GENETIC PARAMETERS AND QUANTITATIVE TRAIT LOCI MAPPING IN TEA, *Camellia sinensis* (L.) O. Kuntze

Samson Machohi Kamunya

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DECLARATION AND RECOMMENDATION

DECLARATION

This thesis is my original work and has not wholly or in parts been previously presented for the award of a degree in this or any other university.

Signature:____________________________ Date:_______________

Samson Machohi Kamunya
(KD12/0158/05)

RECOMMENDATION

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

Signature:__________________________ Date:________________

Prof. F.N. Wachira
Department of Biochemistry and Molecular Biology
Egerton University
P.O. Box 536-20115
Njoro, Kenya.

Signature:__________________________ Date:________________

Prof. R. S. Pathak
Department of Crops, Horticulture and Soil Science
Egerton University
P.O. Box 536-20115
Njoro, Kenya.

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DEDICATION

To my daughter, Evelyne Wanjiru and son, Michael Kamunya

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ABSTRACT

Efficient breeding and selection of elite tea (*Camellia sinensis* (L.) O. Kuntze) clones require sound knowledge and understanding of genetics associated with yield, quality and tolerance to biotic and abiotic stresses. A series of experiments were carried out to elucidate the genetic parameters and map quantitative trait loci (QTL) for agronomic and quality attributes in tea. The combining abilities and heterosis for yield, drought tolerance (DT), and quality traits (percent total polyphenols (%TP), fermentability (FERM), theaflavins (TF), thearubigins (TR) and pubescence (PUB)) in tea were estimated in a 4 x 4 full diallel analysis. Generally, parents with good combining ability produced progeny with above average performance for all the evaluated traits. The general combining ability (GCA) effects were significant for yield, %TP, FERM, DT, TF, TR, PUB and bud weight, while specific combining ability (SCA) effects were significant for %TP, FERM, PUB and bud weight. Strong maternal effect for all traits was evident except for thearubigins and bud weight signifying the importance of female parents in breeding for yield, DT, and diversified tea products such as silvery tips.

The heterosis analysis revealed that the mid-parent heterosis (MPH) and the better-parent heterosis (BPH) averaged across the families, were high for fermentability (MPH: 108.5%; BPH: -12.82) and DT (MPH: 16.44 %; BPH: 2.30%) but not so for yield (MPH: 2.17%; BPH: -5.09%) and total polyphenols (MPH: -2.81%; BPH: -5.71%). The heritability estimates for yield (h^2 = 0. 44 ± 0.16; H^2 = 0.56 ± 0.15), DT (h^2 = 0.61 ± 0.06; H^2 = 0.96 ± 0.01) and fermentability (h^2 = 0.45 ± 0.04; H^2 = 0.98 ± 0.01) were high indicating that the three traits are highly heritable and could be improved through hybridization and judicious clonal selection. Estimates of genetic gains indicated that moderate gains are achievable on clonal selection for characters such as yield and fermentability at 14.1% and 8.9%, respectively, while DT and PUB registered higher gains at 20.6% and 30%, respectively, based on h^2.

Mapping of QTL linked to yield, %TP, DT and shoot traits was assessed on a pseudo-test cross comprising 42 clonal progeny between clones TRFCA SFS150 and AHP S15/10. QTL associated with root knot nematode resistance were investigated on a different cross consisting of 41 clonal progeny arising from TRFCA SFS150 and TRFK 303/577. Bulk segregant analysis was performed followed by complete genotyping. Out of 260 informative markers, 100 markers that showed 1:1 segregation were used to construct a linkage map. The map contained 30 (19 maternal and 11 paternal) linkage groups that spanned 1411.5 cM with mean interval of 14.7 cM between loci. A total of 64 QTLs controlling various traits across the two sites were detected. Of these, QTLs linked to YLD-T, YLD-K, DT-K and PUB were localised at 2 cM, 2.7 cM, 3 cM and 1.4 cM from markers OPG-07-2800, E-AGC/M-CAG-725, OPT-18-2500 and OPO-02-650, respectively. No QTL was detected at both sites, which showed strong genotype x site interaction (G x E). Marker OPF-09-600 that co-segregated ($P < 0.0000$) with susceptibility to the root knot nematode was mapped at 0 cM from locus OPF-09-600 on linkage group 8. Considering the long time expended in developing improved tea varieties, the identification of putative QTLs tightly linked to agronomic traits augmented by known genetic parameters provide room for marker-assisted selection thereby hastening tea improvement efforts.

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LIST OF ABBREVIATIONS AND ACRONYMS

AFLP	Amplified Fragment Length Polymorphism
AHP	African Highlands Produce
ANOVA	Analysis of variance
BC	Backcross
CAT	Clonal adaptability trial
CFT	Clonal field trial
CSIR	Council of Scientific Industrial Research, India
Epistasis	Interaction of non-allelic genes (viz. gene interaction where one gene pair affects the expression of another gene pair)
F_1	The first filial generation produced by crossing two parental lines
GCA	General combining ability
h^2	Narrow sense heritability
H^2	Broad sense heritability
IHBT	Institute of Himalayan Bioresource Technology, India
LOD	Log of odds
LRS	Likelihood ratio statistic which is equal to 1/4.6 base-10 LOD
MAS	Marker assisted selection
PCR	Polymerase chain reaction
PT	Progeny testing
QTL	Quantitative trait locus
r	Coefficient of correlation
R^2	Coefficient of determination
RAPD	Random Amplified Polymorphic DNA
SCA	Specific combining ability
SSR	Simple Sequence Repeats
St	Stock
TBK	Tea Board of Kenya
TRFK	Tea Research Foundation of Kenya
TWAS	Academy of Sciences for the Developing World
Type I error	The error that results when the null hypothesis is falsely rejected
Type II error	The error that results when the null hypothesis is falsely accepted

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background information

The tea plant, *Camellia sinensis* (L.) O. Kuntze, provides different types of beverages, which are the most popular non-alcoholic and soft healthy drinks across the world. The beverages include black, green and oolong teas which constitute about 78%, 20% and 2%, respectively, of the total world production (Basu, 2003). The tea plant is indigenous throughout the forests of south-east Asia, where in its natural state, grows to a height of 30-40 feet. Its centre of origin is thought to be the indefinite belt to the south-east of the Tibetan plateau encompassing Sze-Chuan, Yu-Nan, North Vietnam, Burma, Siam and Assam in north-east of India. The tea plant has been introduced into and become naturalized in many areas of the world and is currently found in many continents. It can be found growing near all old trading routes between China and India and in the islands of south-east Asia, Japan, Europe, North and South America, Africa and Australia. It is cultivated as far north as Georgia (42°N) on the eastern shores of the black sea in southern Russia and as far south as Argentina (27°S) in South America and South Africa (Weatherstone, 1992).

The tea industry plays a significant role in the economy of Kenya where the crop is the leading foreign exchange earner and export commodity. In 2007, Kenya exported over 360 million kilograms of made tea, which earned the country over KSh 43 billion in foreign exchange (www.teaboard.or.ke; TBK, 2008). Tea contributes approximately 26% of the export earnings and 4% of the Gross Domestic Product (GDP) (TBK, 2004). As tea growing is a rural based enterprise, it has contributed to enhanced living standards of rural communities and has led to the development of infrastructure such as tea manufacturing factories, better road networks, schools, hospitals and other amenities that would otherwise have remained under-developed. Since the introduction of tea in Kenya, it has steadily continued to expand in acreage under cultivation with the current land occupied by the crop reaching 149,196 ha by 2007 (TBK, 2008). The sector supports other sectors of the economy and provides a source of livelihood to over 3 million people (TBK, 2004). Being a rural based industry, tea enterprises have therefore contributed to stemming rural-urban migration. Sustainability of tea and its potential to produce diversified products are therefore crucial to the country's economic wellbeing.

Tea was introduced and cultivated commercially in Kenya during the first quarter of the 20th century using seeds from Assam in India. The early tea plantations comprised seedling tea types, which later formed the basal populations from which improved seedling-type (Jat) selections were made. The great demand for planting material that ensued coupled with lack of suitable vegetative propagation techniques led the major planters to establish seed gardens using improved Jat selections. As seedling teas are mixed genotypes, uniformity and sustainability (stability) in yield and black tea quality could not be attained. Meanwhile research on creating uniform tea fields by vegetative propagation was going on in various tea growing countries (Goodchild, 1960; Green, 1964). This subsequently elicited the search for superior bushes culminating in the development and release of clonal teas with high yield and black tea quality from the mid 1960s. Clonal plants currently account for about 60% of all the tea in the country, which has resulted in the instant fixation of few genotypes and consequently narrowing of the genetic base of the commercial germplasm (Wachira, 2002). Over-dependence on a limited number of clones probably of a common ancestry has not only minimised the on-farm diversity but also increased the risks posed by such co-evolving biotic factors such as pathogens and pests as well as the ever changing abiotic stress factors. The dangers of utilizing varieties with a narrow genetic background can be demonstrated by the fact that of all released clonal teas for commercial use, 67% share the same female parent, clone TRFK 6/8 which has been observed to be susceptible to root knot nematodes (Otieno *et al.*, 2002). Emerging information has indeed revealed that of all the tested TRFK 6/8 progeny for root knot nematodes resistance, none showed reliable level of tolerance. This indicated that majority of them might have inherited the nematode susceptibility genes from their maternal parent, clone TRFK 6/8 (TRFK, 2004; Kamunya *et al.*, 2008).

Tea production in Kenya has improved tremendously over the years with the notable replacement of low yielding seedling teas with high yielding and better quality clones (Wachira, 2002). Kenyan tea has imprinted a place in the world for its high black tea quality and yields. Despite this, it has not yet been possible to produce clones with optimum leaf yield, black tea quality and tolerance to important biotic and abiotic stress factors. The early tea improvement efforts hardly paid adequate attention to such secondary traits as tolerance/resistance to abiotic or biotic stress factors. Yet, changing global climatic trends accompanied by unprecedented weather phenomena and agricultural preferences have resulted in emergence of new or increase in virulence of endemic pathogens and pests. Similarly, prolonged drought periods are more common now, for example the 1997 and 2000 *La nina* related drought and freak frost incidences. In a localized area around Mt Kenya, tea

mites, particularly the red crevice (scarlet) mite (*Brevipalpus phoenicis*), cause serious infestation on tea with yield losses of 14 to 30% being recorded (Sudoi, 1995; Sudoi *et al.*, 1996). Incidences of root knot nematodes (*Meloidogyne* sp.) damage on clone TRFK 303/577, a popular variety with high yields and drought tolerance, are now widespread (Otieno *et al.*, 2002; Kamunya *et al.*, 2008) and damages associated with diseases such as *Hypoxylon* wood rot and *Armillaria* root rot are increasing by the day.

The tea plant is a highly outcrossing and strongly but not absolutely self-incompatible tree species (Rogers, 1975; Wachira and Kamunya, 2005a; Muoki *et al.*, 2007), owing to which it is highly heterogeneous and heterozygous (Banerjee, 1992). The allogamous nature of tea coupled with its long generation time and large plant size severely limit development of true hybrids through conventional breeding. The doubled haploid technique is attracting a lot of attention at overcoming this constraint although no useful results have been generated to date (Banerjee, 1986; Mondal *et al.*, 2004). It is currently held that integrating both the biotechnological methods and conventional breeding approaches could result into further improvement of the existing cultivars that otherwise possess one or two undesirable traits. More importantly, the integration of molecular markers in breeding programmes provides a powerful tool for enhancing the efficiency and effectiveness of selection based on identification of quantitative trait loci linked to important agronomic traits. Though a genetic linkage map for tea has been constructed (Hackett *et al.*, 2000), no reports on quantitative trait loci analysis (QTLs) have been published to date.

The above-mentioned shortcomings notwithstanding, significant strides have been made in tea improvement, although faster progress in the development of improved cultivars would have been achievable had it not been for the general lack of knowledge in gene action. Little is known on the combining abilities for the primary desirable traits like yield and quality as well as other secondary attributes. As such, the success of obtaining desirable crosses is unpredictable since crossing activities are based on chance rather than informed choices of progenitors. The acquisition of information on combining abilities for the most important traits is a prerequisite in determining the most suitable mating designs and appropriate parents to involve in the hybridization programmes. By analysing the combining abilities, clues of the nature of gene action and appropriate parents for the target traits could be revealed (Can *et al.*, 1997).

The role of good combiners in all aspects of tea breeding including the use of heterosis coupled to genetic distance of the target parents has rarely been emphasized which are a prerequisite that cannot be ignored. Fortunately for tea, numerous such data (Wachira *et*

al., 1995, 1997, 2001) exist and thus directed breeding approaches are likely to yield encouraging results.

Owing to lack of knowledge about the combining abilities of different progenitors, linkage and inheritance of many characters of economic importance, tea breeders have traditionally relied on phenotypic selection to develop new varieties. However, phenotype is determined by genetic and environmental factors, and usually the effects of the environment mask those of the genes. As such the true genetic potential of plants is not expressed. Moreover, hybridization of progenitor clones with unknown combining abilities for all traits of interest has resulted in development of segregating populations of inferior breeding value. These populations demand huge tracts of land and time needed to be able to extract an elite progeny from field tests. To circumvent this problem, it is imperative to develop methods of identifying genetic markers linked to genes controlling economically important agronomic and physiological traits. There are numerous types of genetic markers that can be used to characterize plant germplasm. These include; morphological markers, cytogenetic markers, biochemical markers (isozymes/allozymes), and molecular markers. The first three markers are amenable to environmental influences and low in abundance. Recent efforts, therefore, have concentrated on detection of molecular markers which are abundant in number, independent of environmental effects, can be assayed easily at any stage of the plant's development, and are free from pleiotropic or epistatic effects.

Molecular markers can be used to elucidate the relationship between heterosis and genetic distance and thereby contribute to the development of heterotic hybrid clones. For majority of the agronomic characters, heterosis can only occur if the character in question is under the influence of substantial non-additive gene effects (namely dominance, overdominance or epistasis) or additive gene effects with high heritability.

1.2 Statement of the problem

Tea breeding is a highly protracted activity due to the crop’s long generation time from seed to flower (3-6 years), allogamous nature and large genome (2n = 30). To obtain an improved seedling population it takes about 21-26 years, while superior clonal plants may take 8-10 years to be extracted from such a population. Additionally, it takes 21-30 years for tea to attain optimum yield potential (Gazi, 1978). The likelihood of obtaining superior recombinants is low when parents of unknown combining abilities are crossed. This is further complicated by the lack of knowledge on inheritance patterns of quantitative traits and polymorphism of molecular markers linked to desirable traits and heterosis. Since most of the

agronomic traits of tea are quantitative in nature and therefore are governed by several genes, they are not easily manipulated in breeding programmes without elaborate and long-term field testing, often in more than one environment in order to determine their inheritance, adaptability and stability. However, such experiments are expensive to maintain in terms of manpower and time consumed as well as land resources required for field testing. If simple molecular markers linked to major quantitative trait loci (QTLs) for the most desirable traits are identified, they could be utilized for the early selection of elite clones thereby saving on time, cost of maintenance of field trials and land resources that would otherwise be required. Further, the use of QTLs in breeding would permit the discrimination of clones having the same phenotype for a quantitative trait but determined by different genotypes (genes).

1.3 Justification

Owing to lack of understanding of the genes controlling characters of economic importance, the development of new varieties of tea is carried out by selecting plants with desirable phenotypes. A plant's phenotype is however determined by interaction of its genetic constitution and the environment in which it is grown. As the influence of the environment occasionally masks those of the genotype, the phenotypic measurement does not reveal the true status of the plant's genetic potential. Many desirable traits of tea require several years to develop and the long duration of testing coupled with highly heterozygous and heterogeneous populations make breeding a highly protracted procedure. Thus, any tool that can help in shortening it could be quite valuable. Genetic linkage maps serve as vital tools for relating genotype to phenotype, which enable the identification and selection of recombinant individuals with desirable attributes based on associations of these attributes and some more easily determined character.

A base genetic linkage map for tea using two heterozygous parents and an F_1 segregating population has been constructed (Hackett *et al.*, 2000). However, the map was assembled using markers from the female parents only. Exploratory statistical analysis based on molecular data revealed patterns of markers which could easily be explained by the hypothesis of three male parents contributing pollen to the cross and hence construction of only the female map (Hackett *et al.*, 2000). The map therefore has 15 linkage groups corresponding to the haploid number of tea. Preliminary quantitative trait loci (QTL) analysis was carried out which revealed that markers and marker regions with significant effects on quantitative trait expression could be identified (Wachira, 1996b). The quantitative phenotypic measurements were however only undertaken on individual bush basis but

replicated in time. As such the number of effective factors (alleles) segregating in the cross for each quantitative trait could not be fully established. As reliable detection of DNA markers associated with quantitative trait loci (QTL) critically depends on precise and accurate determination of phenotypic values of individuals in segregating populations, the use of replicated trials becomes mandatory (Ortiz, 1996). From an applied plant breeding perspective, the biggest immediate reward from QTL mapping would be the development of marker-assisted selection (MAS) for superior genotypes. This would involve the indirect selection for the desired phenotype using informative markers flanking the QTLs. Since MAS can be performed at the seedling stage, years or decades before the selected trait is fully expressed in the adult phenotype, the savings of time, space, and testing effort are considerable. As tea has a long juvenile period and generation interval, the applicability of MAS can significantly and positively impact tea improvement efforts. Besides MAS, other significant short-term and long-term benefits accruing from QTL mapping include the use of markers developed for genome mapping in assessment of clonal identity and study of natural populations from which germplasm for breeding are sought.

Determination of the combining abilities of potential progenitor clones will aid in identification of appropriate parents for recombination through hybridization and hence contribute to generation of superior segregating populations (heterotic crosses) which will require fewer resources to screen for extraction of elite bushes.

1.4 Objectives

1.41 Broad objective

The broad objective of the study was to develop techniques for enhanced tea productivity, quality and product diversification in Kenya.

1.4.2 Specific objectives

1. To estimate general and specific combing abilities and heterosis for yield, quality, drought tolerance and pubescence of four tea clones in a diallel cross.
2. To determine maternal effects in four reciprocal crosses of tea.
3. To identify and map out molecular markers (RAPDs, SSRs, and AFLPs) linked to important quantitative trait loci (QTLs) for yield, quality, drought tolerance and root knot nematode resistance/susceptibility.

1.5 Null hypotheses (H_o)

1. The estimated general and specific combing abilities and heterosis for yield, quality, drought tolerance and pubescence of four tea clones are not significantly different.
2. Maternal effects in four reciprocal crosses of tea are not significantly different.
3. Molecular markers are not linked to important quantitative trait loci (QTLs) for yield, quality, drought tolerance and root knot nematode resistance/susceptibility in tea.

CHAPTER TWO

LITERATURE REVIEW

2.1 Botany of tea

The tea plant, *Camellia sinensis*, belongs to the genus *Camellia*. According to Sealy (1958), the genus contains 12 sub-generic sections, one of which (*Thea*) contains species of cultivated tea. Sealy (1958) reported some 82 species in the genus *Camellia* of which *Camellia sinensis* is the most important both commercially and taxonomically. Chang and Bartholomew (1984), however, recognized over 200 species after revising earlier works. They divided the genus into four subgenera; *Protocamellia, Camellia*, *Thea* and *Metacamellia* and twenty sections.

Owing to the heterogeneity and many overlapping morphological, biological, biochemical and physiological attributes (Purseglove, 1968; Wickremasinghe, 1978), the taxonomy of tea has been very complex (Banerjee, 1988). This has been complicated by the free hybridization between species of the genus, which has led to formation of many interspecific hybrids (Chuangxing, 1988). Thus, genetic relationships, taxonomy and discovery of many new species have remained controversial (Lu and Yang, 1987; Chuangxing, 1988; Tien-Lu, 1992). Apart from *C. sinensis*, numerous other *Camellia* species are used as beverage in parts of China and Indo-China. These include *C. taliensis, C. irrawadiensis, C. grandibractiata, C. kwangsiensis, C. gymnogyna, C. crassicolumna, C. tachangensis* and *C. ptilophylia* (Chang and Bartholomew, 1984; Banerjee, 1992). Several other species including *C. fraternal* are being exploited for seed oil, which is used for cooking and in pharmaceutical and cosmetic industries. Additionally, many *Camellia* species are of great ornamental value.

At species level, several intergrades resulting from unrestricted intercrossing between disparate parents have been documented, but these have not been assigned the status of separate species (Sealy, 1958). However, three distinct tea varieties namely the China variety, *Camellia sinensis* var *sinensis* (L), the Assam variety, *Camellia sinensis* var *assamica* (Masters) Kitamura and the southern form known as the Cambod race, *C. sinensis* var *assamica ssp. Lasiocalyx* (Panchon ex Watt) have been identified based on leaf features such as size, pose and growth habit (Sealy, 1958). The three main taxa can also be differentiated by foliar, floral and growth features (Sealy, 1958; Hadfield, 1974) and by biochemical affinities (Robert *et al.,* 1958; Sanderson, 1963; Ozawa *et al*., 1969; Takeo, 1983; Hazarika *et*

*al.,*1984; Nagata, 1986; Owuor *et al*., 1986; Magoma *et al*., 2000). While the China variety is a dwarf and slow growing shrub, the Assam variety is a tall and quick growing tree. The Cambod variety is thought to be an intermediate between the main taxa, which has now been confirmed by molecular marker studies (Wachira *et al.,* 1997; 2001). Owing to the outbreeding nature of tea and the resultant heterogeneity, most cultivars exhibit a cline extending from extreme China-like plants to those of Assam origin. Thus, it is doubtful whether archetypal (original) *C. sinensis, C. assamica* or *C. assamica ssp. Lasiocalyx* still exist following years of extensive hybridization among the three taxa (Visser, 1969). However, the numerous tea hybrids currently available are still referred to as Assam, Cambod, or China varieties depending on their morphological proximity to the main taxa (Banerjee, 1992).

