiration rates could have been caused by the low soil moisture contents; hence there was no water for the plant to loss (Beardsell *et al.,* 1973). The rates of transpiration and photosynthesis has been reported to became independent of the atmospheric conditions and this is attributed to slow movement of water into the root zone from the surrounding soil with associated stomatal closure limiting the rates of water uptake and transpiration.

In tea, high transpiration rates causes significant soil water deficits and decreased leaf expansion rates (Stephen and Carr, 1993). In high soil moisture contents, the excessive transpiration rates resulting from high irradiance and saturation deficits around mid-day could cause transient water deficits within the plant (Smith *et al*., 1994). Transpiration is linked to photosynthesis, which is the primary process responsible for growth of young tealeaves. Anandacosmaraswamy *et al*. (2003) reported maximum transpiration rates between 10.00am and 15.00hrs when soil moisture content was near field capacity and a rapid decline with reduced moisture content.

3.3.4. Net CO_2 assimilation

The net CO_2 assimilation values varied within the clones and between soil moisture treatments. The net CO_2 assimilation was higher in watered treatments, as it ranged from 4.3 to 8.0 µmol $m^{-2}s^{-1}$ with little fluctuation within clones. The water stress treatments showed reduced net CO_2 assimilation, which was accompanied by high variability within clones. Clone TRFK 31/8 had the highest net CO_2 assimilation of 8.0 µmol $m^{-2}s^{-1}$ followed by TRFK 6/8 on 303/577, 6/8 on EPK TN 14-3, 31/8 on EPK TN 14-3, with 6.67, 5.67, 5.33, µmol $m^{-2}s^{-1}$, respectively. Clone TRFK 31/8 on EPK TN 14-3 had the lowest G_s of 218.58-mmol $m^{-2}s^{-1}$ with a high net CO_2 assimilation suggesting that it was able to conserve water. The results also imply that rootstock EPK TN14-3 is drought tolerant. On the other hand, clone TRFK 6/8 had the lowest net CO_2 assimilation suggesting that it is susceptible to water stress. These results compares well with those of Burgess, (1992), Ng’etich and Wachira, (2003).

There were linear negative correlations between Carbon dioxide assimilation (A) and G_s. Both the adequately watered and stressed treatments had no significant (R^2 =0.073; and R^2 =0.03; $P>0.05$) effects on the relationship between A and G_s. Ng’etich and Wachira (2003) found a positive correlation between net CO_2 assimilation and stomatal conductance. However, in this study we found weak correlation and this may have been caused by environmental differences and clones studied. Searson *et al*. (2004) reported variation in photosynthetic capacity of Eucalyptus and negative correlation between G_s and WUE.

Reduced net CO_2 assimilation rate under water stress in many plants has been reported by many workers such as Read and Farquhar (1991) and Ngugi *et al*. (2003). The decrease could be attributed to the dynamic photoinhibition effects caused by high temperatures, which involve down regulation of photosystem II efficiency and a large amount of dissipation of excess excitation energy as heat (Souza *et al*., 2004). Similarly, changes in rainfall patterns directly impact on plant water status resulting in modifications that affect carbon assimilation rate (Briggs *et al*., 1986; Lowenstein and Pallardy, 1998)

3.3.5. Water use efficiency (WUE)

The water use efficiency varied significantly within the clones and watering regimes. The water stressed treatments had higher WUE than the well watered. The high WUE was particularly on scions clones grafted to TRFCASFS 150, EPK TN14-3 and TRFK 57/15. Scion clone TRFK 6/8 had more than 50% higher WUE than the watered on the three rootstocks. On the other hand, TRFK 31/8 also had higher WUE only on EPKTN14-3 and TRFK 57/15 (Figure 3.2). The results reported in this study concur with those reported on other perennial tree crops. All the clones exhibited quite low WUE probably due to the low water applied which was more restricted than under the field conditions, where the tea could be able to explore deeper horizons in search of water.

In water limited environment, the stomatal conductance decreases as a result of the stomatal closure. This closure has a greater effect on photosynthesis than on transpiration because of the additional resistance associated with diffusion of CO_2 relative to water in the leaf (Nobel, 1991). An increase in WUE caused by stomatal closure results in decrease in carbon dioxide assimilation, and therefore plants that have high WUE in a dry environment could have a higher productivity or ability to survive. In this study, the observation that clone TRFK 6/8 had a high WUE when grafted to TRFCA SFS 150, EPKTN14-3 and TRFK57/15 could be related the drought tolerance of these rootstocks probably due to their deep roots and root length density. However, the clonal variation in well watered composites was less than that of the stressed plants suggesting that the relationship between WUE and drought tolerance could have been complicated by gas exchange or external factors such as water availability (Zhang *et al.*, 1997).

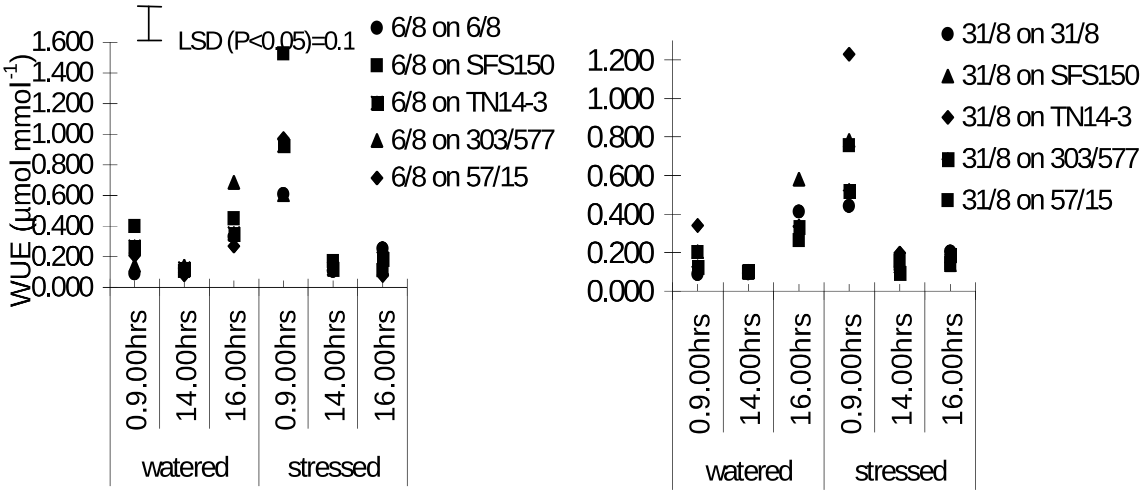

Figure 3.2. The dynamics of diurnal WUE for scion clones TRFK6/8 and TRFK31/8 composite tea subjected to two watering regimes.

High WUE has been considered to be an important adaptive feature in plants that are either exposed to longer periods of drought or subjected to intense competition from the neighbouring plants (Pugnaire *et al.*, 1999). Changes of WUE at leaf level are always associated with intercellular CO_2 partial pressure (C_i), net photosynthetic rate and transpiration rate. The changes in this parameter can occur through numerous mechanisms such as changes in stomatal conductance, and carboxylation efficiency. Stomatal regulation is a complex process involving several pathways (Grabov and Blatt, 1998; Pei *et al*., 1998) all of which can affect physiological WUE (WUE_t the ratio of CO_2 assimilation rate to transpiration rate). Also stomatal closure in response to water stress restricts CO_2 entry into leaves thereby decreasing CO_2 assimilation as well as decreasing water loss from the leaves (Cornic, 1994; Parry *et al*., 2002). Plants differ in their capacity to regulate how much water is lost per unit carbon gained (Condon *et al*., 2002) under different environmental conditions, for example under water stress conditions, higher plants might keep a higher carbon gain by a relative CO_2 assimilation capacity (Condon *et al*., 2002). Thus, improving WUE_t might be one means of achieving more yields per unit water (WUE) under water stress conditions.

Generally, WUE is linearly correlated to the biomass under water stress condition (Condon *et al*., 2002), while it is not always linearly correlated to the biomass or economic yield under non-water stress condition. However, the relationship between WUE_t and biomass production is dependent on environmental factors and variation in genetics and physiological mechanisms. Burgess (1992b) reported that there was a linear relationship between tea yield and transpiration. However, there were variation between clones and the results indicated that under drought, plants would transpire without any commensurate yields. The WUE of clones TRFK6/8 and AHPS15/10 were in the range of 2.7 – 2.8 Kg (ha mm)$^{-1}$. From results in many cases that have been examined, WUE_t has been positively correlated with yield and/or biomass (Guo *et al*., 2006). This can be expected to increase WUE under moderate water stress and when variation in WUE is primarily driven by variation in carboxylation capacity

(Livingston *et al.*, 1999). The behaviour of the diurnal WUE of the clones was at its peak at 0900 to 11.00hrs in the stressed clones similar to observations at 16.00hrs though with some variation within the clones. At 14.00hrs, all the clones at both stressed and watered treatments had very low WUE. This could have been caused by the low G_s that could have reduced the CO_2 assimilation as earlier discussed in the stomatal conductance. There was a little relationship between WUE and A/G_S where, high values of A/G_S indicate drought resistance and low values drought susceptibility. From the results, rootstocks TRFCA SFS150 had high values followed by EPK TN14-3 and the least was TRFK 303/577. The correlation between clonal values of A/G_S and drought resistance suggests that measurements of A/G_S could provide a means of screening clones for drought resistance. Burgess (1992b) had reported that this relationship could be used to screen clones for drought tolerance.

3.3.6. Leaf water potential

There were significant ($P<0.05$) differences in water potentials between tea plants subjected to water stress and those that were adequately watered. Under adequately watered conditions, there were no differences between clones, with the values ranging between –0.4 to –0.5 MPa. Under water stress, water potential ranged between –0.7 to – 0.85 MPa, with the exception of TRFK 303/577 and TRFK 6/8 grafted on EPK TN 14-3, where values were higher. The higher values for TRFK 303/577 and 6/8 on EPK TN14-3 may indicate better tolerance to water stress (Figure 3.3).

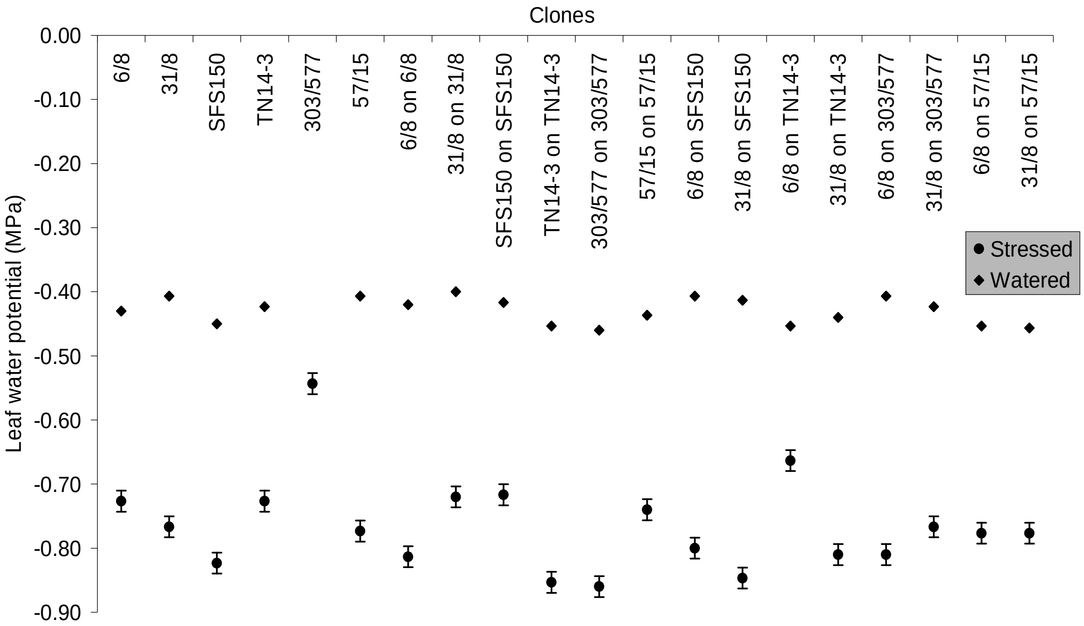

Figure 3.3. Leaf water potentials of composite tea plants subjected to water stress at the Tea Research Foundation, Kericho. Vertical bars are standard errors.

Perry *et al.* (1994) reported that stomatal conductance was influenced by competition for water; with competitive effects being more evident during drought. G_s were most often related to bulk leaf water potential, which in turn was related to competitive effects on soil moisture availability. Water stress avoidance has also been attributed to deep root systems. The deep root development is an escape mechanism that enables the plant to increase the length of water absorption (Levit, 1980; Larcher, 1995). Other mechanisms may, however, also be involved. Li *et al.* (2000) for example reported that trees grown under drought stress had smaller root to leaf water potential gradient. Tea soils in Kenya are generally homogeneous and such differences attributed to soil uniformity may not have applied in this study.

3.3.7. Leaf area (LA)

Leaf Area (LA) is a key variable for most agronomic and physiological studies involving plant growth, light interception, photosynthetic efficiency, evapotranspiration and responses to fertilizers and irrigation (Blanco and Folegatti, 2005). Therefore, LA strongly influences crop growth and productivity, and estimation of LA is a fundamental component of crop growth models (Lizaso *et al.*, 2003). In this study, watering regimes significantly affected the leaf area of most clones, with exception of clone TRFK 31/8, TRFK 6/8 on EPK TN14-3, and TRFK 6/8 on TRFK 57/15 which had similar leaf area, compared to the others. There was however, a general trend where water stressed plants tended to have a lower leaf area than well watered ones (Figure 3.4).

Leaf area in Poplar was reduced by water stress, although the effects upon leaf number, weight, and leaf area differed between treatments, which concur with other findings (Ibrahim *et al.*, 1997). A larger leaf area resulted in larger interception of light and transpiration losses in spruce, which could lead to severe water stress as alluded by Alavi (2002). It is necessary to reduce leaf area in order to minimize excessive water loss especially during periods of water stress (Aminah *et al.*, 1997). The differences in leaf morphology, including leaf mass to leaf area ratio, inclination, and leaf growth, account for differences in leaf performance. Limited water supplies may cause major effects on the development, activity and duration of various source and sink organs by reducing the rates of leaf and shoot expansion, and inducing changes in root to shoot ratio and growth rate in many woody plants (Hall *et al.*, 1993).

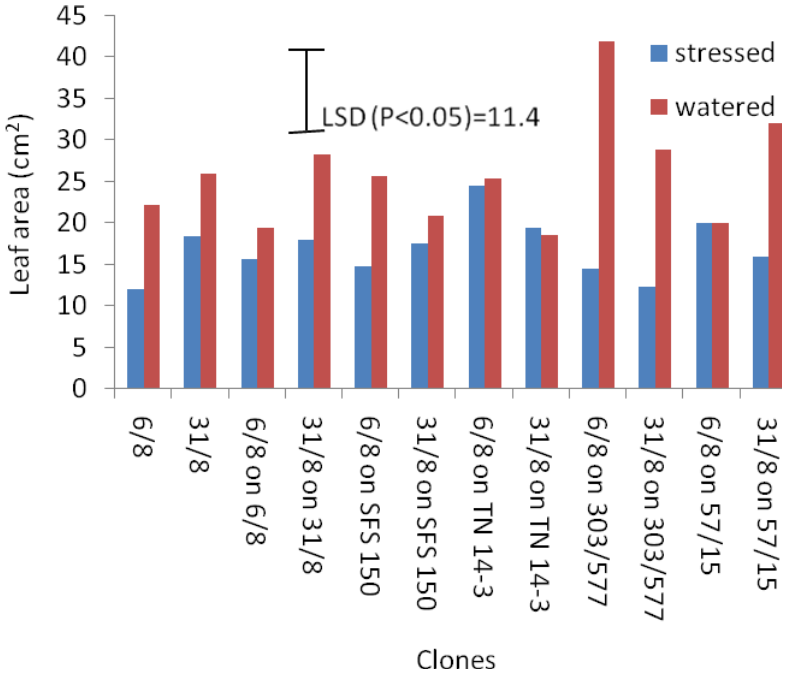

Figure 3.4. Leaf area of first normal leaf for composite tea subjected to different watering regimes at Tea Research Foundation, Kericho, Kenya.

Assam clones of tea have larger leaves compared to chinery teas with erect small leaves. The clones with these latter leaf characteristics tend to survive drought better as they do not loss much water through transpiration, due to reduced leaf area suggesting that small leaf characteristic is an avoidance mechanism to avoid water loss.

Perry *et al.* (1994) found an increased pine growth after a significant drying period, accounting for more than half of the variation in stem volume. Conversely, Schultz and Matthews (1993) reported an inhibition of leaf expansion caused by water deficits. The significant variation as a result of water stress affected; total biomass, height, root mass/foliage area ratio, foliage area/stem cross sectional area ratio, leaf area and water-use efficiency. The observed clonal variation in this study was more pronounced in the water-stressed treatment than in the well-watered ones similar to studies done by Li *et al.* (2000).

Measurements of surface area of a large number of leaves is costly, time consuming and destructive. A modelling approach involving linear relationships between LA and one or more dimensions of the leaf is inexpensive, rapid, reliable and non-destructive (Lu *et al.*, 2004). The results reported in this study showed that LA could be accurately estimated. A similar model has been used in coffee where a power model incorporating either the leaf length (L) alone or both L and W was developed (Antunes *et al.*, 2008). Linear allometric models are inaccurate and under estimates LA, particularly in small leaves with L/W ratio greater than 3.0. The power model based on two leaf dimensions could be used to estimate LA with accuracy and high precision independently of leaf size. This model has been considered adequate for estimating LA of other perennial crops like grapevine (Williams and Martinson, 2003) and dwarf coconut tree (Sousa *et al.*, 2005).

Following a drought period, (Silberstein *et al.* (2001) found a reduction of leaf area in *Eucalyptus marginata* by one-third of the spring value and a reduction of 30% in soil moisture, high leaf conductances during summer, with evapotranspiration rates at around or above 78% of equilibrium evapotranspiration. The results showed that leaf area index (LAI) was not significantly different for each rootstock even though measured LA per tree differed greatly. Also, Li ChunYang *et al*. (2000) made an evaluation of the intra-specific variation in foliage area/stem cross sectional area ratio in the context of tree hydraulic architecture: provenances from dry areas. The results were that trees grown under drought stress had more foliage per stem area ratio. However, their transpiration and the length of their hydraulic pathway were smaller and therefore the root to leaf water potential gradient might be smaller.

A study by Farrell *et al*. (1996) concluded that clones differed significantly in their ability to produce leaf and root tissue during stress periods. Clones which produced the greatest numbers of leaves had leaves of low specific weight, while clones which produced fewer leaves had leaves of relatively high specific weight. Clonal lines that produced the greatest leaf area also produced the greatest root weight. Comparisons of water use produced fewer differences, although some clones, which produced the greatest number of leaves and the greatest total leaf area, also used the most water in the drought trial due to high transpiration rates and aggravates severity to water stress (Alavi, 2002).

The importance of reducing leaf area to minimize excessive water loss especially during periods of water stress is essential and thus, trimming leaf areas for optimum rooting of cuttings is emphasized, which also avoids wastage of space in rooting beds (Aminah *et al.*, 1997).

3.3.8. Root length density (RLD)

Soil water deficits affected the distribution of the RLD, although the trends were variable. For TRFK 6/8 and TRFK 31/8 on their own roots or grafted on the same rootstock, response of RLD to water stress was inconsistent (Figure 3.5). When TRFCA SFS 150 was used as a rootstock for 6/8 and 31/8 there was no significant difference. However, when TRFK 6/8 and TRFK 31/8 were grafted on EPK TN 14-3, TRFK 303/577 and TRFK 57/15, RLD was higher in water stressed plants than well watered ones, with exception of TRFK 6/8 on EPK TN 14-3. Mulching of young tea encourages more superficial root development and these are killed during drought because of significantly high soil temperatures and low soil moisture. Othieno (1982) and Ng'etich and Stephens (2001a) reported that, increasing soil temperatures with mulches had a direct effect on root length. The variation in RLD may indicate non-homogeneity in root distribution within the soil.

Tree management and spacing are known to influence the density of the fine roots in tree species (Atkinson, 1980). Rootstock effects on fine root lengths also depend on soil type, age of plant and soil moisture content and irrigation. Soil water deficits stimulated fine-root growth in *Fagus sylvatica* (Leuschner *et al.*, 2001). This is in agreement with the present study, where RLD was high in rootstocks EPK TN 14-3, TRFK 303/577 and TRFK 57/15 under water stress (Figure 3.5). Patterns of root growth, soil water use, and biomass allocation, may then be attributed to differences in tolerance to water stress.

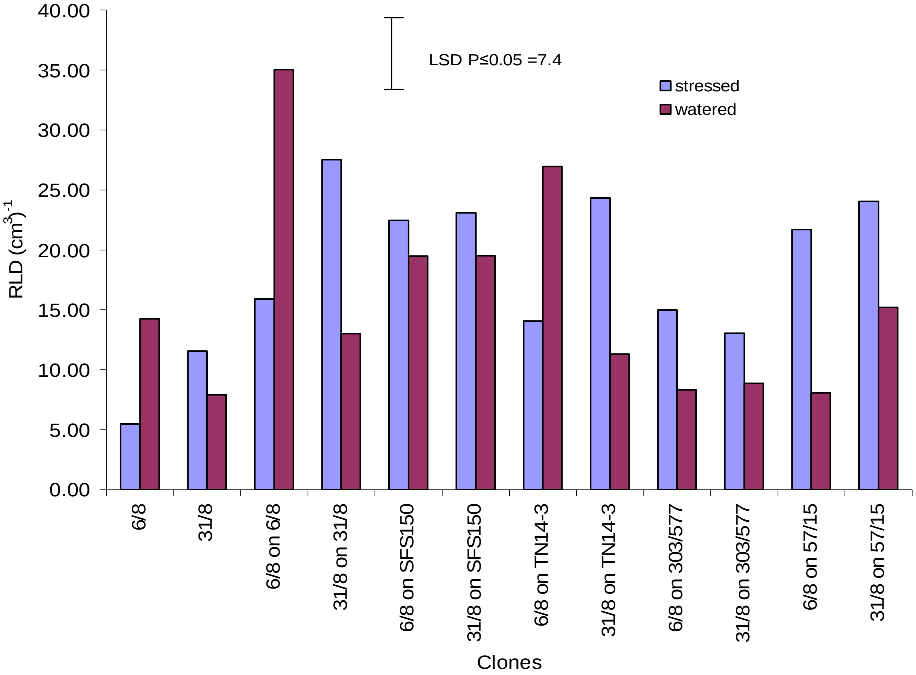

Figure 3.5. Root length density (RLD) of tea plants subjected to different watering regimes at Tea Research Foundation, Kericho – Kenya.

Low root mass in tea, would make the plants to succumb to drought as has been noted by other workers (Ng’etich and Stephens, 2001a). These differential responses in different rootstocks showing such characters were noted to have low dry matter partitioned to roots and low root length density. According to Hsiao and Acevedo (1974), soil drying does not inhibit growth of roots, but stimulates an increase in root: shoot ratio. Many woody plant species respond to water deficits by changing their assimilate partitioning in favour of the structures that are involved in water uptake and transport, and by an increase in water use efficiency (Li *et al.*, 2000; Zhang *et al.*, 1996), probably through expansive root system.

3.3.9. Basal stem diameter

Water stress significantly (P=0.003) reduced the basal stem diameter of the tea clones with the exception of both TRFK 31/8 on TRFK 303/577, 6/8 on TRFK 57/15 and TRFK 31/8 and 6/8 grafted on EPK TN 14-3 (Figure 3.6).

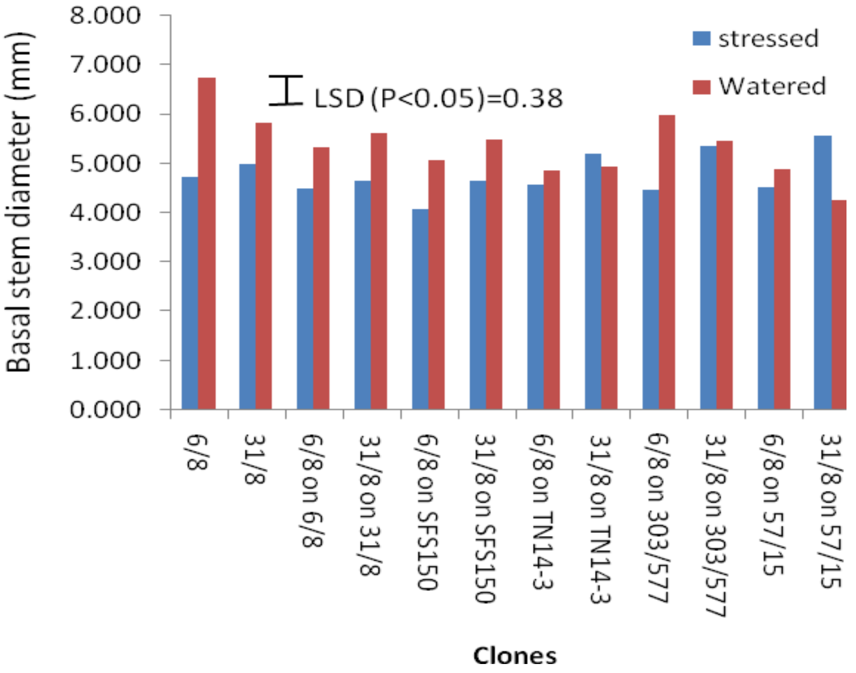

Figure 3.6. Basal stem diameter (mm) of tea plants subjected to different watering regimes at Tea Research Foundation, Kericho- Kenya.

Similar findings were reported for Fraser fir (Hollingsworth and Hain, 1994) and *Fagus sylvatica* (Leuschner *et al.*, 2001). For *Quercus petraea,* however, a clear stem growth / moisture relationship was not detected.

Trunk growth rate has been reported to be most sensitive to water deficits and could be a useful indicator for irrigation scheduling (Moriana and Fereres, 2002; Goldhamer and Fereres, 2001). Also in tea, Othieno (1982) and Ng'etich and Stephens (2001b) reported that, increasing soil temperatures with mulches had a direct effect on stem diameter and yield.

Fordham (1971) found a satisfactory linear relationship between total dry matter and stem diameter when the stem diameter was 20mm. The relationship between total dry mass and stem diameter varied with clones in this study. This indicates that basal stem diameter should not be used to determine clonal differences in dry matter production. This variation could have been due to differences in the proportion of dry matter partitioned to stems (Burgess, 1992b).

3.3.10. Dry matter partitioning

Watering regime influenced distribution of dry matter partitioned to various plant portions. Water stressed plants allocated more dry matter to the roots. Highest allocation to the roots was in scion clones TRFK 31/8 and TRFK6/8 grafted on TRFK 57/15 (75% and 78%), respectively. Allocation to stems was 20% and 20-35% to the leaves in all other clones (Figure 3.7a and b). The well-watered plants unlike the stressed allocated less than 60% dry matter to the roots, and 20% each to stems and leaves, respectively. The result suggests that when water is not limiting, tea plants would allocate less dry matter to roots. Grafting improved dm allocation to the roots of clone 6/8 than clone TRFK 31/8; for example, TRFK 6/8 on TRFK 6/8 allocated 38% to leaves, 40% to roots and

22% to leaves. This improvement suggests that the plant would be able to perform better due to its capability to explore a wider soil area. The predominant way that plants compensate for stress is through various shifts in C allocation, with shifts toward the roots occurring in response to stresses like drought and nutrients (Pell *et al.*, 1994). Tree root systems, play a major role in belowground carbon (C) dynamics. In addition to regulating major carbon fluxes; tree root systems potentially hold numerous controls over responses to a changing environment (Rasse *et al.*, 2001).

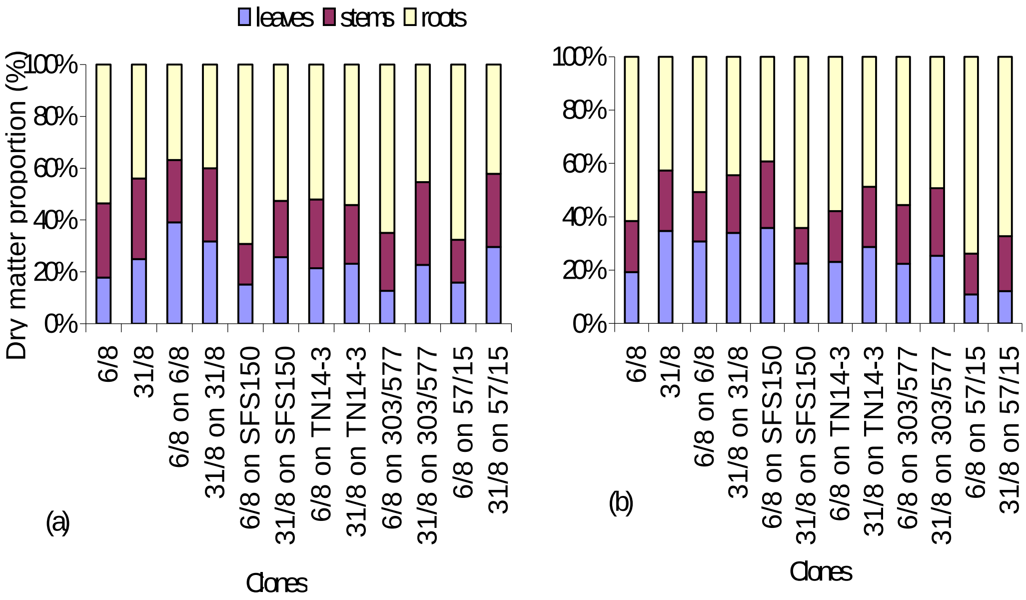

Figure 3.7. Proportion of dry matter partitioning of (a) well watered and (b) stressed tea plants at Tea Research Foundation, Kericho - Kenya

This implies that when water is not limiting, there is a varied allocation to roots as evidenced by this clone. This behaviour could be a modified adaptation to suit a particular environment. The tea soils have adequate capability to retain soil moisture and the importance of a deep soil profile, which retains moisture from rainfall and provides the plants with an adequate supply through the long dry period, is clearly displayed (Silberstein *et al.*, 2001).