2.2 History of tea improvement in Kenya

2.2.1 Tea introduction into Kenya

Tea was reportedly introduced in Kenya by the Caine brothers (British colonial farmers) who imported dark-leafed “Manipuri” hybrid seed from Assam in 1904 and 1905 to establish a plantation in Limuru, Central Kenya (Greenway, 1945; Matheson, 1950; Singh 1979; Wilson *et al.*, 1962). The tea seedling populations arising from this planting became the source of seed for subsequent planting. In 1912, Chinary (*sinensis*) seed was imported from Sri-Lanka to establish a plantation of tea with high quality and yield (Matheson 1950). According to Matheson (1950), little interest existed over the next 12 years except for several small plantations that were established in Limuru in the East of Great Rift Valley and in the West of Great Rift Valley mainly at Kericho and Kaimosi (Figure 1). However, advice given by Howland brothers in 1924 on the use of quality seed from the light coloured leaf Assam or Manipuri types for drought resistance stimulated serious planting by several companies. The planting expanded rapidly and by 1929, there were 2,162 ha of tea in Kenya (Greenway, 1945). By 1963, the acreage increased to 21,448 ha and in 2007, the acreage stood at 149,196 ha (TBK Statistics, www.teaboard.or.ke; TBK, 2008).

Some hybrid seed was introduced from the Mt Vernon estate in Sri Lanka (Anon., 1962) although the total contribution of this seedlot to the pioneer plantations is unknown. From 1960s, tea seed was also introduced from Uganda (Wachira, 2002). These seed was originally derived from germplasm introduced from Dangri Manipuri, Betjan Assam and Rajghur in India (Anon., 1962). In all cases of seed introduction, no data on collection and

passport descriptors were kept (Wachira, 2002). Scanty information on actual seed source notwithstanding, it is generally acknowledged that the origins of the pioneer seed are in the North East of India. Even though precise information of collection is unavailable, it is highly possible that initial germplasm was obtained from restricted source and therefore commercial plantations may be of narrow genetic base. This is evident through an appraisal survey carried out in 1999 (Wachira, 2002), which revealed that clonal tea accounted for 38% and 80% of all tea in the estate and smallholder sectors, respectively in Kenya. The study showed that despite the wide availability of a wide range of clonal varieties to chose from, most growers cultivated only a narrow band most of which are closely related genetically and therefore were not accessing sufficient diversity at the farm level. Arising from the survey and other studies (Wachira *et al.,* 1997, 2001) deliberate efforts to introduce new germplasm mainly to expand diversity and develop novel varieties that hitherto had not been thought of were made (Anon., 2005). Thus, green tea varieties from Japan, China and Taiwan have been introduced into the TRFK tea breeding programme, which is currently under review to make it more adaptable to the emerging challenges in global tea arena.

The early industry was dominated by colonial settlers who solely had the right to seed access. In 1960, the Special Crop Development Authority (SCDA) was founded to promote the cultivation of the crop within the small holder agricultural subsector (M'Imwere, 1997). This was to later evolve into the Kenya Tea Development Authority (KTDA) whose major early task was to facilitate expansion of tea cultivation into native lands. The sector later saw rapid expansion and currently it accounts for more than 60% of all tea produced in Kenya (TBK Statistics, www.teaboard.or.ke; TBK, 2008).

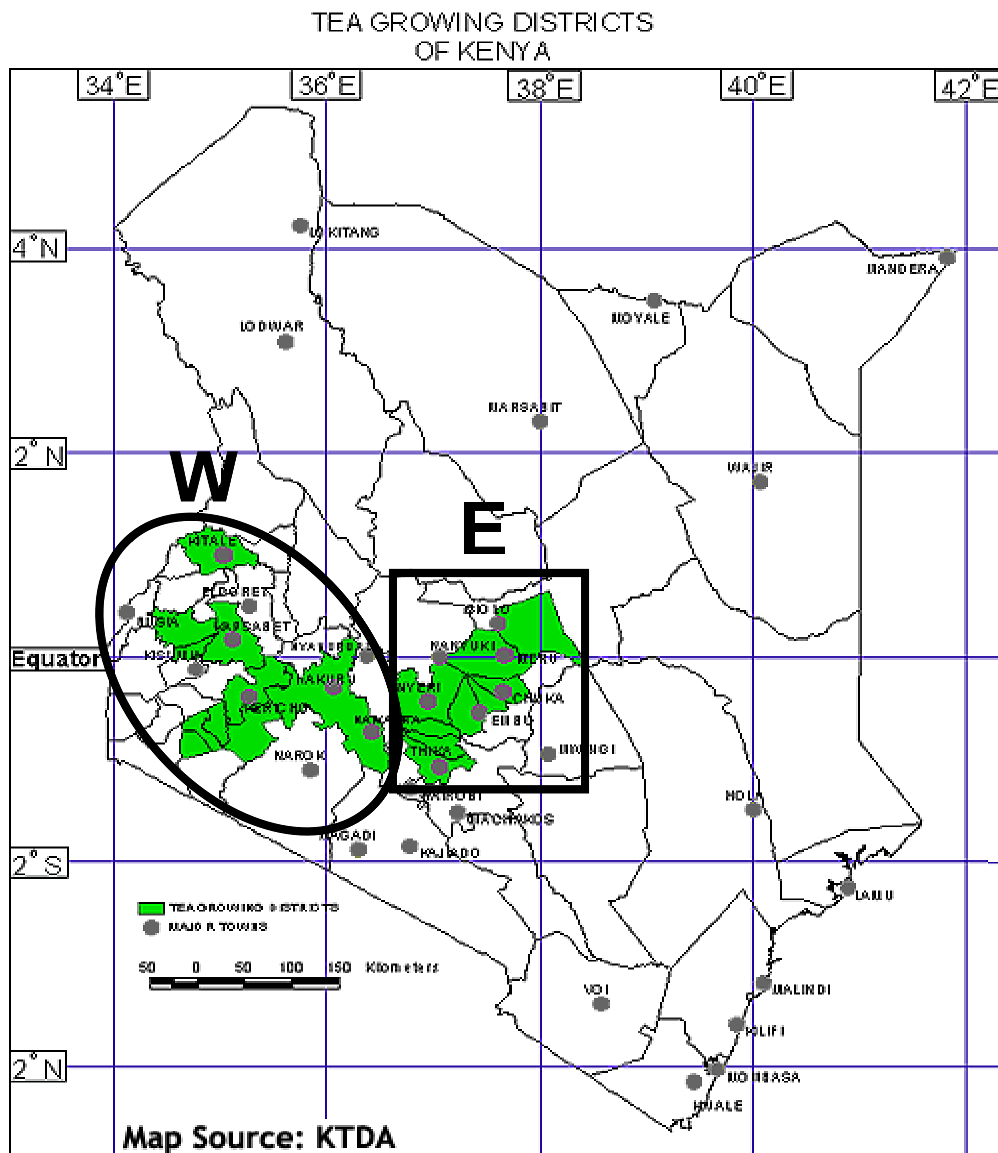

Figure 1. Tea growing regions in Kenya; East (E) and West (W) of the Rift Valley.

2.2.2 Early tea improvement activities

The history of tea breeding is as chequered and as old as the discovery of wild tea in Assam and elsewhere. From the very early days of tea growing, it was recognized that breeding of tea entails addressing breeding challenges that are somewhat unique to the plant. This is so because firstly, unlike in other woody perennials, only a part of the total biomass constitutes the harvest (Wachira and Ng'etich, 1999; Magambo and Cannel, 1981), and secondly the plant is highly heterogeneous and strongly but not absolutely self-incompatible tree species (Rogers, 1975; Wachira and Kamunya, 2005a). Consequently, the seed set is higher with pollen from another bush. The average set of the plant with its own pollen is much lower than that obtained by cross pollination (Wight and Barua, 1939; Muoki *et al.*,

2007). Where selfing occurs, the seeds are smaller with reduced germinability or no germination at all (Mamedov, 1961; Sebastiampillai, 1963). Consequently, the earlier breeding strategy relied on artificial pollination between plants that differed in some morphological features as a way of producing superior planting materials (Sebastiampillai, 1963).

The early phase of tea breeding concentrated more on production of sufficient planting material rather than on high yield and quality (Cannell *et al.*, 1977). In Assam and other parts of north-east India, the emphasis was on mass-selection which involved random crosses between plants apparently varying in leaf shape, size, texture and growth features (Wight, 1956). Mass-selection however often failed not only to produce tea of high quality but also the uniform morphological attributes essential for high yields and quality (Barua, 1963). It however resulted in the development of several seed varieties in Assam that were superior in yield and quality to 'jats' that had earlier been randomly planted.

In Sri Lanka, mass-selection was restricted to choosing the outstanding mother bushes which could be propagated vegetatively to produce high yielding uniform stands (Visser and Kehl, 1958). Unlike in Assam, the emphasis in Sri Lanka was not so much in the selection of seed bearers (Visser, 1969). However, in Africa most tea populations were initially grown from open pollinated seeds (Cannell *et al.*, 1977). However, owing to marked environmental heterogeneity (Hasselo, 1964), and continuous exploitation of the same population for further improvement and expansion, very little progress could be realized (Green, 1971).

Just as in other African countries where tea was introduced, the early tea plantations comprising of seedling tea types, later formed the base populations from which 'jat' selections were made. The great demand for planting material that ensued coupled with lack of suitable vegetative propagation techniques led the major planters to establish seed gardens using the improved 'jat' selections. Early breeders therefore were able to select seed parents, which, to them contained outstanding attributes. The early planters practiced mass selections whereby visual selection for 'jats' similar to *Assamica* in the seedling populations were sought for, selection was based on general vigour, plucking point density and large shoot size (Green, 1966). Selected 'jats' later became the seed bearers (progenitors) used to raise future seedling populations by open pollination. This breeding approach however resulted in slow progress in yield and black tea quality improvement even though the later generations of seedling populations were much better than the ancestral pioneer stocks. Lack of uniformity further compounded the breeding problem as seedling populations comprised unique genotypes. Furthermore, initial selection was biased towards yield with little attention being

accorded other attributes such as black tea quality and resistance/tolerance to biotic and abiotic stress factors.

The attempt to raise more uniform progenies from selected seed parents based on arbitrary criteria and ability to produce many seed only succeeded in production of more variable populations as breeders were not fully in control of the pollination process. Visser (1969), for example, observed that good seed producers might not necessarily result in good progenies in terms of tea production especially where the trait of interest has low heritability.

The foregoing challenges notwithstanding, the prospects of producing highly productive tea plants have greatly improved in the last two decades or so, with greater emphasis on improved methods of selection, hybridization and clonal propagation. These latter efforts have also contributed to narrowing of the genetic variability of tea because most attempts were restricted to selecting elite mother bushes or progenitors from within a few natural hybrid populations of tea. A partial solution was sought by utilizing diverse indigenous tea varieties in developing clonal and seed varieties (Bezbaruah, 1974; Satyanarayana and Sharma, 1986).

Until recently, tea has traditionally been cultivated in highly favourable environments with little pressure from abiotic and biotic stresses. Consequently, basic information on the defense mechanisms to stress has hardly been obtained. Lack of this vital information has circumvented attempts to develop pest or disease and abiotic stress resistant cultivars, or development of ideotypes with an ideal architecture in terms of leaf arrangements (Yao *et al.*, 1987). Besides, dearth in knowledge of inheritance patterns and combining abilities of the desirable traits like yield and quality as well as other secondary attributes has resulted in slow progress in tea improvement. Emphasis has mostly been placed on good adaptations for varying growing conditions. More recently, studies have been carried out to better elucidate the genetics of tea plant, based on molecular techniques (Wachira *et al.*, 1995; Wachira *et al.*, 1997; Hackett *et al.*, 2000; Wachira, 2002), for the purpose of enhancing tea improvement.

2.2.3 The tea genome

Although the actual genome size of tea is still unknown, diploid tea is recognized to have a chromosome number of 2n = 2x = 30 in all the varieties studied (Longley and Tourje, 1959; Bezbaruah, 1971; Kondo, 1977). Some natural polyploids have however been reported (Simura, 1935; Bezbaruah, 1971; Wachira and Kiplang'at, 1991). Although these polyploids have the advantage of high vigor and resistance to environmental stresses, particularly winter hardiness, they do not always contribute to high yield (Bezbaruah, 1968). The tea

chromosomes are generally small and possess median centromere, indicating their primitiveness and very little evolution in karyotype (Bezbaruah, 1971). Thus, the use of karyotype in tea improvement is limited to polyploidy screening. The genome size of tea and other *Camellias* has recently been estimated to be 4.0G bases (Tanaka, 2006). This corroborates earlier studies that had revealed that most other species within genus *Camellia* are also diploid (2x = 30) (Bezbaruah, 1971). *C. sasanqua* has been shown to form stable polyploidy series of tetraploid (4x = 60) and hexaploid (6x = 90) plants and *C. rosaeflora* is a triploid (3x = 45) (Bezbaruah, 1971).

2.2.4 Criteria of selection and clonal release

Like any other woody perennial tree crop, tea has a lengthy juvenile period, poor juvenile-mature correlation especially for growth characteristics and large plant size (Gazi, 1978). Upon development of vegetative propagation as the preferred method of producing uniform tea fields that are easy to manage for uniform tea quality and yield, initial releases were based on rootability, nursery growth, fast fermentation and field performance (Hainsworth, 1965). The best seedling fields were initially used as checks for yield and cup quality with the emerging clones being released upon outperforming these fields (Hainsworth, 1965; Green, 1969; Njuguna, 1985). Other selection criteria adopted included leaf colour (Goodchild, 1960; Todd, 1955), with light green colour being thought to be associated with cup quality of processed black tea. Leaf pose particularly erect to semi-erect, and preference by pluckers for tea bushes with large and heavy shoots were also considered as important attributes for selection and release for commercial use (Njuguna, 1987, 1989).

2.2.5 Current tea breeding strategies in Kenya

Tea improvement essentially consists of four phases; generation of genetic variability, selection of useful genotypes and comparative tests to demonstrate the superiority of the selected genotypes. A fourth phase that involves exposing pre-released and promising genotypes to multiple sites (genotype-environment interaction) for stability and adaptability is always the final phase in tea improvement programmes. Generally, the first three phases have been given adequate attention even though with some limitation. Most on-station trials are carried out at one site in Timbilil, Kericho. Simultaneous comparative testing of genotypes has been hampered by lack of testing sites in different tea growing areas. The fourth phase has been undergoing rationalization as earlier effort to expose promising clones to other sites were haphazardly done by asking interested factories (as farmers

representatives) to collect 500 free single-whole leaf cuttings for propagation and testing in their farms. Where technical follow ups were undertaken they were insufficient and in most cases there were simply none. Consequently, the performance of earlier released clones in different tea growing regions could not be ascertained. It is currently held that involvement of farmers and other end-users right from selection of breeding materials to multilocational testing is the most cost-effective way of developing new elite cultivars with wider acceptability and adaptability.

In East Africa, tea breeding commenced with establishment of a breeding seed barie (orchard) at Rwebitaba Estate in Uganda in 1967 (Green, 1973). Two more polyclonal seed baries were established at Kangaita (Anon., 1980) and Timbilil (Anon., 1990) after the incorporation of TRFK in 1980. Additionally, the major tea companies in East Africa, notably, James Finlay, Brooke Bond, Eastern Produce and George Williamson initiated their own tea improvement programmes by establishing seed baries. Successive breeding efforts at TRFK have seen the expansion of the existing polyclonal seed barie as well as establishment of new biclonal seed baries using elite commercial and promising cultivars. The various parental materials used in the existing polyclonal and biclonal seed orchards are shown in Tables 1 and 2 (Anon., 2006).

Initial breeding in the east African region involved parents consisting of the then elite Tea Research Institute of East Africa (TRIEA) clones which were used as the breeding stocks, with clone TRFK 6/8 being the common parent (Green, 1973). As a result a total of 27 clones related to TRFK 6/8 were released for commercial utilization from this breeding programme at the Timbilil Estate, Kericho. The clones constitute 60% of released clones to date and some of them have given comparable or better yields to TRFK 31/8 and AHP S15/10 (e.g. TRFK 303/577 and TRFK 303/1199), which are currently used as standard checks for high yield. Subsequent breeding and selection efforts have led to the injection into the tea industry a further 20 clones to date (Wachira *et al.,* 2006). While only a small portion of all released clones is currently being utilized by growers, the earlier released unutilized ones may have been rendered irrelevant by the more recently developed elite clones that have shown markedly better performance in combination of preferred attributes.

Table 1. Breeding stocks and their expected genetic contribution in the breeding programme

High yield potential	High quality potential	Pest tolerance/ resistance	Drought tolerance	High soil pH tolerance	Cold tolerance	Genetic study*
TRFK 31/8	TRFK 6/8[9]	TRFK7/9[3]	TRFCA SFS150	EPK TN14-3	EPK TN14-3	TRFK 12/2[1]
TRFK 303/577[8]	GW Ejulu-L	TRFK 57/15[3]	TRFK 303/577[8]	NDT Tai	TRFCA SFS150	TRFK K-Purple
TRFK 301/4	EPK TN 15-23	AHP SC31/37[3]			EPK C12	TRFK 31/30[2]
TRFK 301/5		AHP S15/10[3]			NRIT Yabukita[6]	TRFK 311/287[2]
EPK C12		EPK TN14-3[5]			NRIT Yutakamidori[6]	TRFK 382/1[7]
BBLK 35		TRFK 303/1199[3]				TRFK 382/2[7]
AHP S15/10[9]		TRFK 54/40[4]				TRFK 386/2[7]
AHP SC12/28[9]		TRFCA SFS150[3]				TRFK 371/1[7]
AHP SC31/37		AHP CG28U864[4]				
AHP CG28V929[9]		TRFK 301/1[4]				
AHP CG28U864		TRFK L/16[4]				

[1]Non fermenter; [2]Tetraploid; [3]Resistant to Red Crevice Mite [4]Susceptible to Scales; [5]Preferred but highly tolerant to Red Crevice Mite; [6] Green tea varieties- low catechin content; [7]Triploid; [8]Susceptible to root knot nematodes; [9]Very susceptible to water stress; *Progenitors possess unique genetic characteristics for use in genetic studies.

Table 2. Isolated biclonal seed garden progenitor clones and their attributes

Clones	Location	Attributes
TRFK 6/8 and AHP SC31/37	Timbilil	High quality and yield
TRFCA SFS150 and GW Ejulu-L	Timbilil	Drought tolerance, high yield and quality
TRFCA SFS150 and AHP CG28V929	Timbilil	Drought tolerance and high yield
TRFK 301/4 and EPK C12	Timbilil	High yield and cold tolerance
TRFK 31/30 and AHP SC12/28	Timbilil	Tetraploid and diploid
EPK TN14-3 and AHP CG28U864	Timbilil	High soil pH, cold and pest tolerance and high yield
GW Ejulu –L and TRFK 301/5	Kangaita	High quality and yield
TRFK 301/4 and AHP SC31/37	Kangaita	High yield
TRFK 311/287 and AHP S15/10	Kangaita	Tetraploid and diploid
TRFK 31/8 and NDT Tai	Kangaita	High yield and high soil pH tolerance
TRFK 12/2 and AHP SC12/28	Kangaita	Non-fermenter and high yield

Furthermore, the design and composition of current seed orchards may not be appropriate in that they have been established in the middle of commercial tea plantations using progenitor materials whose combining abilities and genetic worth are unknown. Even though these surrounding plantations are maintained in vegetative phase, some clones flower profusely even under the plucking table with accruing pollen grains being transferred to the breeding materials by foraging insects and hence contributing to illegitimate matings (Chalo *et al.*, 2007). This might have led to production of inferior genotypes as some of the adjoining commercial plantations still comprise of low yielding seedling accessions. As pollination in tea is predominantly entomophilous, cross pollination may be enhanced by the establishment of bee cages in newly designed seed orchards, strategically placed, and surrounded by strips

of other multi-storeyed vegetation such as broad leaved indigenous trees, which would effectively act as buffer zones against extraneous pollen, besides conserving the environment.

2.2.5.1 Breeding and clonal selection

In the recent past, breeding programmes have been intensified and expanded to include improvement of more than one economic trait in a single clone with fruits borne from such efforts expected to be harvested soon. Hybridization programmes take advantage of existence and/or creation of tremendous genetic variability for desirable traits involving choice and crossing of disparate parents possessing the traits. Numerous crosses employing complementary mating design based on existing information have been undertaken since inception of the rationalized tea breeding programme at the TRFK. Seeds resulting from such crosses are collected alongside open pollinated ones and used in formation of basal populations for future selection. Seedlings and clonal descendants of half-sib or full-sib origin are currently at different stages of testing, either in progeny tests or replicated clonal field trials (Anon., 2006). The response of the progenies in relation to yield and black tea quality and other secondary traits as well as estimation of their parental combining abilities would help in determining the best mating design for fruitful tea improvement.

Studies so far undertaken show that Kenya's tea germplasm that is predominantly of the Assam type (i.e. *Camellia sinensis var. assamica*) is highly diverse although many of the commercial clones are genealogically related (Wachira *et al*., 2001). It has been thought that the risks to abiotic and biotic stresses associated with having a population with narrow genetic base may be high, and huge losses can be encountered in event that such constraints happen to arise. There has been a thrust in the last few years to rationalize the breeding strategy in an effort to buffer the existing and future tea germplasm against the emergence of such risk factors by deliberately crossing disparate parents through intra-specific and/or interspecific hybridization (Anon., 2006). This strategy has been aimed at broadening the genetic base as well as introgressing new genes controlling useful traits that were otherwise not present in the base populations. Several introgressants are at different stages of screening, which are a result of direct crosses among Chinese germplasm (*Camellia sinensis* var. *sinensis*), Cambod type (*Camellia sinensis* var *assamica spp lasiocalyx*) and Assam germplasm (*Camellia sinensis* var *assamica*). A few elite clones like TRFK 301/4 and TRFK 301/5 (Cambod varieties) have been released to the industry for on-farm diversification (Wachira, 1994a; Mamati *et al*., 2001). Besides, two new clones, TRFK 430/90 and TRFK 371/3, with combined optimum yield and black tea quality, and which are able to survive

under adverse abiotic and biotic stress factors have recently been released to the industry (Kamunya and Wachira, 2006).

Once hybrid seedlings are raised from the crosses, they are established in progeny trials and evaluated for a period of seven years following which intense selection for desirable traits (usually yield and black tea quality) is imposed with only 2-5% of the best progeny being selected and advanced to the next phase of testing called clonal field trial in which all the entries are replicated (Kamunya, 2003). The seven-year progeny testing period consists of two years during which seedlings form a plucking table, four years of assessment of yield and response to invasion by pests, diseases and drought effects and one year assessment of recovery from prune. The progeny trials are usually not replicated but are planted as hedges owing to variable numbers of offspring per family obtainable for evaluation. Thus, it is not possible to dissociate the effects of the environment from that of the genes while determining the performance of the progenies (Kamunya, 2003). It is normally assumed that the superior performance or response of the plant for the trait under investigation is genetic in origin. Unfortunately, this is not always true since existence of favorable or unfavourable microhabitats within a test site can exist and ultimately exert their influence on genotype's performance hence masking the true genetic potential of the genotype. Methods of circumventing such obstacles prior to progeny testing possibly through micro-propagation at the earliest opportunity (say nursery stage or two years in hedges) would enable establishment of replicated clonal progeny trials. This then would ensure that only superior genotypes for the traits of interest are selected from one stage and advanced to the next. Studies already undertaken have shown that only a small portion of the seedling population is selected for further evaluation in clonal field trials. Occurrence of superior genotypes in such population may sometimes be as low as 0.0025% (Wight, 1958). It is estimated that one seedling plant in 200-300 has high yield or good quality. Consequently, one seedling per 40,000-100,000 may therefore combine both yield and black tea quality (Wight, 1958, 1961; TRIEA, 1966; Green, 1966; Kulasegaram, 1978). This is a low probability indeed, and indicates the monumental task of successfully selecting a new variety that combines several polygenic traits. Besides, massive tracts of land are needed every year for evaluation of newly generated genotypes for attributes of interest. The lack of suitable land for screening progenies and clones may therefore potentially impact negatively on tea improvement. Currently at the TRFK, there are over 28 progeny trials with over 15,000 offspring at different stages of evaluation (Anon., 2006). Once a potential seedling is selected for advancement into clonal field trials (CFT), it is allowed to produce elongated shoots

(whips) from which single node whole leaf cuttings are collected and vegetatively propagated in the nursery using appropriate rooting media (Anon., 2002). Vegetative propagation is thus far the most cost-effective and rapid means of multiplying tea plants for either commercial use or further evaluation. This is normally the first stage through which selected seedling teas are cloned for advancement into CFTs.

The CFTs are always replicated with test clones being evaluated alongside parental clones and commercial standard checks for yield, quality and other secondary attributes (Anon., 2006). This is normally done in order to ensure that newly developed clones are competitively selected. The evaluation of clones in clonal field trials proceeds for ten years, which is equivalent to two standard pruning cycles in tea. Until recently clonal field trials were only evaluated in one site at the TRFK Headquarters in Timbilil estate, but since inauguration of a TRFK sub-station at the east of Rift, a number of CFTs have now been established in two sites, thereby enabling genotype x environment interaction parameters to be estimated. Over 260 different tea clones at different stages of evaluation in 17 CFTs currently exist at the TRFK tea improvement programme (Anon., 2006).

The long testing period of clones in CFTs is necessitated by the long time that it takes tea plants to attain optimum yield potential that range from 21-30 years (Gazi, 1978). It is however, normally assumed that reliable judgment on the performance of a good clone can be made after 8 years. However, before new clones are released to the industry, they have to be subjected to extra testing, in which case they are exposed to different environments in multi-locational trials to determine clonal adaptability (Anon., 2006). Plant genotypes (G) are known to respond and perform differently at different localities owing to variations in such environmental (E) factors as soils, climate, elevation, geographic location, pests and diseases. The tea growing environment is highly variable across time as well as space, leading to significant and unpredictable G x E interactions. For, example a particular tree genotype may grow well in wet years but be a poor competitor in dry years, while its neighbouring tree might have the opposite response (Bradshaw, 1998).

Environmental variations affecting clonal response in yield (Ng'etich and Stephens, 2001; Ng'etich *et al.*, 2001a; Wachira *et al.*, 2002) and black tea quality (Owuor and Othieno, 1987; Owuor *et al.*, 1988; Owuor *et al.*, 1990) have been observed in Kenya and thus justify the need to test potential clones on multilocational sites. Genotypes that are entered in such G x E trials are tested for adaptability and stability for all traits of interest. Such evaluation programmes have been initiated for all released, pre-released and promising potential clones in the West and East of the Rift Valley in Kenya. These trials are carried out in collaboration

with various stakeholders in the tea industry (Anon., 2006). It is envisaged that a tea map indicating clones ideal for different agro-ecological zones will eventually be developed and made available to farmers. It may however be noted that such a programme will take long to bear fruits due to longevity of the tea plant, and even where certain clones may show early take off, their ability to sustain their performance will call for longer periods of testing.

2.2.5.2 Breeding for combined optimal yield and black or green quality

The need to develop tea cultivars with optimum potential combining yield and black or green quality has recently become the single-most important breeding objective. Being universally the most popular beverage, requirements for the cultivation of tea pose a serious limitation to its expansion in areas that are unsuitable for its optimal growth. Moreover, in Kenya its expansion and/or sustainability in new areas that are suitable for tea production is faced by stiff competition from human settlement and other competing enterprises (TBK, 2004). Secondly, increased world tea production, and shrinking markets call for high quality diversified tea products. The various clones that are currently being used to combine high yield and quality through breeding are shown in Tables 1 and 2. It would be worth noting that both yield and tea quality are complex traits under the control of many genes. For example, while in black tea, theaflavins and thearubigins are the most important biochemical attributes affecting tea quality (Hara, 2001), in green tea, the composition of various green catechins becomes the overriding factor. The amount of theaflavins is closely related to the commercial value of black tea (Owuor *et al.*, 1986; Obanda *et al.*, 1992). From a health point of view, elucidating the most important biochemical components contributing to medicinal properties of the end-product, their genetic background and correlation with yield components would be most useful in guiding breeding work. Such knowledge is currently lacking though the current study has attempted to generate the requisite background information.

2.2.5.3 Polyploidy breeding

There has been a deliberate effort to introduce polyploidy breeding in tea improvement following the discovery of naturally occurring primary polyploids which contain more than the basic number of chromosomes (2x = 2n = 30) (Wachira and Kiplang'at, 1991; Wachira, 1994b). For example, thirty-eight triploids (3n) and two tetraploids (4n) have been identified among seedling populations at Timbilil estate (Table 3) and some of them have been incorporated in the breeding programme in polyclonal and bi-clonal seed baries (Anon., 2002). Observations made so far indicate that in spite of their high

growth vigour and superior shoot size traits, polyploids give consistently lower yields than diploids (Wachira, 1994b, Wachira and Ng'etich, 1999). Polyploids tested so far are however of restricted genetic background, most having been derived from low to medium yielding progenitors like BBK 2, BBK 5, BBK 7 and BBK 35 (Anon., 1999). However, potential exists of undertaking useful selections for diversification and/or for further improvement into secondary polyploids from the ongoing plant improvement programme (Anon., 2001).

Hybridization efforts between triploids and diploids have nonetheless, often not borne good results as they have given poor seed set of which some exhibited low viability. This has been attributed to complications arising from the pairing of chromosomes in meiosis during the process of gametogenesis resulting in production of unbalanced gametes (Singh, 1995). Suitable techniques may need to be devised to be able to effectively utilize polyploidy breeding in tea improvement programmes. Further investigations into their possible utilization in genetic studies is continuing (Anon., 2001).

2.2.5.4 Breeding for pest resistance

Tea mites, especially the red crevice mite (*Brevipalpus phoenicis*) have been reported to cause yield losses estimated to vary between 14% and 30% in Kenya when there is heavy infestation (Sudoi, 1995). Similarly, scale insects (*Aspidiotus* species) can cause yield losses of about 10% (Sudoi *et al*., 1996). Several approaches offer promise for managing tea pests. These include chemical, biological and breeding for pest resistance control options. Chemical control measures are costly and hazardous to human health and the environment. Since Kenyan tea is credited for being free of pesticide residues, it is imperative that this status be safeguarded through the development of cost-effective and environmentally friendly pest management options such as breeding for pest resistant/tolerant clones.

Clones TRFK 7/9, TRFK 57/15, AHP SC31/37, TRFK 303/1199, TRFCA SFS150 and AHP S15/10 have been found to be relatively tolerant to the red crevice mite. Clone EPK TN14-3 has been noted to be less susceptible to scale insects though also moderately preferred by the red crevice mite (Sudoi *et al*., 1995). These clones are among the cultivars that are being used in the breeding programme for pest resistance.

Incidences of root knot nematodes damage on clone TRFK 303/577, a popular high yielding and drought tolerant clone have been noted to be widespread (Otieno *et al*., 2002). A recent study revealed widespread clonal variation in response to infection by root knot nematodes in farmers' fields in Kenya indicating possibility of selection for resistance/tolerance to the pest (Kamunya *et al*., 2008).

Table 3. Identified polyploid clones, their chromosome number and ancestry.

Clone number	Ploidy-Chromosome No.	Original seed source
*TRFK 311/287	4n = 60	TRFK 6/8 x TRFK 31/11 - hand-pollinated from TRIEA Uganda
*TRFK 31/30	4n = 60	Seed from Ambangulu Estate, Tanzania
*TRFK 52/1	3n = 45	"Between" seed from Kanywankoko Estate, Uganda
*TRFK 77/1	3n = 45	Open-pollinated seed from Mimosa estate, Malawi
*Dimbolil 3	3n = 45	Seedling from James Finlay (K) Ltd, Dimbolil Estate
*TRFK 77/2	3n = 45	Open-pollinated seed from Koiwa Estate
*TRFK 383/1	3n = 45	Open-pollinated BB35 X BB5, Koiwa Estate
*TRFK 331/2	3n = 45	Seed from Chemosit Estate barie, Unilever tea
*TRFK 378/1	3n = 45	Open-pollinated BB35 X BB7, Koiwa Estate
*TRFK 412/1	3n = 45	Open-pollinated BB21 X BB5, Koiwa Estate
*TRFK 371/1	3n = 45	Open-pollinated seed of AHP S15/10 from Chepgoiben Estate, James Finlay (K) Ltd
*TRFK 400/1	3n = 45	Seed from Chepgoiben Estate barie
*TRFK 389/1	3n = 45	Open-pollinated BB35 X BB152, Koiwa Estate
*TRFK 392/1	3n = 45	Open-pollinated BB7 X BB35, Koiwa, Estate
*TRFK 394/1	3n = 45	Open-pollinated BB2 X BB35, Koiwa, Estate
*TRFK 395/1	3n = 45	Open-pollinated BB5 X BB35, Koiwa, Estate
*TRFK 54/49	3n = 45	Seed from Ambangulu Estate, Tanzania
TRFK 386/1	3n = 45	Seed from Sotik Tea Co.
TRFK 381/1	3n = 45	Seed from BB5 X BB2
TRFK 84/1	3n = 45	Mixed seed from Congo, Toro and Entebbe, Uganda
TRFK 84/2	3n = 45	Mixed seed from Congo, Toro and Entebbe, Uganda
TRFK 85/1	3n = 45	Seed collected from clones from Kakonde Estate
TRFK 382/2	3n = 45	Seeds from BB5 X BB35
TRFK 382/1	3n = 45	Seeds from BB5 X BB35
TRFK 386/2	3n = 45	Seed from Sotik Tea Co.
TRFK 76/3	3n = 45	Seed from Ramjat, Lugeri Estate, Malawi
TRFK 76/1	3n = 45	Seed from Ramjat, Lugeri Estate, Malawi
TRFK 76/2	3n = 45	Seed from Ramjat, Lugeri Estate, Malawi
TRFK 75/1	3n = 45	Commercial seed from Lugeri Estate, Malawi
TRFK 31/36	3n = 45	Seed from Ambangulu Estate, Tanzania
TRFK 31/38	3n = 45	Seed from Ambangulu Estate, Tanzania
TRFK 31/39	3n = 45	Seed from Ambangulu Estate, Tanzania
TRFK 31/40	3n = 45	Seed from Ambangulu Estate, Tanzania
TRFK 31/41	3n = 45	Seed from Ambangulu Estate, Tanzania
TRFK 18/7	3n = 45	Seed from Ambangulu Estate, Tanzania
TRFK 18/27	3n = 45	Seed from Ambangulu Estate, Tanzania
TRFK 18/26	3n = 45	Seed from Ambangulu Estate, Tanzania
TRFK 18/28	3n = 45	Seed from Ambangulu Estate, Tanzania
TRFK 54/50	3n = 45	Seed from Ambangulu Estate, Tanzania
TRFK 550/1	3n = 45	Open-pollinated seed from polyclonal mixture, Timbilil Estate, Kericho

** From Wachira and Kiplangat (1991); NB: in tea, n = x = 15.*

2.2.5.5 Breeding for tolerance to environmental stress

Due to global climatic changes, the frequency, longevity and severity of drought have increased especially in the traditional tea growing areas (Anon, 2001). Similarly, stress associated with low temperatures (freak-frost incidences) as well as high soil pH has exerted serious constraints to tea production in some parts of the west of the Rift Valley (Anon, 2001). Additionally, tea farming is increasingly being extended into the non-traditional tea-growing areas that were formerly considered marginal and therefore unsuitable for the plant. Therefore, there is need to incorporate inherent tolerance to drought in the tea plant, which is imperative for sustainable tea production. In the breeding programme, clones with tolerance attributes to various abiotic stress factors such as clones TRFCA SFS150, TRFK 303/577, EPK TN14-3 and EPK D99/10 (Table 1) have already been identified and introduced in the breeding programme.

2.2.5.6 Interspecific hybridization

Interspecific hybridization (gene introgression) has lately been initiated with the aim of improving the vegetative and hardiness characters of tea by crossing tea *(Camellia sinensis*) with some closely related 'wild' *Camellia* species (Wachira, 1994c). It has been demonstrated that tea can easily be crossed with 10 different species of the genus *Camellia* (Bezbaruah, 1987). Hybridization work has successfully been undertaken with *C. irrawadiensis, C. taliensis, C. japonica* and *C. kissi* (Ackerman, 1970, 1973; Bezbaruah, 1974, 1987). Two species, *C. irrawadiensis* (Wilson's Camellia) and *C. taliensis* (Forest's Camellia) have merited special attention as they lack caffeine, but their liquors lack the quality of tea. To date, no interspefic hybrids have produced commercially acceptable black tea of good quality though efforts continue to develop elite interspecific hybrids. Currently, up to 100 clonal interspefic hybrids between *C. sinensis* and other *Camellia* species have been developed and are being evaluated at the TRFK (Anon., 2007).

A programme of interspecific hybridization that is being implemented in Kenya at the TRFK, also focus on development of diversified tea products from the resulting hybrids (Anon., 1998). Numerous intraspecific crosses aimed at diversification of tea products have also been carried out at TRFK with Japanese chinary germplasm *C. sinensis* 'Yabukita' and 'Yutakamidori' (Anon., 2001), which are popular commercial cultivars for green tea in Japan. Offspring from these crosses are still young with analytical investigations expected to cast light on their quality. The catechin levels to be determined will form the basis for identification of desirable green tea genotypes in Kenya. In addition, owing to possibilities of

natural hybridization between tea and other *Camellia* species, it is debatable whether all cultivated teas are original (archetypal) varieties (Visser, 1969). Indeed, evidence of presence of potential interspecific hybrids has been obtained by selection in pioneer seedling plantations with morphological traits derived from non-tea *Camellia* species. Such species have been selected in Kenya for further evaluation (Wachira-personal communication). It is widely held that the present varieties in most plantations have emanated from hybridization from the three main taxa as well as other *Camellia* species (Banerjee, 1992). Thus, breeders have a wide field of choice when it comes to choosing traits for which to target in their breeding programme.

2.3 Combining abilities

The parental genetic values are expressed in terms of combining abilities. The two types of combining abilities that are of special interest to plant breeders are the general and specific combining abilities. General combining ability (GCA) is defined as the average performance of the progeny of an individual when it is mated to a number of other individuals in the population (Falconer, 1989). Although GCA may be expressed in absolute units, it is usually more convenient and meaningful to express them as deviations from the overall mean. Thus a parent with a GCA of zero has an average general combining ability. A positive GCA indicates a parent that produces above- average progeny, whereas a parent with a negative GCA produces progeny that perform below average for the population.

Specific combining ability (SCA) on the other hand refers to the average performance of the progeny of a cross between two specific parents that are different from what would be expected on the basis of their general combining abilities alone. It can either be positive or negative. Specific combining ability always refers to a specific cross and never to a particular parent by itself (Falconer, 1989). The GCA is a measure of the additive genetic action, while the SCA is assumed to be a deviation from additivity (i.e. non-additive genetic action). Scanty information exists on the combining ability for perennial crops. For instance, combining ability studies for cocoa (*Theobroma cacao* L.) showed SCA effects to be greater than those of GCA for yield (Dias and Kageyama, 1995). However, a separate study involving diallel crossings had earlier revealed GCA to be more important than SCA for the same trait (Berry and Cilas, 1994).

Information on combining abilities in tea is grossly lacking and therefore, tea-breeding work at the TRFK has not been taking cognizance of the type of combining abilities of parents involved. Knowledge of combining abilities will help in determining heterotic

patterns and in choosing the appropriate progenitors, design and structure of seed baries and mating designs for future breeding programmes.

2.4 Heterosis

Heterosis is the superiority of an F_1 hybrid over the mid-parent values. When significant improvement in the character of interest is sought, it is appropriate to estimate heterosis over the better of the two parents (Arunachalam, 1988). Although the molecular basis of heterosis is still unknown, genetic explanations often advanced include dominance, over dominance and epistasis (Barth *et al.*, 2003). With two alleles per locus and no epistasis, heterosis is theoretically a quadratic function of the parental genetic distance (GD) at the underlying quantitative trait loci (QTL) for the trait considered (Falconer and Mackay, 1996; Melchinger, 1999). Experiments with maize have shown an increase in heterosis with increasing parental GD (Melchinger, 1999), but an optimum level of parental GD has been suggested after which heterosis and hybrid performance declines (Moll *et al.,* 1965). A study on heterosis for yields and related traits in hybrids of *Arabidopsis thaliana* L. Heyne, revealed that mid parent heterosis (MPH) was not associated with parental genetic distance determined from molecular markers (Barth *et al.*, 2003). The workers however suggested the use of QTL analysis in pursuing heterosis investigation. Heterotic information on tea is currently lacking.

2.5 Future tea breeding strategies

The dwindling revenue base from tea enterprises occasioned by increased costs of production and inputs, glut in the black tea market and an appreciating local currency requires that appropriate raw material for diversification of tea products be developed and made available to farmers to keep the tea business thriving. The appropriate raw material referred to here is the cultivar developed through a well thought out and implemented plant improvement programme. Owing to the prolonged breeding cycle of tea, a careful choice of the progenitor clones is very important. If such parents are lacking in the national improvement programme, efforts must be made to source them from other countries through mutually negotiated material transfer agreements (MTA). Additionally, any technology that may lead to reduced cost of production would also lead to increased incomes for resource poor farmers and marked reduction in poverty levels. Thus, breeding for cultivars that are suitable for mechanical plucking has now gained considerable importance. The same approach has also been adopted by other countries (Apostolides *et al.*, 2006). Similarly,

breeding strategies aimed at developing elite green tea varieties, value-addition by screening existing germplasm and further breeding for high levels of antioxidants and low levels of caffeine have been instituted. These attempts aim at accessing niche markets with new tea products.

Efforts geared towards selection for black tea quality have often made use of fermentability based on chloroform test (Sanderson, 1963). Fast fermenting clones have been assumed to produce high quality black tea though this has not always been the case. Initially, slow fermenters were always discarded and this might have led to inadvertent rejection of elite clones for other types of tea products such as green tea or tea with high levels of antioxidants, which by then were not considered important. Thus, where diversified tea products are targeted, rationalised breeding activities call for a total overhaul of breeding objectives. While revising the breeding objectives, addressing traits that are not demand driven would be costly and an untenable venture. Thus, farmers and consumers need to be involved while formulating the breeding objectives. For example, where consumers seek for specific tastes, changes impacting positively from breeding and relevant selection criteria for elite cultivars must be considered. In Malawi, at Limbe tea auction, buyers look for two distinct types of tea; one for red coppery tea, and the other for yellow teas (Apostolides *et al.*, 2006). Selection of these attributes requires establishment of rapid and reliable selection criteria and methods.

There has been tremendous improvement in Kenyan tea over the years with notable replacement of seedling teas with high yielding and better quality clones. Kenyan tea has imprinted a place in world tea trade for high black tea quality and yields. However, it has not yet been possible to produce clones with combined optimum yield and black tea quality. However, one of the current objectives in tea breeding programmes is geared at coming up with such a clone. Careful parental choices, well thought out breeding strategies as well as greater understanding of tea genetics, are key prerequisites to achieving greater genetic progress while maintaining a broad genetic base. A combination of earlier identified morphophysiological and recently developed molecular markers for early selection of potential clones is expected to shorten the breeding cycle and allow easier and more accurate selection of disparate stocks possessing high yield and black tea quality (Sanderson, 1963; Wachira, 1994c). In an attempt to come up with clones that will result in marked improvement in yields and quality as well as tolerance to biological and environmental stresses, the following strategies are envisaged.