Laurence *et al.*, (1994) found that species with high relative growth rates readily changed allocation in response to stress: e.g., radish and aspen allocated C to produce new leaves in response to ozone and allocated C to roots in response to water and nitrogen deficits. Ng'etich and Stephens (2001b) observed that root growth in warm areas was greater, and genotypic variation was evident and high yielding clones partitioned more dm to shoot growth. This implies that in the current scenario where high yielding clones are planted, there may be an eminent risk in case of insufficient water, as may be affected by global climate change.

The water stress treatment affected the shoot: root ratio such that apart from TRFK 6/8 on TRFCA SFS150, TRFK 31/8 on EPK TN 14-3, and TRFK 6/8 on TRFK 303/577, all other scion clone combinations had reduced shoot: root ratio while TRFK 6/8 on TRFK 6/8 had the highest ratio (Figure 3.8). Drought reduced shoot to root ratios in most varieties, confirming the results of Splunder *et al.*, (1996). Plant physiological strategies for coping with stress can also lead to shifts in shoot/root ratio; these include accelerated senescence of injured leaves in favour of production of new foliage, altered leaf area and rates of photosynthesis, and N and C allocation (Pell *et al.*, 1994). The mechanisms for induction of compensatory shifts are not known; but, it is likely that growth regulator changes lead to altered genetic expression of protein synthesis that modify metabolism within tissues or organelles (Pell *et al.*, 1994).

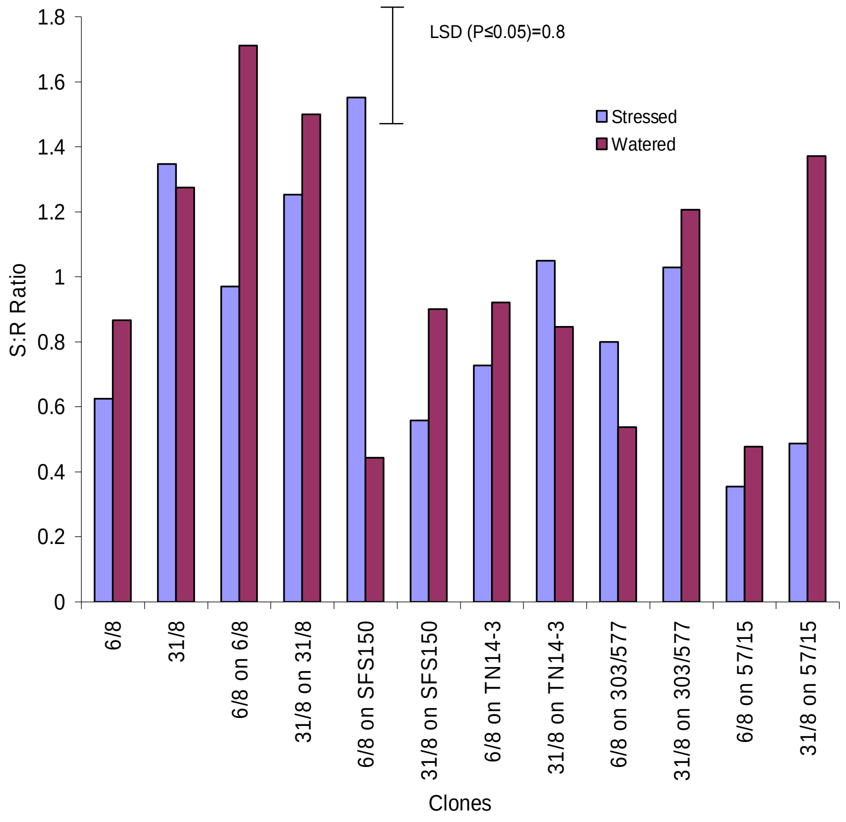

Figure 3.8. Effect of different watering regimes on shoot:root ratio of composite plants at Tea Research Foundation, Kericho- Kenya.

Low nitrogen supply and drought stress both reduce tree growth and increased dry matter allocation to roots (Ibrahim, *et al.*, 1997). Soil water deficits apparently stimulate fine-root growth during the dry periods, thus compensating for root biomass losses due to high root mortalities in this period (Leuschner *et al.*, 2001).

Highly significant correlation existed between the total dry matter partitioned and that of roots ($R^2=0.9075$, $P<0.001$) in the stressed treatments compared to the controls ($R^2=0.7950$, $P<0.001$) (Table 3.4). There was less partitioning of dry matter to roots in watered controls, implying that source –sink relationship takes prominence. Tea yields have been reported to be high during wet periods due to greater partitioning of dm to stems and leaves (Ng'etich and Stephens, 2001a; Burgess, 1992a).

Table 3.4. Correlation coefficients relating to clonal dry matter components of composite tea plants to overall dry matter with respect to water stress, at Tea Research Foundation, Kericho - Kenya

Stressed				
	Leaves	Stems	Roots	Total
Leaves				
Stems	0.4167			
Roots	0.4167	1***		
Total	0.7599***	0.9075***	0.9075***	

Watered				
	Leaves	Stems	Roots	Total
Leaves				
Stems	0.5528*			
Roots	-01318	0.3696		
Total	0.4477*	0.8006***	0.7950***	

At whole plant level, responses to water stress may increase root/shoot ratio, which results in better ability to extract water and nutrients for growth. However, if this improvement occurs at the expense of reducing the amount of biomass allocated to the harvested portion of the plant, then the economic yield will be much less. Also high root: shoot ratio increases the proportion of non-photosynthetic tissues of the plant, thus reducing the subsequent net carbon and energy gain.

4.0. CONCLUSION

In this study the results showed that there were significant variations in different attributes among clones and water regimes. Reduced water contents significantly ($P \leq 0.05$) reduced stomatal conductance (G_s) and transpiration (E), with very consistent diurnal trends. Clonal variations were also evident. Maintenance of low G_s by scion clones grafted on rootstocks EPK TN 14-3, TRFK 57/15 and TRFK 303/577 under water stress regimes, and high root length density of these rootstocks indicates some tolerance to low water contents. Leaf water potential was lower in water stressed plants than well watered ones. Water stress significantly ($P \leq 0.05$) affected dm partitioning, with more dm being allocated to roots under water stress conditions. Leaf area and stem diameter declined with water stress, while root length density and WUE increased. It is concluded that physiological parameters and dry matter allocation are influenced by water stress and rootstocks.

CHAPTER FOUR

4.0. PHYSIOLOGICAL RESPONSES OF COMPOSITE TEA TO REDUCING SOIL WATER CONTENTS

4.1. Introduction

Tea growth and productivity is mainly controlled by water availability. Water availability is a primary factor that controls plant growth processes in all plants (Hsiao, 1973; Kramer and Kozlowski, 1979). The physiological and morphological adjustment that takes place in a plant during a water deficit situation can have either short or long term responses. These responses and their influences affect general field performances, and are either genetically or environmentally controlled (Jones, 1980). Physiological processes include plant water tolerance, water absorption, sap, stomatal opening, cell enlargement, photosynthesis and carbohydrate and nitrogen metabolism. These processes significantly influence plant partitioning and economic yield.

Plants adapt to drought by improving their water uptake, reducing water loss or by maintaining large internal storage of water (Larcher, 1983). According to Kramer (1980), adaptations are heritable changes in plants that help them survive in their environments. These heritable potentiality and plant adaptations impose limits in various environments, and together with environmental factors such as precipitation, soil water storage, temperature and others that affect transpiration and other processes influence physiological processes.

Drought tolerance has been used to describe plants ability to cope with dry conditions (Kozlowski, 1976). Jones (1992) reported that drought tolerance refers to all the mechanisms that maintain the plant survival or productivity during drought. He listed a number of adaptations that have developed in plants to confer selective advantage to survival as follows: 1) Avoidance of plant water deficits 2) Tolerance to plant water deficits and 3) Efficiency mechanisms. Passioura (1982) described the two types of water use behaviour as prodigal use and conservative behaviour. Prodical use is where the plant is not in serious danger of desiccation despite fast water use. This is common where the water supply is interrupted by short dry spell. High stomatal conductance that decreases instantaneous water use efficiency is typical of this prodigal behaviour. They also have low diurnal water potential due to lack of efficient stomatal control (Ritchie and Hinckley, 1975).

Conservative behaviour is appropriate if annual dry periods prevail for long. This enables the plants to use the available water efficiently, and conserve reasonable soil water content for latter use (Passioura, 1982; Jones, 1980). Many woody plant species respond to water deficits by changing their assimilate partitioning in favour of the structures that are involved in water uptake and transport and by increasing their water use efficiency (Zhang *et al.*, 1996; Li *et al.*, 2000) probably through expansive root system. Tea is generally grown in Kenya as a rain fed perennial crop, at altitudes ranging from 1500 to 2700 m amsl. It requires a minimum annual rainfall of 1200mm although amounts ranging from 2500 - 3000mm are considered optimum (Carr, 1972; Callander and Woodhead 1981). Average rainfall in Kenya in most tea growing areas is about 1000mm/year. However, the distribution is bi-modal with long rains falling within March - May, and short rains in October - November.

Consequently, some tea growing areas experience drought periods of 2 - 3 months annually, and during this period, green leaf yields are significantly reduced. The existence of high transpiration rates from tea canopies causes significant soil water deficits, which decrease leaf expansion rates (Squire, 1990; Stephens and Carr, 1993). At times when the soil is wet the excess transpiration at midday could cause transient water deficits within the plant (Smith *et al.*, 1994). The physiological processes that are affected by soil water deficits need to be quantified.

According to Li *et al.,* (2004), physiological responses of black willow (*Salix nigra*) to soil moisture regimes were significantly affected by reduced soil moisture content. Decreased chlorophyll content and stomatal limitation led to reduced photosynthetic capacity. However, Smith *et al.,* (1993) reported that drought stress affected rate of photosynthesis in tea less than in other crops by 13% although Squire (1977) reported a value of 30% in a dry season compared to a wet season. Chlorophyll fluorescence can provide insights into the plants ability to tolerate environmental stresses, and the extent to which the stresses have damaged the photosynthetic apparatus (Maxwell and Johnson, 2000). Damages to photosynthetic apparatus may impose additional non-stomatal limitation to photosynthesis under water deficit (Souza *et al.*, 2004).

By quantifying the effects of soil water deficits on key physiological factors affecting growth and development, we attempt to predict the performance of these important clonal teas in the tea growing environments, which are often characterized by a short or long dry season. The studies on water relations and the responses of plants to drought are becoming important because the frequencies of water deficits are expected to increase in future due to global environmental changes (Chaves *et al.*, 2003).

In order to achieve the general goal of the study, the following objectives were set: a) to determine the diurnal and seasonal physiological responses among tea composite clones as affected by varying soil water contents, b) to explain the diurnal physiological behaviour of tea on the basis of their water relations, c) to compare the physiological behaviour and survival of *Camellia sinensis* composites under semi-controlled conditions.

4.2. MATERIALS AND METHODS

4.2.1. Plant material and experimental site

The study was conducted on two year old composite tea plants containing scion clones TRFK 303/577, TRFK 31/8; TRFK 6/8 and AHP S15/10 grafted on rootstocks; TRFCA SFS150; EPK TN 14-3; TRFK 8/112; TRFK 57/15, ungrafted and on own rootstock. The composites were developed in 2002, grafted in 2003, and allowed to undergo a nursery period of 18 months after which they were hardened according to Tea Research Foundation of Kenya recommendations (Anonymous, 2002). The potting medium consisted of a mixture of topsoil obtained from a virgin forest mixed with subsoil in a ratio of 25:75 (Anonymous, 2002). The topsoil contained 26% sand, 20% silt, and 64% clay with a bulk density of 0.8gcm^{-3} whereas the subsoil contained 10% sand, 17% silt, 73% clay and a bulk density of 1.0 gcm^{-3}. The medium had a mean moisture content of 65 –70%v/v.

The plants were potted in 4.8 litre polyethylene pots and placed in a rain out shelter constructed with poles. The roof was covered with an ultra violet treated 200-micron film clear polyethylene sheet (Sunselector AD – IR 504) which transmits 82% of photo synthetically active radiation (PAR), 65% of diffused light and with 88% thermicity. The rain out shelter structure was oriented at East - West aspect. The plants received adequate watering as they acclimatized before treatment applications. The experimental site was located at TRFK; altitude 2178 m amsl, Latitude 0° 22' S, Longitude 35° 21' E.

4.2.2. Measurements

Plant and soil water status

The plants were arranged in a complete randomized block design, with main plots being four soil moisture contents of field capacity (FC), 75% FC, 50% FC, 25% FC and 12.5% FC equivalent to TDR values of 40%v/v, 30%v/v, 20%v/v, and 12.5%v/v) (+/- 2%) and subplots being six rootstocks; replicated three times. The plants were all irrigated to field capacity, and allowed to dry to respective soil moisture contents within a period of one to three weeks. Their respective soil moisture contents were determined and maintained at their desired levels by adding water and the soil moisture was determined by use of a Time Domain Reflectometer (TDR) soil moisture meter (TRIME-FM-2, Eijkelkamp Agrisearch Equipment, The Netherlands) with the 50 mm 2- rod probe. Shoot water potential was measured by cutting shoots from each treatment and measuring it three times a day, between 09.00- 11.00 hrs, 12.00-

13.00 hrs and 14.00- 16.00 hrs using a pressure chamber (PMS Instruments, Co., Corvallis, OR., USA).

Relative water content

Relative water content of fully developed young tea leaves was calculated from the same shoots as for shoot water potential:

$$RWC (\%) = (FW-DW)/SFW-DW)*100 \qquad \text{Equation 4.1}$$

where FW is fresh weight, DW is dry weight, and SFW is saturated fresh weight of leaves after re-hydrating samples for 24 hours (Turner, 1981).

Determination of chlorophyll and SPAD calibration

Determination of gas exchange parameters and comparison of the data obtained may provide mechanistic information regarding the causes of differences species in photosynthetic activity and growth rates. A possible source of interspecific variation in photosynthetic activity may be differences in the constitution of the photosynthetic apparatus, particularly chlorophyll content. It was therefore considered essential to relate water stress parameters to concurrent estimates of leaf greenness in the form of SPAD readings in order to establish the relationship between the variables in all the tested clones.

SPAD measurements have commonly been made and the link between these and chlorophyll content is species dependent (Marquard and Tipton, 1987) and therefore specific calibration is necessary.

SPAD measurements

Chlorophyll content was determined non-destructively using N-tester (Hydro International, Hydro Agri Deutschland GmbH, Hanninghof 35, D-48249 Dulmen). The third leaf was used to quantify SPAD values in each clone under the four water treatments as outlined by Wanyoko *et al*., (2000). Determination was done once a day for two days in the month of November 2004. The measurements provide numerical values related to chlorophyll content (Lawson, *et al*., 2001). Close linear correlation between SPAD values and extractable chlorophyll content have been reported for a wide range of plant species (Marquard and Tipton, 1987; Finnan, *et al.,* 1998).

SPAD calibration

In order to derive the estimates of chlorophyll content from SPAD values, it was necessary to construct a calibration curve. Leaves from 18 month old composite tea were sampled to represent a range of colour extending one mature leaf and an immature bud to three mature leaves and an immature bud. Three SPAD measurements were made for the selected leaves. The leaves were immediately sealed in labelled polyethylene bags and either stored in a refrigerator or analysed immediately.

Chlorophyll determination was done by spectrophotometric analysis following extraction in acetone (Leegood, 1993). One gram fresh weight sub-sample from the sampled leaves were ground with a pestle using a mortar and pure quartz and 40ml of 80% acetone as the extraction solvent. The resulting suspension was diluted to 100ml using 80% acetone. Absorbance was read and recorded using a 10ml aliquot placed in a quartz cuvette using a digital grating spectrophotometer (Cecil CE: 393) set at 470nm, 653nm and 666nm using methanol as a blank.

Chlorophyll a, b, and total chlorophyll contents (µgg^{-1} tissue) were determined using Taylors (1994) equations as follows:

Chlorophyll $_a = 15.65A_{666} - 7.34A_{653}$ Equation 4.2

Chlorophyll $_b = 27.05A_{653} - 11.21A_{666}$ Equation 4.3

Total chlorophyll = Chlorophyll $_a$ + Chlorophyll $_b$ Equation 4.4

Where A represents absorbance.

The values obtained were used to construct a calibration curve relating the SPAD values and their chlorophyll content. This calibration was used to convert SPAD values to the corresponding chlorophyll contents.

4.2.3. Data analysis

The recorded data were subjected to statistical analyses using an M-STAT-C package, and where the means were significant at $P \leq 0.05$, they were separated using Least Significant Differences (LSD).

4.3. RESULTS AND DISCUSSIONS

4.3.1. Shoot water potential

The shoot water potential varied between varieties and with the prevailing soil moisture contents. The reduced soil moisture content reduced the mean shoot water potential of all the plants (Figure 4.1). There were diurnal differences in different soil moisture regimes. Generally, the morning water potential values were more negative (lower) under low soil moisture levels than they were in high moisture regimes. During periods of water stress, midday depression of leaf water potential occurred after the morning maximum (Correia *et al.,* 1995; Grelle *et al.*, 1999). Other factors such as radiation and vapour pressure deficit are the most important factors in determining shoot water potential when soil water was not limiting.

Water availability is a primary factor that controls plant growth processes (Hsiao, 1973; Kramer and Kozlowski, 1979) and in case of drought plants adapt by improving their water uptake, reducing water loss or by maintaining large internal storage of water (Larcher, 1983). Several indicators can be used to estimate responses to water stress, the most widely available of which is leaf water potential as measured with a pressure chamber.

The minimum values of shoot water potential were observed at solar noon and the differences in values observed at this time between the water treatments were as high as 0.1 MPa. Similar results were reported by Olufayo *et al.* (1993). Moreshet *et al.* (1996) observed that afternoon leaf water potential values were below those of the morning, suggesting a lowering of the soil water potential near the roots. Vadell *et al.* (1995) found that the instantaneous water-use efficiency was much higher in drought stressed plants during the early morning and late evening while in the middle of the day water use efficiency values were similar to those of irrigated plants. Coupled with the morphological changes such as reduced leaf area, as a result of acclimation to progressive drought, there were modifications of diurnal patterns. It is suggested that these modifications significantly improved water-use efficiency, especially at periods with high humidity such as the mornings.

Leaf water potential was related to soil volumetric water content. Midday leaf water potential values presented very large diurnal variations particularly the low moisture levels which had values ranging from 1.43 MPa for 12.5%v/v and 0.442 MPa for 40%v/v moisture contents. The low leaf water potentials observed for some composites during low soil water content suggest that this parameter may be related to the evaporative demand (Correia *et al.*, 1995).

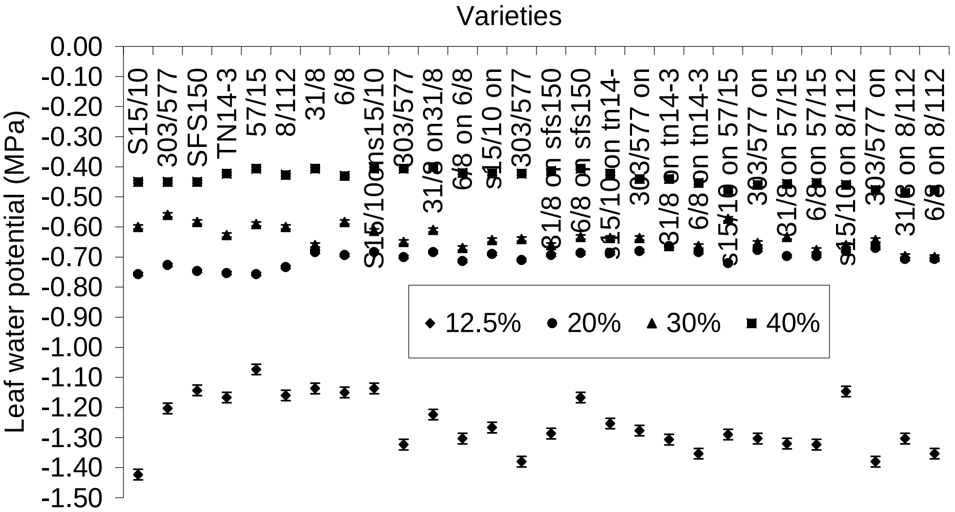

Figure 4.1. Mean shoot water potential (MPa) of all composite tea grown under four different soil moisture content (%v/v) in 2005 at Kericho. Vertical bars indicate standard error

There were significant ($P \leq 0.05$) varietal differences in leaf water potential in the low soil moisture regimes as compared to high soil water contents. S15/10 was the worst affected by the low soil water content as its leaf water potential was close to wilting point of –1.5 MPa, thus indicating that its survival is threatened under low soil water content (Figure 4.1).

There was a high significant relationship (R^2=0.9619, $P<0.001$) between varietal soil moisture content and the reduced water potential implying that as the water content in the soil reduces, the plant has to exert more force to extract the amount of available water. All the scions had similar low water potentials in the morning which increased in mid afternoon and declined in late afternoon. What this shows is that although varietal differences occurred, the most drought effect was at midday. Munne Bosch and Alegre (2000) found that summer drought caused a marked decrease in water potential of Melissa leaves to ca. 35% and -3 MPa indicating severe stress. Lower leaf water potential (more negative) indicates a steeper water-potential gradient between the leaves and soil and thus probably faster water-uptake on a daily basis. It is proposed that the diverse behaviour in diurnal leaf water potential among tea varieties can be attributed to differences in stomatal conductance. Some varieties maintain higher leaf water potential by keeping stomata less open than others similar to observations in some *Eucalyptus* species (Tuomela, 1997). Among the atmospheric factors observed, the vapour pressure deficit (VPD) proved to be the most relevant variable in predicting diurnal changes. The leaf water status was more sensitive to VPD at sufficient soil water storage.

The rootstocks, soil moisture and their interactions had significant ($P \leq 0.05$) diurnal leaf water potentials. There was an increase in leaf water potential with a decrease in soil moisture content. The high moisture content of 40(%v/v) had low values, followed by 30, 20 and 12.5 (% v/v) with 0.439; 0.635; 0.701 and 1.315 MPa (Figure 4.2 a-d).

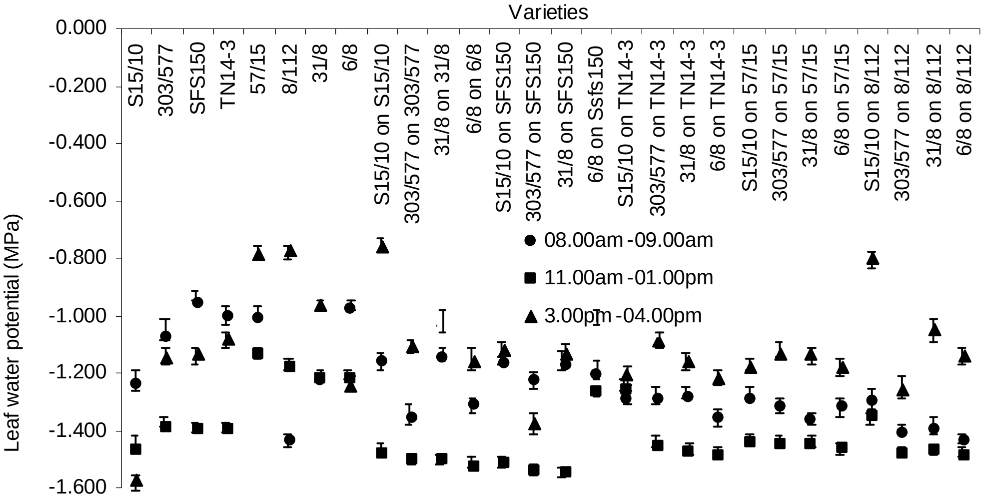

Figure 4.2a. Changes in leaf water potential (MPa) values composite tea in response to 12.5 (%v/v) soil moisture content, at Tea Research Foundation, Kericho - Kenya. Vertical bars indicate standard error

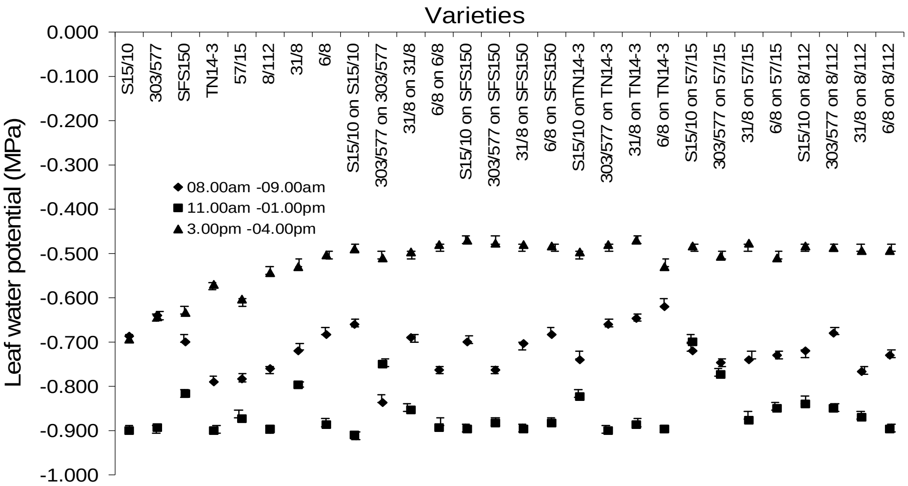

Figure 4.2b. Changes in leaf water potential (MPa) values of composite tea in response to 20% (v/v) soil moisture content at Tea Research Foundation, Kericho - Kenya. Vertical bars indicate standard error

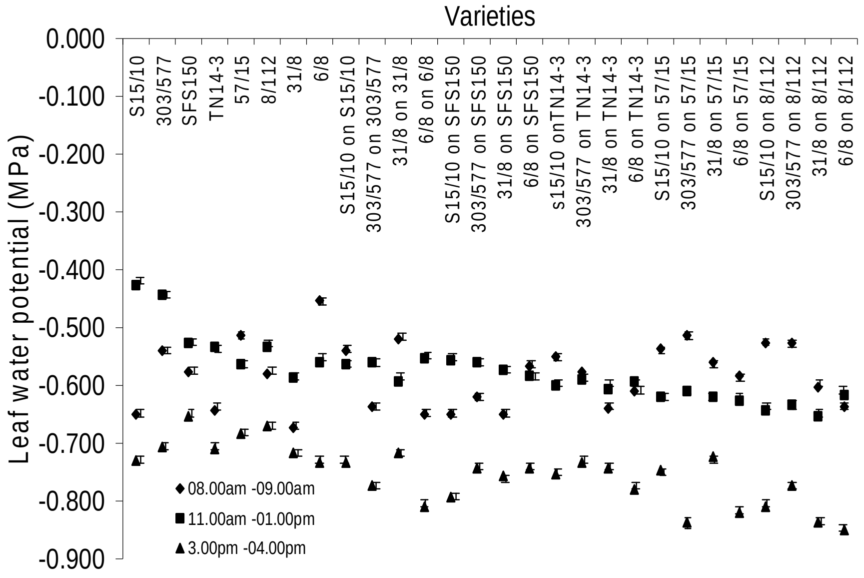

Figure 4.2c. Changes in leaf water potential (MPa) values of composite tea in response to 30% (v/v) soil moisture content at Tea Reasearch Foundation, Kericho- Kenya. Vertical bars indicate standard error

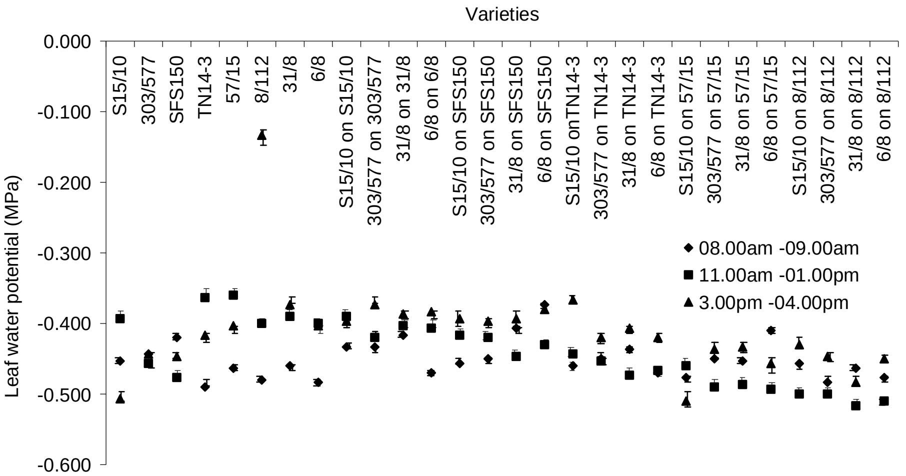

Figure 4.2d. Changes in leaf water potential (MPa) values of composite tea in response to 40% (v/v) soil moisture content at Tea Research Foundation, Kericho - Kenya. Vertical bars indicate standard error

The varieties had significantly lower water potentials in the morning hours and increased at midday, and further reduced between 15.00 - 16.00hrs to slightly lower values than the morning values (Figure 4.3). From these results, the diurnal changes in leaf water potential begin at 11.00hours and declines to lowest levels by 13.00 hours and again increases from 14.00 hours. The reductions in the leaf water potential seem to decline much earlier from 15.00hrs regardless of the soil water content, thus showing that it is influenced by temperatures which are higher from around midday and persist till 16.00hrs when it declines. The leaf water potential was lower throughout the day for well-watered plants. However, mid-morning and mid-afternoon values of leaf water potential were slightly lower for stressed plants (Eamus *et al.*, 1995). These values depended on the soil moisture level. The ascending order was 40%>30%>20%and 12.5%, respectively.