2.5.1 Tissue culture

Plant tissue culture entails cultivation of organs, tissues or cells in test tubes on artificial media (Singh, 1995). Often the techniques of plant cell and tissue culture are also called *in vitro* techniques (Singh, 1995). The development of true hybrids particularly from crosses in *C. sinensis* using conventional breeding methods has often been hampered by poor fertility and low viability of hybrid embryos arising from crosses aimed at producing new hybrids (Wachira and Ogada, 1995). Micropropagation by somatic embryogenesis from immature zygotic embryos before abortion provides an embryo rescue technique that get around the stated shortcoming besides providing a method of plant multiplication (Singh, 1995; Wachira and Ogada, 1995). Successful *In vitro* regeneration of *Camellia sinensis* by somatic embryogenesis has been reported (Wachira and Ogada, 1995). In a review by Wachira (1990, 1994), the applicability of tissue and cell culture in tea improvement was mostly observed to be useful in assisting research rather than being an alternative method of propagation due to its cost implication. However, a case of successful multiplication of elite clone in Sri Lanka with up to 30,000-35,000 plantlets produced from 50 nodal explants in one year has been reported (Arulpragasam, 1990).

Since production of purelines or inbreds involves six to seven generations of selfing, development of haploids through distant crosses or through pollen culture, followed by chromosome doubling, reduces this time to two generations (Deepak *et al*., 2004; Singh, 1995). Doubled haploids are produced by spontaneous or artificial doubling of the chromosomes of haploid plants. As doubled haploid plants have the exact copies of chromosomes as in the haploid plants, attainment of homozygosity is achieved in a single step thus significantly reducing the breeding cycle (Deepak *et al*., 2004). Tissue culture utilization in developing pure lines of tea through generation of doubled haploids is currently under investigation (Anon., 2001), with useful information to help elucidate tea genetics expected to be generated soon. It is also expected that somatic hybridization, through protoplast fusion, once put in place can also provide a means through which genetic variation, genetic recombination at the cellular level, and genetic transformation will lead to realization of new tea genotypes. This will enable the poor flowering but high yielding and pest resistant clones like TRFK 31/8 to be included in hybridization programmes in future tea improvement.

2.5.2 Mutation breeding

The objective of mutation breeding is to induce desirable genetic changes to enhance both quality and yield. But in the absence of information on the precise location of gene loci responsible for these characteristics, preliminary investigations were restricted mostly to irradiating cuttings, pollen grains and seeds anticipating that some of the treated plants would do better than their untreated predecessors (Singh and Sharma, 1982). The mutagens used include ionizing radiation such as χ-rays and γ-rays, and chemicals such as ethyl methane sulphonate and clastogens such as colchicine. However, these treatments failed not only to produce superior mutants, but those treated had reduced vigour, stunted growth, and a lesser number of foliage and branches (Singh and Sharma, 1982). According to Sharma and Ranganathan (1985), use of irradiated pollens caused fruit drop. It has also been reported that tea clones differ in their responses to γ-radiation. Clones of China and Assam origins are generally more tolerant to γ-radiation than those of Cambod origin. However 2 Krad appears to be the upper limit for survival (Singh, 1980). The significance of these findings is not clear at present, but the apparent genetic variation in the response to mutagens suggests scope for further exploitation of this strategy in broadening the genetic diversity of tea.

In the TRFK tea improvement programme, mutagens have been incorporated in *vitro* cultured materials with the aim of developing new genotypes that are able to tolerate stress factors as well as secondary polyploids (Anon. 2001). The mutagens used include colchicine, hydroxyquinoline and sulfanimide but since investigations are still at the earliest stage, no useful results have been reported. Some plantlets have however been raised from these investigations and have been transplanted to the fields for further evaluation (Anon. 2001).

2.5.3 Breeding for medicinal tea

Tea is increasingly becoming recognized as a health drink with research on its pharmacological properties focusing on the possible components that make it biologically active (Zongmao, 1995). Tea breeding therefore is currently targeting the selection of populations with high functional components such as catechins, flavanols, theanine, b-carotene, 2-amino-5 (N-ethylcarboxyamido)- pentanoic acid, and polysaccharides (Zongmao, 1995). These chemicals have also been known to have antioxidant properties and so help in combating chronic diseases like cancer as well as cardiovascular diseases. Other prophylactic properties of tea are also attributed to caffeine, which is found in young flush shoots of tea. Since caffeine acts as stimulant to both the central nervous and cardiovascular systems (Macrae, 1985; Marks, 1992), considerable consumer preferences in relation to caffeine

contents have been noted. As different tea varieties harbour varying levels of caffeine (Magoma *et al.*, 2001), potential exists to have pharmacological tea formulations with predetermined levels of caffeine in order to satisfy different consumer needs. Tea has been linked to lower heart disease and cancer risk (Hara, 2001; Weisburger, 2006) through the action of flavonoids. Tea also relieves some allergy symptoms and has recently been shown to boost the body's immune system (Anon., 2003a&b; Basu, 2002-2003).

Tea, as drunk derives its pharmacological properties largely from its polyphenols content. The polyphenols include catechins: (+) catechin (C), (-) epichatechin (EC), (-) epigallocatechin (EGC), (-)-epigallocatechin gallate (EGCG) and (-) epicatechin gallate (ECG). These biochemicals are formed through intermediary glucose metabolism comprising the pentose, shikimate and the prephenelate pathways (Magoma *et al.*, 2000; Hara, 2001). They play a prominent role in green and black tea quality (Nakagawa, 1970; Obanda *et al.*, 1992) and have been reported to have important medical properties, which include the ability to reduce serum cholesterol levels (Ohtsura, 1991), alleviation of hypertension and vascular disorders (Matsubara *et al.*, 1985; Yilddizole–Ari *et al.*, 1991), prevention of breast and prostate cancers (Ohtsura, 1991) and inhibition of inflammation (Maeda, 1989; Sugiyama, 1995; Yamada, 1995; Karori *et al.*, 2008). EGCG has recently been found to boost the body's immune system by warding off HIV (Shearer, 2003) and inhibition of HIV reverse transcriptase activity (Nakane and Ono, 1990). Epidemiological studies have also demonstrated that catechins in tea inhibit diabetes including hyperglycemia by reducing elevated sorbital, decreasing protein glycosylation and lipid peroxidation and by inhibiting diabetic cataracts (Vinson *et al.*, 2001). Epidemiological studies done in Europe revealed that drinkers of black tea had a lower incidence of heart diseases (Weisburger, 2006). These findings were attributed to tea polyphenols acting as effective antioxidants, inhibiting the oxidation of low-density lipoprotein (LDL) cholesterol caused by reactive oxygen species which lead to antherogenesis. Studies involving constituents of the polyphenolic fractions of green and black tea (theaflavins and thearubugins) showed that polyphenols reduced the mutagenic capacity of different types of carcinogens (review by Weisburger, 2006) as well as exhibiting powerful antibacterial action (Hara, 2001). Other investigators reported that tea and tea polyphenols decrease the rate of growth of tumour cells through mechanisms involving alterations in gene expression (Hara, 2001, 2006). Additionally, tea polyphenols increase the rate of apoptosis (cell death) of tumour cells leading to their elimination (Hara, 2006). As tea polyphenols have antiviral and antibacterial properties, regular tea drinkers have been found to have healthier intestinal bacterial flora than those that drink less or no tea

at all (Hara, 2001). According to Hara (2006), catechins have been found to inhibit the growth of food-borne pathogenic bacteria, while they do not have adverse effects on beneficial bacteria as *Bifidobacterium/Lactobacillus*. Further, tea catechins, particularly EGCG, were confirmed to interact with the influenza virus in a way that rendered the virus non-infective to the cells (Hara, 2006). Owing to the suppressive power of tea polyphenols over reactive oxygen species formed during normal cellular metabolism, and which also causes premature ageing, various studies found that regular daily intake of five or more cups of tea facilitates healthier ageing process (Weisburger, 2006). Black tea, the chief tea production from Kenya was recently established to be as efficacious as green tea from other tea producing countries in its antioxidative properties (Hara, 2001; Karori *et al*., 2007).

In India, tea is classified as a health food akin to Rasayanas known to ancient Indians (Dhawan, 2006). Rasayanas is a general term encompassing a group of health foods and herbal based drugs, which if regularly used, convey the concept of attainment of positive health, increased resistance to diseases and assured longevity (Dhawan, 2006). Rasayanas re-establish youth, strengthen life and brain function and provide capability to counteract diseases.

Because of its rich phenolic composition, tea is increasingly being put to other uses in products other than in foods and drinks. For example, numerous environment-friendly industrial cleaning agents, deodorizers and antimicrobial agents have been formulated using tea (Yayabe, 2001). Owing to the health promotive and disease preventive properties of tea, some extracts of green tea, known as green tea polyphenols (GTP) are finding commercial application in several substances such as antioxidants for foods and cosmetics, anti-tooth-decay agents and deodorants (Hara, 2001; Basu, 2002-2003).

Other polyphenols with potential pharmaceutical properties are the anthocyanins (purple pigmentation, Figure 2) (Walker 1975; Clifford, 2000) and flavanoid pigments (Dufresne and Farnsworth, 2001). Tea has one of the highest total flavanoid of all plants at 15% of the leaf by dry weight and is also the major source of flavonoids in the UK diet, providing 80% of dietary flavonoids for the population as a whole (UK Tea Trade Technical Committee).

Figure 2. Anthocyanin-rich tea clone at the foreground of a clonal trial.

As great variation has been revealed in the expression of these important biochemicals in different tea germplasm (Takeda, 1994; Magoma *et al*., 2001), large-scale efforts in tea product diversification demand that the inheritance patterns of the native biochemicals be properly understood and potential molecular markers identified to hasten development of superior varieties that are not only high yielding but also of high pharmacological value. Thus, breeding strategies geared towards meeting the anticipated future demand for clones with more health attributes have been initiated and would be further strengthened even through germplasm exchange.

Studies involving animals and humans have shown that both green and black teas are equally beneficial in their pharmacological properties (Hara, 2001; Karori *et al.,* 2008). A study carried out to compare total polyphenols of some selected Kenyan teas with teas from other countries revealed that Kenyan teas had 7% to 27% more total polyphenols than those from China, Japan and Taiwan that are traditionally used for green , oolong and pouching tea manufacture and extraction of total polyphenols (Wachira and Kamunya, 2005b). Total polyphenols are important in black tea quality determination as well as potential health enhancement (Obanda *et al*., 1992; Kamunya and Wachira, 2006). Owing to their potent

antioxidant properties, total polyphenols can be used to market and bargain for premium prices for Kenyan tea.

2.5.4 Genetic transformation in tea

Foreign genes have been introduced in several woody crops including the rubber tree by using the *Agrobacterium tumefaciens* Ti plasmid (Horsch *et al*., 1985; Venkatachalam *et al*., 2006). Although the transgenic technology has immense potential for the genetic improvement of tea (Mondal *et al*., 2004; Lopez *et al*., 2004), the technique was hardly tried prior to 2000. This may have been caused by initial challenges in developing a viable protocol for gene transfer as well as efficient protocols for the *in vitro* regeneration of tea. Mondal *et al*. (2001) were able to optimize transformation conditions and production of transgenic tea via *Agrobacterium tumefaciens*. While research interest in genetic transformation in tea was highly motivated by the need to develop blister blight resistant cultivars in India, little interest has been evoked in Kenya largely due to lack of capacity to perform such work. Further, the assurance needed by Kenyan tea consumers abroad that tea products are not derived from genetically modified cultivars strongly negate the need to conduct research in this area. However, owing to the current production constraints occasioned by severe incidences of prolonged drought and frost and increased susceptibility to pests and diseases, a review of tea improvement efforts from the largely conventional approaches to modern biotechnology is now warranted for faster genetic improvement of tea.

2.5.5 Quantitative trait loci (QTL) mapping

Most attributes of agricultural importance frequently manipulated by plant breeders (e.g. size, shape, yield, quality, tolerance to abiotic and sometimes biotic stresses) display a quantitative mode of inheritance and normally exhibit continuous variation (Collard *et al*., 2005). A phenotype that is continuously expressed can be explained by the independent actions of many distinct genetic factors (polygenes), each having a small effect on the overall phenotype (Thompson and Thoday, 1974). These polygenes differ from genes affecting simply inherited traits in that they cannot be monitored directly by conventional methods, but a biometrical approach which partitions the total variation into genetic and non-genetic components has been devised (Jinks, 1981; Kearsy and Pooni, 1996). Procedures for estimating the number of effective genes controlling a quantitative trait (Mather and Jinks, 1982; Becker, 1984; Kearsy and Pooni, 1996) and the theoretical basis for interpreting the association of marker loci with QTL have been developed (Tanksley *et al*., 1982). Molecular

markers have also been used for studying quantitative inheritance (Tanksley, 1993; Kearsey *et al.,* 2003; Elberse *et al.,* 2004).

The principle behind detecting linkage between a marker and QTL involves evaluation of progeny for a character of interest and for their genotypes at marker loci at regular intervals (say 10 - 20 cM) throughout the genome. A search is then made for associations between the segregating marker and trait and any such associations discovered are owed to linkage. One-way analysis of variance on marker-genotype classes is the simplest way to look for QTLs (Soller *et al.,* 1976). Other more complex methods are those that involve identification of two linked markers flanking a QTL on either side (Lander and Botstein, 1989) using Log of odds (LOD) and joint effects of several QTLs on a trait using stepwise multiple regressions (Cowen, 1989; Stam, 1991).

Linkage between genetic markers and quantitative traits of economic importance has now been documented in a number of plants. For example in tomato, selection for specific QTLs in segregating progeny has led to the development of insect resistant lines (Niehuis *et al*., 1987) and cold tolerant lines (Vallejos and Tanksley, 1983). Elberse *et al*., (2004) detected a number of QTLs affecting growth related traits in wild barley. Saintagne *et al.* (2004), demonstrated the applicability of QTL variation in discriminating between some species of oak. QTLs associated with wood property traits in pine have been identified and verified (Brown *et al*., 2003). Work on Cacao detected stable yield QTLs in a study that spanned 15 years (Crouzillat *et al*., 2000). In tea, Wachira (1996) attempted to demonstrate the existence of various markers that significantly associated with QTLs influencing the nine traits measured. However, as the study involved only single-tree progeny that were not replicated, the precise measurement of phenotypic trait expression in the cross for each quantitative character could not be fully established. Replicated field trials of the mapping populations are currently available and coupled with more suitable models for resolving QTLs, identification of complex agronomic traits based on molecular markers is expected to be easier.

2.5.6 Marker-assisted breeding

One of the principal uses of DNA markers and genetic maps is marker-assisted selection (MAS) that is also known as marker-based selection or marker-mediated selection (Weising *et al*., 2005). MAS entails identification of individuals in germplasm banks or plant improvement programmes with DNA markers closely linked to traits of interest, and use such markers in indirect selection and breeding. Close linkage infers that the markers are flanking

the tagged gene(s) at a resolution of at least 2 cM and even better at zero recombination (an extremely rare thing). It is expected that the diagnostic DNA marker(s) will tag the gene(s) underlying the trait, so that no circumstantial and time-consuming field tests have to be performed. Thus, instead of selecting for the trait, which can cause undue environmental effect, the breeder selects for a DNA marker. An already successful procedure to tag agronomically relevant genes is given by Mohan *et al.* (1997). Such markers can also be identified by bulk segregant analysis (BSA), and therefore independently of any genetic map. Moreover, such markers can be detected very early in the selection procedure, so that the breeder can significantly reduce the number of seedlings grown and screened, thereby reducing expenses and enhancing efficiency of breeding. If seedlings are screened for the presence of a closely linked molecular marker, there is high probability that the seedlings carrying the marker will also carry the desirable trait, allowing them to be selected at a much earlier stage than would otherwise have been possible.

MAS applies for monogenic, oligogenic, or qualitative traits, and polygenic or QTL characters, and can be used to pyramid major genes for a trait (e.g., resistance) to produce varieties with improved properties (Kearsy and Pooni, 1996). For, example, pyramiding of *Xanthomonas* blight-resistance genes *Xa1, Xa3, Xa4, Xa5* and *Xa10* in different combinations using molecular marker tags proved to be efficient for developing resistant rice cultivars (Yoshimura *et al.*, 1995).

2.5.7 Bulk segregant analysis

Bulk segregant analysis (BSA) is one of the most dominant mapping techniques (Giovanonni *et al.*, 1992; Michelmore *et al.*, 1991). In this procedure, two bulked DNA samples from at least 10 individuals of an F_2 or a backcross population originating from a single cross are drawn. The bulks are homogeneous for a particular trait (e.g. resistant or susceptible to a specific pathogen, respectively), but heterogeneous at all unlinked regions. The bulks are screened for DNA polymorphisms, and detected differences compared with a randomized genetic background of unlinked loci. Any differences between these bulks (.e.g. presence vs. absence of a band on a gel) represent a candidate for a marker linked closely to the trait in question. Linkage has ultimately to be verified in a segregating population. BSA is widely applied to generate markers for marker-assisted selection, especially for those crops for which classical mapping procedures are complicated by huge genomes and long generation times such as forest trees.

Barua *et al*. (1993) were able to identify a RAPD marker linked to *Rhycosporium secalis* resistance gene in barley using BSA. Similarly, Chalmers *et al*. (1993) identified markers linked to genetic factors controlling the milling energy requirement of barley using the same approach. A 2.7 kb RAPD marker linked to leaf rust resistance in barley has also been identified using BSA (Poulsen *et al*., 1995). A study by Yang *et al*. (1997) found a PCR marker, RAPD-OPK/1300, linked to scab resistance gene V_f in apples. The marker was then cloned and sequenced leading to development of a sequence characterized amplified region (SCAR), which was then used to detect polymorphism as well as identify individuals resistant to the disease. Although much has concentrated on diseases, inroads have been made in other agronomic traits like yield and drought tolerance. For example, a QTL for grain yield in wheat associated with a locus SSR psp 3094 has been reported by Quarrie *et al*. (2007). Additionally, two RAPD markers, OPAE-09-1800bp and OPAE-14-1600bp were specific to high and low yielding individuals, respectively, in rice (*Oryza sativa*) (Shashidar *et al*., 2007). Identification of molecular markers linked to some genes of interest would enable prediction of presence or absence of the genes without actually perceiving the phenotype (trait). Thus, traits may be indirectly selected for using DNA markers instead of relying on the phenotype, which is largely influenced by the environment. Molecular markers come in handy especially when a trait is difficult to score, recessive or is hard to monitor in the presence of other genes (Wachira, 1996a). The demonstrated usefulness for these markers in marker-aided selection has elicited efforts in converting the markers into SCARs.

2.5.8 Saturated linkage maps

One of the main uses of DNA markers in agricultural research has been in the construction of linkage maps for diverse crop species. Linkage maps have been utilized for identifying chromosomal regions that contain genes which control simple traits mainly through a single gene and quantitative traits using QTL analysis. DNA markers that are tightly linked to agronomically important genes (called gene ‘tagging’) may be used as molecular tools for marker-assisted selection (MAS) in plant breeding (Collard *et al*., 2005). As earlier mentioned, MAS involves the presence / absence of a marker as a substitute for or to assist in phenotypic selection, in a way which may make it more efficient, effective, reliable and cost-effective compared to the conventional plant breeding methodology. Because of the long juvenile period and generation interval of most perennial crops such as tea and forest trees, it is arguable that MAS has more to offer in the improvement of such

crops than in short-lived annuals (Bradshaw, 1998). For example, if MAS proves useful not just in identifying clones for propagation in the current generation, but also in choosing superior parents with complementary multilocus genotypes for the next generation, 10 years or more might be saved in each breeding cycle (Bradshaw, 1998). Studies on construction of linkage maps in tree crops have only gained relevance recently owing to difficulties inherent and unique to such crops (Bradshaw, 1998). The first linkage map for tea was constructed by Wachira (1996) using RAPD and AFLP markers and published by Hackett *et al.* (2000). The map covered 1349.7 cM, with an average distance of 11.7 cM between loci. However, to date no comprehensive QTL analysis has been carried out in tea.

Linkage maps are constructed from analysis of many segregating markers. Essentially, linkage map construction involves three main steps; (1) production of mapping population; (2) identification of polymorphism and (3) linkage analysis of markers (Collard *et al.*, 2005). Since linkage analysis is based on meiotic recombination, genetic mapping can be accomplished in all plant species that undergo sexual reproduction. A mapping population in which parents segregate for a trait of importance is a prerequisite for the visualization of linkage relationships (Bradshaw, 1998).

Linkage map construction is normally accomplished by five main steps (Berg *et al.*, 1994).

- Analysis of segregation at individual loci
- Detection of linkage between loci
- Estimation of recombination frequencies and map distance between loci
- Assembly of loci into linkage groups, and
- Estimation of the linear order of loci within linkage groups.