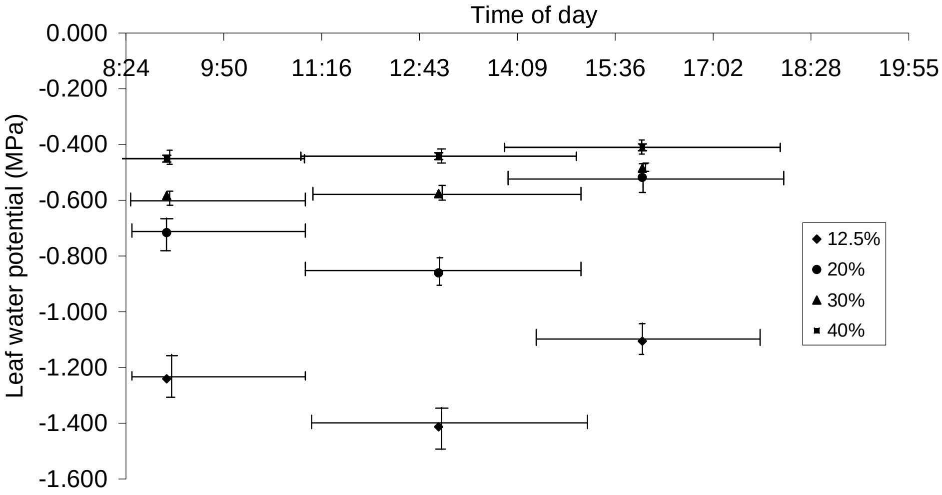

Figure 4.3. Changes in mean leaf water potential (MPa) of composite tea within a day (Nov. 2004) in response to four varying soil moisture content (%v/v) at Tea Research Foundation, Kericho- Kenya. Vertical and horizontal bars indicate standard error

The rootstocks had significantly ($P \leq 0.05$) different mean water potentials as shown in Table 4.1. Both own rootstocks and TRFCA SFS150 were similar and better but different from TRFK 57/15 and 8/112, which were intermediate. The ungrafted clones had significantly lower water potential, thus showing that grafting the selected scions assisted in improving and maintaining low water potentials. The soil water contents had significant influence on the mean water potentials. The lowest water potentials were achieved at high soil moisture levels with a mean of -0.439 MPa, whereas on the extreme end, low water potential of -1.315 MPa was recorded. These low water potentials were close to permanent wilting point.

Generally, the mean water potential significantly (R^2=0.99, P<0.001) increased with a decline in soil moisture content (Figure 4.4). The results suggests that the composite tea plants that are subjected to low soil moisture levels below 20%v/v are likely to suffer moisture stress depending on the varieties, and possibly the duration of exposure. These results indicate that, the critical soil moisture content lies between 20 and 30% v/v for the tested clones.

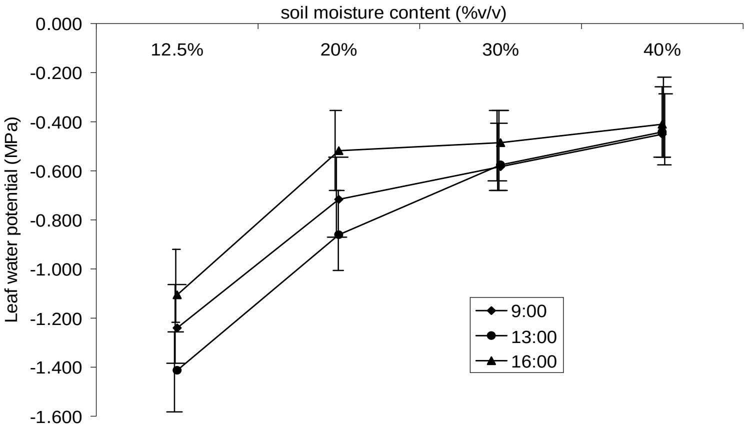

Figure 4.4. Relationship between diurnal changes of mean water potential (MPa) and mean soil water content (%v/v) by composite tea plants at Tea Research Foundation, Kericho- Kenya. Vertical bars indicate standard error

Table 4.1. Leaf water potential (MPa) values of rootstocks subjected to four varying soil moisture content (%v/v) at Tea Research Foundation, Kericho

	Rootstocks						
Soil moisture (%v/v)	Ungrafted	Own	SFS 150	TN 14-3	57/15	8/112	Mean
40	0.434	0.49	0.417	0.439	0.462	0.475	0.439
30	0.572	0.636	0.645	0.650	0.634	0.675	0.635
20	0.761	0.695	0.695	0.679	0.698	0.694	0.704
12.5	1.434	1.247	1.275	1.298	1.309	1.328	1.315
Mean	0.800	0.747	0.758	0.767	0.776	0.793	

C V (%) 2.15
LSD (P≤0.05) Rootstocks 0.0137
Soil Moisture 0.0112
Interaction 0.0274

Although all the rootstock had a variable response on the scion clones, they were within the same range of water potential (0.755 - 0.7770 MPa). AHP S15/10 had an outstanding performance on all the rootstocks, followed by TRFK 6/8 (Table 4.2).

Table 4.2. Mean leaf water potential (MPa) values of scion clones grafted on different rootstocks at Tea Research Foundation, Kericho- Kenya

	Rootstocks						
Scions	Ungrafted	Own	SFS 150	TN 14-3	57/15	8/112	Mean
S15/10	0.808	0.710	0.756	0.750	0.765	0.737	0.755
303/577	0.735	0.770	0.788	0.759	0.774	0.793	0.770
6/8	0.722	0.729	0.763	0.768	0.777	0.799	0.760
31/8	0.715	0.777	0.724	0.789	0.788	0.809	0.761
Mean	0.745	0.747	0.758	0.767	0.776	0.785	

C V (%) 2.25
LSD (P≤0.05) Rootstocks 0.0141
Clones 0.0115
Interaction 0.028

Considering all the rootstocks potential to withstand water stress assed on their own, the pooled soil moisture levels revealed that the order in terms of varietal performance was; TRFK 57/15>8/112>TRFCA SFS 150>EPK TN14-3 with values of 0.706, 0.730, 0.731 >0.743 MPa, respectively (Table 4.3).

Table 4.3. Changes of leaf water potential (MPa) values of rootstocks at four different soil moisture (%v/v) contents during the day at three different periods in a day at Tea Research Foundation, Kericho- Kenya

Soil moisture (%v/v)	Rootstocks (a) 8.00-9.00am						
	ungrafted	Own	SFS150	TN 14-3	57/15	8/112	Mean
40	0.452	0.472	0.439	0.422	0.454	0.448	0.448
30	0.603	0.556	0.587	0.622	0.594	0.549	0.585
20	0.704	0.738	0.738	0.713	0.667	0.734	0.715
12.5	1.069	1.161	1.246	1.193	1.307	1.324	1.217
Mean	0.707	0.731	0.752	0.737	0.756	0.764	
CV (%) 3.88							
LSD (P≤0.05) Rootstocks (R) 0.024; Soil moisture (SM) 0.019; (RXSM) 0.047							
	(b) 11.00-01.00pm						
40	0.423	0.388	0.405	0.429	0.459	0.483	0.431
30	0.483	0.561	0.568	0.569	0.598	0.620	0.566
20	0.878	0.864	0.852	0.890	0.877	0.800	0.860
12.5	1.414	1.188	1.503	1.468	1.419	1.451	1.407
Mean	0.799	0.750	0.832	0.839	0.838	0.839	
CV (%) 2.87							
LSD (P≤ 0.05) Rootstocks (R) 0.019; Soil moisture (SM) 0.016; (RXSM) 0.039							
	(c) 3.00-4.00pm						
40	0.454	0.329	0.385	0.391	0.404	0.459	0.404
30	0.700	0.701	0.759	0.760	0.753	0.782	0.742
20	0.635	0.5.45	0.494	0.478	0.494	0.494	0.524
12.5	1.234	0.944	1.011	1.164	1.167	1.158	1.113
Mean	0.756	0.630	0.662	0.698	0.704	0.723	
CV (%) 6.97							
LSD (P≤ 0.05) Rootstocks (R) 0.040; Soil moisture (SM) 0.033; (RXSM) 0.080							

These results show that rootstocks with the lowest water potential at different soil moisture levels are able to withstand moisture stress better than others.

The grafted combinations with all scion across all soil moisture levels indicated the following order; own rootstocks>TRFCA SFS 150>EPK TN 14-3 >TRFK 57/15>TRFK8/112 with water potential values of 0.746, 0.758, 0.766, 0.7776, and 0.784 MPa, respectively. Conclusively, other varietal attributes held constant, it would be prudent to plant clone 57/15, 8/112, SFS150 and TN 14-3 as straight (ungrafted) clones. However, if other reasons are considered, then, own rootstocks, followed by SFS 150, TN 14-3 and 57/15 could be better alternatives.

Considering water potential in isolation, grafting would not be of benefit to tea plants because the results indicate that soil moisture content has an upper hand in influencing the water potential. The influence of adequate water application in tea gives a response that was closely correlated with the severity of the stress (Romero *et al.*, 2004). David *et al.* (1997) observed that as soil moisture deficit increased, the daily maximum stomatal conductance decreased and showed that, on a seasonal basis, stomatal conductance and daily transpiration were mainly related to predawn leaf water potential and, thus, to soil moisture content.

The environment of plants native habitat also plays a role in influencing traits that are associated to water stress tolerance e.g. low relative water content (RWC) (Zine *et al.*, 1994). These results suggest that the plant origin and genotype is important in plant water relations and therefore support the view that their parental rootstock - scion characteristics are important and hinges on hybridization and selection criteria that may modify the performance of composite plants.

While monitoring tissue water relations of potted ponderosa pine (*Pinus ponderosa*) and green leaf manzanita (*Arctostaphylos patula*) seedlings subjected to three levels of soil moisture availability, Anderson and Helms (1994) observed that moisture regime had little effect on the osmotic potentials except for apoplasmic water content, which increased, at moderate and high stress levels for both species. The results suggested that osmotic adjustment occurred, at least partially, as a result of other factors rather than moisture availability. Differences between plants may also have an influence in water relations (Wilson and Clark, 1998). Leaf water potential was related to soil volumetric water content. Midday leaf water potential values presented very large diurnal variations and very low values independent of treatments. The low leaf water potentials observed for some composites during low soil water content suggest that this parameter may be related to the evaporative demand and concurs with the findings of Correia *et al.* (1995). The scion varieties showed variable water potential values and had the ascending order of TRFK 6/8>31/8>303/577>AHP S15/10 (Table 4.4).

Similarly, the differences in results reported herein from composite treatments may actually be attributed to the genotypes constituting the composites. For many annual crops, the predawn leaf water potential is, assumed to represent the mean soil water potential next to the roots and is closely correlated to the relative transpiration rate, except when soil water content is markedly heterogeneous (Ameglio *et al.*, 1999). It is suggested that in addition to water stress avoidance due to deep root systems, some mechanisms of water stress tolerance may operate among tea varieties.

Table 4.4. Changes of diurnal water potential (MPa) of scions grafted on different rootstocks determined at three different hours of the day at Tea Research Foundation, Kericho- Kenya

Scions	Rootstocks (a) 8.00-9.00am						
	ungrafted	Own	SFS150	TN 14-3	57/15	8/112	Mean
S 15/10	0.757	0.692	0.699	0.743	0.761	0.756	0.735
303/577	0.675	0.814	0.816	0.764	0.745	0.758	0.762
6/8	0.664	0.769	0.694	0.734	0.753	0.780	0.732
31/8	0.732	0.650	0.799	0.708	0.765	0.760	0.736
Mean	0.707	0.731	0.752	0.737	0.756	0.764	
CV (%) 2.06							
LSD (P=0.001) Rootstocks (R) 0.022; Scions (S) 0.018; (RXS) 0.044							
	(b) 11.00-01.00pm						
S15/10	0.797	0.734	0.836	0.846	0.781	0.805	0.799
303/577	0.797	0.753	0.808	0.852	0.849	0.831	0.815
6/8	0.804	0.748	0.838	0.867	0.861	0.859	0.829
31/8	0.799	0.766	0.845	0.790	0.862	0.859	0.820
Mean	0.799	0.750	0.832	0.839	0.838	0.838	
CV (%) 3.00							
LSD (P≤ 0.05) Rootstocks (R) 0.020; Scions (S) 0.017; (RXS) 0.040							
	c) 3.00-4.00pm)						
S15/10	0.876	0.620	0.596	0.695	0.705	0.730	0.704
303/577	0.735	0.530	0.691	0.748	0.680	0.729	0.686
6/8	0.717	0.647	0.654	0.691	0.695	0.692	0.683
31/8	0.694	0.722	0.708	0.657	0.737	0.743	0.710
Mean	0.756	0.630	0.662	0.698	0.704	0.723	
CV (%) 6.46							
LSD (P≤ 0.05) Rootstocks (R) 0.037; Scions (S) NS; (RXS) 0.074							

Diurnal variability's in water status parameters were large for some clones that regulated water loss conservatively with only limited reductions in leaf water potential in high or moderately low water contents but large decreases in low water contents. In dry environments, tea water relations should be studied for at least three or four seasons to account for large inter seasonal variability's in water status parameters (Backes and Leuschner, 2000).

4.3.2. Relative water content

The rootstocks had mean relative water content (RWC) of 82%, thus no effect on the leaf relative water contents, but the soil moisture contents had a significant ($P<0.001$) effect. The RWC reduced with reduction in the soil water content. The treatments subjected to the high soil water content of 40%v/v had the highest RWC of 92.7% and the least being 12.5% v/v having RWC of 74.9%. The scion varieties also had similar RWC ranging from 82.0 - 82.5% but were not significant. There were significant rootstocks and scion interactions (Table 4.5).

The variety with the highest RWC was TRFK 6/8 on 57/15 and TRFK 6/8 on TRFCA SFS150 with RWC of 86.1% while the least was AHP S15/10 on TRFK 57/15 with RWC of 78.9% (Figure 6.7). Gadallah (2000) reported a significantly ($P\leq0.05$) reduced RWC in soybeans but Medina *at al.* (1998) found no decline in RWC, in potted 2-year-old Valencia orange trees grafted on Rangpur lime (Citrus *limonia*) or *Poncirus trifoliata* rootstock, but there were varietal responses.

Table 4.5. Relative water contents (%) values of rootstocks subjected to different soil moisture (%v/v), and scions grafted on different rootstocks

Soil water content (%v/v)	Rootstocks						
	ungrafted	Own	SFS150	TN 14-3	57/15	8/112	Means
40	87.71	94.75	93.96	93.45	92.91	93.19	92.66
30	88.17	87.56	86.06	85.20	85.51	84.94	86.24
20	78.33	74.08	77.42	75.42	79.13	75.50	76.65
12.5	76.34	76.36	73.71	74.84	74.23	74.17	74.94
Means	82.64	83.19	82.79	82.23	82.94	81.95	
CV (%) 3.34 LSD (P≤0.05) Rootstocks (R) NS; Soil Moisture (SM) 1.86; (RXS) NS							
Scions							
S15/10	81.08	85.50	84.45	81.87	78.95	80.40	82.04
303/577	81.71	84.41	80.86	84.64	83.26	79.99	83.02
6/8	82.15	80.57	86.12	80.59	86.12	82.57	83.02
31/8	85.61	82.28	79.72	81.81	83.44	84.84	82.95
Mean	82.64	83.19	82.79	82.22	82.94	81.95	
CV (%) 2.77 LSD (P≤ 0.05) Rootstocks (R) NS; Scions (S) NS; (RXS) 3.78							

The relative water content was highest for clone TRFK 6/8 at soil moisture content of 12.5% and the least was AHP S15/10. Also, AHP S15/10 had the lowest RWC at soil moisture content of 40% (Figure 4.5). In wheat, water stress was found to reduce relative water content (Tambussi *et al.*, 2002). The mean RWC of all clones decreased linearly (R^2=0.9915, P<0.001) with decrease in the soil moisture contents. Varietal maintenance of higher RWC at a specific soil moisture contents suggests that they are resistant to desiccation and confers drought resistance. From the data, it is evident that AHP S15/10 is more droughts susceptible than all the tested scion materials (Figure 4.6). However, grafting on most rootstocks except TRFK 57/15 improved their RWC.

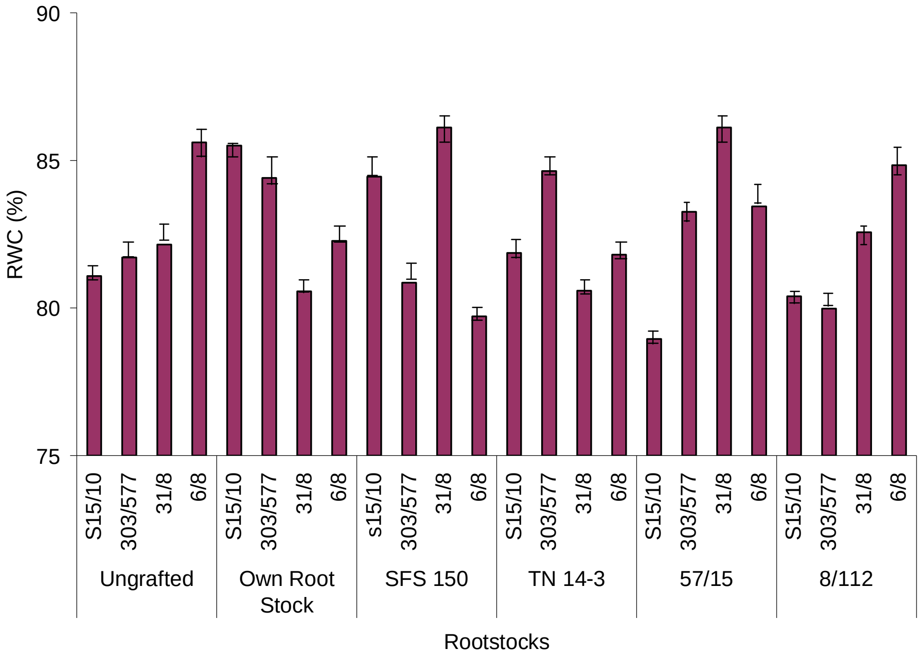

Figure 4.5. Mean relative water content (%) of scion clones grafted to six different rootstocks at Tea Reasearch Foundation, Kericho- Kenya.Vertical bars indicate standard error

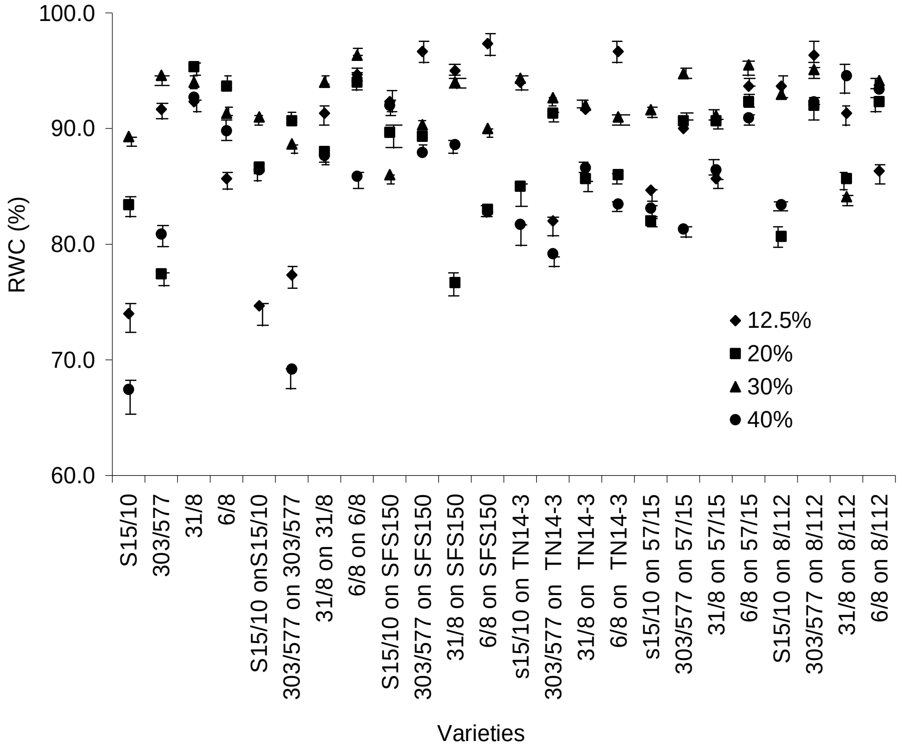

Figure 4.6. Relative water content (%) of composite tea plants subjected to four different soil moisture content (%v/v) at Tea Research Foundation, Kericho-Kenya. Vertical bars indicate standard error

4.3.3. Chlorophyll

Drought induces several responses in plants including leaf senescence, which plays a major role in the survival of several species. Drought-induced leaf senescence contributes to nutrient remobilization during stress, thus allowing the rest of the plant (i.e. the youngest leaves, fruits or flowers) to benefit from the nutrients accumulated during the life span of the leaf. The SPAD measurements were made for the leaves from the treatment plants and the values obtained were correlated with absolute values for chlorophyll contents for the same leaves expressed on a fresh weight basis after solvent extraction. The results were all analyzed in order to establish the best functional relationship; between the SPAD and the chlorophyll contents. Both linear and polynomial relationships were tested. There were similar polynomial and linear relationships for chlorophyll a with $R^2=0.511$ ($P<0.001$) whereas for chlorophyll b, and total chlorophyll contents only polynomial was slightly better than the linear relationship (Figure 4.7).

The concentration levels of chlorophyll a were found to be twice as much as chlorophyll b and the total contents in all clones was ranging between 4 – 6 μgg^{-1} of tissue although the concentration varied with the varieties tested and decreased with increase in soil moisture content. The decrease could either be attributed to high leaching of soil nitrogen, by the high soil water in high soil water treatments or reduced leaf area in low moisture treatments, which makes chlorophylls, be concentrated in a small leaf area. Sutinen *et al.* (2000) studied physiological condition of yellow and visually green Scots pine (*Pinus sylvestris*) needles and found that the recovery of green colour was accompanied by an increase in the chlorophyll a and b content. From our results, it showed that the yellowing of leaf colour of tea is an indication of a deeper state of photo-inhibition and slower deacclimation and is not directly related to desiccation stress. Leaf yellowing (i.e. chlorophyll degradation) and specific changes in cell ultrastructure (e.g. chromatin condensation, thylakoid swelling, plastoglobuli accumulation), metabolism (e.g. protein degradation, lipid peroxidation) and gene expression occur during leaf senescence in drought-stressed plants (Munne Bosch and Alegre, 2004).

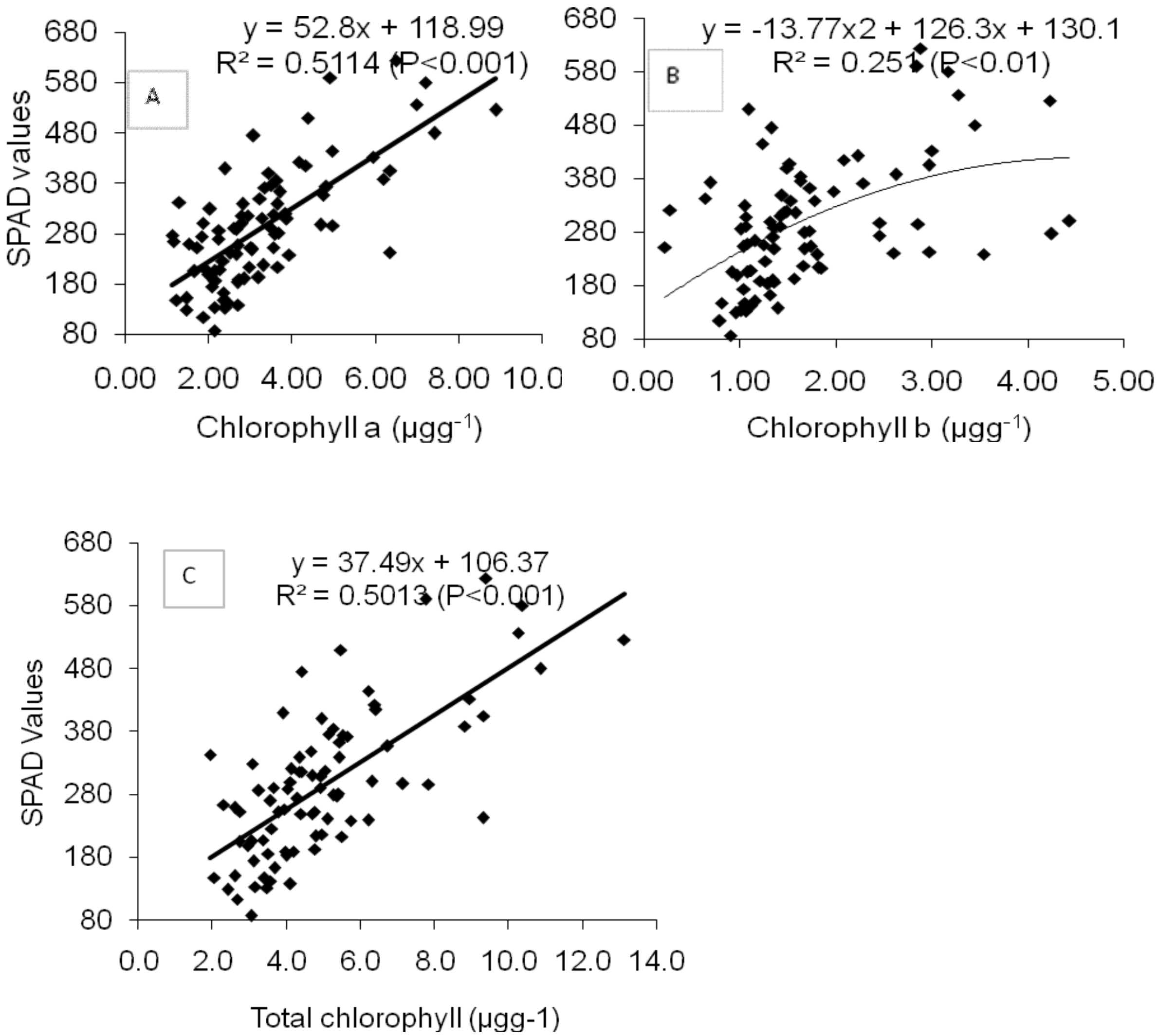

Figure 4.7. Relationship between SPAD values and chlorophyll a (A), chlorophyll b (B) and total chlorophyll (C) contents for leaves of grafted tea scions and rootstocks under four different soil moisture levels (%v/v) at Tea Research Foundation, Kericho.

Gadallah (2000) reported that, soybean (*Glycine max*) plants under control conditions with increasing soil drying progressively retarded shoot and root growth (length and dry mass production), reduced RWC and decreased the contents of chlorophyll and lowered osmotic water potential of shoots and roots (osmotic adjustment). Similarly, Munne Bosch and Alegre (2000) reported that Chlorophyll and carotenoid (particularly beta -carotene) levels decreased progressively with drought. Li *et al.* (2004) found that decreased chlorophyll content and stomatal limitation lead to reduced photosynthetic capacity in a low soil moisture regime. The decreased water content may also have led to damages to photosynthetic apparatus thus imposing additional non-stomatal limitation to photosynthesis under water deficit (Souza *et al.*, 2004).

Further analyses were done to find the contribution of individual rootstock and scions. Results showed that all the rootstocks did not significantly affect the total chlorophyll contents. For the scions, AHP S 15/10 had low chlorophyll contents, whereas TRFK 303/577 had high levels. Both soil moisture levels and clones had no significant interactions indicating that clonal differences do not contribute to significant chlorophylls. However, there were notable chlorophyll contents in ungrafted and SFS 150 at 40% and 30% soil moisture contents (Table 4.6). Both clones TRFK 303/577 and TRFK 6/8 naturally have lighter colours, and this could have contributed to the high chlorophyll contents noted in this experiment.

Table 4.6. Total chlorophyll contents (µgg^{-1} of tissue) in rootstock tea clones and scions subjected to four varying levels of soil moisture stress (%v/v), at Tea Research Foundation, Kericho- Kenya

	Rootstocks						
Soil moisture (%v/v)	ungrafted	Own	SFS150	TN 14-3	57/15	8/112	Mean
40	9.78	12.84	12.77	12.66	12.00	12.00	12.34
30	14.71	12.58	9.73	12.02	12.31	12.02	12.23
20	13.14	17.51	15.70	16.85	16.10	14.95	15.70
12.5	16.47	17.48	16.79	17.27	15.83	15.03	16.47
Mean	13.51	15.11	13.75	14.71	14.31	13.75	
CV (%) 8.62							
LSD (P≤0.05) Rootstocks (R) NS; Soil moisture (SM) 1.85; (RXSM) NS							
Scions							
S15/10	12.15	15.33	12.26	12.61	12.13	12.50	12.82
303/577	15.51	13.46	12.88	16.66	16.18	15.30	14.98
6/8	13.17	15.09	14.37	15.27	16.10	14.12	14.69
31/8	13.27	16.55	15.49	14.26	12.82	13.11	14.26
Mean	13.54	15.11	13.75	14.69	14.31	13.75	
CV (%) 12.50							
LSD (P≤ 0.05) Rootstocks (R) NS; Scions (S) 1.40; (RxS) NS							

In this study, SPAD values and the corresponding total chlorophyll contents were higher in water deficit treatments. The high chlorophyll content under water stress here confirms similar results reported for other crops such as cotton (Pettigrew, 2004) and *Acacia saligna* (Nativ, 1999). Water stress condition had a 19% greater leaf chlorophyll content which contributed to their higher CO_2 exchange rates during the morning and thus supports the results of high WUE earlier reported in this study. Cabrera *et al.* (1995) found that drought stress in barley plants reduced chlorophyll contents and caused higher chlorosis. Chlorosis arises from drought-stress and it is concluded that water-stress caused by drought would probably affect the development due to the effects on the chemical composition of the plant.