Linkage is detected when the proportion of progeny inheriting recombinant combinations of any pair of markers falls significantly below a chosen threshold (usually 0.4) (Berg *et al.*, 1994). Several statistical tests have been suggested and applied in detection of linkage but they differ in their power to detect linkage (Berg *et al.*, 1994; Kearsey and Pooni, 1996). Several of these tests employ the principle of comparison between given sets of expected phenotypic frequencies and the actual observed frequencies and assume Mendelian single-locus segregation (Garcia-Dorado and Gallego, 1992). A different approach, known as logarithm of "odds" (LOD) score test by Morton (1955) was employed. The "odds" refer to the probability that two loci are linked with a given recombination value over the probability that they are not linked. With this method, pair wise recombination frequencies are first

calculated. The corresponding LOD values are also calculated. A LOD value of 3.0 between two markers indicates that linkage is 1000 times more likely (1000.1) than no linkage (null hypothesis) (Collard *et al*., 2005). LOD values of > 3.0 are typically used to construct linkage maps. LOD values may be lowered in order to detect a greater level of linkage or to place additional markers within maps constructed at higher LOD value. LOD values are therefore seen as a measure of linkage in the data and the score value normally increases with sample size and decreases with increasing recombination value. Several computer softwares that can be used to calculate pair wise recombination frequencies, linkage distances and associated likelihood have now become available and they include; Mapmaker (Lander *et al*., 1987), Joinmap (Stam, 1993) and Map Manager QTX (Manly *et al*., 2001).

2.6 Development of markers in crop breeding

Three types of genetic markers have been used in genomic analysis in general, and plant breeding in particular. These are morphological markers, protein based markers and DNA based markers. Variation among genotypes within a species is the raw material for genomic analysis. To get a genetic marker, the marker locus has to show experimentally detectable variation among individuals in the test population. The variation can be considered at different biological levels, from the simple heritable phenotype to detection of variation at the single nucleotide. Once the variation is identified and the genotypes of all individuals known, the frequency of recombination events between loci is used to estimate linkage distances between markers (Liu, 1998). A genetic marker has to be a polymorphic marker. However, the inverse is not true that a polymorphic marker may not be a genetic marker if it is not operationally defined as a heritable polymorphic marker with clear genetic interpretation and repeatability. According to Liu (1998), genomic analysis using genetic markers should be based on well established genetic models. If the underlying genetics of a marker is not clear, then the analysis may be misleading. More importantly, the marker assay should be repeatable at different times in the same or different laboratories.

2.6.1 Morphological markers

Early mapping studies concentrated on discreet traits with simple Mendelian inheritance, such as shape, colour, size or height (Stadler, 1929). Morphological traits often have one to one correspondence with genes controlling the traits. Due to that, morphological characters can be used as reliable indicators for specific genes and are useful as genetic

markers on chromosomes. Most morphological markers have originated from mutation studies (Wettstein *et al.*, 1992; Waycott *et al*., 1999). The variations represented through these mutations were observed as altered plant phenotypes that range from pigment differences and gross changes in development such as vernalization habit or dwarf versus tall habit, to disease resistance response. Nevertheless, morphological markers have not been used extensively in practical plant breeding because of the limited availability of different mutants and those available were not neutral in their effect on agronomic phenotype (Worland *et al.,* 1987). To obtain a reasonable number of polymorphic morphological markers, many mapping populations are needed (Liu, 1998). Additionally, the complexity of genotype by environment interaction that governs the trait of interest might lead to misleading identification of a phenotype.

2.6.2 Protein and isozyme markers

Efforts to counter the aforementioned limitations led to development of protein and isozyme markers (Markert and Moller, 1959). However, it took about two decades before a tight genetic linkage between a nematode resistance gene and an *Aps* isozyme allele in tomato was established (Medina-Finho, 1980). This subsequently opened the avenue of tagging gene(s) of agronomic importance (Tanksley and Rick, 1980). The effect of isozymes and proteins on the plant's phenotype is usually neutral and both of them are often expressed codominantly making the discrimination between homozygote and heterozygote possible. The utilization of these markers in plant breeding programmes however is limited owing to low number of isozyme and protein markers and requirement of different protocols for each isozyme system (Markert, 1975). These problems have, fortunately, been overcome with advent of DNA marker system (Botstein *et al*., 1980).

2.6.3 DNA Markers

The application of DNA markers in crop breeding includes the DNA polymorphic assays for genetic mapping, marker-assisted plant breeding, genome analysis, parasite diagnosis and genotyping (Mignouna *et al*., 1996). These molecular technologies include RFLPs (i.e. Restriction Fragment Length Polymorphism), RAPD (Randomly Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), SSRP (Simple Sequence Repeat Polymorphism), SCARS (Sequence Characterized Amplified Regions), STS (Sequence Tag Sites), CAPS (Cleaved Amplified Polymorphic Regions), SSCP (Single

Strand Conformational Polymorphism), DCSP (Double Strand Conformational Polymorphism) and SNP (Single Nucleotide Polymorphisms).

Since different DNA marker technologies detect different types of variations, the choice of marker(s) to use in a study is crucial to the study objectives. The polymerase chain reaction (PCR) based DNA amplification techniques are in general more advantageous than classical markers such as RFLPs, but all have their limitations. The two most important factors to consider include the multiplex ratio (the number of markers that can be generated in a single reaction) and the information content (the effective number of alleles that can be detected per marker in a set of individuals) (Gysel *et al*., 1996).

2.6.3.1 Restriction Fragment Length Polymorphism (RFLP)

The RFLP has been found to elicit considerable polymorphism on the basis of the restriction size variation in the genomic DNA (Tanksley *et al*., 1989; Paterson *et al.,* 1991). Generally, RFLP are robust genetic markers, which are inherited in a Mendelian manner, are abundant in many plant genomes and encompass both coding and no-coding sequences (Mignouna *et al*., 1996). They are highly heritable and do not display epistatic or pleiotropic effects (Mignouna *et al*., 1996). The RFLP multilocus probes have been used to fingerprint crop species and also establish linkage to the quantitative trait loci important in breeding (Melfin *et al*., 1999; Powell *et al*., 1991). Their use on perennial crops has however been constrained by high costs, lack of technical expertise and the quantity and quality of DNA extractable from species under study. Additionally, RFLPs are labour intensive and have low throughput. The use of short-lived radioisotopes also makes this approach unsuitable for use in studies involving large numbers of samples (Wachira, 1996b; Mignouna *et al*., 1996).

2.6.3.2 Polymerase Chain Reaction (PCR)

The PCR technique was first devised by Mullis and Faloona (1987) for *in vitro* amplification of specific DNA sequences by the simultaneous primer extension of complementary strands of DNA. It involves three steps. DNA denaturation, primer binding and DNA synthesis. Target duplex DNA is first denatured, followed by hybridization and annealing to two oligonucleotide primers that flank the specific target DNA segment. DNA polymerase then extends the annealed primers on template strands. Repeated cycles generate an exponential accumulation of multiple copies of discrete DNA fragments. The efficiency of PCR has been greatly improved by thermostable DNA polymerases like the Taq polymerase from the bacterium *Thermus aquaticus* (Saiki *et al.,* 1988).

2.6.3.3 Randomly Amplified Polymorphic DNA (RAPD)

The RAPD technique is based on the amplification of random DNA sequences by the Polymerase Chain Reaction (PCR) with arbitrary primers. The assay is technically simple, fast and requires only small quantities of crude DNA preparations (Williams *et al.*, 1990). The RAPD markers have found use in many types of genetic analyses. They have been used in construction of linkage maps (Williams *et al.*, 1990; Carlson *et al.*, 1991; Hackett *et al.*, 2000). They have also been used in estimation of genetic diversity and population differentiation (Chalmers *et al.,* 1992; Sharma *et al.*, 1995; Wachira *et al.,* 1995; Wachira *et al.,* 2001; Wachira, 2002). The advantage of RAPD over RFLP is due to its high multiplex ratio and can yield up to 20 informative markers per primer (Powell *et al.*, 1996). RAPD markers are however sensitive to changes in quality and quantity of DNA and reagents. Thus, amplification conditions must be optimized and kept under very strict control to avoid inconsistencies (Williams *et al.*, 1993). Moreover, the resulting amplified DNA fragments have the characteristics of any Mendelian dominant genetic marker. A further drawback is that amplification fragments of indistinguishable size may not represent homologous loci (Weising *et al.*, 2005). Due to this, the application of RAPD markers in comparative mapping projects may be limited.

2.6.3.4 Amplified Fragment Length Polymorphims (AFLP)

The AFLP technique combines the robustness of RFLP analysis with the reliability of stringent PCR reactions. The DNA of the target organism is digested with a combination of two restriction enzymes (one with a 6 bp recognition sequence such as *Eco* RI and another one with a 4 bp recognition sequence such as *MSe* I). The resulting fragments are ligated to oligonucleotide adapters of known sequences, followed by amplification of a specific set of restriction fragments. Fragment amplification is determined by primers, which include the adapter sequence plus a number of arbitrary nucleotides at the 3' ends of the primers. This technique allows the simultaneous amplification of a very large number of fragments of which 10-50 may be polymorphic depending on the genome size and target species. The total number of fragments amplified can be adjusted by altering the total number of arbitrary nucleotides used in the primers. Additionally, AFLP allows dominant as well as co dominant markers to be analyzed. However, scoring different alleles of a particular locus is not obvious, implying that information content is rather low but this problem is overcome by other molecular marker systems, such as the SSR. AFLP has been used to establish the extent of genetic variation, population differentiation in plants and linkage mapping (Vos *et al.*,

1995; Paul *et al*., 1997; Loh *et al*., 2000; Hackett *et al*., 2000; Wachira *et al*., 2001). AFLP are particularly useful owing to their high multiplex ratio (Wachira *et al*., 2001).

2.6.3.5 Sequence Tag Sites (STS)

This is a general term applied to any unique genome fragment amplified with 1824 bp primers derived from known sequences or end sequenced RFLP probes (Farooq and Azam, 2002). Polymorphism can be recognized as on or off (where one primer does not work in variety and no PCR product is produced), or length variants where part of the intervening sequences are deleted or inserted. Though robust, STS are beset with low polymorphism that can be enhanced by development of four base pair cutter and later converted into a specific polymorphic marker such as single polymorphic amplified test (SPLAT) (Farooq and Azam, 2002). Sequence Tag Sites of mitochondrial and chloroplast sequences have been used to study phylogenetic relationships of species within the genus *Camellia* to which tea belongs (Wachira *et al*., 1997).

2.6.3.6 Sequence Characterized Amplified Region (SCARS)

This technique is used to generate reliable markers from RAPD and AFLP polymorphisms. The RAPD band, for example, is identified as a candidate marker, excised from the gel and the ends of this fragment are sequenced. It is then possible to design sequence specific primers, which will reliably amplify a single locus in several genotypes (Mignouna *et al*., 1996). This technique has been used to identify markers linked to the downy mildew resistance genes in lettuce (Paran and Milchelmore, 1993) and scab resistance gene in apple (Yang *et al*., 1997; Boudichevskaia *et al*., 2006).

2.6.3.7 Simple Sequence Repeats (SSR)

Most plant genomes include regions consisting of a simple sequence repeated many times. These repeated motifs have been classified into two groups. Motifs 10-50 bp in length are termed minisatellites or variables number tandem repeat (VNTR) while motifs which range 2-4 bp in length are termed micro satellites or simple sequence repeats (SSR) (Jacob *et al.,* 1991). It is variation in the number of times that these elements are repeated that provides the basis for length polymorphisms which can be used in genetic studies. SSRs, in particular, have been found to be highly polymorphic even between closely related individuals. In addition, SSR markers are simple and quick to analyze, are robust and reliable, and, can detect heterozygotes. However, the cost and time required to generate primers are major

drawbacks. SSR markers are considered excellent genetic markers for genetic fingerprinting, population genetic studies particularly for examination of gene flow and molecular breeding (Powell *et al.*, 1996). Owing to their codominant nature, SSRs are particularly useful in determining parentage.

2.6.3.8 Information content of molecular marker system

It has been shown that the utility of a given marker system is a function of its marker index; (MI) = H X E; where H= diversity index and E= effective multiplex ratio (proportion of polymorphic loci x number of loci analyzed per gel) (Powell *et al.*, 1996). A comparison of the various marker systems in use shows informativeness to be in the following descending order AFLP>RAPD>SSR>RFLP (Wachira *et al.*, 2001).

2.7 From conventional to molecular breeding

Quantitative trait describes a character for which the observed variation is due to the segregation of several genes and where, for each gene, the effects of the allelic differences on the phenotype are generally small compared with the effects of the environment (Kearsey and Pooni, 1996). Genetic mapping of QTLs involves identifying and determining the degree of association between the continuous traits and sets of genetic markers.

The ability to assess complex phenotypes such as yield, quality, drought tolerance and susceptibility to pests and diseases in tea at the seedling stage using genetic markers would greatly accelerate new variety development. In addition to the selection of desired traits, markers linked to complex traits could be used to select against negative characteristics, and could even be used to select the combination of parents that would give rise to progeny with the desired genotype.

An essential requisite for accurate QTL identification in any plant species is a saturated genetic map covering the entire genome. If certain regions of the genome are not adequately represented by genetic markers, the QTL located in such regions will not be reliably mapped, because it will be difficult to determine if the QTL has genuinely small phenotypic effect, or is merely weakly linked to flanking markers (Lander and Botstein, 1989).

2.7.1 Map-based cloning

In plants, some traits are controlled by a single gene (major gene), while others are polygenically influenced. The location of the gene controlling a trait of interest is deduced by

following the inheritance of the trait relative to the inheritance of linked molecular markers. Markers that are located very close to the DNA region controlling the trait are identified by virtue of co-inheritance with the trait in the progeny of a cross between two plants differing in the trait (but not necessarily in the heterozygote species) (Weising *et al*., 2005). By identifying two such markers that are very close and flank the trait of interest (fine-mapping) (Weising *et al*., 2005), a small DNA fragment that contains the genes can be isolated (positional or map-based cloning). Once isolated, the DNA sequence can be determined and the function and organization of the gene can be studied.

Map-based cloning has been used to isolate disease resistance genes in many crop plants, for example the gene controlling resistance to the bacterial pathogen *Pseudomonas syringae*, in tomato (Martin *et al*., 1993). This gene product was determined to be a protein kinase, and when this gene was transferred to susceptible plants, they became resistant. A gene controlling resistance to *Xanthomonas oryza* was also identified with the map-based cloning approach (Song *et al*., 1995).

2.8 Participatory Crop Improvement. Involvement of farmers in tea improvement

Participatory Crop Improvement (PCI) emerged in the past decade as an alternative plant breeding approach for developing countries in response to the recognition that conventional breeding of the formal institutions had brought little significant crop improvement to small-scale farmers in agro-ecologically and socio-economically marginal and variable environments (Virk *et al*., 2003; Witcombe *et al*., 2003). A major reason for this is the fact that Formal Crop Improvement (FCI) in developing countries concentrated on cereals and cash crops such as tea in favourable high input agricultural systems. It was expected that at least some of the materials, which were developed for high input production systems, would also be successful in low input environments. However, farming systems in marginal environments are too different from those in the more favourable production areas (Lipton and Longhurst, 1989).

In developing countries, FCI programmes are largely carried out on-station under well-controlled conditions, thus reducing environmental variation and increasing heritability and expected genetic gain. However, majority of the small-scale farmers operate in environments in which variable complex stresses have a dominating effect on crop performance (Banziger *et al*., 1997). The importance of adaptation to variable and risky low-input rain fed conditions, secondary crop uses and cultural preferences have received little or no attention. As breeding work focuses more on breeding for high yields and adaptation, the

need to emphasize on other characteristics of importance for small-scale farmers calls for closer attention. The need to produce a stable variety demands that selection be carried out in marginal environments, preferably with farmers. Generally, if a breeder wishes to produce a strain or variety that should perform well in a particular environment, then selection should be carried out in that environment (Kearsy and Pooni, 1996). On the other hand, in order to produce a stable variety, selection should be carried out in a poor environment.

The common usage of relatively high input levels to minimize abiotic and biotic stresses and to target moderate to high input agriculture in FCI, does not only reduce the ratio of environmental variance versus genotypic variance in comparison with the use of lower input levels, but also increases the discrepancy between on-station and on-farm conditions (Ceccarelli *et al*., 1992). If environments are sufficiently different, GxE interactions can result in different ranking of evaluated germplasm representing the so-called crossover effect. Thus, products from FCI programmes are not necessarily adapted to the marginal environments. On-station selection, therefore, does not in such a case result in the most productive materials for the specific conditions in the farmer's fields.

Differences in selection criteria contribute to diversity in materials selected by breeders and farmers. While farmers pay more attention than the breeder to yield stability and characters of quality and secondary uses, the breeder may be paying attention to high yields (Thiele *et al*., 1997). Because the criteria other than yield appear very important factors in variety adoptions and rejection, it is logical to include them explicitly in the analysis of GxE interaction and related issues in the context of PCI. Another drawback of the FCI system is its slow release of relatively few genetically homogeneous genotypes. It takes a FCI programme 12-18 years to develop a new variety. A breeding programme easily works with thousands of heterogeneous or homogeneous entries in different stages, of which only a fraction reaches the on-farm testing phase, of which in turn only few varieties are released. Much material that could potentially have been valuable for other conditions and preferences is eliminated in the process. The released varieties are usually genetically uniform, which is not a necessity for small-scale farmers. On the contrary, materials that contain some genetic diversity may be more suitable for variable and heterogeneous environments, providing them with an increased buffering capacity and potential to adapt. The rights of variety registration and plant variety protection add to the time needed for release and involve costs that form an additional drawback to respond to the needs for diversity within and among crop varieties.

2.8.1 Strategy and justification of participatory crop improvement

Participatory Crop Improvement aims to link formal and local systems of crop improvement, combining the complementary capacities and expertise, seeking to combine the improvement of productivity with the supply of agrobiodiversity needed by farmers (Hardon, 1995). The PCI-strategy aims to insert useful genetic diversity into the local systems and build on farmers' capacity on seed selection and exchange. Rather than trying to improve the impact of conventional breeding programmes that generate at the end of breeding pipeline a limited number of genetically uniform varieties, the idea is to flush out into farmers' fields larger number of materials, representing a wider range of genetic diversity. PCI builds on the recognition of farmers' capacity to select what best fits their environment and improved development of local crop adaptation through farmers' variety and seed selection. It relies on farmers' seed production and exchange to maintain and diffuse varieties.

The main advantage of PCI over conventional breeding is that it involves farmers in developing, adapting and adopting new varieties; setting breeding goals; and selecting parents according to their requirements. Level of participation, however, varies with the nature and objectives of the project and availability of resources. It develops among different organizations and farmers the spirit of working closely together and appreciating each other's capability and contributions. The strengths and capabilities of different stakeholders are fully utilized in an integrated form.

A common functional distinction within PCI is Participatory Variety Selection (PVS), which is the selection among advanced or genetically stable populations and lines, and Participatory Plant Breeding (PPB), which is selection within segregating populations (Witcombe *et al.*, 1996). In PVS, farmers are given varieties (finished products from plant breeding) for testing in their own fields. After successful PVS programme, the varieties preferred by farmers can be used as parents in breeding programme where farmers participate as active collaborators. This involves breeding and selection to create new varieties and is called PPB. However, the distinction between PVS and PPB is not always clear. In case of cross-pollinating populations, selection among populations (PVS) is usually combined with within-population selection (PPB). On-farm evaluation allows weighing of preferences and needs by the end-user of the products, and enables exploitation of G x E interaction through seeking location-specific adaptation to the complex and variable environment.

2.8.2 Success cases of PCI

The success of the reported cases so far supports the promises of impact that participatory approaches can have on crop improvement in marginal environments (Sperling *et al*., 2001). The success of these cases is largely based on the fact that through collaboration with farmers and on-farm selection in the target area, selection criteria and characters that were not given sufficient weight in the selection in FCI, are now identified and incorporated in the material. The farmers' willingness and capacity to invest time and resources in selection and participation with breeders will depend strongly on the benefits they derive from it. Benefits to farmers are access to materials with increased yield; yield stability or other improvement status, knowledge and increased capacities (empowerment), and benefits from seed exchange. The latter benefit however assumes that locally selected materials have a wide agro-ecological adaptation and are attractive to a larger group of farmers (Sperling *et al*., 2001).

The farmers' empowerment is considered as an important social benefit from PCI since it presumably influences their empowerment impact. If farmers are only consulted and are not allowed to make decisions in the identification of material, setting of selection criteria and selection itself, there is no true participation or an empowerment benefit. Empowerment or the capacity of farmers to work on improving their own is recognized as an important condition for sustainable agricultural development.

2.8.3 Situation of tea improvement in relation to PCI

Studies conducted in the recent past have revealed that the performance of tea clones relative to each other considerably vary with environments so that clones which are superior in one environment are not correspondingly superior elsewhere (Ng'etich *et al*., 2001b; Wachira *et al*., 2002). Such genetic variation in response to environmental changes and in adaptation has not been adequately studied in tea. Earlier tea improvement efforts had limited involvement of farmers' role in selection. Tea varieties were developed in one site, usually, at the Tea Research Foundation's (TRFK) headquarters in Timbilil estate, Kericho with all the stages in the selection process being evaluated at the site. The elite varieties were then released to farmers in the country in order to test their adaptability to the local niche environments. Owing to the perennial nature of the tea growth cycle, the farmers eventually adopted a few of released varieties. In deed one clone TRFK 6/8 endeared itself to the farmers owing to its high black tea quality and consequently it has been adopted by more than 60% of small scale farmers (Wachira, 2002).

The current tea improvement programmes have fully embraced the concept of modified PCI by involving the farmers in clonal adaptability studies, through in part, their factories tea extension agents and partly through the small-holder management agency, the Kenya Tea Development Agency (KTDA). Some farmers have volunteered their fields as testing sites and even their resources to maintain and collect data from the trials. Extension officers supervise data collection activities on weekly basis and later forward them to the plant breeder at TRFK.

To ensure that quality and reliable data is collected, training sessions on clonal identification, labeling, data collection, recording and reporting are held on-farm. The initial data recording is done by a team comprising TRFK technical staff, KTDA Tea Extension staff and the farmer. Subsequently, the Tea Extension Coordinators and their assistants conduct supervision and provide linkage between the farmer and TRFK staff. The breeder and his technical staff carry out follow up visits on quarterly basis to hold talks with parties involved, which include finding out the problems the farmers are facing and how best to solve them.