Li *et al.* (2004) reported a decreased chlorophyll content and stomatal limitation that lead to reduced photosynthetic capacity of black willow (*Salix nigra*) in different soil moisture regimes. The results obtained in our study did not concurred with those reported elsewhere as chlorophyll decreased with increase in soil water contents. The probable reason may have been due to the dilution effect of water in the pots which could have leached nitrogen which is an important component of chlorophyll. Also, the reduced leaf area in low moisture contents which could have lead to higher concentration of chloroplasts may not be over ruled. Based on this study, it evident that chlorophyll contents may be involved in regulating the tea response and productivity under water stress and further studies along the same line are suggested. During the study, ABA and cytokinins were not determined and their concentration varies depending on water stress level. Under water stress there were a substantial increased ABA concentration in the root exudates and leaves in rice suggesting that both ABA and cytokinins are involved in controlling plant senescence, and an enhanced carbon remobilization is attributed to an elevated ABA level in rice plants (Yang JianChang *et al.,* 2002). In our view, quantification of IBA and cytokinins could also be included in the suggested studies.

4.3.4. Relationship between water potential and leaf relative water contents

The physiological response of the tea plant to water stress is important in that it allows identification of traits that form a basis of selection of varieties that are drought tolerant. Differences in drought tolerance between different plant species or between varieties of a specie depend on the relationship between relative water content and low leaf water potentials (Jarvis and Jarvis, 1963). The results of RWC and water potential relationships (Figure 4.8) show a general decline in both parameters with a decrease in soil moisture content.

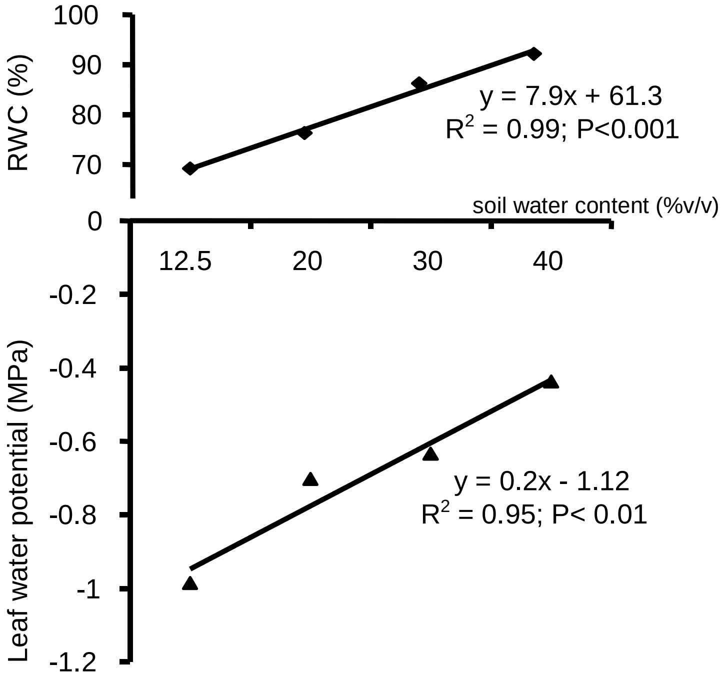

Figure 4.8. Relationship between relative water contents (%) and leaf water potentials (MPa) of composite tea under four different soil moisture contents (%) at Tea Research Foundation, Kericho- Kenya.

Although the age of the leaf was not assessed for tolerance, visually, it was observed that the young tea leaves wilted earlier than the old leaves at various soils moisture levels (Williams, 1971) probably because of their nearness to the water transport pathway. Both the RWC and leaf water potentials can effectively be used as composite tea plant water stress indicators.

4.4. CONCLUSION

The results reported in this chapter provide important information on key physiological parameters of the composite tea examined. The total chlorophyll content varied between clones and decreased significantly ($P<0.05$) with increase in soil moisture content. Rootstocks did not affect the chlorophyll content. The chlorophyll differences between clones could significantly reduce the photosynthetic capacity. The reduced soil moisture content reduced the leaf water potential in all the clones. The diurnal differences in leaf water potential were more pronounced at the 12.5% v/v soil moisture content. Generally, the mean leaf water potential increased with a decline in soil moisture content. Based on this study, the minimum moisture level below which composite tea plants might suffer water stress is below 30% v/v, and the exposure period should be short. Rootstocks identified as being potential for commercialization are TRFK 57/15, TRFK 8/112, TRFCA SFS 150 and EPK TN 14-3. Among the Scions, AHP S15/10 was found to be more susceptible to water stress. Relative water content declined with decrease in soil moisture content ($R^2= 0.9915$, $P<0.001$) in all the scions. Both relative water content and leaf water potential can effectively be used as water stress indicators in composite tea.

CHAPTER FIVE

SEASONAL YIELD DISTRIBUTION AND WATER USE EFFICIENCY OF DIPLOID SCIONS GRAFTED TO ROOTSTOCKS OF VARYING PLOIDY IN RELATION TO SOIL WATER DEFICITS

5.1. Introduction

Tea yields are influenced by many weather variables namely: temperature; the saturation deficit of the air through its influence on plant and soil water deficits; rainfall and evaporation (Carr, 1972; Stephen *et al*., 1992). Tea yields are reduced if the climatic variables are not suitable, for example temperatures should be in the range of as low as 13°C to a maximum of 30°C (Anonymous, 2002). In places where air temperatures below 13°C occur, the tea shoot extension and development is reduced to varying magnitudes depending on the clone and the duration of low temperatures. Some clones have lower base temperatures than others. Conversely, at high temperatures of more than 30°C, large SVPD's occur which lead to temporary stoppage of active growth of tea.

Optimum soil temperatures should also be in the range of 20-25°C, as low soil temperatures reduce yields (Othieno, 1982). Temperatures are inversely related to altitude and although tea performs economically in altitudes between 1500-2250m a.m.s.l, there is a linear yield decrease of 200 Kg mt/ha/yr for every rise of 100m in altitude (Othieno *et al*., 1992). The magnitude of decrease can be more in some clones (Othieno *et al*., 1992; Anonymous, 2002).

Generally, tea yield distribution in Kenya is uniform (Carr and Stephens, 1992; Stephens *et al*., 1992), however, little fluctuations often occur as may be induced by change in seasonal temperature and soil moisture. These fluctuations in tea yields are influenced and determined by factors which affect partitioning of assimilates to harvestable shoots that contribute to yield (Squire, 1979).

A number of researchers have related tea yields to major climatic variables. According to Carr and Stephens (1992), air temperature, soil water deficits, saturation deficits (SD) above 2.3 Kpa in Malawi (Tanton, 1982a) and soil water deficits above 40mm in Tanzania (Stephens and Carr, 1989) reduced yields. Some tea/ clonal yield differences in response to water stress were reported by Burgess (1992b).

The essence of using composite plants is to boost yield of scion materials. However, the choice of rootstocks is critical in that, grafting very vigorous scions on weak rootstock results in poor success (Bezbaruah, 1971), depresses yields (Nyirenda and Ridpath, 1983; Bore *et al*., 1995) and exerts negative growth effects (Nyirenda and Kayange, 1984). Yield increases may be attributed to rootstocks ability to tolerate some level of water stress (Bezbaruah, 1971), and this has been reported in Malawi (Kayange *et al*., 1981) but has failed in very high altitudes of Kenya where there is absence of drought differential. In Tanzania, yield of composites were reported to decrease linearly with maximum soil water deficits, and scion clone AHP S15/10 grafted on TRFK 6/8 and TRFCA PC81 yielded higher by 32% and 11%, respectively (Mizambwa, 2000). Potential yield increase of composites, therefore, depends on the prevailing stress periods, clones etc. Water stress condition exerts differences in the responses of scion materials on different rootstocks.

The proportion of dry matter for tea is affected by several environmental factors and clonal material. The conversion efficiency of intercepted solar radiation to effective dry matter is low, and the ultimate crop production or performance is limited to the economic yield (Squire, 1985). The economic yield of tea consists of tender shoots bearing up to three fully expanded shoots and an immature bud. According to Clowes (1989), these shoots comprise 25%, while green stems and leaves comprise 75%, respectively.

The proportion of economic yield of tea forms the ‘harvest index’, and ranges from 7 to 24% (Magambo and Cannel, 1981; Murty and Sharma, 1986). However, this harvest index has been reported to be as high as 14 - 37% depending on the clones and plant densities (Tanton, 1979; Magambo *et al.*, 1988; Burgess, 1992b; Ng’etich, 1995). The extreme range of harvest index is also influenced by the cultural practices, for instance; Magambo (1983) reported about four-fold difference in plucked and unplucked tea, respectively. In all reported studies, high harvest index was positively related to increase in the ratio of shoot to root partitioning.

The main environmental factor that determines yields is the duration and severity of dry season (Carr and Stephens, 1992). Drought duration varies with locality and within tea growing areas of East Africa the longest is seven months in Southern Tanzania to a few weeks in Kenya. Blum (1983) reported that the yield of a clone depends on both the maximum yield under well-watered condition and the severity of the drought. Burgess and Carr (1993) found that AHP S15/10 had higher yields when irrigated and was highly susceptible to drought. Not much information is available on dry matter partitioning of composite plants with respect to drought. The proportional partitioning of composites to their respective portions need to be quantified with respect to seasonal soil water deficits.

Tea plants form stable polyploids containing a set of 15 chromosomes (Wachira and Kiplangat, 1991). In Kenya, most teas are diploids, and some primary polyploids have been selected from the seedling tea plantations (Wachira *et al.,* 1999). Characteristically, polyploid tea has large leaves, shoots, flowers (Wachira and Ng'etich, 1992), and low rates of dry matter production than diploids. Photosynthesis is high in most polyploid plants such as *Triticum* and *Aegilops* where high photosynthesis is registered in diploids and low in hexaploids (Kaminsky *et al.,* 1990). Generally, photosynthesis is negatively correlated to specific leaf mass (Mediavilla *et al.,* 2001), where high specific leaf mass indicates drought tolerance.

Composite plants developed from rootstocks of different ploidy levels (2n; 3n; and 4n) may boost yield of scions in water stressed environment. This work was aimed at evaluating the effects of rootstocks of different ploidy level on seasonal yield distribution in response to different soil water deficits. The main objective of this experiment was to evaluate the potential of polyploid tea rootstock clones in composite tea. Specific objective were: (i). To compare the performance of some diploid scion clones grafted on diploid, triploid and tetraploid rootstocks in a low altitude site.

(ii). To test some diploid, triploids and tetraploid clones for suitability as rootstocks.

(iii). To evaluate diploid, triploids and tetraploid rootstock clones and their effect on seasonal yield distribution in relation to varying soil water deficits.

5.2. MATERIALS AND METHODS

5.2.1. Establishment of experimental plants

The composite plants used in this study were developed by grafting selected scion clones on rootstocks of different ploidy levels (2n, 3n, and 4n) in 1997. Composite plants were prepared by leaving selected materials to grow freely for five months. The rootstocks and scions were grafted using chip budding, as out lined by Bore *et al*. (1995) and as explained in chapter three.

Scion test clones (all diploids); AHP S15/10; a high yielding and sensitive to environmental changes, TRFK 303/231; a high quality and moderate yielder; GW Ejulu, high quality and low yielder; TRFK 11/26, high quality and very low yielder. Rootstock test clones: diploids; EPK TN14-3, TRFCA SFS150. Triploids; TRFK 382/1, TRFK 421/2, TRFK 54/49, TRFK 371/1, and TRFK 76/2. Tetraploids; TRFK 311/287, TRFK 31/30. The plants were planted in June 1998 in a 122cm x 91cm rectangular spacing (10766 plants/ha), and brought into bearing by formative pruning as recommended by Tea Research Foundation of Kenya (Anonymous, 2002).

The field site where the experiment was planted lies at an altitude of 1740 m, and lies within latitude of 0º 17’ S and 35º 3’ E. According to Jaetzold and Schmidt (1983), the site is a tea-dairy zone (LH 1 p or two), with permanent cropping possibilities, and divisible into two variable cropping seasons. The soils were developed on acid igneous rocks (rhyolites), with volcanic ash admixture, and are well drained, deep to very deep, dark reddish brown, with friable and slightly smeary top soils (andoluvic phaeozems) (Jaetzold and

Schmidt, 1983). The site receives an average of 1618 mm of annual rainfall (13 years average). The mean minimum temperature is 17.8° C, while the mean maximum is 20.5° C.

5.2.2. Treatments

The treatments comprising of rootstocks of different ploidy levels and four scion clones grafted on them were arranged in a complete randomized block design with three replicates. Yield data were collected from 2003 to 2005.

5.2.3. Measurements

Soil water measurement

The soil water balance model was used to quantify the soil water deficits (SWD) during the field experimentation from 2003 to 2005. The model requires inputs such as rainfall, R and reference evapotranspiration, ETo (Hess, 1994). For the model to run, initial crop and soil parameters are required. Plant parameters include dates of planting, 20% emergence and maximum ground cover. Crop coefficients, Kc and Ky are required to relate potential crop transpiration to ETo.

The soil parameters required are topsoil depth, volumetric water content at saturation, field capacity and permanent wilting point at top and subsoil; and easily available water as a fraction of total available water. Bare soil evaporation factor, and drainage factors being the amount of water drained in the first day, expressed, as a fraction of the total water is also required. In the model, evapotranspiration (ETo) was computed using the combination equation of

Penman-Montheith as described by Smith (1991). The model partitions ETo to bare soil evaporation (Es) and crop transpiration (ETc).

Actual evapotranspiration (Eta) can be expressed by the following equation;

$$ETa = ETc + Es \qquad \text{Equation 5.1}$$

The potential soil water deficits or evaporation deficit was calculated using the following equation:

$$SWD_{poti} = SWD_{poti-1} + ETo - R_{i} + D_{poti} \qquad \text{Equation 5.2}$$

where SWD_{poti} is the potential soil water deficit; ETo is the potential transpiration and D_{pot} is the potential drainage assumed to occur at maximum rate once the soil is at saturation and stops at field capacity.

Water use efficiency

Water use efficiency of all tested clonal materials was computed as the monthly yields divided by its respective ETa (evapotranspiration). Seasonal and annual yields were divided by the seasonal and annual ETa to obtain seasonal and annual WUE, respectively. The values obtained were used to evaluate the respective clonal response during the three seasons: warm dry season, cool wet season, and warm wet season (Stephens *et al.*, 1992).

Seasonal yield data collection

The pluckable shoots consisting of mainly two mature leaves and a bud were harvested from the experimental plots on an average period/every 10-14 days as the routine practiced by the tea Estate at TRFK. This interval was maintained constant throughout the period of experimentation. A conversion factor of 0.225 was used to convert the green leaf mass to dry matter (made tea) (Anonymous, 2002). The harvested leaves in the various seasons were analyzed with respect to the prevailing soil water deficits (SWD) in order to determine their respective performance.

Determination of dry matter partitioning

Some plants from each treatment were excavated and their root span and lengths determined before being partitioned in to leaves, stems, and roots; and their respective dry weights determined. The leaves were stripped off and stems cut into small pieces. The roots and soils were washed and sieved through a 10mm^2 mesh. The leaves, stems and roots were dried in an oven at 85°C for 48 hours when a constant weight was achieved. Root excavation has been found to give a high estimation of dry matter partition, hence a good indicator of plant performance (Burgess, 1992a).

The amount of total dry matter produced by each clonal composite combination was calculated. The amount of individual dry matter partitioned were calculated and expressed as a proportion of the total accumulated dry matter.

5.2.4. Data analysis

For each of the parameters assessed, data were subjected to the analysis of variance (ANOVA), and where significant F-values ($P \leq 0.05$) resulted, mean separations were done using either the Least Significant Difference (LSD).

5.3. RESULTS AND DISCUSSION

5.3.1 Soil water deficits

For purposes of monitoring and discussing seasonal variations of various weather parameters and tea crop data, three seasons were recognized, namely: warm dry season (December- March), cool wet season (April- August), and warm wet season (September- November) as described by Stephens *et, al.* (1992). The soil water deficits during the experimental period (2003 - 2005) are presented in Figure 5.1.

There were highly significant differences in SWD within and between the years. Generally, 2003 was the driest period as it recorded 1676.9 mm of annual rainfall and maximum potential SWD of 247mm as compared to 2004, which had 1798.8mm of rainfall, and maximum potential SWD of 198.93mm. Year 2005 had a rainfall of 1630.95mm and maximum potential SWD of 215.61 mm. Therefore, 2004 rainfall was evenly distributed which had a mitigating effect of reducing the potential increase of SWD. The high SWD in the period December 2004 –March 2005 had progressed from mid-to late 2004. On the other hand, 2005 had poor rainfall distribution and excessively high SWD especially the last two weeks towards the end of the year. These significant changes in SWD had a profound effect on the potential yields. There were seasonal variations in SWD. The hot and dry season of 2003 had a maximum SWD of 210mm in March, compared to the same period in 2004 and 2005 where SWD of 100 mm and 161 mm were recorded, respectively. As the season progressed to cool wet in 2003, the SWD decreased from a maximum SWD of 247mm to 67mm in months April –August and Sep- November, respectively. In

2004, it ranged from 77mm in September –November and finally to 135mm in 2005.

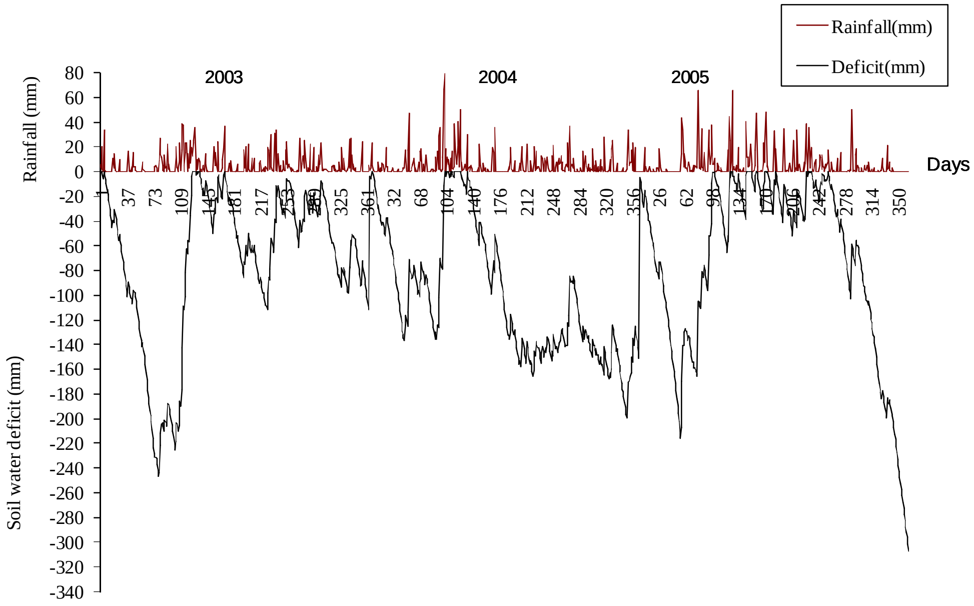

Figure 5.1. Actual soil water deficits (mm) calculated from soil water balance model and rainfall (mm) at Kipkebe, Sotik - Kenya during 2003-2005

Previously, there were attempts to quantify the critical limit of SWD for mature tea above which the total yields are reduced. The values reported vary and range from 40mm for clone TRFK 6/8 (Stephens and Carr, 1989); 70 - 100 mm for seedling tea (Carr, 1974), 120-150mm for clone 1 in Tanzania; and 200-250mm for seedling tea in Malawi (Willat, 1971). In Kenya, yield reduction in seedling tea was reported to occur when the potential SWD exceeded 120mm (Stephens *et al.*, 1991). The results here confirm that whereas the yields were reduced at SWD of more than 120 mm, there were apparent varietal/clone differences. Yield of some varieties were much reduced, and this was attributed to their water stress susceptibility or tolerance. High susceptibility of some clones would cause high yields decline and low yield decline in tolerant clones during drought.

Actual soil water deficits depend on soil type and depth. Ritche (1972) reported that, bare soil evaporation proceeds at a maximum rate when the soil is wet and declines as a function of the square root of time after the initial drying of the topsoil. Transpiration is assumed to proceed at a potential rate while the root zone soil water deficit is less than the easily available water capacity, thereafter it reduces to zero when the deficit reaches the total available water (Hess, 1994).

The environmental variables that influence partition of assimilates are temperature, photoperiod, water stress and nutrition. These seasonal changes in partitioning are related to changes in temperature and water stress between seasons (Cannel, 1985). The seasonal changes in partitioning may also be caused by changes in direction of photosynthates as observed in tea due to changes in season (Manivel and Hussain, 1982). The annual yields were also different (Table 5.1). Year 2003 had almost 50% lower yields than 2004 and 2005.

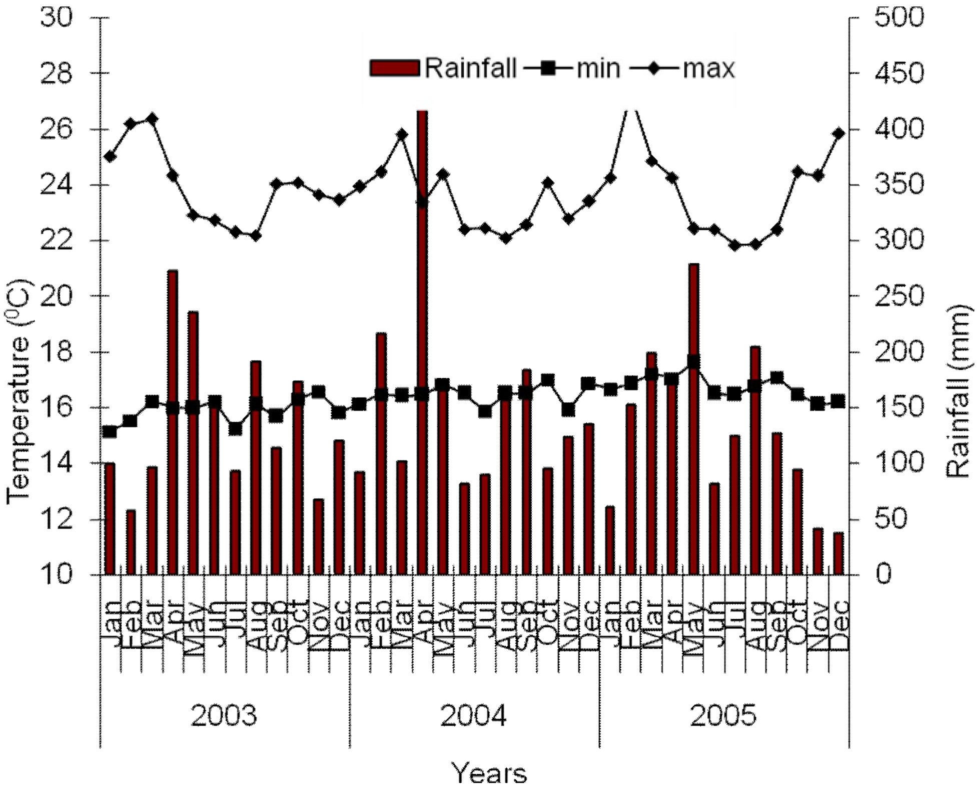

Figure 5.2. Monthly rainfall and average temperature at Kipkebe, Sotik during 2003 – 2005.

Air temperatures

The mean air temperatures for Kipkebe had a peak of 21.2 °C, 21.1 °C, and 20.8°C in March, 2003, 2004 and 2005, respectively. These temperatures were slightly higher and ranged between 20.0 °C to 21.2 °C coupled with the fact that there was less rainfall at this period (Figure 5.2). The maximum temperatures were however, found to be in the range of 26.3, 25.7 and 24.8 °C for 2003, 2004, and 2005, respectively. The mean daily rainfall amount received was 3.1mm, 3.2mm, and 6.4mm for 2003, 2004, and 2005. The lowest mean temperature (15.19 ^{o}C) occurred in July 2003.

The minimum temperatures were found not to fluctuate much unlike the maximum temperatures. The average mean temperatures showed a strong seasonal influence as they were high in early months of the year (January to March) then reduces around April to July, a period characterized by average rainfall and low temperatures in the range of 18.0 to 19.1 ^{o}C. Also at this period, daily rainfall is higher. The seasons beginning around September to November were slightly warmer and drier. In fact maximum temperatures were ranging between 23.0 to 25.0 ^{o}C with very low rainfall.

The temperature amplitude obtained at the time of this study was higher than those recorded in Kericho and slightly closer to those obtained at Changoi, (17 - 20 ^{o}C) (Ng’etich, 1995). The slight difference may have significant effect in influencing plant growth development and yields since these variations are mainly caused by altitude.

Table 5.1. Summary of mean monthly temperatures, rainfall, SWD and Evapotranspiration in 2003-2005 at Kipkebe Estate

		Dec-March	April-August	Sept -Nov.
2003	Temperature °C			
	Maximum	25.85 (2.02)*	22.89 (1.11)	23.92 (1.65)
	Minimum	15.59 (1.39)	15.90 (1.36)	16.18 (1.11)
	Rainfall (mm)	2.818 (6.02)	6.205 (8.59)	3.89 (6.45)
	ETo (mm)	4.631 (0.96)	4.188 (0.47)	4.685 (0.42)
	Potential SWD (mm)	113.875 (76.22)	67.39 (30.45)	39.84 (23.41)
2004	Temperature °C			
	Maximum	24.42 (2.21)	22.94 (1.63)	23.17 (2.49)
	Minimum	16.21 (1.43)	16.43 (0.95)	16.47 (1.76)
	Rainfall (mm)	3.58 (7.09)	6.17 (7.93)	4.421 (6.76)
	ETo (mm)	4.735 (0.59)	4.55 (0.36)	4.476 (0.56)
	Potential SWD (mm)	77.324 (34.12)	82.305 (52.26)	135.458 (25.37)
2005	Temperature °C			
	Maximum	25.44 (2.53)	22.58 (1.58)	23.73 (2.82)
	Minimum	16.91 (1.30)	17.05 (1.17)	16.55 (1.82)
	Rainfall (mm)	3.91 (9.46)	6.40 (12.44)	2.88 (7.71)
	ETo (mm)	4.77 (0.61)	4.34 (0.44)	4.64 (0.69)
	Potential SWD (mm)	160.71 (52.98)	20.72 (13.89)	77.73 (56.39)

* Numbers in parenthesis indicate the standard deviation

The main climatic variables influencing tea yields are temperature, saturation deficit (SD), SWD, rainfall and ETo (Stephens *et al.*, 1992; Carr, 1972). Tea yield decrease with increase in altitude has been associated with low temperatures (Othieno *et al.*, 1992). On the other hand, temperatures tend to be slightly high in lower altitudes as was found in this study. The yields were much reduced during the period January to march, when the air temperatures were higher in comparison to those of higher altitudes. Also, during the cool season, high yields were recorded unlike in other periods. Although annual tea yields in Kenya are uniform, compared to other countries (Carr and Stephens, 1992; Stephens *et al.*, 1992), only short-term fluctuations are induced by changes in temperature and soil moisture. The seasonal yield changes in this study were due to temperature and SWD (Table 5.1).

Since large differences in environmental factors may occur within small distances, (Ng'etich *et al.*, 1995), there is need to test composites in different areas. Bore *et al.* (1995) did not record yield increases in composite tea in high altitudes and alluded to low temperatures, high rainfall and lack of stress differential. Tea yield differences have been identified to be closely correlated to temperatures (Ng'etich *et al.*, 2001a).

Saturation vapour pressure deficit (SVPD)

The saturation vapour pressure deficit varied within the year and followed the same pattern in the three years (Figure 5.3). It was high in the warm dry season (December- march), very low in cool wet season (April- August) and intermediate in warm wet season (September- November). Tea yields were significantly low during the period of high SVPD, and concomitantly high with low SVPD. Tea yields in Kenya have been reported to be uniform although some little fluctuations occur. These fluctuations are induced by changes in temperatures, soil moisture and these affect the partitioning of assimilates to harvestable shoots that contribute to yields (Squire, 1979; Carr and Stephens, 1992). In Malawi, Tanton (1982a) reported that yield of tea are reduced when there is existence of saturation deficits above 2.3 Kpa.

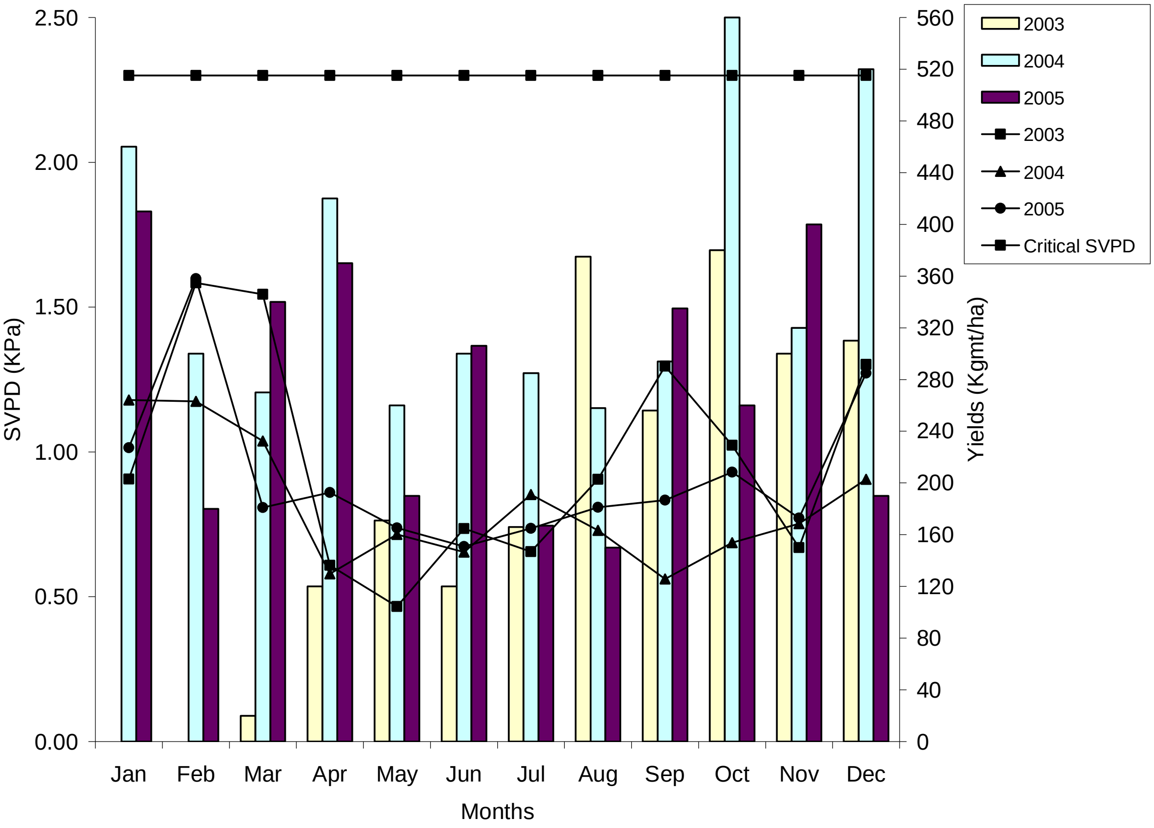

Figure 5.3. Monthly Saturation Vapour Pressure Deficict (KPa), and yields at Kipkebe from 2003 – 2005. Data for January and February 2003 excluded as the plants were recovering from prune.

According to Ng'etich *et al.* (1995) the prevalence of dry air during dry seasons with saturation deficits of more than 2.3Kpa could restrict tea yields. However, the duration of exposure to environments with SD of more than 2.3Kpa is important. This duration varied with seasons, and this explains why the yield decrease was severe in the hot and dry seasons in December – march. In Malawi, Tanton (1982a) concluded that SD greater than 2.3Kpa reduced yields of seedling tea. Response of composite tea would be different, as they constitute clonal teas with varied soil water stress tolerance. The mean annual SD values at the site were higher than those reported by Ng'etich (1995). Exceeding 2.3Kpa often in low altitudes would imply that restrictions of growth and yield of composites caused by low humidity are lower. High SVPD values (1.6Kpa) reported in this study during January- march are 0.7Kpa (30.43%) lower and below the levels considered to cause atmospheric drought stress (Tanton, 1982a). High yield losses attributed to high saturation deficits were reported by Carr *et al.* (1987).

Evapotranspiration

The soil water balance model showed a seasonal variation in evapotranspiration. Low values of 4.188, 4.55, and 4.34 mm d^{-1} were however, recorded and achieved in the same period (April-August) 2003, 2004 and 2005, respectively. High values ranging between 4.59 and 4.77 mm d^{-1} were recorded in Dec-march 2005 although there was some rain, which could have reduced the evapotranspiration rates (Table 5.1). September to November season had evapotranspiration average of 4.685 mm d^{-1} for 2003; 4.476 mm d^{-1} for 2004 and 4.64 mm d^{-1} in 2005, respectively. Grelle *et al.* (1999) in a temperate region found that the overall average evaporation during a 947-days observation period was 1.07 mm d^{-1}. Maximum daily rates were typically 4 mm d^{-1} around mid-summer, the evaporation sometimes reached 0.5 mm d^{-1} in winter, and accounted for 69% of the potential evaporation.

The low evapotranspiration values for 2004 season were attributed to the even rainfall distribution. The results reported here concur with those of Ng'etich (1995). Canopy temperature as well as canopy-air temperature difference were considerably higher in the stressed treatment than in the well-watered treatment during the daytime. High correlation in the linear relationship between canopy-air temperature difference and the air saturation vapour deficit for the well-watered plot was observed (Olufayo *et al.,* 1993).

5.3.2. Yield distribution in response to seasons and rootstocks

The annual mean yields for 2003 to 2005 are presented in Table 5.2. There were significant ($P<0.05$) clonal yield differences. Generally, the entire group of test rootstocks depressed scion yields in 2003 (results not shown). Considering the total yields across the years, (2003-2005), generally, grafting did not confer any advantage to any of the test rootstocks except TRFK 303/231 on its own rootstock which increased yields by a margin of 3.64% in 2003. Also TRFK 11/26 had its yields increased by its own rootstock and TRFK 412/2 by 4.9% and 5.72% in 2003, respectively. Grafting on own rootstock seemed to have advantage in improving yields and this could be due to similarity of polyphenol contents between the rootstock and scions. Also, the tea was recovering from pruning during the year (2003) and probably, low yields may be attributed to the pruning effects of the previous year.

During 2004, variety TRFK 11/26 on TRFCA SFS 150 had its yields increased by 12.02%, while TRFK 303/231 on its own rootstock gained by 9.76%. All other scions were not affected. Variety TRFK 11/26 had the lowest yield decrease by most rootstocks whereas; GW Ejulu L had the highest yield decline (Table 5.2). Differences in drought response were also found in the different scion rootstock combinations. Use of vigorous rootstocks was found to improve yields of low yielding good quality scion clones (Barua and Saikia, 1973; Kayange *et al.,* 1981). However, the results presented here are contrary to this. The probable reason is that the chemical constitution between rootstocks and scions are so different to have caused the observed yield depression (Sreedhar and Satyanarayana, 1996). Variety GW Ejulu has high polyphenol and catechin content while the others have lower contents. Grafting success in rootstocks is related to vigour and inversely related to scion in many woody perennials (Mosse, 1951; Taeotia and Phogat, 1971; Ho, 1981).

Table 5.2. Annual mean yields (Kg mt/ha) of composite tea plants at Kipkebe, Sotik from 2003- 2005.

	Scions				
Rootstocks	S15/10	303/231	Ejulu	11/26	Mean
Ungrafted	5465	4553	3311	3451	4195
Own rootstock	5153	4564	2968	3206	3972
SFS 150	4522	3647	2995	3552	3679
TN 14-3	3611	3987	2668	2933	3300
382/1	4196	4284	2860	2915	3564
54/49	3067	4001	1668	2775	2878
412/2	3097	3371	2447	3097	3003
371/1	4106	3971	2998	3022	3524
76/2	4025	3697	2575	2944	3310
311/287	3596	3081	2279	2578	2884
31/30	1930	2380	1479	2220	2002
Mean	3888	3776	2568	2972	
C.V. (%)	20.10				
LSD ($P<0.05$) Rootstocks 536 Scions 323 Interactions NS					

Data on cumulative mean yields showed that except for the scion clone TRFK 303/231 on its own rootstock, where yield was boosted by 2.32% and TRFK 11/26 on TRFCA SFS 150 by 6.17%, most other scion clones were depressed in yield performance by the test rootstocks by a mean of 21.91%. The variation in the ploidy level of the test rootstocks and seasonality factor with varying environmental differences may have contributed significantly.

Analyses were done to isolate the effect of the various rootstocks on the scions during the different seasons. Results indicated that overall, the three seasons varied and affected the mean yields (Table 5.3a-c). Also, there were significant varietal differences ($P \leq 0.05$) (Figures 5.4 to 5.6). Figure 5.4a shows the results during a more stressful season in December to march where high yielders were AHP S15/10, AHP S 15/10 on TRFCA SFS 150, TRFCA SFS 150, EPK TN 14-3 and TRFK 303/231 on its own rootstock. The low yielders were TRFK 31/30, GW Ejulu, TRFK 311/287, TRFK 11/26 on TRFK 31/30. During the cool and wet period, April –August, (Figure 5.4b), varieties that were high yielding were EPK TN 14-3, TRFCA SFS150, AHP S15/10, TRFK 303/231 and TRFK 11/26 grafted on TRFK 303/231. Low yielders were TRFK311/287, TRFK 31/30, GW Ejulu on TRFK 31/30, and AHP S15/10 on TRFK 31/30. This shows the tetraploids and high quality scions as poor performers. During the warm and wet season in September- November, the high yielder was AHP S15/10 on its rootstock.

Table 5.3. Effect of rootstocks on seasonal annual mean yields (Kg mt/ha) (2003-2005)

a) Dec-March

	Scions				Rootstock mean
Rootstocks	S15/10	303/231	Ejulu	11/26	
Ungrafted	1826	1470	1050	1096	1361
Own rootstock	1799	1390	889	948	1256
SFS 150	1607	1197	969	1131	1226
TN 14-3	1255	1344	884	953	1109
382/1	1392	1366	845	881	1121
54/49	937	1315	494	860	902
412/2	975	1001	768	963	927
371/1	1349	1303	903	871	1107
76/2	1430	1120	824	925	1075
311/287	1152	1017	663	742	893
31/30	603	738	398	671	603
Scion mean	1302	1205	790	913	
C.V. (%)	22.41				
LSD ($P<0.05$)	Rootstocks 191 Scions 115 Interactions NS				

b) April-August

	Scions				Rootstock mean
Rootstocks	S15/10	303/231	Ejulu	11/26	
Ungrafted	1902	1600	1209	1255	1492
Own rootstock	1800	1575	1130	1186	1423
SFS 150	1613	1247	1097	1281	1309
TN 14-3	1333	1398	933	1070	1183
382/1	1417	1524	1090	1127	1289
54/49	1143	1337	603	1032	1029
412/2	1110	1210	901	1106	1082
371/1	1482	1307	1146	1196	1283
76/2	1345	1322	939	1149	1189
311/287	1337	1003	811	968	1030
31/30	695	842	565	834	734
Scion mean	1380	1306	948	1109	
C.V. (%)	20.97				
LSD ($P<0.05$)	Rootstocks 201 Scions 121 Interactions NS				

c) September –November

	Scions				Rootstock mean
Rootstocks	S15/10	303/231	Ejulu	11/26	
Ungrafted	1737	1473	1052	1100	1340
Own rootstock	1555	1599	948	1071	1293
SFS 150	1303	1203	930	1140	1144
TN 14-3	1023	1245	850	910	1007
382/1	1387	1394	925	908	1153
54/49	987	1349	572	883	948
412/2	1012	1160	778	1028	994
371/1	1274	1361	950	955	1135
76/2	1250	1254	811	870	1046
311/287	1106	1061	806	868	960
31/30	632	800	515	715	665
Scion mean	1206	1264	831	950	
C.V. (%)	19.08				
LSD ($P<0.05$)	Rootstocks 170 Scions 103 Interactions NS				

Others were ungrafted AHP S15/10, TRFK 303/231 on its rootstock, TRFCA SFS 150 and EPK TN 14-3 and low yielders were TRFK 311/287, TRFK31/30, GW Ejulu on TRFK 31/30. Generally, the pattern of yields during all seasons and years were similar for the listed varieties as shown in Figures 5.5 and 5.6 thus indicating their yield stability across all seasons. Both ungrafted AHP S15/10 and TRFCA SFS150 were found to be better performers during both warm and dry period, and cool and wet period in 2003 and 2004. The others that were poor performers were tetraploids TRFK 311/287 and TRFK 31/30.

The clonal differences in seasonal yield distribution have quality implications. Tea produced in the cool wet season produces high quality made tea than that produced in the hot dry season. Therefore, the economic value of made tea may increase during the cool season provided clones produce high yields during that period. Maintenance of high yields during cool seasons tends to even out the tea yields production in the seasonal distribution of yields and reduces the large peak yields and deep troughs of yields when growth is inhibited (Fordham and Palmer-Jones, 1977).

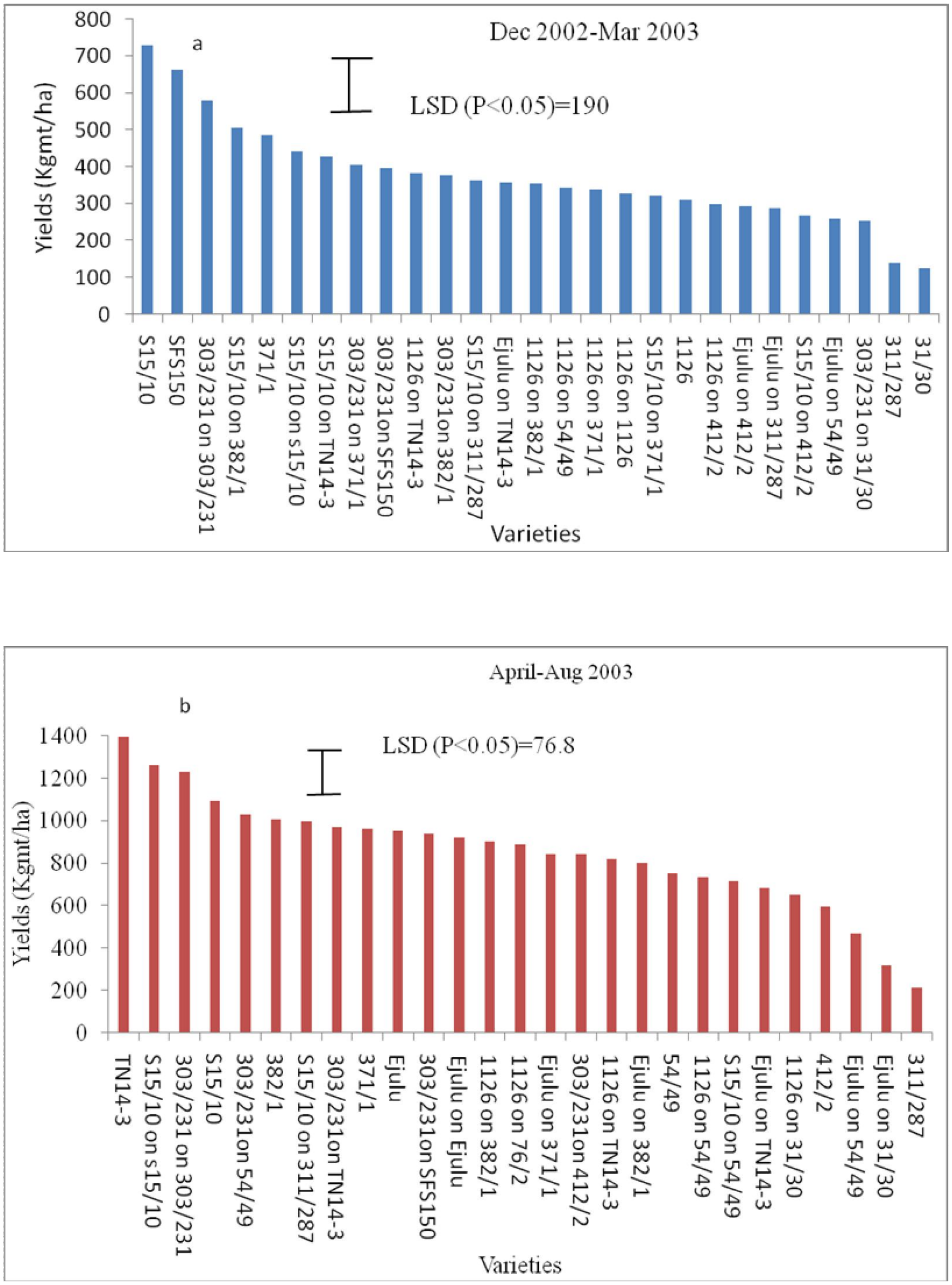

Figure 5.4. Effect of rootstocks on seasonal yield of composite tea during (a) Dec 2002 -March 2003, (b) April- August 2003 at Kipkebe, Sotik-Kenya

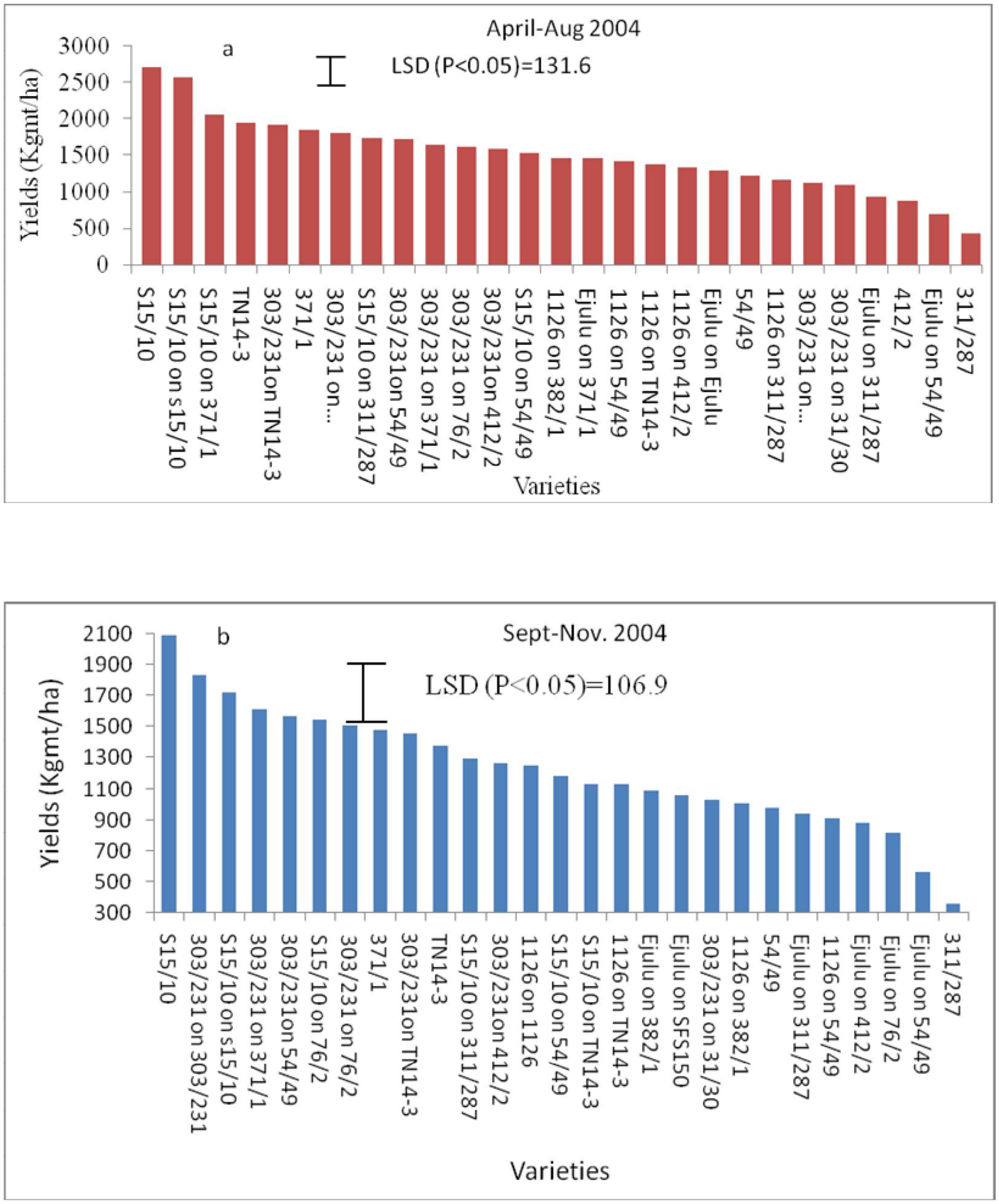

Figure 5.5. Effect of rootstocks on seasonal yield of composite tea during (a) April- August 2004 and (b) Sept.- November 2004 at Kipkebe, Sotik-Kenya

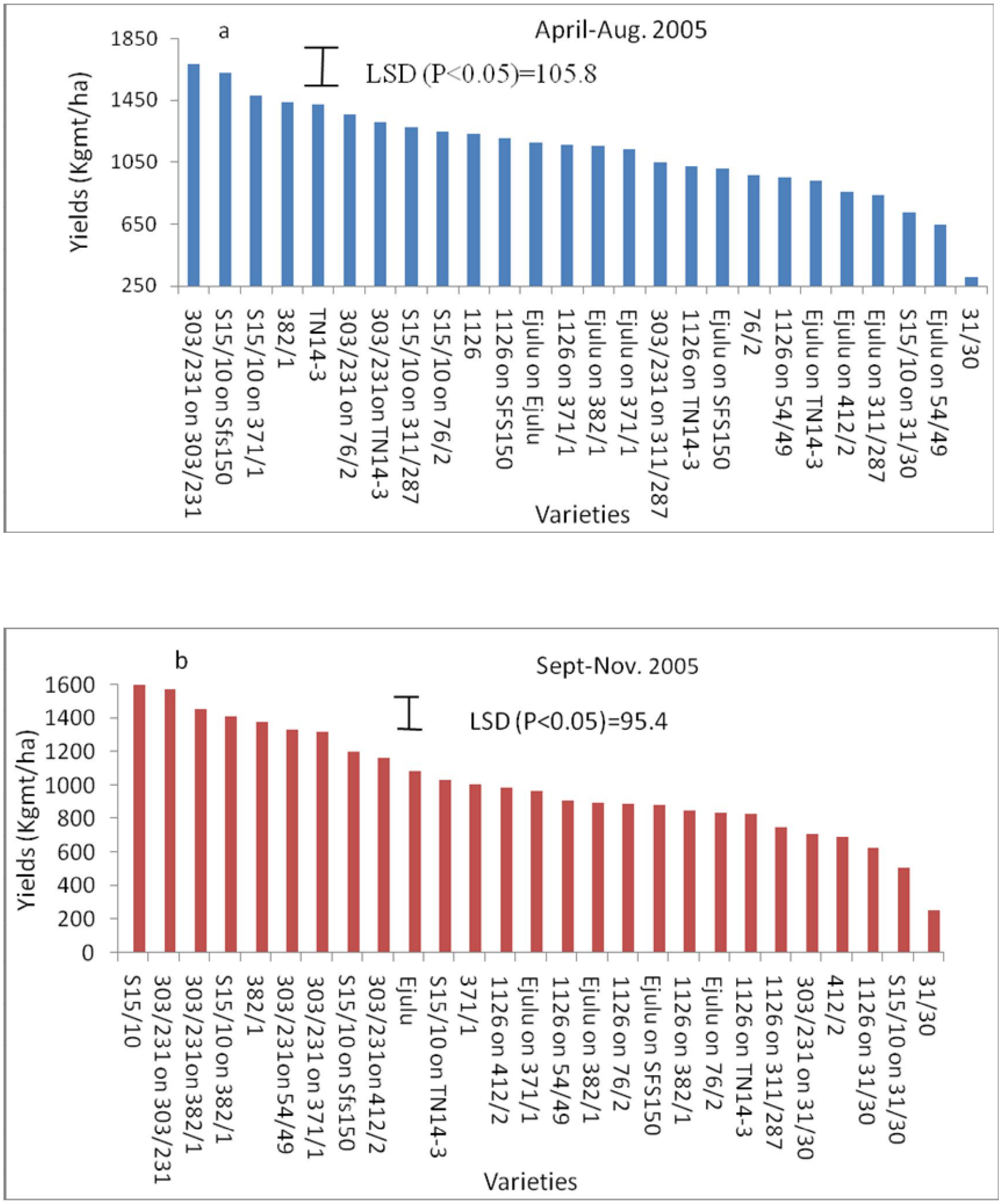

Figure 5.6. Effect of rootstocks on seasonal yield of composite tea during (a) April- August 2005 and (b) Sept.- November 2005 at Kipkebe, Sotik- Kenya

When all the yield and SWD data were pooled for the three years, 2003 – 2005, (Figure 5.7), it was found that yield decreased significantly ($P\leq 0.05$) by 60 kg mt/ha /season (mm SWD) $^{-1}$. Varietal differences occurred, with AHP S15/10 being the most affected during Dec-March season as the decrease in yield was 0.97 kg mt/ha /week (mm SWD) $^{-1}$, followed by AHP S15/10 on its own rootstock with 0.96 kg mt/ha /week (mm SWD) $^{-1}$ and TRFCA SFS 150 with 0.90 kg mt/ha /week (mm SWD) $^{-1}$. The least affected clones were TRFK 31/30, 311/287 (tetraploids) and GW Ejulu L (high quality) with 0.16, 0.19, and 0.26 kg mt/ha /week (mm SWD) $^{-1}$, respectively. These results imply that tetraploids were less affected by stress, hence more hardy.

During the cool wet (April-August) season, the response of clones was in the following order; TRFCA SFS 150, AHP S15/10, AHP S15/10 on AHP S15/10 with 1.80, 1.67, 1.58 kg mt/ha /week (mm SWD) $^{-1}$, respectively. Low response was from TRFK 31/30, 311/287 and GW Ejulu with 0.31, 0.34, and 0.49 kg mt/ha /week (mm SWD) $^{-1}$, respectively. September- November season registered a clonal variation which was not much different from each other; as AHP S 15/10, TRFCA SFS 150, and TRFK 303/231 lost 1.72, 1.59, and 1.58 kg mt/ha (week mm SWD)$^{-1}$, and similarly, TRFK 31/30, 311/287 and GW Ejulu with 0.3, 0.32 and 0.51 kg mt/ha (week mm SWD) $^{-1}$, respectively. Ng’etich (1995) reported similar yield reductions with increase in SWD. Clonal variation showed that only diploid GW Ejulu and tetraploids TRFK 31/30 and TRFK 311/287 were the least affected, confirming the earlier postulation that polyploids are vigorous.

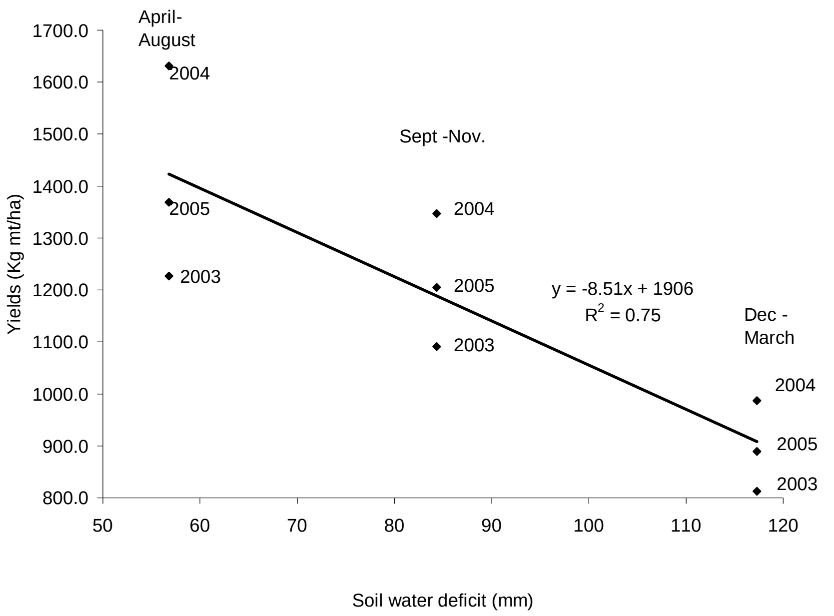

Figure 5.7. Effect of seasonal soil water deficit (mm) on annual seasonal yields, at Kipkebe, Sotik during the year 2003 to 2005. Data points represents individual years.

The seasonal fluctuation in yield of tea is determined by factors affecting partitioning of assimilates between young shoots and the rest of the plant components such as frames and roots (Squire, 1979). These fluctuations are induced by temperature and soil moisture. Yields varied between clones and seasons with variable SWD that significantly influenced the yields. Scion clone AHP S15/10 consistently gave high yields. Interactions between seasons and clones were significant. During the warm and dry season, the clones had the lowest yields, and high during cool-wet season. The significantly linear yield response to SWD may have been due to relatively longer water stress periods.

The yield loss to SWD were, however, lower than those reported by Stephens and Carr, (1991a) and Burgess, (1992). The explanation for this variation could be that the effect of temperature and tea response to SWD at low altitudes means that yield losses are higher. Ng'etich *et al.* (2001b) found that yields loss to water stress were about 20%. In this study, the losses were higher than 20% given that the altitude is much lower. Soil water content was not controlled, thus, it is difficult to accurately quantify the water use of composites. The limitation of this study was that the experiments were carried out under field conditions where several other variables apply, therefore the exact SWD range needs to be quantified under controlled environments.

5.3.3. Yield distribution in response to ploidy level of rootstocks

Compared to the diploid test rootstocks, the triploid and tetraploid rootstocks depressed the yields of the scion clone GW Ejulu-L by bigger margins. A comparison of average long-term performance of the entire scion clones grafted on rootstocks of different ploidy revealed that overall, grafting depressed yields. The effect increased with increasing ploidy of rootstocks. When annual data was considered, there were few significant yield increases (Figure 5.8). The diploid rootstocks TRFCA SFS150 reduced the yields of all tested scions except TRFK 11/26. EPK TN 14-3 also increased yields of TRFK 303/231 by 10%, and TRFK 11/26 by 38%. Although yield of scion GW Ejulu was reduced, the proportional decrease was less than that exhibited on the triploid and tetraploids rootstocks, respectively.