A success case of PCI in Kenya can be demonstrated by three clonal trials planted in 2003 in some selected smallholder farms with the aim of testing tea germplasm for tolerance and/or resistance to root knot nematodes (Kamunya *et al*., 2008). The three farms, situated in Kirinyaga district, East of Rift Valley, were chosen on the basis of harboring high levels of nematode populations and their owners' willingness to surrender them for experimentation until sufficient data were collected. A total of 58 clones (17 released; 41 promising clones) were screened for nematode tolerance/ resistance in the three sites. Evaluation carried out over 2-year period from 2004 revealed that the resistant/tolerant varieties gave significantly higher yields than the susceptible clones (Anon., 2006).

Similar trials have been set up in Trans Nzoia, Mt Elgon, Gucha, Nandi and Meru North districts. Preliminary results show remarkable variability in clonal performance and preferences by the farmers (Anon., 2006).

From earlier discussion on success cases of PCI the role of farmers emerged to be as:

(i) Identification of traits, which are considered to be minor for the FCI to address.

(ii) Recognition of traits that correspond with the farmers' preferences.

(iii) Identification of characters that better suit their farming systems.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Plant materials for quantitative genetic parameters

The plant material consisting of four parental clones involved in the 4 x 4 full diallel cross were among the most popular Kenyan commercial tea clones that were selected based on diverse attributes (Table 4). The generated 16 clonal full-sib crosses (F_1s) including reciprocals and selfs were derived from full diallel crosses carried out between 1983 and 1993. Seeds were collected into muslin bags tied to the artificially pollinated flowers upon maturity and germinated in a germination chamber before transferring them to the nursery. Seedlings were reared in the nursery for one year after which they were transplanted in the field as single bush progeny tests. Upon establishment, the seedlings were then brought into bearing and by the end of third year the bushes had formed a closed canopy which enabled subsequent cloning of selected bushes. Owing to variable number of bushes per cross, five plants were randomly selected to represent each full-sib progeny except for two selfs belonging to TRFK 6/8 and EPK TN14-3 that had two surviving sib families each. The bushes were left to run for cuttings for about five months, following which healthy cuttings were collected and prepared according to the recommended method (Anon., 2002). Cuttings were collected from selected progeny, rooted and raised in the nursery for one year prior to field transplanting.

Table 4. Attributes of diploid parental clones used to generate full-sib families

Clone	Varietal type	Special attributes
EPK TN14-3	Kenyan Chinary local selection	Tolerant to high soil pH and cold; Susceptible to Red Crevice Mites (*Brevipalpus phoenicis*); Moderate levels of caffeine (2.7%)
TRFCA SFS150	Malawian Assam type	Drought, cold and pest tolerant; Moderate levels of caffeine (2.9%)
AHP S15/10	Assam type Kenyan local selection	High yielding; Highly pubescent; Susceptible to water stress; Moderate levels of caffeine (3.0%)
TRFK 6/8	Assam type Kenyan local selection	High black tea quality (fast fermentability and high levels of polyphenols (25%)); Average yielding; Susceptible to water stress with low levels of caffeine (1.7%)

3.1.2 Test site

The 4 x 4 full diallel cross trial comprising twelve clonal full-sib families and four parental clones was established in the year 2000 at the Timbilil Estate of the TRFK, Kericho (Table 5). The field trial was set up in a randomized complete block design with three replications in plots of 30 plants spaced at 0.61 m within rows and 1.22 m between rows (i.e. 13448 plants/ha). The trial had been receiving 150 Kg N per hectare per year in the form of NPKS 25:5:5:5 compound fertilizer. Each replicate was surrounded by a guard row of clone TRFK 303/1199. The tea was brought into bearing following the recommended management practices (Anon., 2002).

Table 5. Location, elevation and climatic characteristics of the two experimental sites in Kenya

Site	Latitude	Longitude	Elevation (m)	Annual average temperature (^oC)	Annual rainfall (mm)	Solar radiation (MJ $m^{-2}d^{-1}$)
Timbilil	0° 22’ S	35° 21’ E	2180	16.28	2043	20.72
Kangaita	0° 30’ S	37^o18’E	2100	15.27	2009	-

3.2 Plant material for QTL mapping of yield, total polyphenols, drought tolerance and pubescence

Genetic materials for construction of a linkage map and mapping of QTL governing the above traits included the 42 clonal progeny of two heterozygous parental clones TRFCA SFS150 (female) and AHP S15/10 (male). Although the two clones belong to the Assam taxa, the cross was chosen on the basis of the differing parental attributes and only a moderate genetic similarity of 67% (Wachira, 2002).

3.2.1 Test sites

The cross comprising 42 clonal progenies and their parents were established in 2000 in two sites, one each at the two research stations of TRFK, in Timbilil (Kericho district) and Kangaita (Kirinyaga district) (Table 5). The trial was set up as a completely randomized block design with three replications in plots of 30 ramets spaced at 0.61 m within rows and 1.22 m between rows.

3.3 Plant material for QTL mapping of resistance to root knot nematode

A nursery experiment aimed at testing the host-plant resistance/tolerance to root knot nematode was established at the Kangaita tea nursery in December 2005. The propagation

materials used in the trial were collected from 41 progeny of a cross involving highly tolerant parental and susceptible clones, TRFCA SFS150 (female) and TRFK 303/577 (male), respectively. The F_1 single bushes arising from the cross were allowed to grow freely for about five months, following which healthy cuttings were collected and prepared (Anon., 2002). A hundred cuttings from each progeny were then propagated into root knot nematode infested soil in nursery micro-plots and replicated three times as a randomized complete block design. Cuttings from the two parental clones were also included in the experiment. The infected soil had been mined from various farmers fields on which intensive horticultural farming had been practiced with most of the crops being nematode susceptible plants like spinach (*Spinacia oleracea*), bananas (*Musa spp.*) and passion fruits (*Passiflora edulis*). Micro-plots were first planted with spinach 5 months ahead of propagation in order to boost the population of nematodes.

Assessment undertaken one year after propagation indicated lack of significant infection by root knot nematode (RKN) owing to low number of the pest in the soil. As the nursery plants were ready for transplanting, it was decided that a farm infested with root knot nematodes be sought instead within farmers' fields and negotiations held with the owners for a 2-year collaborative study entailing testing host-plant resistance. Three such farms were availed but only one was found suitable for field testing owing to its high numbers of nematodes. Other than what was evident from soil sampling results, the farmer had severally tried but in vain to establish the popular commercial cultivar, TRFK 303/577. A clonal progeny trial was superimposed on the farm in January 2007 with 6-plants clonal plots planted inter-rows and replicated three times (Figure 3 and 4). All the 41 clonal progenies and parental clones were included in the trial.

3.4 Measurements of phenotypic traits

3.4.1 Green leaf yield (Kg mt/ha)

Data collection on yield in form of plucked two leaves and a bud commenced in February, 2001 and continued up to December, 2007. Harvesting was carried out at intervals of 7 to 10 days depending on availability of crop. The cumulative yield data was converted from green leaf weight to annual mean yield by dividing it with the number of years since first plucking. The green leaf yield (Kg) was converted to made tea per hectare (mt/ha) by a conversion factor of 0.225 prior to statistical analyses.

Figure 3. Root knot nematode susceptible clone TRFK 303/577 showing poor establishment

Figure 4. Robust root knot nematode resistant clone TRFCA SFS150 showing good establishment

3.4.2 Total polyphenolic contents of green leaf (%)

The total polyphenols content (%) was determined from 0.5 g of steamed and milled fresh tea shoots collected from each of five randomly selected bushes of each clonal full-sib progeny. The amounts of polyphenols from the test samples were determined from a standard curve generated using gallic acid as a standard, and were expressed as the amount of gallic acid equivalent. The total polyphenol content was expressed as per cent by mass on a dry matter basis following procedures outlined in the British Standard ISO document (BS ISO 14502-1:2005(E)).

3.4.3 Fermentability of green leaf

Chloroform test (Sanderson, 1963) was used to determine rate of leaf fermentability. This was carried out on harvested two leaves and a bud sampled from five randomly selected bushes per plot to determine the fermentability of the test array as well as the parent clones and one inherently non-fermenting clone, TRFK 12/2 as control. Fermentability was scored based on the change in colour after four hours using a 4-point scale as: 1 - bright red brown (fast fermenter); 2- dull brown (moderate fermenter); 3- greenish tinge (poor fermenter); 4- green (non-fermenter).

3.4.4 Drought damage assessment

Drought damage was scored during periods of severe water stress that were accompanied by frost incidences between January and April, 2003 and November 2005 to April 2006. Damage due to drought was scored on five randomly selected plants per plot using a 5-point scale as: 1. (0-10% scorch with prolific flushing with no dormant shoots); 2. (11-25% scorch and wilting with few dormant, few flushing shoots and some leaf fall); 3. (26-50% scorch with many dormant shoots, wilting leaves and moderate leaf fall); 4. (51-75% scorch with many dormant shoots, wilting leaves, severe leaf defoliation and die back); 5. (76-100% scorch with severe defoliation and die back, all shoots dormant and sometimes death).

3.4.5 Bud pubescence

The degree of pubescence (PUB) on the leading bud (“tip”) and abaxial surface of the leaf is an important morphological marker for quality especially in orthodox and white tea. This character was scored on bud and first unfurled leaf from three randomly selected bushes using a modified 5-point scale that was first described by Wight and Barua (1954). Thus,

assessment of pubescence was carried out under a light dissecting microscope as follows. 1. glabrous buds and leaves with hair only on the mid-rib; 2. buds and leaves with a few scattered hairs on the lamina; 3. hairiness extending about halfway to the margin; 4. leaves and buds with entire surface of lamina pubescent; 5. leaves and buds where pubescence formed a dense indumentum.

3.4.6 Bud size and weight

Bud width (BWd) was measured at 1 mm above the basal end of the selected buds, while bud length (Blth) was measured from tip to the basal end of the same buds. The buds used for scoring pubescence were also used for bud size measurements. Bud weight (BWt) was recorded as dry weight for 20 randomly selected buds among 50 plucked ones which had been dried at 70°C for 48 hours.

3.4.7 Total theaflavins (TF) content

Black tea quality analysis was conducted from miniature manufactured tea samples obtained from each of the clones represented in the trial. The tea leaf was withered at room temperature for 18 hours and then processed by crush, tear and curl (CTC) maceration method using miniature CTC machine. The leaf was passed through the CTC rollers four times to achieve maceration equivalent to that obtained using commercial CTC rollers in black tea manufacture (Owuor and Othieno, 1991). Upon maceration, the tea (dhool) was fermented for 90 minutes under ambient temperature (22-26°C) and 100% relative humidity. A bench top fluid drier system (Tea Craft Ltd) was utilized in firing the tea, initially at 120°C for about 20 minutes then lowered to 100°C for 10 minutes. The unsorted black teas were then subjected to chemical analysis. Total TF were determined by the Flavognost method (Hilton, 1973) as follows: a tea infusion was made with 375 ml of boiling water, added from an overhead boiler into tared flask, and 9 g of tea. The flask was shaken for 10 min, the infusion filtered through rough cotton wool, and allowed to cool to room temperature, and then 10 ml were pipetted into 10 ml of isobutylmethyl ketone; (4-methylpentan-2-one, IBMK). The mixture was shaken for 10 min and allowed to stand until the layers separated. Two milliliters of the upper layer were pipetted into a test tube, followed by 4 ml of ethanol and 2 ml of Flavognost reagent (2 g diphenylboric acid-2-aminoethyl ester dissolved in 100 ml). The contents were mixed and colour was allowed to develop for 15 min. The absorbance (*A*) was read at 625 nm against an IBMK/ethanol (1:1 v/v) blank.

Theaflavin (µmol/g) = A_{625} x 47.9 x 100/DM

3.4.8 Total Thearubigins (TR)

Total thearubigins (TR) were determined using the protocol of Roberts and Smith (1963). Fifty milliliters of the cooled, well-shaken and filtered standard tea infusion from theaflavin analysis were mixed with 50 ml of isobutylmethyl ketone (IBMK) and gently shaken to avoid formation of an infusion. The layers were allowed to separate and a 4 ml portion of IBMK layer was taken and topped up to 25 ml with methanol in a volumetric flask (Solution A).

Twenty-five millilitres of the remaining initial IBMK layer were taken in a separate flask and mixed with 25 ml of 2.5% aqueous sodium hydrogen carbonate. The mixture was vigorously shaken before the layers were allowed to separate and the aqueous layer discarded. A 4 ml portion of the washed IBMK layer was made to 25 ml with methanol (Solution B).

Two millilitres of a saturated oxalic acid aqueous solution and 6 ml of water were added to a 2 ml portion of the aqueous layer left from the first extraction with IBMK, and diluted to 25 ml with methanol (Solution C).

The absorbances A_A, A_B and A_C, of solutions A, B and C, at 380 nm were obtained using a CE 393 Cecil Digital grating spectrophotometer with distilled water as the blank.

Considering the fact that mean absorbance of the thearubigin fractions at 380 nm was 0.733 (Roberts and Smith, 1963), the following equation was used in deriving the total thearubigins.

$\%TR = (375 \times 0.02 \times 6.25[2 A_C + A_A - A_B,]/(0.733 \times 9 \times DM/100)$

Measurements from the mapping family were averaged over the three replicates prior to QTL analysis.

3.4.9 Assessment of root knot nematode (RKN) susceptibility

Assessment of susceptibility to root knot nematode (*Meloidogyne* sp) was carried out in July 2007. Binary data was collected for presence (1) or absence (0) of knotting on roots of breeding Stock 526 (TRFCA SFS150 x TRFK 303/577).

Figure 5. Root knot nematode infected roots of a susceptible clone TRFK 303/577. Knots are shown by arrows

3.5 Statistical analysis of quantitative parameters

3.5.1 Analysis of combining abilities

Combining abilities were estimated according to Griffing's (1956) diallel Model I Method 1 assuming random effects.

The statistical model for Griffing (1956) analysis is:

$Y_{ij} = m + g_i + g_j + s_{ij} + r_{ij} + 1/bc\Sigma\Sigma e_{ijkl}$

$i, j = 1,2, \ldots, n$

$k = 1, 2, \ldots, b$

$l = 1, 2, \ldots, c$

where,

Y_{ij} is the mean of i x jth genotype over k and l,

g_i is the general combining ability (gca) effect of the ith parent,

g_j is the gca effect of the jth parent,

s_{ij} is the interaction, i.e. specific combining ability effect,

r_{ij} is the reciprocal effect and,

$1/bc\Sigma\Sigma e_{ijkl}$ is the mean error effect.

The gca and sca mean squares were tested for significance against the error means. Partitioning of various genetic components namely, *a* (additive), *b* (non-additive, sub-divided further into *b1* (with 1 df, contrasted the mean of the n(n-1) crosses with the mean of the n selfs and is a test for directional dominance or inbreeding depression), *b2* (n-1 df essentially tested the variation in the difference between the selfs and crosses among the parents) and *b3* (remaining 1/2n(n-3) df, measured the residual dominance variation)), *c* (maternal) and *d* (reciprocal) was carried out according to Hayman's approach (1954a). Graphic analysis of %TP, FERM, TF and PUB data using Vr (variance of each cross) and Wr (covariance between parents and their progeny) approach of Hayman (1954b) was done because of significance of item *b* (non-additive variance) and their potential basis for tea product diversification. The distance between the origin and the point where the regression line cuts the *Wr* axis provided a measure of average degree of dominance (Singh and Chaudhary, 1985):

(i) $D > H_1$ (partial dominance) when intercept is positive;

(ii) $D > H_1$ (complete dominance) when line passes through the origin;

(iv) $D > H_1$ (overdominance) when intercept is negative, and

(v) No dominance when the regression line touches parabola limit.

The underlying model for Hayman's analysis is:

$$Y_{rs} = m + j_r + j_{rs} + l + l_r + l_s + l_{rs} + k_r - k_s + k_{rs}$$

where,

Y_{rs} = Entry in *r*th row and *s*th column,

m = Grand mean,

j_r = Mean deviation of *r*th parents from grand mean,

j_{rs} = Remaining discrepancy due to *rs*th reciprocal sum,

l = Mean dominance deviation,

l_r = Dominance deviation (additional) due to *r*th parent,

l_{rs} = Remaining discrepancy due to *rs*th reciprocal sum,

$2k_r$ = Difference when rth line is used as male and female, and

$2k_{rs}$ = Discrepancy in *rs*th reciprocal differences.

Data on all the measured traits were analysed based on the two models using a DIAL98 statistical software by Ukai (2002: http://www.asahi-net.or.jp/~fh6y.uki) based on the assumptions of absence of non-allelic interaction and independent distribution of genes among the parents.

3.5.2 Estimation of other genetic parameters

Both narrow-sense heritability (h^2) and broad-sense heritability (H^2) estimates were derived from the above statistical model by obtaining the variances of the various effects by equating the mean squares to the corresponding components of variance and data analyzed using DIAL98 statistical software by Ukai (2002). Genetic correlations among the measured characters were calculated on MSTAT-C statistical software, while genetic gains following the expected family and clonal selection for the same characters were calculated according to Williams and Matheson (1994) using their respective phenotypic standard deviation, heritability and clonal means. The following formula was applied for calculation of genetic gain:

$G = i \sigma_p h^2$

where G = genetic gain, i is selection intensity which is the difference between the mean of the selected individuals with at least 20% better performance than MPV and the overall mean divided by σ_p, and σ_p is the phenotypic standard deviation. The data for genetic gain is presented as a percentage of means for the characters evaluated.

Heterosis was calculated as: mid-parent heterosis (MPH): MPH = $F_1 - P$; better-parent heterosis (BPH): BPH = $F_1 - P_{max}$. Mid-parent value (MPV = P) was derived from the means of the two parents involved in a cross [i.e. $(P_1 + P_2)/2$]. In addition, calculations were done for the relative MPH as MPH = $100^* (F_1 - P)/P$ and relative BPH as BPH = $100^* (F_1 - P_{max})/P_{max}$ where P_{max} refers to the better performing parent while F_1 is the family mean. For drought tolerance and fermentability, however, the relative MPH and BPH were calculated as $(P-F_1)/P * 100$ and $(P_{min} - F_1)/P_{min} * 100$, respectively, where P_{min} refers to the better parent in terms of the two traits. The contrasts were tested with the appropriate two-tailed tests (Snedecor and Cochran, 1974).

3.6 Molecular techniques

3.6.1 DNA extraction, purification and quantification

Young fresh leaf (two leaves and a terminal bud) was harvested from the experiments and frozen at -20°C prior to DNA extraction using a modified protocol of Gawel and Jarret (1991). About 4 g of the freeze-dried leaf material was ground to a fine powder in liquid nitrogen using a pestle and mortar with addition of 400 mg of insoluble polyclar AT. 100 ml of pre-heated (60°C) extraction buffer (2% CTAB, 100 mM Tris-HCL, pH 8, 1.4 M Nacl, 20mM EDTA, 0.1% DTT) was then added. Samples were then incubated for 20 min at 60°C

with constant shaking, extracted with 75 ml of chloroform:isoamyl alcohol (24:1), centrifuged (Sorvall RC5C) for 25 min at 3000 rpm at 10 °C and the resultant supernatant collected by filtration through several layers of muslin cloth. The aqueous phase was mixed with an equal volume of ice-cold propan-2-ol and left at room temperature for 10 min to precipitate the DNA. Following centrifugation at 5000 rpm for 10 min, the supernatant was discarded and the DNA pellet drained on the bench for 3 hrs. The dried DNA pellet was resuspended in 400 µl of TE buffer (10 mM Tris-HCL, pH 7.5, 1 mM EDTA). After addition of 4 µl RNase A (10 mg/ml), the samples were incubated for 1 hr at 65°C and the supernatant removed into clean 1.5 ml microcentrifuge tubes. The DNA was then reprecipitated in 2 volumes of ice-cold ethanol and then recovered by centrifugation. The pellet was washed in 70% ethanol, then air-dried on bench and resuspended in 100 µl sterilized distilled water. The final samples were stored at -20°C.

DNA quantification was carried out using NanoDrop spectrophotometer (NanoDrop Technologies) which gave the concentration of DNA in ng/µl and secondly by 0.8% agarose gel electrophoresis where the extracted DNA along with a dilution series of a standard unmethylated, uncut DNA from phage lambda was run in the gel. The gel was stained with ethidium bromide and visualized under UV-light (312 nm). The agarose gel electrophoresis method doubled up in estimation of DNA quantity and checking the integrity of DNA. High quality DNA samples gave high molecular weight sharp bands, whereas sheared or DNase-digested DNA resulted in no bands or smears. DNase-digested samples were excluded from further molecular analysis.

3.6.2 Bulk segregant analysis

Bulk Segregant Analysis (BSA) (Michelmore *et al.*, 1991) was carried out using polymorphic primers (Table 6) to target the genomic regions associated with yield, total polyphenols, pubescence, and drought (for St 463), and total polyphenols and resistance to root knot nematode (for St 526) QTLs. For each trait, two bulk DNA samples were constructed using equal amounts of DNA from 10 parental-type high performers and 10 parental-type poor performers judged based on phenotypic assessments during the field experiments (see appendices 2 and 3). Two hundred and fifty two (252) RAPD 10-mer primers, 96 AFLP primer combinations and 15 SSR primers pairs (Freeman *et al.*, 2004), were then screened on the parents and all the bulked DNA samples. Primers distinguishing both the parents, corresponding bulks and individuals making up the bulks were selected for further BSA and fingerprinting of the entire populations.

3.6.3 RAPD-Polymerase Chain Reaction (PCR) Protocol

Two DNA bulks comprising of 10 good and 10 poor performers were prepared and screened for polymorphism of molecular markers. For RAPD analysis, modified PCR conditions first described by William *et al*., (1990) were used. The reaction was conducted in a 25 µl volume containing 20 ng genomic DNA template, 20 ng of a single oligomer-primer (Operon Technologies, Alameda, CA), 200 µM each of dNTPs, 1X Taq polymerase buffer with 1.5 mM $MgCl_2$ and 0.5 U of Taq DNA polymerase (Bangalore Genei Pvt Ltd., Bangalore, India). All the PCR reactions were performed on 1-Cycler PCR system (Bio-Rad, Australia) as follows: 94°C for 5 min, 45 cycles of 94°C for 1 min, 37°C for 1 min, 72°C for 2 min and one final extension cycle of 72°C for 7 min. After amplification, 2.5 µl of a mixture of bromophenol blue (0.25%), glycerol (60%) and water (39.75%) were added to each sample. The samples were then electrophoretically resolved on 1.5% agarose gels containing 0.1g/ml ethidium bromide with 1 X TBE buffer (89 uM Tris HCL pH 8.3, 89 uM boric acid, 5 mM EDTA) and sized with 50 bp and 100 bp Plus DNA ladders as size standards (MBI Fermentas, Lithuania). The electrophoresis lasted for at least four hours at 65 volts. DNA fragments were visualized under UV light and documented with the Gel Doc™ XR system (Bio-Rad, Australia). Out of the 252 RAPD primers screened, 60 were polymorphic and were used in subsequent experiments.

Table 6. RAPD primers used for the detection of polymorphism in the QTL mapping families

Primer	**Sequence (5'-3')**
OPÚ-15	ÁCGGGCCAGT
OPW-18	TTCAGGGCAC
OPW-11	ÇTGATGCGTG
AB4-13	GTCAGAGTCC
OPW-06	ÁGGCCCGATG
OPV-15	ÇAGTGCCGGT
OPV-17	ÁCCGGCTTGT
OPV-20	ÁCAGCCCCCA
OPW-03	GTCCGGAGTG
OPW-04	ÇAGAAGCGGA
OPW-07	ÇTGGACGTCA
OPD-18	GAGAGCCAAC
AB4-16	TCGGCGGTTC
OPF-01	ACGGATCCTG
OPF-02	GAGGATCCCT
OPF-03	CCTGATCACC

Table 6 (Ctd.)

OPF-05	CCGAATTCCC
OPF-06	GGGAATTCGG
OPF-09	CCAAGCTTCC
OPF-15	CCAGTACTCC
OPF-16	GGAGTACTGG
OPT-01	GGGCCACTCA
OPT-02	GGAGAGACTC
OPT-03	TCCACTCCTG
OPT-04	CACAGAGGGA
OPT-17	CCAACGTCGT
OPT-18	GATGCCAGAC
OPO-02	ACGTAGCGTC
OPO-03	CTGTTGCTAC
OPO-05	CCCAGTCACT
OPO-06	CCACGGGAAG
OPO-07	CAGCACTGAC
OPO-10	TCAGAGCGCC
OPO-11	GACAGGAGGT
OP-26-05	GGAACCAATC
OP-26-07	TCGATACAGG
OP-26-08	TGGTAAAGGG
OP-26-09	TCGGTCATAG
OP-26-15	GATCCAGTAC
OP-26-16	GATCACGTAC
OPE-06	AAGACCCCTC
OPE-09	CTTCACCCGA
OPE-11	GAGTCTCAGG
OPE-18	GGACTGGAGA
OPE-19	ACGGCGTATG
OPM-05	GGGAACGTGT
OPM-07	CCGTGACTCA
OPA-01	CAGGCCCTTC
OPA-07	GAAACGGGTG
OPA-10	GTGATCGCAG
G-8	TCACGTCCAC
G-12	CAGCTCACGA
G-15	ACTGGGACTC
OPG-07	GAACCTGCGG
OPG-11	TGCCCGTCGT
OPG-17	ACGACCGAGA
OPV-01	TGACGCATGG
OPV-06	ACGCCCAGGT

3.6.4 AFLP

3.6.4.1 Digestion of DNA

The AFLP procedure was performed following the protocol of Key-gene N.V. (Zabeau and Vos, 1993) with modification as described in invitrogen™ AFLP® Analysis System 1 and AFLP® Starter Primer Kit (Catalog nos. 10544-013 and 10483-014). Between 200 – 500 ng template DNA was digested on I-Cycler PCR system (Bio-Rad, Australia) at 37°C for 1 hr using restriction enzymes *Eco*RI/*Mse*I (1.25 units/µl each in 10 mM Tris-HCL (pH 7.5), 50 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1 mg/ml BSA, 50% (v/v) glycerol, 0.1% Triton® X-100), 5X reaction buffer (50 mM Tris-HCL (pH 7.5), 50 mM Mg-acetate, 250 mM K- acetate) and double distilled water in a final volume of 12.5 µl.

3.6.4.2 Adaptor ligation

Adaptor ligation was achieved by adding 12.0 µl of adapter ligation solution (*Eco*RI/*Mse*I adapters, 0.4 mM ATP, 10 mM Tris-HCL (pH 7.5), 10 mM Mg-acetate, 50 mM K-acetate)) and 0.5 µl T_4-DNA ligase (1 U in 10 mM Tris-HCL (pH 7.5), 1 mM DTT, 50 mM KCL, 50% glycerol (v/v)) into restriction-digestion mixture immediately restriction of genomic DNA was over to make 25.0 µl final volume. The adaptor-ligation reaction was carried out in I-Cycler PCR system (Bio-Rad, Australia) at 20°C for 2 hrs. The reaction mixture was then diluted at 1:8 by transferring 10 µl of the reaction mixture into fresh PCR tubes into which was added 70 µl of TE buffer (10 mM Tris-HCL (pH 8.0), 0.1 mM EDTA) and mixed well. The unused portion of the reaction mixture was stored at -20°C.

3.6.4.3 Pre-amplification

Pre-amplification of the diluted DNA template was performed with primers complementary to the core of the adaptor sequences (Table 7). 2.5 µl of digested-ligated and diluted DNA was mixed with 20 µl pre-amp primer mix, 2.5 µl 10X PCR buffer plus Mg^{2+} and 1 µl 3 U Taq DNA polymerase making up a total reaction volume of 26 µl. The PCR reaction was performed on I-Cycler PCR system (Bio-Rad, Australia) using the following temperature profile: 30 cycles of 30 s at 94°C, 30 s at 60°C, 60 s at 72°C. After pre-amplification, a 1:50 dilution was performed by transferring 2 µl of reaction mixture into new 0.2 ml microcentrifuge tubes into which was added 98 µl of TE buffer. Ten microlitre of the pre-amplified DNA (8 µl of pre-amplification product and 2 µl of 5 X loading buffer) was

checked on 0.8% agarose in 1X TBE buffer for visibility of smear within the 100 – 1500 bp range. Both the unused diluted and undiluted reactions were stored at -20°C.

Table 7. Adaptor sequences used for pre-amplification of DNA template

Adaptor	Sequence
*Eco*RI	5'-CTCGTAGACTGCGTACC-3' 3'- CTGACGCATGGTTAA-5'
*Mse*I	5'-GACGATGAGTCCTGAG-3' 3'-TACTCAGGACTCAT-5'

3.6.4.4 End-labelling of primers

Sufficient *Eco*RI compatible primers with three selective nucleotides for screening as well as for final complete genotyping were labeled with ^{33}P. Each reaction mixture comprised 0.09 µl *Eco*RI primer, 0.09 µl sterile distilled water, 5X kinase buffer (350 mM Tris-HCL (pH 7.6)), 50 mM $MgCl_2$, 500 mM KCL, 5 mM 2-mercapethanol), 0.01 µl T_4 kinase (10 units/µl in 50 mM Tris-HCL (pH 7.6), 25 mM KCL, 1 mM 2-mercaptoethanol, 0.1 µM ATP, 50% (v/v) glycerol) and 0.01 µl [γ-^{33}P]ATP (2,000 Ci/mmol). Labelling was carried out on I-Cycler PCR system (Bio-Rad, Australia) at 37°C for 1 hr.

3.6.4.5 Selective PCR amplifications

Selective restriction fragment amplification was performed with [γ-^{33}P]-labeled *Eco*RI + 3 primer and unlabelled *Mse*I + 3 primer (containing dNTPs (see primer pair sequences used in appendix 14). Each 10 µl PCR reaction mixture consisted of 2.5 µl pre-amplified diluted DNA, 5.85 µl sterilized distilled water, 1 µl 10X PCR buffer plus Mg^{2+}, 0.1 µl *Taq* polymerase (3 U), 0.3 µl of unlabelled *Mse*I primer and 0.25 µl labeled *Eco*RI primer. PCR was conducted on I-Cycler PCR system (Bio-Rad, Australia) as: 1 cycle of 30 s at 94°C, 30 s at 65°C, 60 s at 72°C followed by 11 cycles of 0.7^{o}C lower annealing temperature each cycle and 23 cycles of 30 s at 94°C, 30 s at 56°C and 60 s at 72°C.

3.6.4.6 Polyacrylamide gel electrophoresis

The PCR product was mixed with 10 µl formamide loading buffer (98% formamide, 10 mM EDTA, 0.005% each of xylene cyanol IT and bromophenol blue) and denatured on I-Cycler PCR system (Bio-Rad, Australia) for 5 min at 94°C and immediately snap-cooled on ice. The 6% polyacrylamide gels were prepared by mixing 45g of urea, 30 ml of acrylamide:bisacrylamide solution (19:1), 20 ml of sterilized distilled water, 20 ml of 5X

TBE buffer, 750 µl 10% ammonium persulphate (APS) and 44 µl TEMED. The gels were prepared at least 2 hrs before use and pre-ran in 1X TBE buffer at 65 W for 20 min to 30 min. 3.5 µl samples of the PCR products were loaded into individual wells and gels ran for approximately 1.5 hrs or until just before the dark blue front ran off the bottom of the gel.

3.6.4.7 Gel-drying and documentation

The gels were transferred onto filter papers (Whatman Chromatography papers grade CP 3M) and vacuum-dried at 70°C for 2 hrs before exposing them to Kodak X-ray films. The gels were then exposed to phosphorimaging screen for 3 hrs and the radioactive patterns transferred to the computer via phosphorimaging scanner. They were then placed in x-ray cassettes and put in a deep-freezer at -70°C for at least 15 days or until development.

3.6.5 ISSR

Twelve ISSR primers (Biotech, University of British Colombia) were screened for polymorphism using some 10 samples of the target populations. Seven polymorphic ISSR primers producing informative markers are listed in Table 8. The simple sequence repeat anchored polymerase chain reaction (SSR-anchored PCR) or Inter SSR-PCR (ISSR) procedure described by Mondal (2002) was used. PCR was performed in a 20 µl reaction volume that contained 5X PCR buffer (500 mM KCL, 100 mM Tris-HCL, 1.5% $MgCl_2$, 1.3% BSA, 2% formamide and 1.0% TritonX-100), 2 mM of each dNTP, 3 mM $MgCl_2$, 1 U of *Taq* DNA polymerase and 10-25 ng template DNA.

Table 8. ISSR primers used for the detection of polymorphism in the QTL mapping families

Primer	Primer sequences (5' - 3')
808	AGA GAG AGA GAG AGA GC
849	GTG TGT GTG TGT GTG TCA
817	CAC ACA CAC ACA CAC AA
842	GAG AGA GAG AGA GAG ATG
810	GAG AGA GAG AGA GAG AT
861	ACC ACC ACC ACC ACC ACC
857	ACA CAC ACA CAC ACA CTG

Samples were overlaid with10 µl of mineral oil and reactions carried out on a Perkin Elmer 480 thermal cycler using the following reactions conditions: 94°C for 7 min, 1 cycle, 94°C for 30 s, 52°C for 45 s, 72°C for 2 min, 45 cycles, 72°C for 7 min, 1 cycle. On completion of PCR, the reaction was stopped using 6 µl of stop solution (95% formamide, 20mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol). PCR products were analysed on 2%

agarose gels stained with ethidium bromide in 1X TBE buffer (89 mM Tris-HCL pH 8.3, 89 mM boric acid, 5 mM EDTA). The gels were viewed and photographed under UV light (312 nm) on a transilluminator.

3.6.6 SSR

Fifteen tea SSR primers (Table 9) developed by Freeman *et al* (2004) were utilized in the current study. PCR was performed in a 10 µl reaction volume containing 15-25 ng of template DNA, 15 ng of each primer, 200 µmol/L of each dNTP, 10 mmol/L Tris-HCL (pH 8.3), 50 mmol/L KCL, 1.5 mmol/L $MgCl_2$, 0.01% gelatin, and 0.5 U of Taq DNA polymerase Bangalore Genei Pvt Ltd., Bangalore, India). All PCR reactions were performed using an I-Cycler PCR system (Bio-Rad, Australia) as follows: 94°C for 5 min, 35 cycles of 1 min at 94°C, 1 min at 55°C/60°C/65°C (as per the published annealing temperature of each primer), 2 min at 72°C and a final extension of 7 min at 72°C.

Table 9. Microsatellite primers used in tea linkage mapping

Locus	Primer sequences (5' – 3')	Repeat motif	T_a (°C)
CamsinM1	F:GAATCAGGACATTATAGGAATTAA R:GGCCGAATGTTGTCTTTTGT	(GT)16	50
CamsinM2	F:CCTCTGGTGGTCCTACACCT R:AAAGCCTTGATGCCTTTCG	(GT(17	55
CamsinM3	F:GGTGTGGTGTTTTGAAGAAA R:TGTTAAGCCGCTTCAATGC	(CA)18	65
CamsinM4	F:ACATTCAAGCANTCCACATATGTGAAA R:CCTGNTGCAGGACTGTCTATAGATGA	(GA)19	60
CamsinM5	F:AAACTTCAACAACCAGCTCTGGTA R:ATTATAGGATGCAAACAGGCATGA	(GT)15(GA)8	60
CamsinM6	F:TGTTTTCTTAGGGTTGGATAAAGG R:TTTTGTTGTAATGACGAAAATTC	(TG)12(T)15	55
CamsinM7	F:TGGTAAGGGTCCTAAGAGGTACAC R:TTCCAATCTTTTTCTATAACATCTGC	(GT)16	55
CamsinM8	F:CCATCATTGGCCATTACTACAA R:CCATATGTGTGTGAATGATAAAACC	(CA)17(TA)5	65
CamsinM9	F:CTCATGGAGTCCAAGGAAGC R:AAAGCAGTCTGGAACCTTGC	(CT)15(CA)12	55
CamsinM10	F:TTACATCTCTTTTGCAGCTGTCGG R:CTTCGGGAACTTCTGCTTCATC	(GT)16	65
CamsinM11	F:GCATCATTCCACCACTCACC R:GTCATCAAACCAGTGGCTCA	(CA)12	65
CamsinM12	F:CATTATCGTCACTTGCAAAGAGGT R:CGAGAAGAAGAGCTCTATTGGTT	(GT)12(GA)18	65
CamsinM13	F:CACATTGTGGCGTGTTATTAATTT R:ACATTGGCTATCTCTCATCATGG	(TG)13	60
CamsinM14	F:TGGACTTGGAAGGACTGAGG R:ACAAAGCTCAACCTGCCATT	(GA)16	65
CamsinM15	F:CAACTTGAGCATCAAACGTTCA R:TGAAGCTGTGGGAGATGTCA	(CT)13(CA)23	55

Amplified products were electrophoresed on 3% Metaphor® agarose gel (Cambrex Bio Science Rockland, Inc., USA) using 1X TBE buffer and sized with 50 bp and 100 bp plus DNA ladders as size standards (MBI Fermentas, Lithuania). DNA fragments were visualized under UV light and documented with the Gel Doc™ XR system (Bio-Rad, Australia) (See appendices 21 and 22).

PCR products were also fractionated in preheated 6% w/v denaturing polyacrylamide gel to be resolved further. The PCR product was mixed with 10 µl formamide loading buffer (98% formamide, 10 mM EDTA, 0.005% each of xylene cyanol IT and bromophenol blue) and denatured on I-Cycler PCR system (Bio-Rad, Australia) for 5 min at 94°C and immediately snap-cooled on ice. 4.5 µl samples were loaded into individual wells and gels ran for approximately 1.5 hrs or until just before the dark blue front ran off the bottom of the gel at constant voltage of 1500 V. Silver Sequencing® kit (Promega, Madison, Wis) was used to visualize the DNA fingerprints, according to protocol provided by the manufacturer. The 50 bp DNA ladder (MBI Fermentas, Lithuania) was used to size the bands. The gel images were documented using a digital camera (See appendices 23 to 27).

3.7 DNA data analysis

3.7.1 Marker scoring and Nomenclature

Scoring of bands followed Joinmap/Map Manager/Mapmaker coding schemes. Thus, letters "a" and "b" were used to denote markers from female and male parents, respectively, while codominant observations for F_1 were denoted by "h". Some marker types such as RAPD, AFLP and ISSR, however, could only be observed in the dominant state, i.e. heterozygotes could not be distinguished from one of the homozygous genotypes, and therefore, "c" was used to denote genotype "h-or-b" or not "a" and "d" to denote "h-or-a" or not "b". Missing genotype observations, where they arose, were denoted by "-". Only data from intensely stained unambiguous bands was used for statistical analysis. Markers were scored according to three types: (1) those showing segregation only for female parent, (2) those showing segregation only for male parent and (3) those showing segregation for both parents (heterozygous in both parents). Markers of type 1 were used to construct a separate genetic linkage map for female parent, while markers of type 2 were used to construct a separate map for male parent using a two-way pseudo-testcross mapping strategy (Grattapaglia and Sederoff, 1994). The names of individual marker loci were described using the manufacturer primer code name followed by the relative marker size from the largest to

the smallest using alphabetical letters a, b, c, etc, and where determined, the actual size in base pairs.

3.7.2 Linkage analysis

As the pedigrees used for linkage analysis were outcrossed, a mixture of backcross (BC) and intercross (F_2) mating types at some marker loci were obtained. In this case, informative mating types are the backcross with a heterozygous maternal parent, i.e. Aa x aa (AA) or heterozygous paternal parent aa (AA) x Aa, leading to a segregation ratio 1:1. In the intercross mating type, both P_1 and P_2 are in heterozygous state Aa x Aa leading to a 3:1 segregation ratio for dominant markers and 1:2:1 for codominant markers. Thus, all informative markers were individually examined and categorized according to mating type. Single locus segregation analysis was then carried out to identify distortions from Mendelian ratios that are larger than that likely to occur by random fluctuation alone. Each marker was tested against the expected segregation ratio using a chi-square (X^2) goodness of fit test as:

$$X^2 = \sum_{i=1} \frac{(O_i - E_i)^2}{E_i}$$

where $\sum$ is the summation over all phenotypic classes, O_i and E_i are the observed and expected phenotypic frequencies for class i, respectively. Only marker loci that did not show segregation distortion ($P < 0.01$) from the expected 1:1 were used for map construction to eliminate spurious linkages and subsequently, QTL analysis and mapping.

Linkage maps were constructed using Map Manager QTXb2.0 (Manly *et al.*, 2001). The Kosambi mapping function (Kosambi, 1944; Lander *et al.*, 1987) was used with the threshold value of $P < 0.001$ to convert the recombination fraction into map distances. For this purpose, the “make linkage groups” function was used. Additionally, the “ripple” function was used to improve the order of loci in a linkage group by testing alternative orders created by local permutations of the locus order.

3.7.3 Quantitative trait loci analysis

Each of the measured traits was assessed for normal distribution by a chi-square (X^2) goodness of fit test using the computer software StatistiXl. Quantitative trait loci (QTL) analysis was performed using genome-wide single marker regression and composite interval mapping in Map Manager QTX2.0 (Manly *et al.*, 2001). Thus, mean phenotypic trait data for each site were computed and entered along with the mapping data. The “marker regression”

function ($P < 0.01$) was used to declare the presence of a putative QTL associated with each set of trait data. With this threshold, an overall false positive rate of ~5% was expected, given the average marker distance per linkage group. The locus with the highest likelihood ratio statistic (LRS) for each set of trait data was added to the background. Composite interval mapping was then applied using the "interval mapping" function by scanning each linkage group at 1-cM intervals to detect regions explaining proportion of phenotypic variance. The locus in the background for each trait was used to control for other QTLs. For each linkage group carrying significant QTL, confidence intervals were estimated by bootstrap re-sampling, and interval map figures and histograms representing the confidence intervals of peak LRS values were generated. Suggestive, significant, and highly significant effects were generated by determining LRS thresholds for each trait and linkage group by at least 1000 permutations using the "permutation test" function at $P < 0.01$ (Churchill and Doerge, 1994). Composite interval mapping was used to test for multiple QTLs and ascertain the position of QTLs in the linkage groups (Zeng, 1993, 1994) via Map Manager QTX2.0. Epistatic gene action was inferred from interaction between QTLs as established by Map Manager QTX2.0 (Manly *et al*., 2001). In case of BSA generated data, the total percentage of phenotypic variance explained for each trait by all QTLs was calculated with a multiple regression analysis using the trait as dependent variable and the previously identified markers, linked to the QTL, as treatments.

CHAPTER FOUR

RESULTS

COMBINING ABILITIES FOR YIELD, DROUGHT TOLERANCE AND QUALITY TRAITS IN TEA

4.1 Introduction

Knowledge and understanding of the underlying genetics associated with yield, quality and tolerance to biotic and abiotic stresses would be desirable for efficient tea breeding programme. Development of tea varieties that are high yielding and acceptable quality with potential for diversified tea products, meeting grower demands and satisfying consumer requests is a time-consuming and challenging task (Gill, 1992). Besides, strong self-incompatibility (Rogers, 1975; Wachira and Kamunya, 2005a; Muoki *et al.*, 2007), slow growth, allogamous characteristic and a long juvenile phase of the crop hamper efficient controlled crossing and selection of desired genotypes (Banerjee, 1992).