Triploid rootstocks TRFK 54/49 only increased the yields of AHP S15/10 by 35%, TRFK 11/26 by 10%. TRFK 371/1 increased TRFK 11/26 yields by 10%, but decreased that of AHP S15/10 by 35%. Both tetraploids rootstocks TRFK 311/287 and 31/30 decreased all scion yields in differing proportions. TRFK 31/30 rootstock depressed AHP S15/10 by more than 45% GW Ejulu, 38%, TRFK303/231 18% and TRFK 11/26 10%. Comparing the two, it was concluded that though both reduced yields of all scions, the worst was TRFK 31/30 (Figure 5.9).

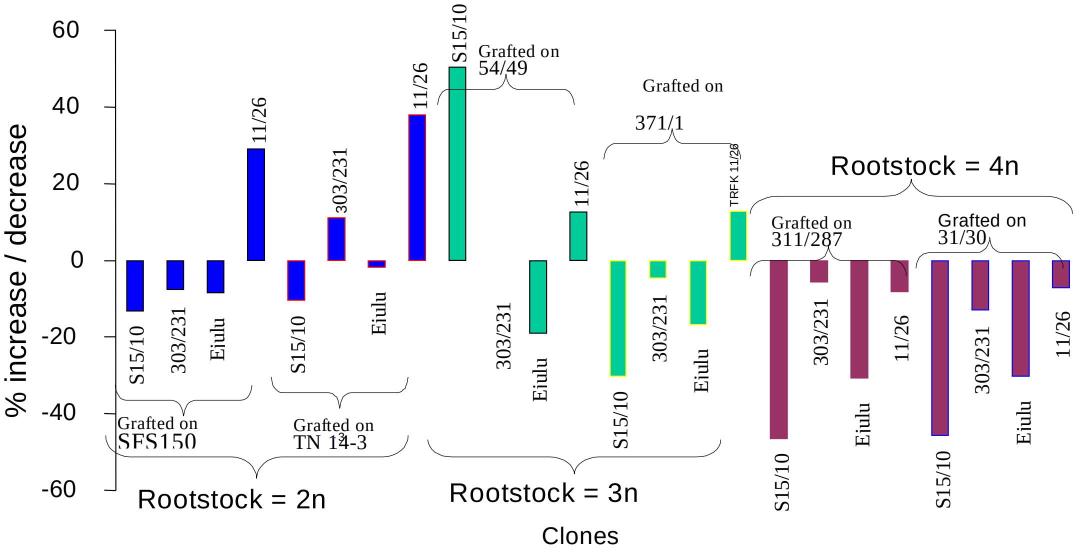

Figure 5.8. Effect on yields (Kg mt/ha) of composite tea plants by rootstocks of different ploidy levels at Kipkebe, Sotik

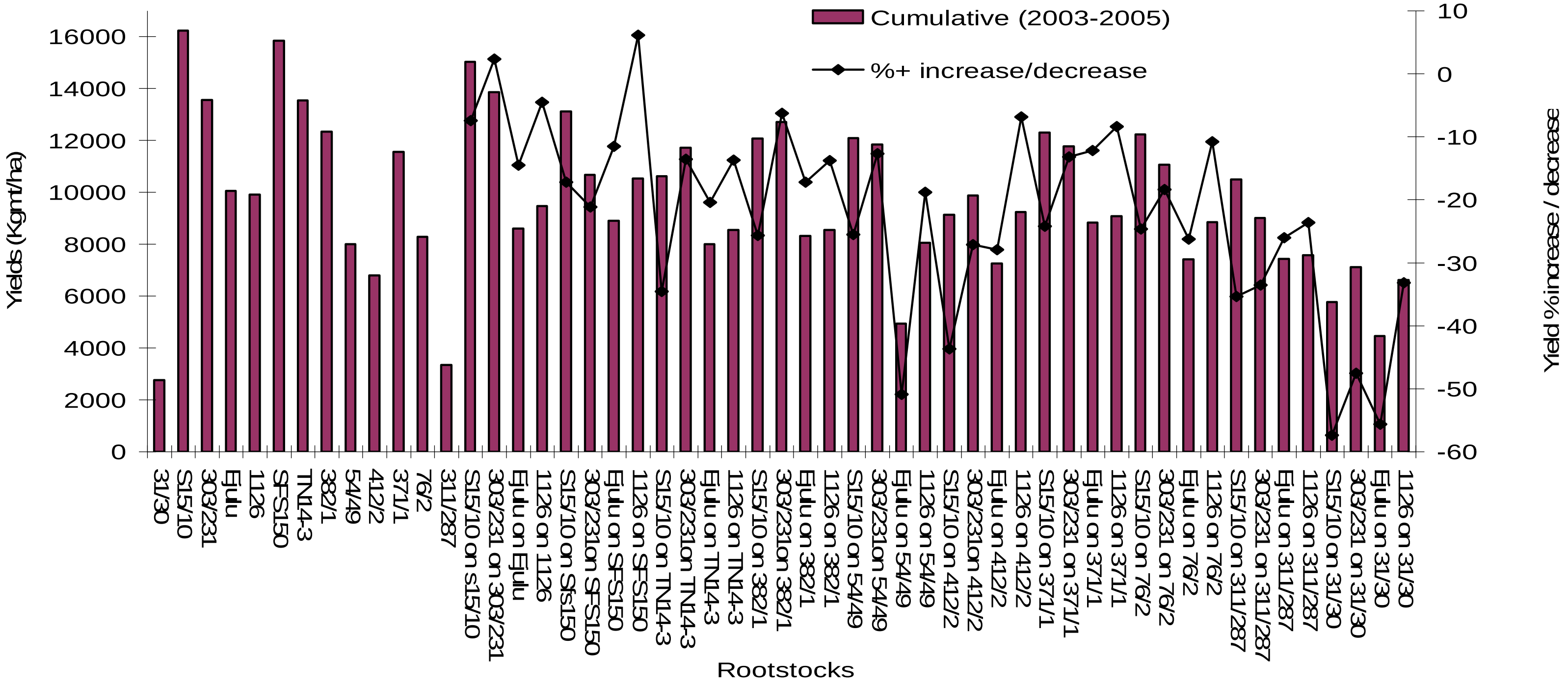

Figure 5.9. Effect of rootstocks (increase / decrease) on cumulative yields of tea at Kipkebe, Sotik during 2003-2005.

Annual yield results showed that year 2004 was a better year as far as the yields were concerned. This was attributed to good rainfall distribution. However, 2003 had low yields due to very high SWD and poor rainfall. This was also a prune year and the decrease in yields was attributed to pruning effects of the previous year (2002). On the other hand, 2005 produced moderate yields. Overall, there were varietal differences. Clones TRFK 31/30 and TRFK 311/287 had low yields showing that both were inherently low yielders (Figure 5.10). Perhaps their inability to increase yield of scions could be attributed to either their inherent low yields or other factors. Drought score of the test materials was assessed and it was observed that there were differential responses, which were reflected in the yields (Figure 5.11).

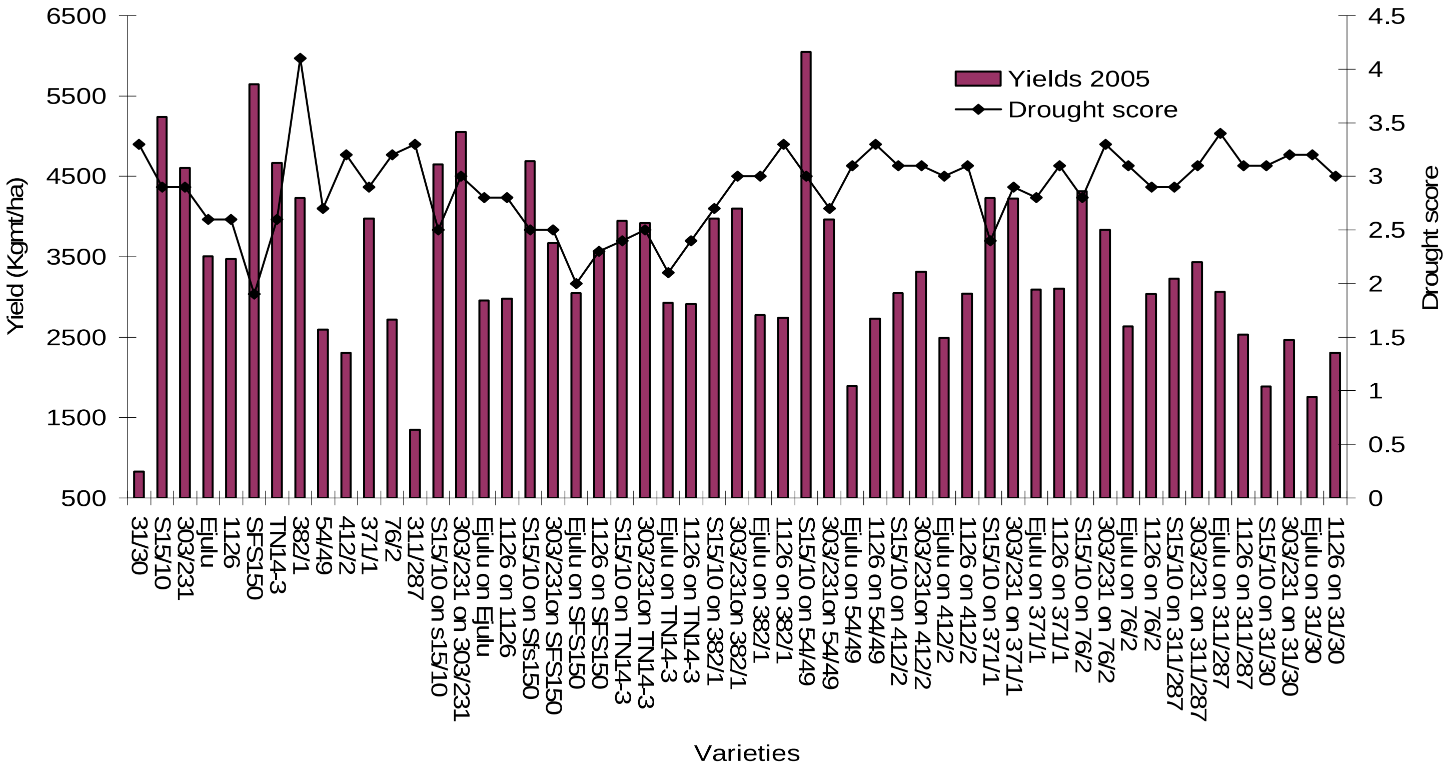

Figure 5.10. Annual yields and drought score of composite tea at Kipkebe, Sotik during 2005.

Drought score: 1- Not affected, 2- slightly affected, 3-Moderately affected, 4- Severely affected, 5- Very severely affected.

The pooled data on effect of ploidy and rootstocks on scion yields during warm and dry season (Dec-March) showed that both rootstocks and scions had significant ($P \leq 0.05$) effect on yields. Both diploids and triploids were similar but better than the tetraploids, and had yield decrease ranging from 37.16% to 56.01% within triploids (Table 5.4). Both scions AHP S15/10 and TRFK 303/231 yielded similarly but significantly different from GW Ejulu and TRFK 11/26. There were no significant interactions. However, the results revealed that all scions tested responded similarly and yields decreased with the increase in rootstock ploidy.

Table 5.4. Effect of rootstock ploidy on seasonal annual mean yields (Kg mt/ha) in the 1st season of Dec 2002- March 2005

	Scion Varieties				Means
Rootstocks	AHP S15/10	TRFK 303/231	GW Ejulu	TRFK 11/26	
Diploids	1431	1271	926	1042	1167
Triploids	1216	1221	767	900	1026
Tetraploids	878	877	530	706	748
Means	1175	1123	741	823	
CV (%)	17.43				
LSD ($P \leq 0.05$)	Rootstocks 143 Scion 165 Interactions NS				

Table 5.5 shows the results of the cool and wet season (April- August). There were significant ($P \leq 0.05$) yield differences, and the triploid rootstocks had an edge over the others. Both AHP S15/10 and TRFK 303/231 had similar and

significant yields as compared to GW Ejulu and TRFK 11/26. Yields of GW Ejulu were lower by 27.5% as compared to AHP S15/10 (Table 5.5). No significant interactions were found. Variety AHP S15/10 on tetraploids had its yields reduced by 53.5% from that on triploids whereas; TRFK 303/231, GW Ejulu and TRFK 11/26 were reduced by 61.4, 59.0, and 54.0%, respectively. The triploid rootstocks TRFK 371/1 and 76/2 were better among the triploid rootstocks and therefore significantly increased the scion yields. These two triploid rootstocks were noted to be better performers.

Table 5.5. Effect of rootstock ploidy on seasonal annual mean yields (Kg mt/ha) in the 2nd season of April- August 2003

	Scion Varieties				Means
Rootstocks	AHP S15/10	TRFK 303/231	GW Ejulu	TRFK 11/26	
Diploids	1473	1322	1015	1176	1246
Triploids	2186	2390	1684	2025	2071
Tetraploids	1016	922	688	901	882
Means	1558	1545	1129	1367	
CV (%)	15.17				
LSD (P≤0.05)	Rootstocks 177 Scion 206 Interactions NS				

The warm wet season (September to November) results showed that main treatment and sub treatment had significant yield effects (Table 5.6). Variety TRFK 303/231 had the highest yields, followed by AHP S15/10 though not significantly different. GW Ejulu L was the lowest yielder and for all other scions; the interactions indicate the high yield trends in the ascending order of diploids> triploids> tetraploids.

Table 5.6. Effect of rootstock ploidy on seasonal annual mean yields (Kg mt/ha) in the 3rd season of Sept 2003-Nov.2005.

	Scion Varieties				Means
Rootstocks	AHP S15/10	TRFK 303/231	GW Ejulu	TRFK 11/26	
Diploids	1163	1224	890	1025	1075
Triploids	1182	1305	806	929	1055
Tetraploids	869	930	660	792	812
Means	1071	1153	785	915	
CV (%)	15.51				
LSD ($P \leq 0.05$)	Rootstocks 128 Scion 145 Interaction NS				

The total annual mean yields of the varieties during the three-year period show that rootstocks and scions had significant yield differences. Diploid rootstocks out-yielded the triploids and tetraploid rootstocks, respectively. There were clonal differences during this period and both AHP S15/10, TRFK 303/231 were better performers and GW Ejulu L being the lowest yielder (Table 5.7). Generally, GW Ejulu L is the best quality clone and whether there is a link between quality and yield requires to be quantified. However, earlier reports indicate that, some chemicals present in both scions and rootstocks need to be similar for even take and successful graft (Sreedhar and Satyanarayana, 1996).

These chemical variations may lead to delayed incompatibility, which causes depressed yields.

Table 5.7. Effect of rootstock ploidy on total annual mean yields (Kg mt/ha) from Dec. 2002- Nov. 2005.

	Scion Varieties				Means
Rootstocks	AHP S15/10	TRFK 303/231	GW Ejulu	TRFK 11/26	
Diploids	4066	3817	2831	3242	3489
Triploids	3698	3865	2509	2951	3256
Tetraploids	2412	2369	1646	2082	2128
Means	3392	3350	2329	2758	
CV (%)	15.81				
LSD (P<0.05) Rootstocks 391 Scion 453 Interaction NS					

5.3.4. Water use efficiency

Water use efficiency (WUE) was evaluated in order to identify the clones that use water effectively for dry matter production. This could be used to determine those clones or combinations that could withstand some level of water deficit. WUE of the rootstock clones are shown (Figure 5.11). The results show that there were variations in the WUE of the different rootstocks in different years. Growing season rainfall had a significant ($P \leq 0.05$) influence on biomass and yield of tea in rain-fed conditions during the 3 years. Rainfall amounts had a significant influence on total yields and it greatly affected distribution. The yields were high in year 2004 and this influenced the WUE. This was attributed to high rainfall and annual yields as earlier shown. Yields were very low and they varied significantly due to the low and significant variability of growing season rainfall. Water-use efficiency for tea yield also increased with increasing rainfall. The results suggest that higher crop yields in Kipkebe may be achieved during the high rainfall period. Yield loss due to high soil water deficits under dry conditions is possible and this could be ascribed to high evaporative loss of water which occurred thus considerably reducing crop water availability.

Seasonal dependence of biomass production on transpiration has been previously reported for a number of crops under drought. Linear yield to transpiration relationships have been utilized in plant-growth and water-uptake models to estimate yield based on predicted transpiration values (Ben Gal *et al.*, 2003). The relationships are valid throughout the life span of crops under varied natural conditions and levels of environmental stress. The effects of water supply on growth, water use and yields of tea studied indicated linear relationships between yields and SWD, which were consistent throughout the seasons.

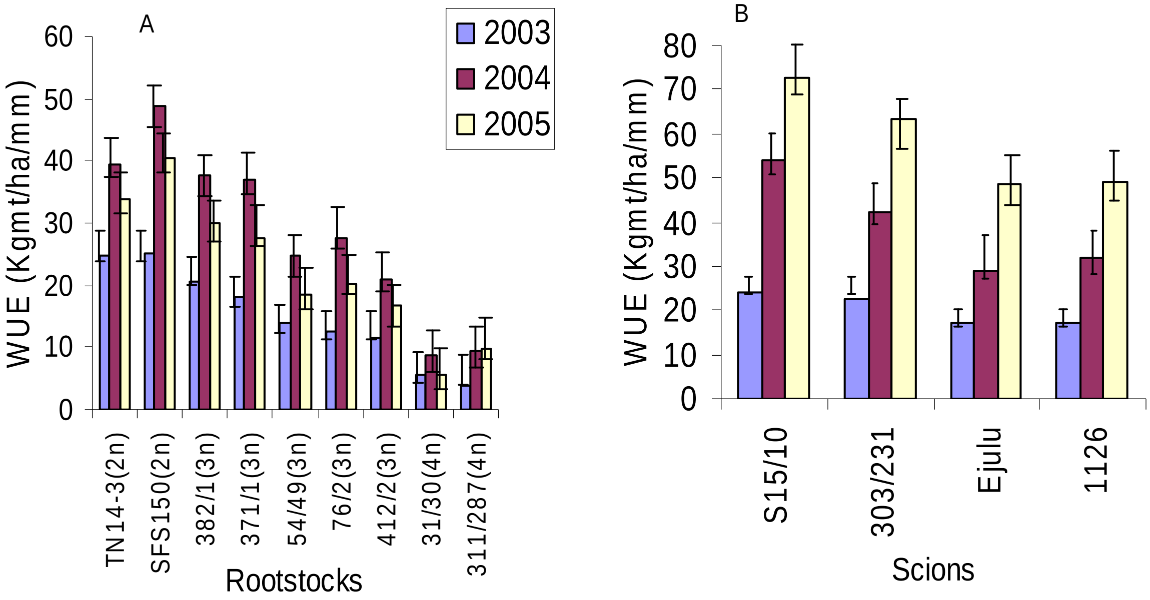

Figure 5.11. Effects of (A) rootstock and ploidy and (B) scions on annual water use efficiency at Kipkebe, Sotik during 2003 –2005. Vertical bars indicate standard errors

The WUE of the scions were different and followed the ascending order of AHP S15/10> TRFK 303/231 > TRFK11/26> GW Ejulu L. This order of performance was similar in years, 2003 and 2004 (Figure 5.11). The WUE of variety AHP S15/10 ranged between 16 kg mt (ha mm) $^{-1}$ in cool and wet season to 14.5 kg mt (ha mm) $^{-1}$ in warm and wet season.

For variety TRFK 303/231, the WUE ranged between 18kg mt (ha mm) $^{-1}$ in cool and wet season to 12.5 kg mt (ha mm) $^{-1}$ in warm and wet season. Both TRFK 11/26 and GW Ejulu L had low WUE of 9 to 12 kg mt (ha mm) $^{-1}$ in warm and dry season and warm and wet season; while GW Ejulu L had 7.5

to 12.5 kg mt (ha mm)$^{-1}$ for warm and wet, and cool and wet seasons, respectively. There was a varied proportional decrease in scion WUE in different seasons.

The decrease was 66%, 45%, 44%, and 33% for GW Ejulu L, TRFK 303/231, AHP S15/10 and TRFK 11/26. The low response of TRFK 11/26 WUE was also reflected in its seasonal yields. Due to the seasonal differences in WUE, it is possible that partial drought affects seasonal yield distribution. Once stress is alleviated, there is an increase in amplitude of yield fluctuations, which also depend on variety. The WUE found in this study are higher than those found in Southern Tanzania (Stephens *et al.,* 1994). These large differences are accounted for by the fact that S. Tanzania experience longer drought periods resulting in low potential yields coupled with higher SWD than those found in Kenya.

When seasonal factor was introduced, it was evident that seasonal variation affected the scions WUE. The cool and wet season (April – August) had the highest WUE for all the scions, whereas the warm and wet (September – November), the least and warm and dry season (December – March) were intermediate (Figure 5.12). Stephens and Carr (1991a) reported that there is a low WUE during the cool period, (June- August), and he ascribed it to low prevailing temperature. These temperatures restrict the rate of shoot extension and during this time, partitioning of assimilates to roots is common, and are stored as starch for latter use once the stress is alleviated. Dry matter produced during the cool wet season enhanced the WUE of tea in warm dry season.

Burgess (1992b) reported that clone 1 and SFS150 had high WUE values and were the most drought resistant while clone BBK207 and S15/10 with low values were drought susceptible. Correlation between WUE and drought suggests that measurements of WUE could provide a means of screening clones for drought resistance. However, the potential yield is also the main determinant of WUE for yield.

The factors transcribe high potential productivity, for example, high rate of carbon assimilation, are actually behind most of the intra species genetic variation in WUE for total biomass. Therefore, WUE has little to do with either drought adaptation or drought resistance, and in certain cases, selection for high WUE may even shift the population towards drought susceptibility (Read *et al.*, 1993). A good case in point is clone AHP S15/10 which is high yielding but drought susceptible. WUE is basically a constitutive trait derived from the capacity of the plant for potential productivity and it may interact with the environment, including the water regime (Hall *et al.*, 1994). The direction of the interaction is such that selection for higher WUE in certain stress environments may result in a shift towards reduced yields in such environments (Blum, 1992).

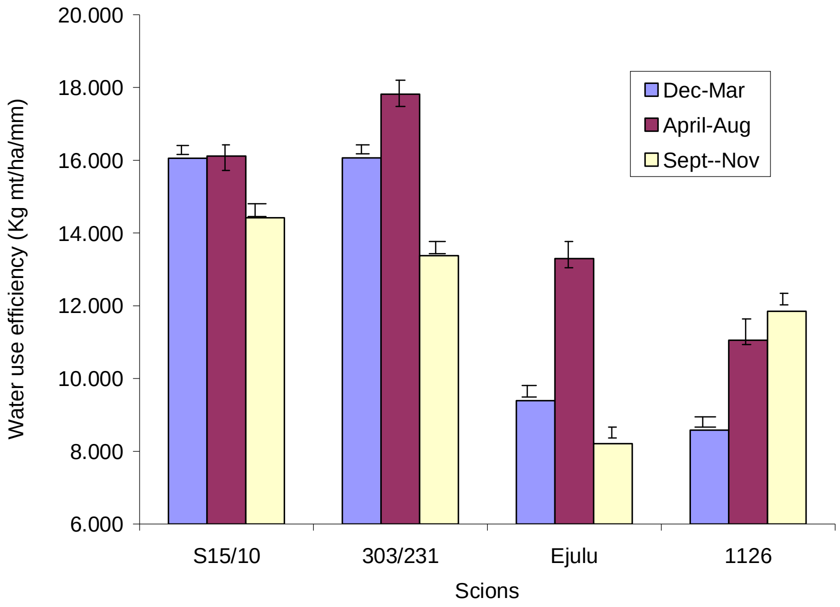

Figure 5.12. Seasonal water use efficiency of scion clones at Kipkebe, Sotik. Vertical bars indicate standard errors.

The annual WUE of the rootstocks as affected by the ploidy level showed that the diploids had high WUE in the range of 25 to 45 kg mt (ha mm)$^{-1}$ for 2003 and 2004, respectively. On the other hand, the triploids and tetraploids had 12 - 25, and 5 - 8 kg mt (ha mm)$^{-1}$ for 2003 and 2004, respectively. Year 2005 had significantly higher WUE that ranged from 65 - 78, 35 - 78, and 11 - 18 kg mt (ha mm)$^{-1}$ for diploids, triploids and tetraploids rootstocks, respectively (Figure 5.13). The variation between the three years was attributed to the level of annual dry matter that was produced. Based on this scenario, diploid rootstocks outperformed the others and therefore are recommended for selective use in commercial tea plantations in Kenya.

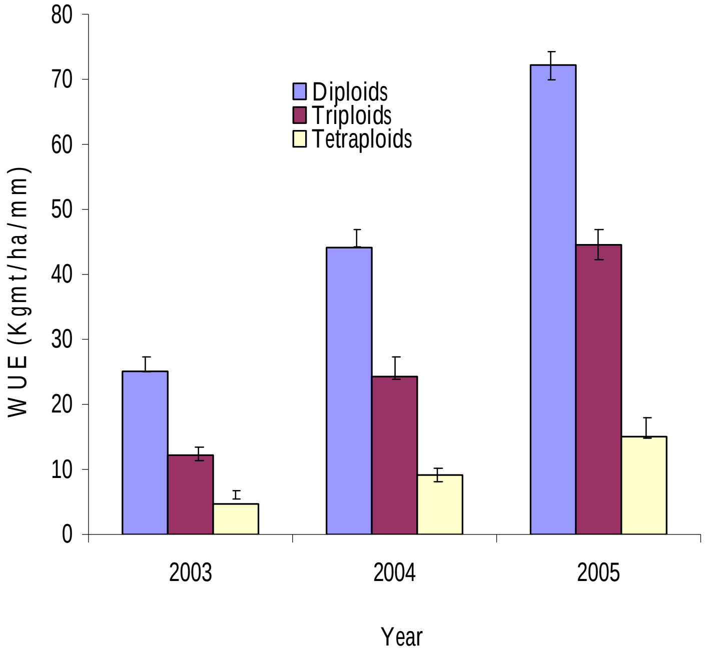

Figure 5.13. Effect of rootstock ploidy on annual water use efficiency during 2003-2005 at Kipkebe, Sotik. Vertical bars indicate standard errors

There were significant ($P \leq 0.05$) WUE fluctuations in diploids of 23.5 kg mt (ha mm)$^{-1}$ between the years than in both triploids (16.17 kg mt (ha mm)$^{-1}$ and 5.17 kg mt (ha mm)$^{-1}$ for tetraploids. The results showed that WUE of diploids was six fold that of tetraploids and twice that of triploids. Comparatively, triploids had twice WUE of tetraploids. The substantial increase in WUE in the different seasons must have been due to improved conversion efficiency of solar radiation to dry matter or to a larger harvest index (Stephens *et al.*, 1994). Selection of rootstocks is a key factor in solving any soil stress problem but complete resistance is still a problem. Agrotechnical measures should be applied parallel to use of resistant rootstocks. Good productivity and WUE of a cultivar as induced by the best rootstock can be achieved only if the essential factors are present at optimum levels. The WUE determination in this study was based on the HI, therefore the results concur with those reported by Stephens *et al.* (1994).

5.3.5. Dry matter partitioning

There were significant ($P \leq 0.05$) differences between dm in the leaves, frames and roots within and between the tested rootstock varieties. The dry matter partitioning within the different ploidy levels was also clearly evident (Table 5.8). There was a trend in ploidy levels in the ascending order of diploids> triploids> tetraploids.

Table 5.8. Dry matter production and partitioning of the test rootstocks plants after 48 months since planting at Kipkebe, Sotik.

Rootstock clone	Ploidy	Leaves/ plant (gm)	Frames/ plant (gm)	Roots/ plant (gm)	Root: shoot ratio (r: s)	Root depth (cm)	Root spread (cm)
TRFCA SFS150	2X	588^2	1097^2	742^2	0.440	99.3^1	114.55
EPK TN 14-3	2X	866^1	1928^1	765^1	0.274	93.8^2	118.73
TRFK 382/1	3X	557^3	686^5	571^5	0.459	86.2^3	132.81
TRFK 54/49	3X	486^6	657^6	528^7	0.462	74.5^7	130.82
TRFK 412/2	3X	780*	923*	443*	0.260	67.5*	96.8*
TRFK 371/1	3X	556^4	987^4	709^3	0.459	76.5^6	117.74
TRFK 76/2	3X	200^8	413^8	463^8	0.755	80.8^4	112.86
TRFK 311/287	4X	512^5	991^3	560^6	0.373	78.0^5	101.27
TRFK 31/30	4X	385^7	436^7	579^4	0.705	65.3^8	98.08
Mean		516	899	615	0.435	82.6	115.8
CV (%)	7.2						
LSD ($P \leq 0.05$)		35	41	52	0.08	10.2	12
0.01		321	657	NS	0.16	14.7	NS
0.001		NS	912		0.20	20.4	

**Only two replicates, hence not included in the analysis*

Superscripts 1-8 indicate ranks

Harvest Index (HI) is defined as the fraction of the total dry matter partitioned to harvestable shoots. This proportion for tea ranges between 7 – 24% (Magambo and Cannell, 1981; Murty and Sharma, 1986). In this study, the test rootstocks apportioned between 24 - 30% of their dry matter to the leaves (Figure 5.14). The triploid rootstock TRFK 382/1 partitioned 30% of dm to leaves, while for the lowest yielding triploid TRFK 76/2 it was only 18%. Leaves as a proportion of total dm decreased marginally with increasing rootstock ploidy. The leaves dm did not seem to change much within the ploidy level, showing that, though polyploids are low yielding when mature two leaves and an immature bud are plucked, there would be good or improved yields if more than two leaves and a bud were considered for plucking provided there was no compromise in quality. Wachira and Ng'etich (1999) also found that leaf dm did not differ much within a ploidy level.

The frames formed the largest proportion of the dm for all varieties, which was within a range of 46-48%. This proportion was highest for diploid rootstocks and lowest for tetraploids. It was suggested that the high accumulation of dm in the frames may reflect the spread of the individual tea plant e.g. TRFCA SFS 150 and EPK TN 14-3 that contributes to critical and significant yields (Wachira and Ng'etich, 1999). Magambo and Cannel (1981) linked the large development of wood frames with reduced yields in tea. In this study, however, TRFCA SFS150 and EPK TN 14-3 rootstocks contributed to the highest yield responses in most of the scions tested.

Seasonal changes in dm production may have been due to environmental factors. There was increased dm during cool wet season and this was similar to what was observed by Ng'etich and Stephens (2001b). Water stress also reduced dm in leaves particularly in December-march season. The rate of dm production was clearly related to seasons. The economic yield (HI) of tea is small as compared to that of forest trees, whose HI range from 40 - 85% (Cannel, 1985) and 42% for oil palm (Corley, 1983). The HI for tea is known to decrease with age, probably because of secondary thickening (Magambo and Waithaka, 1983).

Root biomass was highest in the diploid test rootstock clone EPK TN14-3 (diploid) followed by clones TRFCA SFS150, (diploid); TRFK 371/1 (triploid), and TRFK 31/30 (tetraploid). Rooting depth was highest for clone TRFCA SFS150 followed by clones EPK TN14-3; TRFK 382/1 (triploid). The root spread was largest in clones TRFK 382/1, TRFK 54/49 and EPK TN14-3.

When the biomass of the different plant parts of the rootstocks was regressed against data on scion performance, only rooting depths had a significant correlation (R^2=0.732; $P \leq 0.05$) with performance despite the fact that the net average effect of grafting was negative. Rooting depth can, therefore, be used as an important trait for evaluating rootstocks.

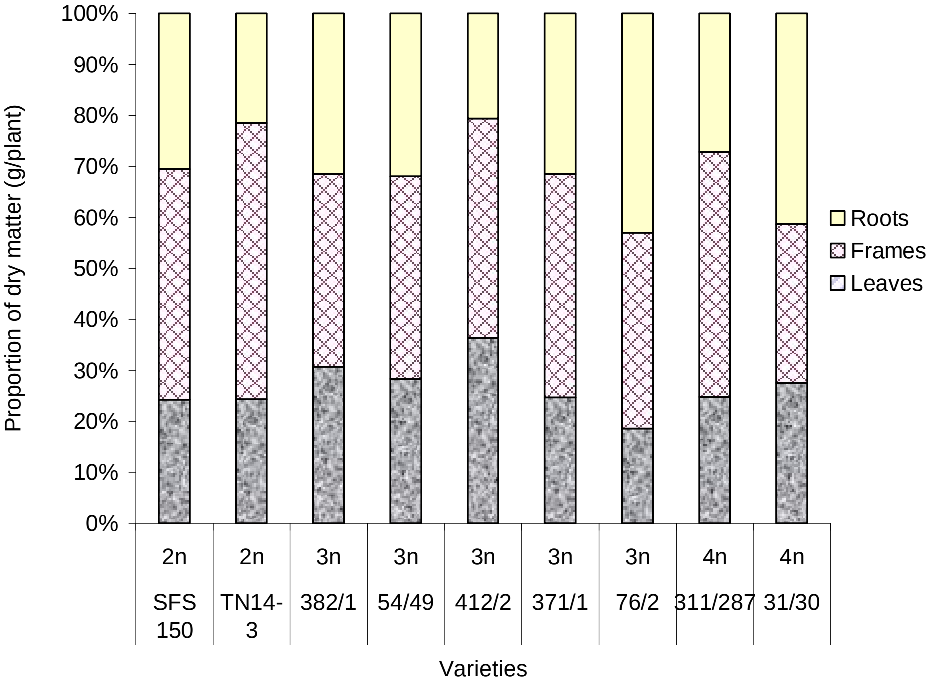

Figure 5.14. Proportional partitioning (%) of dry matter of nine rootstock clones to leaves, frames and roots at Kipkebe, Sotik, after 48 months since planting

Root depth is an important trait in rootstock performance as it allows the plant to explore extensively for nutrients and water particularly if it is in a water stressed environment. Diploid rootstocks EPK TN14-3 and TRFCA SFS 150 had greater root depths, than those of triploids and tetraploids, respectively (Figure 5.15). Only triploid TRFK 382/1 had a comparable depth with that of the diploid rootstocks. This characteristic explains why the scion materials grafted to these rootstocks were out- performing all other scions grafted to triploid and tetraploid rootstocks.

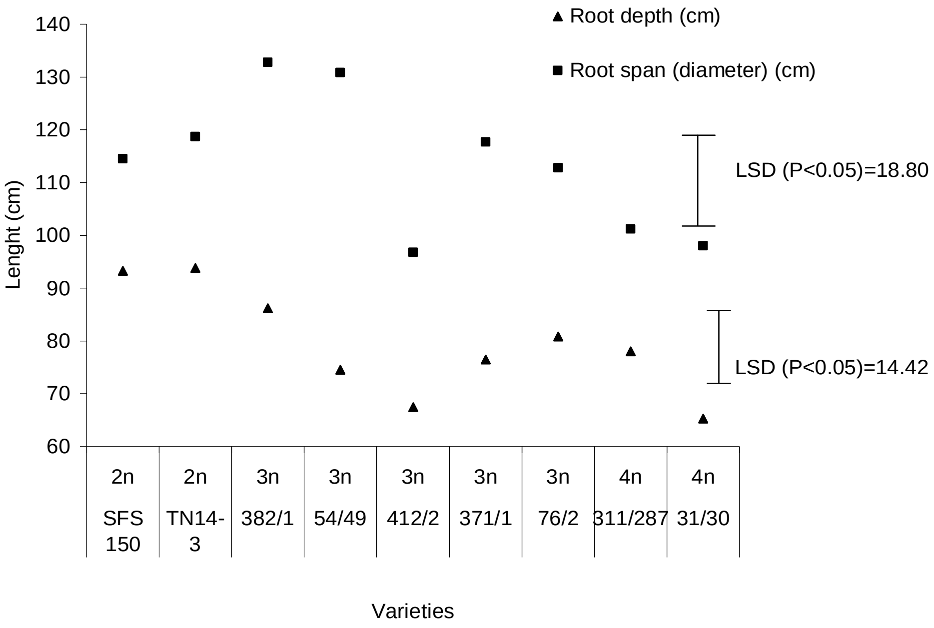

Figure 5.15. Effect of rootstock ploidy on root length (cm) and span (cm). Vertical bars indicate standard error

On the other hand, tetraploid TRFK 31/30 was shallow -rooted and had a poor root span. This also explains its significant influence in reducing the scion yields because of its inability to explore and possibly extract nutrients and water.

Within the growing season, the root systems of plants are subjected to fast renewal and decay. In the water- stressed condition, the turnover is strategic for plant survival and productivity in a mechanism, which is still unclear (Vamerali *et al.*, 2003). The maximum root depth is dependent on the nutrition of the plants. Nutritionally -stressed plants tend to grow slowly but this was not addressed in the current study, therefore, further investigations are suggested. In other studies, it has been found that greater drought resistance of mature tea is due to the greater spread and depth of the roots which can extend to 5.5m (Stephens and Carr, 1991a). Nyirenda (1987, 1990) also identified deep root system as an important clonal characteristic for drought resistance.

The results indicated that both root depth and root span in composite tea depended on the varieties and ploidy level. It was concluded that the responses of the different varieties were inherent in nature. These were governed by the genetic make-up and expressed themselves in varying proportions in different water stress levels.

5.4. CONCLUSION

The results presented in this chapter indicate that, soil water deficit (SWD), water use efficiency (WUE) varied within and between years. There were significant differences in SWD with the maximum being 247, 199.4, and 307.8 mm for 2003, 2004, and 2005, respectively. Air temperatures ranged between 20.8 and 21.2 ^{o}C and had a strong influence on plant growth, development and yields. Yields decreased (R^2=0.74***) with increase in SWD and clonal variation was evident with S15/10 having a decrease of 0.97 kg mt/ha/week (mm SWD)$^{-1}$. Grafting depressed the yield of the scion varieties, and showed a significant ($P \leq 0.05$) decrease with increase in rootstock ploidy. The WUE significantly (R^2=0.86***, R^2=0.72***) varied and was in the order of diploids>triploids>tetraploids rootstocks, for 2003, and 2004, respectively. Although the scions had varying proportion of WUE, it was high for S15/10>303/231>11/26 and Ejulu being the least in all the three seasons. It is also evident that the productivity of composite tea in the study is water-limited. Root biomass was highest for diploid rootstocks; TN 14-3 and SFS150 and intermediate for 371/1 (triploid) and low for 31/30 (tetraploid). Root depth had a significant correlation (R^2=0.73*) with performance despite the net average effect of grafting being negative. Rooting depth is therefore an important trait for rootstocks.

CHAPTER SIX

SEASONAL EFFECTS OF ROOTSTOCKS OF DIFFERENT PLOIDY ON DEVELOPMENT RATE, POPULATION DENSITY AND COMPOSITION OF SCION SHOOT

6.1. Introduction

Dry weight of tea shoots harvested at any time is dependent on the rate of shoot growth, number of shoots per plant, and is influenced by environmental factors. Variation of these yield components depends on the various clones.

Fordham and Palmer-Jones (1977) reported a dynamic and stochastic model that incorporates these components. The model starts with a known shoot population, growing at a certain rate. Shoots of specified length are harvested at a certain time, and their mass calculated from their length and then total yield is calculated. The model has also been used by Cannel, *et al.* (1990) to describe shoot extension and for prediction of effects of changes in harvesting interval on shoot composition.

Temperature has a significant effect on tea growth, and clonal response also varies (Cannell, *et al.*, 1990). Shoot extension rate for clone TRFCA SFS 204 was found to be linearly related to night temperatures in the range of 12.5-20^{0}C (Tanton, 1982b). The relative extension rate of field grown TRFCA SFS 204 was linearly related to weekly mean air temperature. The minimum temperature below, which the shoot extension ceases, is described as base temperature. Obaga *et al.* (1988) defined the value as the increment of extension (mm $d^{-1}\,^{0}C^{-1}$ or mm mm^{-1} $d^{-1}\,^{0}C$) per unit thermal time.

In tea harvesting the time to pluck is dependent on the clone and on the number of fully expanded leaves and a bud that have reached pluckable size. Composite tea plants may follow the same pattern. However, the contribution of rootstock in influencing the plucking regime requires to be quantified, in terms of clonal differences, relation to shoot length, and their respective responses to seasonal factors.

In tea the number of harvested shoots relies on the shoot population density and the clonal differences account for the major variations. Stephen and Carr (1990) reported that the shoot numbers varied with clones, based on the leaf counts per unit area. The fresh mass of harvested shoots can also be determined from the length of the shoot, and relationship between fresh mass and length (Fordham and Palmer-Jones, 1977). This relationship has been described as either linear (Palmer-Jones, 1974), or exponential (Smith *et al.*, 1990).

Although the dry matter content of a harvested shoot is assumed to be constant at 22.5% (Anonymous, 2002), the conversion factor of green leaf to dry weight is largely affected by saturation deficit (Ng'etich, 1995). Shoot population densities have been reported to range from 50-400 shoots m^{-2} for Assam type of tea while Chinery clones range from 350-1200 (Stephens and Carr, 1990).

The objective of the study reported in this chapter was to investigate the effects of seasons on rate of shoot extension, shoot population density and on composition of diploid scion varieties grafted to rootstocks of different ploidy levels.

6.2. MATERIALS AND METHODS

6.2.1. Shoot extension and development

The experiment was carried out in a tea field containing tea that was grafted to diploid, triploid and tetraploid rootstocks. The experimental site details were provided in chapter four. From the experimental materials explained in chapter four, shoot extension was monitored on monthly intervals. These intervals represented periods with different soil water deficits. Every month a shoot with two fully expanded leaves was removed and the remaining stem butt was tagged. The bud length was measured at weekly intervals as the shoot extended and leaf unfurl until the shoot emerged or reached a pluckable size.

6.2.2. Measurements

Although several models for computing growth and development exist, only exponential (Tanton, 1982b), logistic (Bond, 1945) and Gompertz (Smith *et al.*, 1990) models best fit the shoot extension parameter. These three models allow shoot extension to be modelled as continuous after release of apical dominance. The exponential model uses only two variables as initial shoot length (L_o mm) is constant relative extension rate (RER, mm $mm^{-1}d^{-1}$).

$$L=L_oE^{RER.t} \qquad \text{Equation 6.1}$$

Both Logistic and Gompertz models use three variables: a factor to Relative Extension Rate (RER), time taken from apical dominance release to date of maximum shoot extension rate (m; days) and the final shoot length (L_f ; in mm).

Logistic $L=L_f /(1+e^{(-b\,(t-m))})$ Equation 6.2

Gompertz $L= L_f e^{-e\ -b\,(t-m)}$ Equation 6.3

The shoot extension in our study was calculated using the exponential model due its simplicity as described by Stephens and Carr (1993). Shoot growth duration from the release of apical dominance to when it has fully expanded to pluckable size of two leaves and a bud was determined as the Shoot Replacement Cycle (SRC), the rate of shoot development was defined as the inverse of SRC. This was seasonally done to isolate drought and rootstock ploidy effects.

Leaf area

Leaf area measurements were taken during different seasons of the year in 2004 and 2005. The intervals represented periods with different soil water deficits. A shoot with two fully expanded leaves was removed, and the remaining stem butt was tagged. The length and width of an actively growing leaf was measured with vernier calipers at weekly intervals as the leaf unfurled until it reached a pluckable size of two leaves and a bud. These measurements were taken on three bushes per plot and replicated five times. The measured leaf area was multiplied with a factor of 0.623 (Ng'etich and Wachira, 1992).

Shoot population density

During the time when the shoot length was measured, and when tea was due for plucking, the total number of shoots removed at each harvest was determined. The shoot numbers that were either actively growing or dormant (banjhi) were counted in a 625cm^2 (0.25m^2) grid placed randomly at the centre of each bush in each treatment plot. The shoot population density in the grid was converted to an area of m^2 before analysis. The shoots were counted at the time when the terminal auxiliary bud had begun to extend according to method of Stephens and Carr (1994). The duration of shoot replacement cycle (SRC in days) from the removal of apical dominance to when the shoot had developed two fully expanded leaves and a bud were determined. The proportion of either active or dormant shoots in harvested shoots was determined.

Shoot composition

During plucking, a sample of green harvested shoots was removed from each treatment plot. These shoots were separated in to various shoot standard sizes, depending on their growth activity. Those showing active growth, they were further separated into one (1+b), two (2+b), three (3+b) or four (4+b) shoots and an immature bud. The banjhi (dormant) buds were also sorted out.

The various proportions of the different types of shoots were determined as a percentage of the total shoots in the sample.

6.2.3. Data analysis

For each of the parameters assessed: shoot development and extension rate, leaf area, shoot population density and shoot composition, data were subjected to the analysis of variance (ANOVA), and where significant F-values ($P \leq 0.05$) resulted, mean separations were done using the Least Significant Difference (LSD).

6.3. RESULTS AND DISCUSSIONS

6.3.1. Shoot development and extension rate

Shoot extension rate was significantly ($P<0.05$) affected by the seasons. The extension rate was high during both cool wet and warm wet seasons (Table 6.1). The lowest rate of development was registered in warm dry season (results not shown), and this was attributed to high SWD, low temperatures, and high evapotranspiration that were prevailing. There were variations between the clones across seasons. The shoot extension rates have been reported to decrease with increase in altitude (Obaga *et al.*, 1988) and with lowered temperatures (Stephens and Carr, 1990).

Temperature effects on shoot development and extension mainly arise due to their major influence on cell division, differentiation and expansion. Tanton (1982b) suggested an integral of time (in days) and temperature above the base for a bud released from apical dominance to reach pluckable size of three leaves and a bud. The shoot development as well as rate of extension linearly increase with rise in air temperature. Vapour pressure deficit can also influence shoot extension rate.

Table 6.1. Relationships between shoot extension rates (mm mm day)$^{-1}$ and warm wet seasons from release of apical dominance to harvesting at Kipkebe, Sotik during the three seasons between 2004 and 2005

	Scions				
Rootstocks	S15/10	303/231	Ejulu	11/26	Rootstock mean
Ungrafted	0.015	0.014	0.016	0.016	0.015
Own rootstock	0.016	0.013	0.016	0.014	0.015
SFS 150	0.015	0.013	0.016	0.015	0.015
TN 14-3	0.014	0.013	0.015	0.014	0.014
382/1	0.014	0.014	0.015	0.014	0.014
54/49	0.014	0.013	0.014	0.014	0.014
412/2	0.014	0.013	0.015	0.014	0.014
371/1	0.014	0.013	0.015	0.014	0.014
76/2	0.013	0.014	0.014	0.014	0.014
311/287	0.016	0.013	0.016	0.014	0.015
31/30	0.014	0.014	0.014	0.015	0.014
Scion mean	0.014	0.014	0.015	0.014	
C.V. (%)	5.51				
LSD (P<0.05)	Rootstocks 0.0005				
	Scions 0.0003				
	Interactions 0.001				

The results of this study showed that extension rate was slow during the period with high saturation vapour pressure deficit (SVPD) in December to March season unlike when the SVPD values were low in the cool wet and warm wet seasons. The correlations between shoot extension and saturation deficits can explain seasonality of shoot growth and effects of temperature and humidity. Similar results have been reported by Squire, (1979) and Tanton (1982c). The reduced extension rates in this study may have arisen due to high saturation deficits, high air temperatures and water stress.

The seasonal variations observed in our study were much lower than those in Southern Tanzania (Burgess, 1992a), because of the significant temperature differences. There were no marked seasonal variations at the experimental site, although they were slightly more apparent than in Kericho (Ng'etich, 1995). The seasons had different SWD (Chapter 5) which could have influenced the shoot development through reduced extension during hot dry seasons.

The shoot development rate at Kipkebe may have tea harvesting implications because the shoots will reach pluckable size sooner than in higher altitudes. Therefore, tea harvesting at these sites may adopt short plucking intervals that are beneficial in quality determination. Burgess (1992b) developed a harvesting plan based on phyllocron, being the interval of two leaves emerging.

6.3.2. Leaf area

Leaf area is a very important component and has a major bearing on the physiological mechanisms that control yield and dry matter partitioning. Its rate of growth/expansion contributes significantly to yield realization through its indirect influence on photosynthetic activity of its surface area. The number of days taken between the unfurling of the first and the second leaf differed for all the tested clones and seasons. The time taken to reach pluckable size differed in all the tested clones. However, leaf area of scion clones grafted on the diploids took a shorter time compared to those grafted on triploids or tetraploids.

The tetraploids and triploids had bigger leaf area than the diploids. In woody plants, leaf structure is an important determinant of leaf assimilation capacities, and there is an inverse relationship between gas exchange and specific leaf mass (Niinemets, 1999). The high leaf area for tetraploids, especially TRFK 311/287 indicates its drought intolerance as well as being of low productivity (Wachira and Ng'etich, 1999). The diploids had low leaf mass area and this was an indicator of high yields as earlier reported by Ng'etich *et al.* (2001).

The expansion of first leaf area was significantly ($P< 0.05$) faster in warm dry season (Table 6.2) followed by cool wet season, and the least was during warm wet season (results not shown). Development of leaf area is significantly influenced by seasons, and these have carry over effects in that the leaf initiation may start in a different season and be completed in another subsequent season. Initiation in warm dry season becomes faster in that, there are slightly higher temperatures.

Table 6.2. Changes in leaf area (cm^2) of first leaf within the warm dry seasons during 2004-2005, at Kipkebe, Sotik

	Scions				
Rootstocks	S15/10	303/231	Ejulu	11/26	Rootstock mean
Ungrafted	0.527	0.270	0.280	0.280	0.339
Own rootstock	0.337	0.313	0.330	0.357	0.334
SFS 150	0.323	0.600	0.257	0.267	0.362
TN 14-3	0.447	0.657	0.693	0.390	0.547
382/1	0.250	0.383	0.567	0.223	0.356
54/49	0.267	0.300	0.377	0.313	0.314
412/2	0.240	0.363	0.393	0.247	0.311
371/1	0.270	0.407	0.600	0.613	0.473
76/2	0.467	0.417	0.220	0.213	0.329
311/287	0.357	0.490	0.543	0.360	0.437
31/30	0.227	0.207	0.690	0.207	0.332
Scion mean	0.337	0.401	0.450	0.315	
C.V. (%)	43.16				
LSD ($P<0.05$)	Rootstocks 0.131				
	Scions 0.079				
	Interactions 0.262				

Varieties showed variation in their respective leaf area expansion. The ungrafted tetraploids; TRFK 311/287 and 31/30 and triploid TRFK 412/2 had 40% higher leaf area than diploids. Grafting improved the leaf area of the scion clones especially TRFK 303/231, and GW Ejulu L. The large leaf area of AHP S 15/10 compared to other scion varieties was a major contributor to yield differences that were observed and reported in chapter four. Similar results have also been reported by Squire *et al.* (1993). Allowing a leaf to develop to its maximum surface area has significant harvesting implications, because it increases shoot mass of up to 70% at three leaves and a bud stage, and inadvertently causes a decline in quality (Ng'etich, 1995). However, Squire *et al.*

(1993) found that leaf mass area did not vary with altitude and warmer environments had shoots with more dry mass.

Whereas variation in leaf area is related to and/or induced by environmental factors, other characteristics, particularly leaf morphology, are mainly determined by genetic background (Cordell *et al.,* 1998). Changes in leaf area are affected by altitudes and are also common in other tree crops. Leaf mass decrease with increase in altitude in other crops e.g. Hawaiian *Metrosideros polymorpha* to attain and dominate an extremely wide ecological distribution not observed in other tree species. The variation in leaf area reported here therefore contributed to yield variation that was observed in the different clones.

6.3. 3. Shoot population density

There were highly significant ($P<0.001$) seasonal influences on the shoot population density. Tea plants harvested in the warm dry season of Dec-2003 – March 2004 (Table 6.3) and warm wet season in September –November 2005 had the highest shoot density (Table 6.5). Variety AHP S 15/10 had a shoot population of 176 shoots $(m^2)^{-1}$ when grafted on TRFCA SFS 150 and a 20% lower shoot density when grafted on both TRFK 31/30 and 311/287. Generally, the performance of the other scion clones was lower than that of AHP S15/10. The lowest shoot generating variety was GW Ejulu L while TRFK 303/231 and 11/26 were intermediate.

During the warm dry seasons when the shoot density was low, the proportion of dormant shoots was higher (Table 6.4). Since the experimental materials were pruned in 2002, the shoot density also increased with time and away from pruning time and this was attributed to increased plucking surface area (Stephens and Carr, 1994). The shoot densities of some of the clones were higher than those reported by Ng'etich (1995). These differences may have been due to altitudinal differences of the experimental sites. Squire *et al.* (1993) reported that tea shoot density increases with decrease in altitude. The low shoot density in cool wet period was because of low soil water deficits and low mean air temperatures that further decreased the shoot extension rate. In Sri Lanka, Wijeratne (2004) reported that the optimum temperatures for dry matter accumulation in tea shoots were around 26.9 - 27.7°C. These temperatures improve the shoot population density which has been found to be a major yield determinant in tea (Squire and Callander, 1981).

Table 6.3. Changes in seasonal shoot population density $(m^2)^{-1}$ of scions grafted to rootstocks of varying ploidy at Kipkebe, Sotik during warm dry season from 2003-2005

	Scions				
Rootstocks	S15/10	303/231	Ejulu	11/26	Rootstock mean
Ungrafted	131	110	97	95	108
Own rootstock	129	122	87	105	111
SFS 150	124	104	108	103	110
TN 14-3	107	118	104	105	109
382/1	111	116	106	95	107
54/49	108	118	88	95	102
412/2	95	99	94	97	96
371/1	115	116	94	105	108
76/2	118	112	103	110	111
311/287	86	86	90	91	88
31/30	83	100	84	94	90
Scion mean	110	109	96	100	

C.V. (%) 14.46

LSD ($P<0.05$) Rootstocks 12.12

Scions 7.31

Interactions NS

Table 6.4. Changes in seasonal shoot population density (m^2)$^{-1}$ of scions grafted to rootstocks of varying ploidy at Kipkebe, Sotik during cool wet season from 2003-2005

	Scions				
Rootstocks	S15/10	303/231	Ejulu	11/26	Rootstock mean
Ungrafted	134	128	103	103	117
Own rootstock	147	119	99	97	116
SFS 150	131	116	96	93	109
TN 14-3	122	112	97	109	110
382/1	121	115	100	94	107
54/49	113	119	94	98	106
412/2	127	115	89	94	106
371/1	123	116	107	102	112
76/2	126	124	103	108	115
311/287	109	100	91	107	102
31/30	91	106	85	94	94
Scion mean	122	115	97	100	
C.V. (%)	9.56				
LSD (P<0.05)	Rootstocks 8.38				
	Scions 5.06				
	Interactions 16.77				

Variation in seasonal shoot population density resulted from the effects of temperature, SWD, and SVPD on shoot growth rates (Squire, 1979; Tanton, 1982a; Stephens and Carr, 1990). Low shoot population density was mainly recorded during cool wet season and warm dry season (December 2004 to March, 2005) but the shoot density was higher during warm dry season in December 2003 to March 2004, and warm wet season in 2005 (Table 6.5).

Table 6.5. Changes in seasonal shoot population density $(m^2)^{-1}$ of scions grafted to rootstocks of varying ploidy at Kipkebe, Sotik during warm wet season from 2003-2005

	Scions				
Rootstocks	S15/10	303/231	Ejulu	11/26	Rootstock mean
Ungrafted	148	143	143	123	139
Own rootstock	148	139	112	116	129
SFS 150	150	135	129	109	131
TN 14-3	137	121	129	124	128
382/1	139	134	132	113	130
54/49	140	143	135	108	132
412/2	140	124	120	109	123
371/1	139	131	132	116	130
76/2	145	142	129	131	137
311/287	106	120	119	114	115
31/30	102	131	118	98	112
Scion mean	136	133	127	115	
C.V. (%)	9.56				
LSD (P<0.05)	Rootstocks 10.29 Scions 6.20 Interactions NS				

In any single year, there was a significant ($P<0.05$) decrease in shoot population density in July, which improved with time, implying influence of the prevailing low air temperatures (Figure 6.1). There was a significant polynomial decrease in shoot population density with ploidy of rootstock from July 2005, (R^2= 0.8134, $P<0.01$) and August (R^2=0.7504, $P<0.01$) and September (R^2=0.5605, $P>0.05$), from diploids, triploids and tetraploids, respectively.

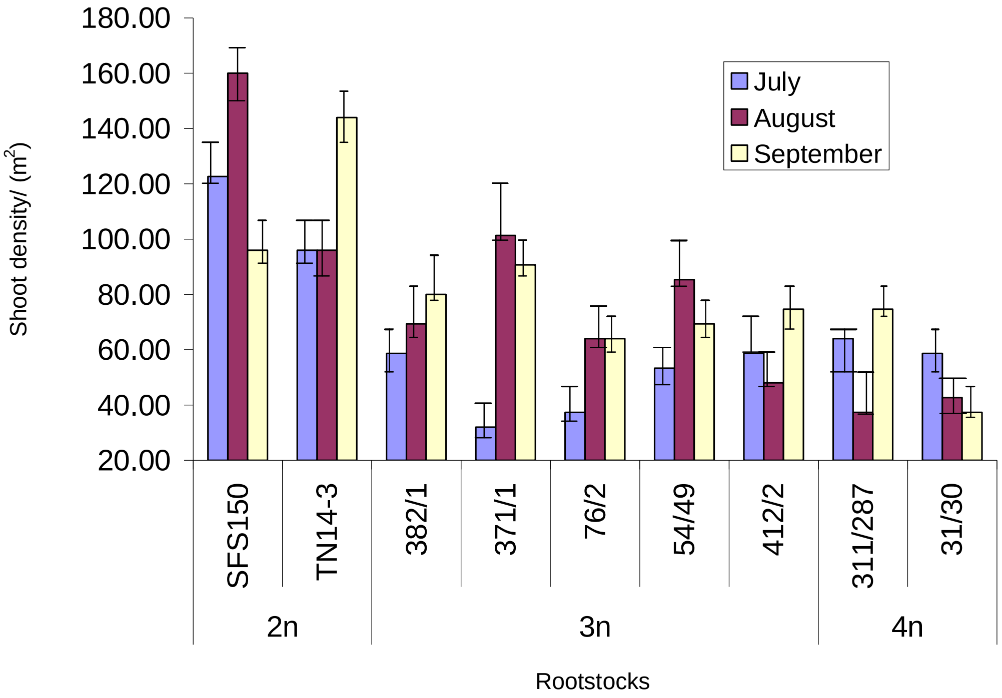

Figure 6.1. Changes in shoot density due to seasonal effects and rootstock ploidy at Kipkebe, Sotik. Vertical bars indicates standard error.

A large proportion of shoots were dormant during the cool wet season thus, leading to reduced population of active shoots. Ng'etich (1995) found differences in between clones and sites. Also in this study, varietal differences were evident as indicated by their various shoot generations.

Several workers have indicated that shoot population density, which is the main component of yield, is mainly affected by environmental factors as well as their interaction (Carr, 1977; Wickramatne, 1981; Wachira *et al.*, 1990; Rono *et al.*, 1991). What this implies is that the seasonal variations in shoot population density results obtained in this study were mainly due to influences of environmental factors such as rainfall, temperatures, saturation deficits of the air (Chapter 5) and composite combinations. These influences affected shoot growth rates thereby affecting the shoot density (Squire, 1979).

The increased differences in shoot population between 2003, 2004 and 2005 were because the surface area of tea bush increased with time from pruning. Since pruning was done in December 2002, the low density observed in 2003 and it increase in 2004 and 2005 agreed with earlier findings by Stephens and Carr (1994). Large population density noted in this study concurred with the results of Squire *et al.* (1993).

During the warm dry season (December, 2003- March, 2004) and warm wet season (September-November, 2005) the rootstock ploidy, scion clones and their interactions had no significant ($P \leq 0.05$) effects on the shoot population density. This may have been caused by the low amount of rainfall, and similar SWD of 77mm (Chapter 5). In the cool wet season (April - August 2004), there was no rootstock ploidy effect on shoot population density. However, shoot population density of scion clones were significantly ($P < 0.001$) affected. The probable explanation is that different clones have different capabilities to generate shoots which is what contributes to differences in yields. The order of varieties in terms of shoot population density was AHP S15/10 > TRFK 303/231 > TRFK 11/26 > GW Ejulu. There were no significant interaction effects.

In the warm wet season (September –November, 2004) and warm dry season (December 2004- March 2005), there were rootstock ploidy effects on shoot density (Figure 6.2). Although the polyploid rootstocks had a disadvantage in production of shoots the diploids performed better than both triploids and tetraploids. Both scion varieties and rootstock interactions were not significant. The increase in shoot density after a drought stress period in December to march could be attributed to synchronization of growth by stress. Ng'etich (1995) observed a significant decrease in shoot population after a drought, and alluded to a decline in air and soil temperature that influenced reduced extension rate.

In the cool wet season (April –August, 2005), the rootstock ploidy, scion varieties and their interactions had significant ($P<0.05$) effect on shoot populations (Figure 6.2). The major differences were between the tetraploids and triploids. Both scion varieties AHP S15/10 and TRFK 303/231 had higher shoot density compared to the others. GW Ejulu L had the least shoot density. AHP S15/10 was the best performer as it produced a significant increase in shoot density of 23.35% more on diploid rootstocks than it did on tetraploid rootstocks. On the other hand, TRFK 303/231 had 6.53% more shoots on triploids rootstocks compared to tetraploids. GW Ejulu L produced 11.25% more shoots on triploids than it did on tetraploids. The shoot density produced during this period was higher than those recorded in the same period the previous year. This variation in performance was due to environmental factors that favoured growth.

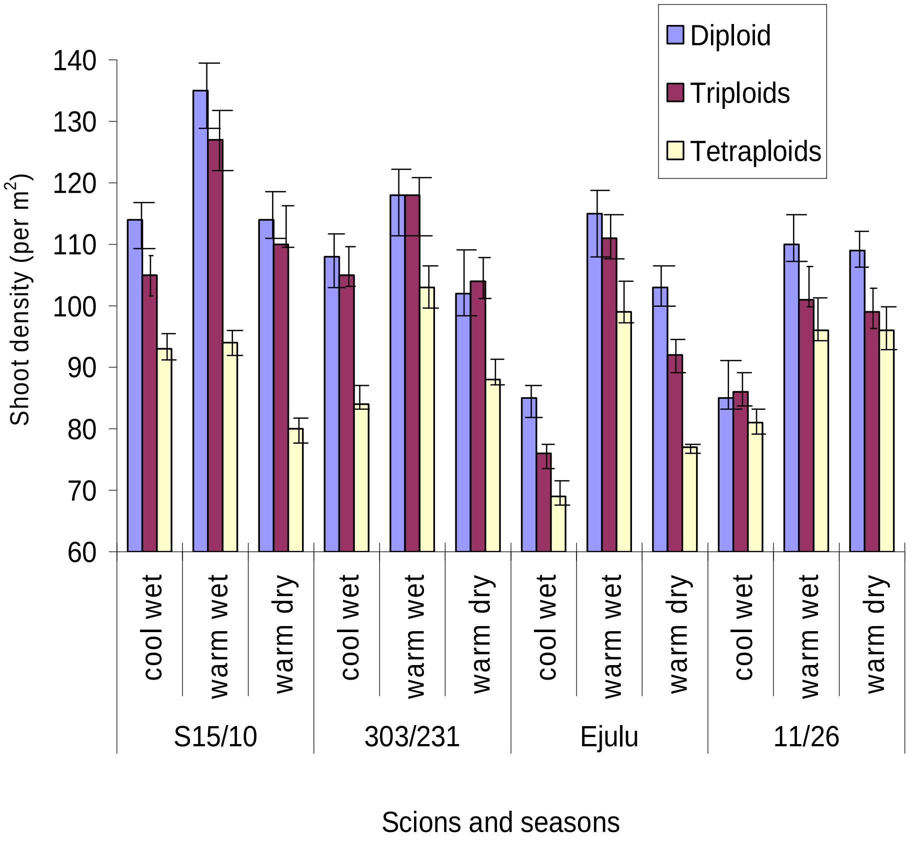

Figure 6.2. Effect of rootstock ploidy and seasons on scions shoot population density (m^{-2}) at Kipkebe, Sotik during 2004 and 2005. Vertical bars indicates standard error.

Shoot population density was found to vary with the tea variety, nutritional status, drought and temperature (Stephens and Carr, 1990). The seasonal variation in shoot density results from air and soil temperature, rainfall as influenced through SWD, and Saturation Deficit (SD) of air on shoot growth rate (Tanton, 1982c; Stephens and Carr, 1990).

The reduced shoot population density during the water stress period in warm dry season (December- March) when temperatures were high (Chapter 5) caused reduced growth because of water deficit. In the cool wet (April August) period there were apparent low temperatures in July which further depressed shoot development, thus further reducing the shoot density (Stephens and Carr, 1994). High dormant shoots proportion characterized this period but when stress was relieved, there was an increase in shoot population.

Grafting on diploid rootstocks had an advantage over grafting on triploids and tetraploids. The contribution of diploid rootstocks to shoot density compared to triploids and tetraploids requires further investigations with a view to identifying the mechanisms for their shoot regeneration, quality parameters and anatomy. Their physiological contributions towards shoot density and yields also need further investigations.

6.3.4. Shoot composition

There were seasonal effects on the shoot composition. During the warm and dry season, the shoots comprised of a low proportion ranging between 5-18% of one mature leaf and a bud. Majority of the plucked leaves contained the same shoot type (one mature leaf plus an immature bud) proportion of 20 to 60% in variety GW Ejulu L on TRFCA SFS150 and AHP S15/10 on TRFK 412/2 (Figure 6.3). The rest of the clones had a mean of 40%. There were variations in the proportions of one mature leaf and a bud, two mature leaves and a bud, three mature leaves and a bud, four mature leaves and a bud and dormant (banjhi) shoots during each harvest.

The ungrafted variety TRFK 54/49 had a lower proportion of one leaf and a bud, and two leaves and a bud that ranged from 4 to 16%. TRFK 311/287 and 31/30, both tetraploids, had the least proportion of one leaf and a bud, four and a bud and majority of dormant buds. GW Ejulu L on most rootstocks had a high proportion of both three, and four mature leaves and a bud. Most scion varieties had less four mature leaves and a bud, except AHP S15/10 on TRFK 382/1, and 76/2 with a proportion of 20% and 15%, respectively. Dormant shoots accounted for a mean of 38% in all the scions shoot composition during this season. However, scion variety TRFK 11/26 on 54/49 had a high proportion of about 70%.

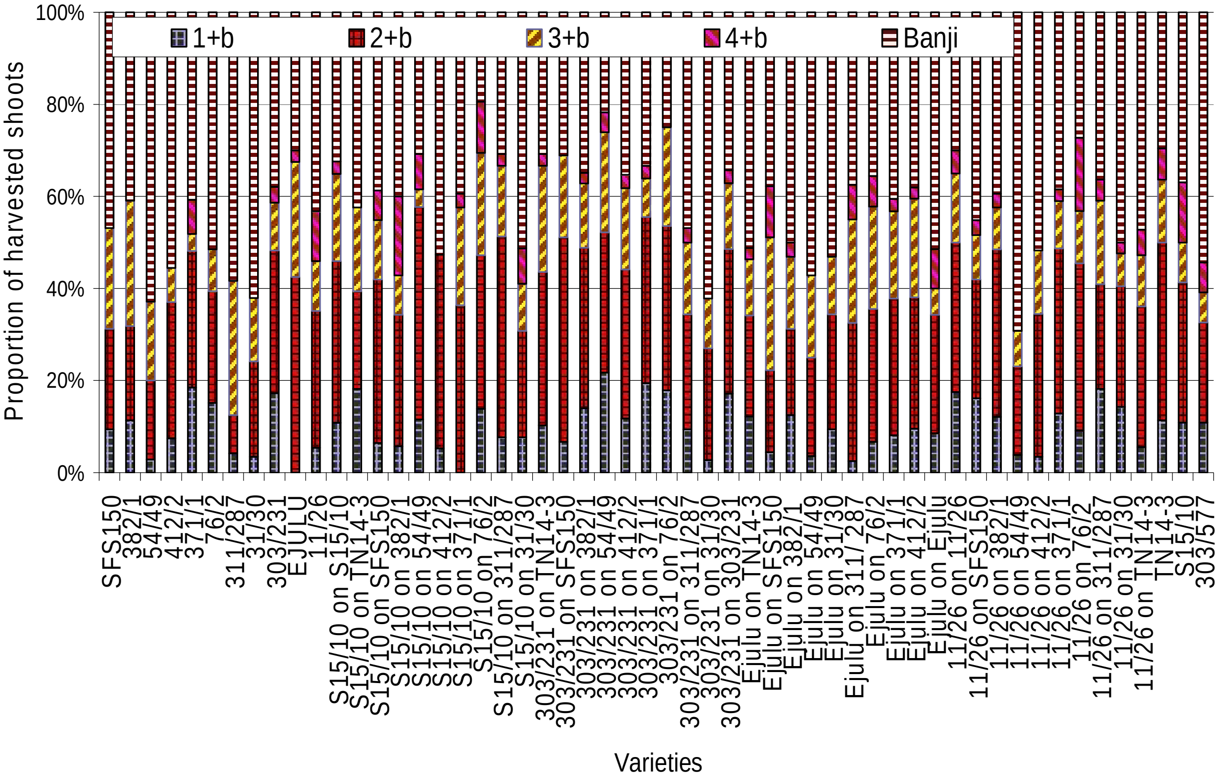

Figure 6.3. Variation in proportions of harvested shoots at various stages of growth of scions grafted to different rootstocks of varying ploidy during the period of December 2004- March 2005 (warm and dry season), at Kipkebe, Sotik.

In this study, there were similar seasonal effects on varietal shoot composition as other studies done in clonal tea in the past. AHP S 15/10 and EPK TN 14-3 had varying seasonal composition, and up to 30% dormant shoots were reported in dry seasons and cooler parts of the year (Ng'etich, 1995). The high dormant shoot composition recorded in this experiment may have been due to low altitude and higher air temperatures and soil water deficits as discussed in

Chapter 5. Stephens and Carr (1994) reported a linear relationship between shoots mass and stages of development of clone TRFK 6/8. These relationships have tea-plucking implications due to the fact that leaving shoots to develop from one mature shoot and a bud to three mature leaves and a bud may increase the shoot mass, because of increased dry matter. On the other hand, these shoots lead to reduced quality of made tea.

During the cool and wet season, there was a significantly ($P \leq 0.05$) higher proportion of two mature leaves and a bud, which accounted for 40% of the shoots, followed by 18% of one leaf and a bud, 10% of three leaves and a bud (Figure 6.4). Although varietal differences occurred, they were minimal. The amount of dormant shoots was larger (>40%) than was noted in the previous warm and dry season. This could be attributed to significantly lower air temperatures. Temperatures play a major role in dormancy release such that as low temperature persists, shoot development is retarded. Also, the large clonal differences observed in tea have been similarly reported for spruce (*Picea sitchensis*). Based on this, cold hardiness demonstrated a potential for selecting cold tolerant clones for tea (Nicoll *et al.*, 1996).

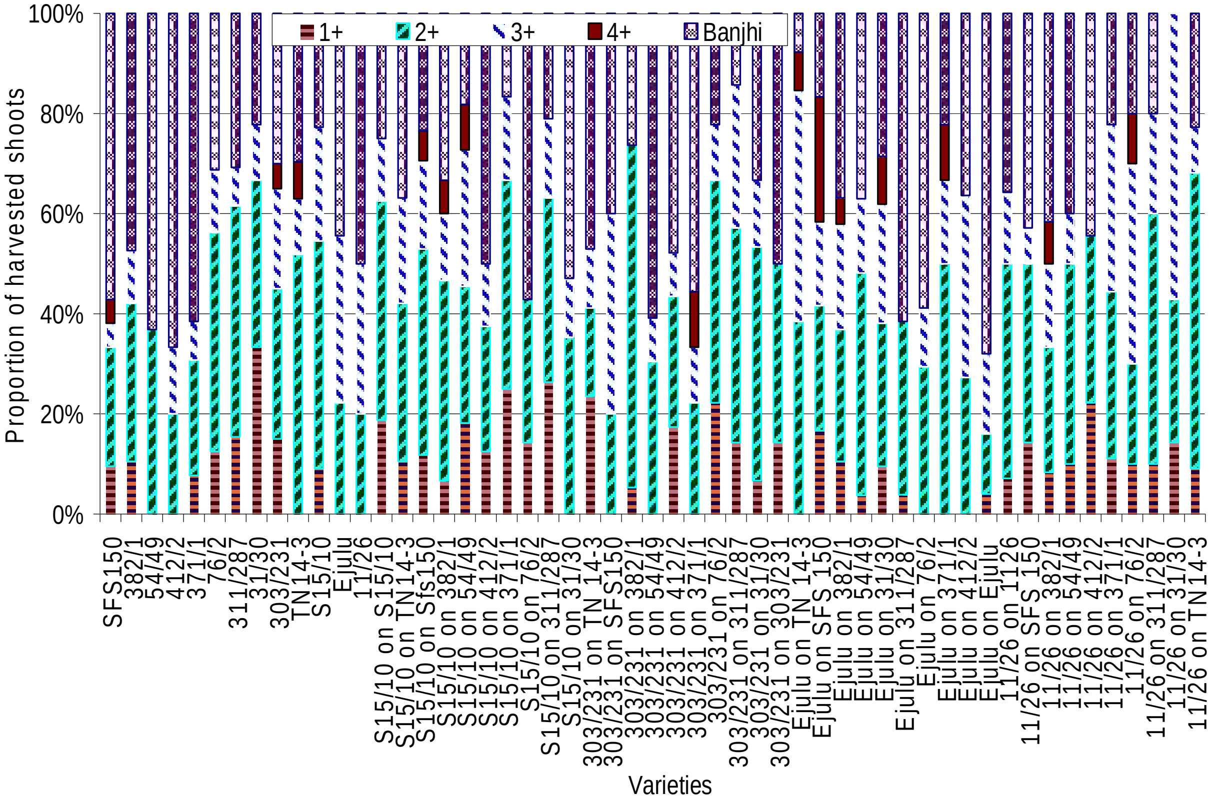

Figure 6.4. Variations in proportions of shoots harvested from scions grafted to rootstocks of varying ploidy during the cool and wet seasons in July 2005, at Kipkebe, Sotik

Varieties differ in response to temperature with low temperatures having significant effects on tea growth (Cannel, *et al.*, 1990). High proportion of dormant shoots in the leaf sampled during this cool season was related to low temperatures experienced during most of the season.

In the warm wet season, there were fewer differences in shoot composition. Other than AHP S15/10 on EPK TN 14-3, which had 22% of the one leaf and a bud, the other clones had a mean of less than 10%. Majority of shoots were mature two leaves and a bud that accounted for 40%. The three leaves and a bud were 20%, four leaves and a bud, less than 10% and the rest were dormant shoots (Figure 6.5).

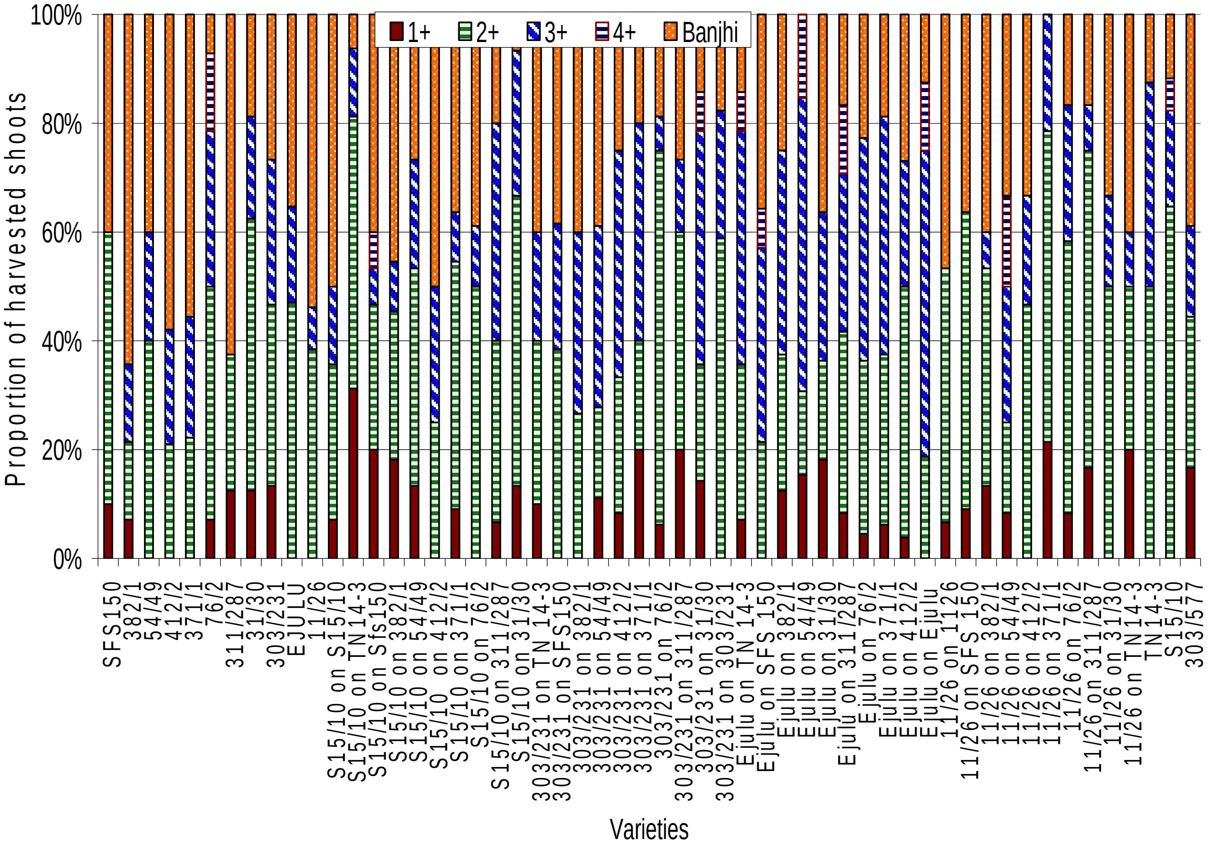

Figure 6.5. Variation in proportions of harvested shoots from scions grafted to rootstocks of different ploidy during the warm wet/ dry season of September-November.

The results obtained in this study show that shoot composition was season dependent, and the composition consisted of over 30% dormant shoots during the cooler part of the year. The smallest proportion of one mature leaf and an immature bud accounted for less than 20%. The results, though some variations occurred concur with those of Ng'etich, (1995) and Burgess (1992b).

The variations could be due to differences in clones and the testing sites, which had different environmental parameters. Differences in environmental factors particularly, temperatures and rainfall, have been mentioned as significantly influencing clonal responses (Ng'etich and Stephens, 2001). The seasonal changes had effects on shoot composition that were similar to patterns of SWD and SVPD (Chapter 5), showing that the responses obtained were most likely to these environmental variables.

6.4. CONCLUSIONS

The study aimed at determining the influence of seasonal changes on shoot development; population density and composition of scion clones grafted on diploid, triploid and tetraploids rootstocks. The results showed that the shoot extension rate was high during the cool wet and warm wet season. The lowest rate of development was in warm dry season, and this was attributed to high soil water deficit, temperatures, and evapotranspiration. The tetraploids TRFK 311/287 and TRFK 31/30 and triploid TRFK 412/2 had 40% higher leaf area than the diploids. Tetraploids had few shoots, hence, registered low yields. The large leaf area and shoot density of scion clone AHP S15/10 significantly contributed to its high yields. Clone GW Ejulu L had low shoot density. The proportion of dormant shoots was high during the warm dry season, while low shoot density was common in cool wet season. Low air temperatures significantly reduced the shoot density. The season and clones affected the shoot composition. The tetraploids were found to be more dormant during the cool wet season. The rootstock ploidy mainly influenced shoot density with diploid rootstocks out performing triploids and tetraploids during warm wet and warm dry seasons. Conclusively, shoot developments and extension is dependent on temperatures, saturation vapour pressure deficit and soil water deficit, therefore directly affecting the yield of composite tea.

CHAPTER SEVEN

7.0. GENERAL CONCLUSIONS AND RECOMMENDATIONS

In Kenya, tea is a major foreign exchange earner and may be threatened by lack of land for future tea expansion. Seedling tea earlier planted between 1920’s – 1950’s accounts for 20% and 38% in both KTDA and KTGA sectors, respectively. The main focus of tea plantations then, is to maximise on the yields and clonal improvement through an understanding of their physiological responses to varying environmental factors such as soil water contents, SVPD and temperatures. The objective of this study was to quantify physiological responses of composite tea to soil moisture deficits. Experiments were conducted both in the field and in semi-controlled rain out shelters to test the composite tea plants that were identified for the study in order to address the key objective.

7.1. Soil water stress and physiological responses and dry matter partitioning

Various physiological parameters were used to quantify the contributions to yield variations. These included the leaf water potential, gas exchange and dry matter. The effects of soil water stress on some physiological parameters and dry matter partitioning in composite tea plants showed that there were significant ($P<0.05$) variations in shoot development, shoot density and yields among scions, rootstocks and soil water deficits. Yields declined with increasing soil water deficits. The yield components such as shoot population density were influenced by the seasons. These variations contributed significantly to yields recorded in various seasons. Shoot composition also varied and had a major bearing on the air temperatures, soil water deficits, and SVPD. There were significant interactions between varieties, seasons, and dry matter partitioning. Dry matter partitioning is therefore is an important component in the choice and development of composites. Water stress significantly ($P<0.05$) affected stomatal conductance and transpiration, and clonal variations were evident. The gas exchange changes were not related in the well-watered treatments ($R^2=0.4181$, $P>0.05$) but were highly significant ($R^2=0.8064$, $P<0.001$) for the stressed treatments.

Maintenance of high stomatal conductance by scion clones on rootstocks EPK TN 14-3, TRFK 57/15 and TRFK 303/577 suggested high tolerance to water stress. Diurnal differences of stomatal conductance were more pronounced in the afternoon suggesting that, soil moisture status had an influence on diurnal stomatal activity. Leaf water potential differed between well watered, stressed plants and clones, with more variations in stressed plants. Water stress significantly ($P<0.05$) affected dry matter partitioning, with more dry matter being allocated to roots in stressed treatments. In watered treatment, less than 60% of the dry matter was allocated to roots, and 20% each to stems and leaves, respectively.

Shoot: root ratio, leaf area and stem diameter declined under water stress. However, water stress increased root length density, but reduced the root depth.

Reduced leaf area is a strategy to minimize further water loss. It is concluded that physiological parameters and dry matter allocation are strongly influenced by water stress.

7.2. Effects of soil water deficit and rootstocks on tea yield distribution

Seasonal changes in yields, soil water deficit, water use efficiency and dry matter partitioning were monitored to evaluate the influence of soil water deficit on scion varieties grafted on diploid, triploid and tetraploids rootstocks. There were significant differences in SWD with the maximum being 247, 198.93, and 215.61 mm for 2003, 2004, and 2005, respectively. Air temperatures ranged between 20.8 and 21.2 ^{o}C and had a strong influence on plant growth, development and yields. Yields decreased (R^2=0.7379, P<0.001) with increase in SWD and clonal variation was evident with AHP S15/10 having a decrease of 0.97 kg mt/ha/week (mm SWD)$^{-1}$. Grafting depressed the yield of the scion varieties, and showed a significant (P<0.05) decrease with increase in rootstock ploidy. The water use efficiency significantly (P<0.001, R^2=0.87, 0.72) varied and was in the order of diploids>triploids>tetraploids rootstocks, for years 2003 and 2004, respectively. Although the scions had varying proportions of WUE, it was in the following ascending order S15/10>303/231>11/26 and Ejulu in all the three seasons.

The varied proportional seasonal decrease in scion performance was in the range of between 33 – 66% between the highest and lowest yielding, respectively. Root biomass was highest for diploid rootstocks; EPK TN 14-3 and TRFCA SFS150 and intermediate for TRFK 371/1 (triploid) and low for TRFK 31/30 (tetraploid). Root depth had a significant correlation ($R^2=0.73$, $P<0.001$) with yield despite the net average effect of grafting being negative. Rooting depth is therefore an important trait for rootstocks, and clones have to be considered for their potential use as rootstocks based on this criterion. In conclusion, it is suggested that where grafting is considered necessary, diploid rootstocks be used.

7.3. Shoot development, population density and composition

The shoot extension rate was high during the cool wet and warm wet season. The lowest rate of development was in warm dry season, and this was attributed to high soil water deficit, high temperatures, and high evapotranspiration. The tetraploids TRFK 311/287 and 31/30 and triploid TRFK 412/2 had 40% higher leaf area than the diploids. Tetraploids had few shoots, hence, registered low yields. The large leaf area and shoot density of scion clone AHP S15/10 significantly contributed to its high yields. Clone GW Ejulu L had low shoot density and yields.

The proportion of dormant shoots was high during the warm dry season, while low shoot density was common in cool wet season. It is suggested that the low air temperatures significantly reduced the shoot density, hence contributing to the low yields observed. The season and clones affected the shoot composition. The tetraploids rootstocks were found to be more dormant during the cool wet season. Conclusively, shoot developments and extension rate was found to be dependent on temperatures, SVPD and SWD, therefore directly affecting the yield of composite tea.

7.4. Physiological responses of composite tea to progressive drought

The total SPAD values together with the corresponding chlorophyll contents varied with varieties and decreased with increase in soil moisture content. Rootstocks did not affect the chlorophyll contents. However, there were differences between clones, and this could significantly affect the photosynthetic capacity. The reduced soil moisture content reduced the leaf water potential of all the clones. The diurnal differences in leaf water potential were more pronounced at the low (12.5%) soil moisture content. Generally, the mean leaf water potential increased with a decline in soil moisture content. Based on this study, the minimum moisture level below which composite tea plants might suffer water stress could be 20% v/v, and the exposure period should be short. Based on the drought tolerance in these results, the rootstocks identified as being potential for commercialization are TRFK 57/15, TRFK 8/112, TRFCA SFS 150 and EPK TN 14-3. Among the Scions, AHP S15/10 was found to be more susceptible to water stress.

Relative water content declined with decrease in soil moisture content (R^2= 0.9915, P<0.001) in all the teas. Both relative water content and leaf water potential could effectively be used as water stress indicators in composite tea.

7.5. Future directions

The study highlighted some areas where significant physiological effects on composite tea are influenced. There are however, areas that require further investigations in order to exploit the use of composite plants in the tea plantations. The progressively increasing shortage of new planting area for tea is crucial considering its potential as a major foreign exchange earner. Potential use of composite tea needs to be considered in uprooted and replanted areas particularly where old seedling tea requires to be uprooted and replaced with high yielding quality clones. These areas constitute an average of 27% of all the tea industry in Kenya. It is recommended that composites should be developed to constitute the high yielders, good quality varieties, and tested over a wide area with varying environmental conditions. Yield variations need to be quantified so long as the key environmental variables such as rainfall, temperature, SVPD, and SWD data are kept for ease of application in modelling and accurate prediction of potential yields.

Extrapolation of physiological responses needs to be treated cautiously because of the high variation between rootstocks and scions. The issue of compatibility also needs to be established within the two components, considering the fact that the chemical quality parameters and constituents are different between scions and rootstocks. This will achieve the desired goals of certain attributes. In this study, SWD and temperature were the main variables determining the potential of dry matter production and indirectly, yields. Dry matter differences were attributed to rate of shoot development in different seasons; shoot extension, and yields with temperatures that strongly affected the varietal performance.

In the warm dry season, the response to SWD was significant. This was common in 2003, where yields were drastically reduced. This SWD variation contributed to WUE differences between clones. More studies are suggested to address yield of composites, with respect to drought and seasons in low altitude sites where there are long drought periods. More studies on composites are likely to increase in the near future especially considering the global warming which is likely to increase overall temperatures and SWD, and therefore significantly reduce tea production. Economics on the potential use of composites need to be quantified to advise accordingly on the foregone yields verses other good attributes of composites. More rootstocks need to be further tested along with scion clones that are needed provided they have attributes as may be desired by the farmers, processors and market forces that influence tea consumption.

It is suggested that controlled experiments be done to clearly define the possible exact SWD range for tea. This was found to be a limitation of this study as the experiment was carried out under field conditions where several other variables were in play.

This study has contributed to a better understanding of physiological responses of composites as may be influenced by SWD, temperatures, and SVPD. Therefore, composites may be developed for marginal areas, provided that diploid rootstocks are used.

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