Despite these limitations, some strides have been made in tea improvement, although faster progress could be achieved with knowledge of nature of gene action and combining abilities for desirable traits like yield and quality attributes. The role of good combiners in all aspects of crop improvement including the use of heterosis has rarely been emphasized particularly in long lived woody species such as tea. Information on combining abilities for the most important traits is a prerequisite in determining the most suitable mating designs and selection of appropriate parents to involve in the hybridization programmes.

A number of methods that are used to determine the inheritance patterns of economically important quantitative traits exist (Becker, 1984). The most popularly mating designs used to determine combining abilities include Line x Tester design (Kempthorne, 1957), partial diallel (Kempthorne and Curnow, 1961) and full diallel (Grifffing, 1956; Hayman, 1954a,b). The general combining ability (GCA) and specific combining ability (SCA) variances derived from these designs could be useful in selecting suitable parents. Although scant data on the use of these designs exist for tea, studies employing diallel analysis have recently been reported by Ikeda and Amma (2004). They studied the inheritance of resistance to Anthracnose disease in tea involving 5 clonal cultivars using a full diallel analysis that identified cultivars with dominant and recessive genes as well as

elucidated the influence of additive and non-additive gene effects in the resistance of the disease.

In this study, a diallel cross was used to study the combining abilities for three important agronomic traits; yield, bud weight and drought tolerance and four traits related to the quality of black tea namely; fermentability, total polyphenols, theaflavins and thearubigins and pubescence. A full diallel design was employed to calculate GCA, SCA and maternal effects in the expression of the traits among the crosses based on Eisenhat's model II (random effects) method 1 and Hayman's approach as quoted in Singh and Chaudhary (1985). The implications of the genetic parameters studied and their utilization in tea breeding strategies and clonal selection are discussed.

4.2 Results

4.2.1 Phenotypic variation for measured traits for parents and F_1 progenies

There was significant phenotypic variation ($P<0.05$) for all the traits measured among the progenies and their parents as shown in Table 10. Results on mean yield indicate that progeny from crosses AHP S15/10 x TRFK 6/8, AHP S15/10 x EPK TN14-3 and TRFCA SFS150 x TRFK 6/8 produced significantly higher yields. Progeny from the cross EPK TN14-3 x TRFCA SFS150 as well as the selfs of EPK TN14-3 and TRFCA SFS150, were relatively inferior. The inbred cross of AHP S15/10 gave comparable yield performance to other hybrids such as TRFK 6/8 x TRFCA SFS150 and TRFCA SFS150 x EPK TN14-3. Progeny from crosses TRFK 6/8 x AHP S15/10, AHPS15/10 x TRFK 6/8 and EPK TN14-3 x TRFK 6/8 had a combination of superior performance for yield, percent total polyphenols, fermentability, drought tolerance, TF and bud weight. Based on family means alone, none of the crosses produced progeny that had outstanding performance for yield, total polyphenols, TR, pubescence and bud weight. The crosses with higher levels of leaf pubescence had AHP S15/10 either as female or male parent. Cross EPK TN14-3 x TRFCA SFS150 had significantly higher levels of TF and TF:TR indicating superior black tea quality even over the highest black tea quality clone TRFK 6/8.

4.2.2 Combining abilities

The means of the nine traits used to derive the respective components of variance and combining abilities are presented in Tables 11a to 11i. The ANOVAs for the various genetic components and combining ability effects are presented in Tables 12 and 13, respectively. All

the traits demonstrated predominant additive effects ($P < 0.05$). The non-additive effects were significant for all but TF:TR and bud weight (Table 12). Table 13 further shows that SCA effects for yield, DT, TF, TR and TF:TR were not significant. However, once SCA was partitioned into individual components, significant dominance effects were observed for DT, TF and PUB as shown by *b1,* which indicates directional dominance (Table 12). Further, significant GCA effects were observed for all traits save for TF:TR (Table 13). Although GCA and SCA were mutually significant for %TP, FERM and PUB, these traits were predominantly influenced by additive genes as accentuated by GCA/SCA ratios of 1.8, 8 and 16.5, respectively.

Variances of various crosses (Vr) were plotted against covariances between parents and their progeny (Wr) to reveal which parents harboured more dominant genes. Results of %TP, FERM, TF and PUB are presented in Figures 6 to 9, respectively. Based on the Vr-Wr graph and considering the assumption of absence of non-allelic interaction and independent distribution of genes among parents (Jinks, 1954; Hayman, 1954b), trait-dependent interpretation could be derived. In the case of %TP (Figure 6) and FERM (Figure 7), parents 1 (TRFK 6/8) and 3 (TRFCA SFS150) displayed overdominance effects. Similarly, overdominance was expressed by parents 1 and 4 (EPK TN14-3) for TF (Figure 8). Contrary to what has always been assumed, parents 3 and 4 (Figure 9) seemed to carry more dominant genes for PUB, even though they are known to be least pubescent.

Significant maternal effects were revealed for all traits except TR and bud weight (Table 12). Non-maternal effects were significant for %TP, FERM, TF, TF:TR and PUB (Table 13). Generally, the importance of reciprocal effects for yield, %TP, FERM, DT and PUB is confirmed by their significance in the two approaches of analysis employed.

Tables 14a and 14b show that all the GCA effects of yield, %TP and DT were contributed by parents AHP S15/10 and TRFK 6/8, as the other parents in the diallel, TRFCA SFS150 and EPK TN14-3, scored negative GCA values. Parents TRFK 6/8 and EPK TN14-3 produced progeny with above average GCA effects for FERM (Table 14c), TF (Table 14e), TR (Table 14f) and TF:TR (Table 14g). Above-average progeny for pubescence descended from parents AHP S15/10 and EPK TN14-3 (Table 14h), while AHP S15/10 and TRFCA SFS150 gave above-average progeny for bud weight, as can be confirmed by their positive GCA (Table 14i). The SCA and reciprocal effects for the nine traits are also presented in Tables 14a to 14i as above-diagonal and below-diagonal values, respectively.

Table 10. Means of F_1 hybrids for yield, %TP (percent total polyphenols, Ferm (rate of fermentability), DT (drought tolerance), TF (total theaflavins levels), TR (total thearubigins levels), TF:TR, leaf pubescence and bud weight of *C. sinensis* at Timbilil estate.

		Family mean (F_1)								
Family code	**Pedigree** ♀ ♂	**Yield**	**%TP**	**Ferm**	**DT**	**TF(umol/g)**	**TR(%)**	**TF:TR**	**Pubescence**	**Bud wt(gm)**
467	TRFK 6/8 self	2347	21.07	1.50	2.00	19.99	15.94	0.11	3.00	0.37
475	TRFK 6/8 X AHP S15/10	2486	22.33	1.50	1.80	22.21	15.23	0.11	4.00	0.40
482	TRFK 6/8 X TRFCA SFS150	2440	21.53	1.05	1.30	21.72	15.82	0.10	1.80	0.36
476	TRFK 6/8 X EPK TN14-3	2381	23.90	1.53	1.50	22.85	16.99	0.10	3.00	0.38
456	AHP S15/10 X TRFK 6/8	2609	20.70	1.21	1.97	22.53	14.52	0.11	2.60	0.39
478	AHP S15/10 self	2499	23.63	1.09	2.00	19.85	15.15	0.10	2.60	0.44
485	AHP S15/10 X TRFCA SFS150	2375	21.60	1.11	1.40	19.40	15.12	0.09	2.20	0.44
474	AHP S15/10 X EPK TN 14-3	2533	22.20	1.30	1.63	20.05	15.52	0.10	3.80	0.36
420	TRFCA SFS150 X TRFK 6/8	2525	22.23	1.39	1.46	22.47	15.93	0.09	1.80	0.37
463	TRFCA SFS150 X AHP S15/10	2451	20.60	1.20	1.57	18.57	14.98	0.09	3.80	0.47
471	TRFCA SFS150 self	2171	21.93	1.40	1.80	18.68	14.95	0.09	3.00	0.38
430	TRFCA SFS150 X EPK TN14-3	2470	21.30	1.15	1.13	18.83	15.75	0.08	2.47	0.30
443	EPK TN14-3 X TRFK 6/8	2510	22.63	1.77	1.70	23.66	16.53	0.11	2.20	0.32
447	EPK TN14-3 X AHP S15/10	2434	21.20	1.32	1.63	21.71	16.60	0.09	4.20	0.38
488	EPK TN14-3 X TFFCA SFS150	1966	20.53	1.00	1.27	25.63	15.50	0.12	3.40	0.36
490	EPK TN14-3 self	2102	20.33	1.50	2.33	20.46	15.27	0.11	3.30	0.32
	Overall mean	2394	21.06	1.31	1.66	21.16	15.61	0.10	2.95	0.38
	Significance of t-test (p = 0.05)	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	S

Note. *S denotes significance level at P < 0.05*

Table 11a. Means of full-sib families for yield (Kg mt/ha)

Female parents	Male parents				Progeny mean
	TRFK 6/8	AHP S15/10	TRFCA SFS150	EPK TN14-3	
TRFK 6/8	2347	2486	2440	2381	2414
AHP S15/10	2609	2499	2375	2533	2504
TRFCA SFS150	2525	2451	2171	2470	2404
EPK TN14-3	2510	2434	1966	2102	2253
Progeny mean	2498	2468	2238	2372	**2394**

Table 11b. Means of full-sib families for %TP

Female parents	Male parents				Progeny mean
	TRFK 6/8	AHP S15/10	TRFCA SFS150	EPK TN14-3	
TRFK 6/8	21.07	22.33	21.53	23.90	22.21
AHP S15/10	20.70	23.63	21.60	22.20	22.03
TRFCA SFS150	22.23	20.60	21.93	21.30	21.52
EPK TN14-3	22.63	21.20	20.53	20.33	21.17
Progeny mean	21.66	21.94	21.40	21.93	**21.73**

Table 11c. Means of full-sib families for FERM

Female parents	Male parents				Progeny mean
	TRFK 6/8	AHP S15/10	TRFCA SFS150	EPK TN14-3	
TRFK 6/8	1.50	1.50	1.05	1.53	1.40
AHP S15/10	1.21	1.09	1.11	1.30	1.18
TRFCA SFS150	1.39	1.20	1.40	1.15	1.29
EPK TN14-3	1.77	1.32	1.00	1.50	1.40
Progeny mean	1.47	1.28	1.14	1.37	**1.31**

Table 11d. Means of full-sib families for DT

Female parents	Male parents				Progeny mean
	TRFK 6/8	AHP S15/10	TRFCA SFS150	EPK TN14-3	
TRFK 6/8	2.00	1.80	1.30	1.50	1.65
AHP S15/10	1.97	2.00	1.40	1.63	1.75
TRFCA SFS150	1.46	1.57	1.80	1.13	1.49
EPK TN14-3	1.70	1.63	1.27	2.33	1.73
Progeny mean	1.78	1.75	1.44	1.65	**1.66**

Table 11e. Means of full-sib families for TF(umol/g)

Female parents	Male parents				Progeny mean
	TRFK 6/8	AHP S15/10	TRFCA SFS150	EPK TN14-3	
TRFK 6/8	19.99	22.21	21.72	22.85	21.69
AHP S15/10	22.53	19.85	19.40	20.05	20.46
TRFCA SFS150	22.47	18.57	18.68	18.83	19.64
EPK TN14-3	23.66	21.71	25.63	20.46	22.87
Progeny mean	22.16	20.59	21.36	20.55	**21.16**

Table 11f. Means of full-sib families for TR(%)

Female parents	Male parents				Progeny mean
	TRFK 6/8	AHP S15/10	TRFCA SFS150	EPK TN14-3	
TRFK 6/8	15.94	15.23	15.82	16.99	16.00
AHP S15/10	14.52	15.15	15.12	15.52	15.08
TRFCA SFS150	15.93	14.98	14.95	15.75	15.40
EPK TN14-3	16.53	16.60	15.50	15.27	15.98
Progeny mean	15.73	15.49	15.35	15.88	**15.61**

Table 11g. Means of full-sib families for TF:TR

Female parents	Male parents				Progeny mean
	TRFK 6/8	AHP S15/10	TRFCA SFS150	EPK TN14-3	
TRFK 6/8	0.11	0.11	0.10	0.10	0.11
AHP S15/10	0.11	0.10	0.09	0.10	0.10
TRFCA SFS150	0.09	0.09	0.09	0.08	0.09
EPK TN14-3	0.11	0.09	0.12	0.11	0.11
Progeny mean	0.11	0.10	0.10	0.10	**0.10**

Table 11h. Means of full-sib families for PUB

Female parents	Male parents				Progeny mean
	TRFK 6/8	AHP S15/10	TRFCA SFS150	EPK TN14-3	
TRFK 6/8	3.00	4.00	1.80	3.00	2.95
AHP S15/10	2.60	2.60	2.20	3.80	2.80
TRFCA SFS150	1.80	3.80	3.00	2.47	2.77
EPK TN14-3	2.20	4.20	3.40	3.30	3.28
Progeny mean	2.40	3.65	2.60	3.14	**2.95**

Table 11i. Means of full-sib families for Bud wt(g)

Female parents	Male parents				Progeny mean
	TRFK 6/8	AHP S15/10	TRFCA SFS150	EPK TN14-3	
TRFK 6/8	0.37	0.40	0.36	0.38	0.38
AHP S15/10	0.39	0.44	0.44	0.36	0.41
TRFCA SFS150	0.37	0.47	0.38	0.3	0.38
EPK TN14-3	0.32	0.38	0.36	0.32	0.35
Progeny mean	0.36	0.42	0.39	0.34	**0.38**

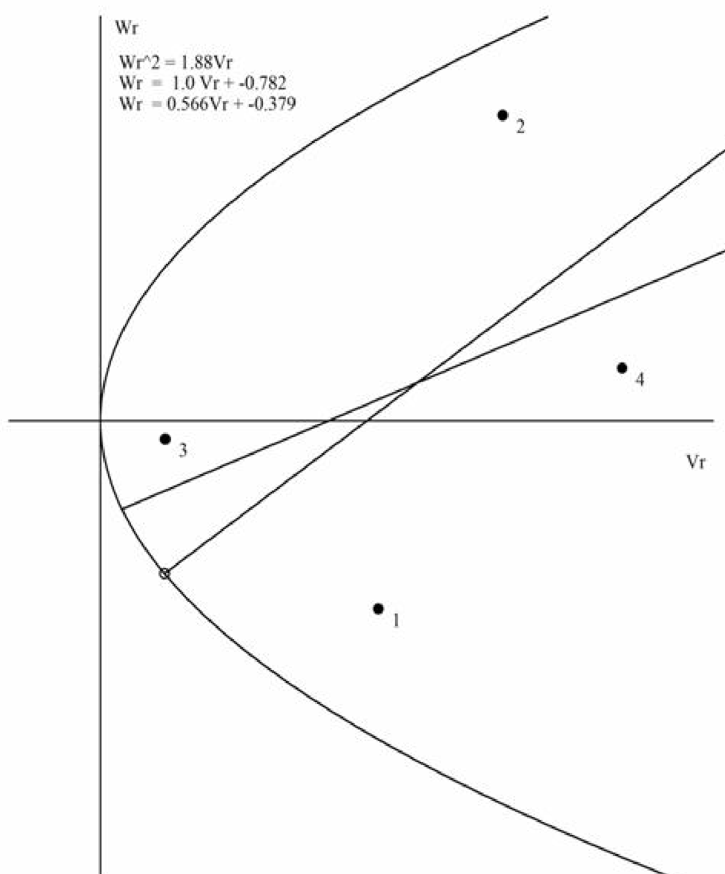

Figure 6. Relationship between Wr (covariance between parents and their progeny) and Vr (variance of each cross) for %TP. Nos 1, 2, 3 and 4 denote TRFK 6/8, AHP S15/10, TRFCA SFS150 and EPK TN14-3, respectively.

Table 12. Mean squares for the genetic parameters of the nine assayed traits

Source	df	Mean squares								
		Yield	%TP	FERM	DT	TF(umol/g)	TR(%)	TF:TR	PUB	Bud Wt(gm)
Rep	2	80672	0.6	0.02	0.03	7.54	10.97	0.030	0.04**	0.005
a	3	129600**	1.7*	0.40**	0.67**	13.83**	2.62*	0.008*	2.4**	0.02*
b	6	37390	5.7**	0.30**	0.20**	10.42**	1.60*	0.003	1.56**	0.002
B1	1	53631	0.0	0.00	0.50**	31.51**	1.28	0.005	0.13**	0.00002
B2	3	36841	9.4**	0.40**	0.23**	7.35	1.48	0.001	2.87**	0.001
B3	2	30094	2.8**	0.10**	0.00	4.48	1.93	0.005	0.31**	0.01*
c	3	100287*	1.8*	0.10**	0.05**	17.43**	0.52	0.008*	2.14**	0.001
d	3	54071	1.9**	0.02*	0.02	7.92*	0.49	0.009*	0.95**	0.005
Error	30	24698	0.4	0.01	0.01	2.69	0.61	0.002	0.02	0.0024
Total	47									

*NB: * and ** denote significance at P < 0.05 and P < 0.01, respectively;* a denotes additive variance, b non-additive which is sub-divided into *b1, b2* and *b3* indicating directional dominance, extent of directional dominance and residual dominance, respectively, c maternal and d reciprocal differences other than maternal. Genetic parameters were derived using the method of Hayman's approach (1954a).

Table 13. Mean squares for combining abilities† of the nine assayed traits

Source	df	Mean squares								
		Yield	%TP	FERM	DT	TF(umol/g)	TR(%)	TF:TR	PUB	Bud Wt(gm)
Rep	2	17028	0.7	0.04	0.02	7.89	4.88	0.004	0.0045	0.01
gca	3	109402**	5.1**	0.8***	0.54**	19.46**	3.54*	0.001	5.13***	0.01**
sca	6	30147	2.8**	0.1**	0.004	4.49	1.93	0.001	0.31**	0.01**
Reciprocal	6	77179**	1.8*	0.1**	0.04*	12.68*	0.50	0.002**	1.55**	0.003
Error	22	11086	0.5	0.011	0.01	2.59	0.61	0.0004	0.0045	0.0017
Total	35									

†Combining abilities were determined using the method 1, model I of Griffing (1956)

Table 14a. General combining ability effects (diagonal values), specific combining ability effects (above diagonal values) and reciprocal effects (below diagonal) for yield (Kg mt/ha)

Female parents	Male parents			
	TRFK 6/8	AHP S15/10	TRFCA SFS150	EPK TN14-3
TRFK 6/8	**23.13**	-0.1875	99.4375	71.3125
AHP S15/10	-123	**67.50**	78.8125	79.1875
TRFCA SFS150	-85	-76	**-45.75**	-21.6875
EPK TN14-3	-129	99	504	**-44.88**

Table 14b. General combining ability effects (diagonal values), specific combining ability effects (above diagonal values) and reciprocal effects (below diagonal) for %TP

Female parents	Male parents			
	TRFK 6/8	AHP S15/10	TRFCA SFS150	EPK TN14-3
TRFK 6/8	**0.61**	-0.671875	0.223125	1.511875
AHP S15/10	1.63	**0.48**	-0.560625	-0.106875
TRFCA SFS150	-0.7	1	**-0.89**	-0.361875
EPK TN14-3	1.27	1	0.77	**-0.20**

Table 14c. General combining ability effects (diagonal values), specific combining ability effects (above diagonal values) and reciprocal effects (below diagonal) for FERM

Female parents	Male parents			
	TRFK 6/8	AHP S15/10	TRFCA SFS150	EPK TN14-3
TRFK 6/8	**0.09**	0.01	-0.11	0.14875
AHP S15/10	0.29	**-0.08**	0.03375	0.0125
TRFCA SFS150	-0.34	-0.09	**-0.08**	-0.2075
EPK TN14-3	-0.24	-0.02	0.15	**0.07**

Table 14d. General combining ability effects (diagonal values), specific combining ability effects (above diagonal values) and reciprocal effects (below diagonal) for DT

Female parents	Male parents			
	TRFK 6/8	AHP S15/10	TRFCA SFS150	EPK TN14-3
TRFK 6/8	**0.06**	0.074375	-0.146875	-0.150625
AHP S15/10	-0.17	**0.10**	0.124375	-0.154375
TRFCA SFS150	-0.16	-0.17	**-0.10**	-0.300625
EPK TN14-3	-0.2	0	-0.14	**-0.06**

Table 14e. General combining ability effects (diagonal values), specific combining ability effects (above diagonal values) and reciprocal effects (below diagonal) for TF (umol/g)

	Male parents			
Female parents	TRFK 6/8	AHP S15/10	TRFCA SFS150	EPK TN14-3
TRFK 6/8	**0.76**	1.084375	0.833125	0.784375
AHP S15/10	-0.32	**-0.64**	1.109375	-0.184375
TRFCA SFS150	-0.75	0.83	**-0.67**	1.189375
EPK TN14-3	-0.81	-1.66	-6.8	**0.54**

Table 14f. General combining ability effects (diagonal values), specific combining ability effects (above diagonal values) and reciprocal effects (below diagonal) for TR (%)

	Male parents			
Female parents	TRFK 6/8	AHP S15/10	TRFCA SFS150	EPK TN14-3
TRFK 6/8	**0.25**	-0.65875	0.25	0.58125
AHP S15/10	0.71	**-0.33**	-0.22625	0.46
TRFCA SFS150	-0.11	0.14	**-0.24**	-0.06625
EPK TN14-3	0.46	-1.08	0.25	**0.32**

Table 14g. General combining ability effects (diagonal values), specific combining ability effects (above diagonal values) and reciprocal effects (below diagonal) for TF:TR

	Male parents			
Female parents	TRFK 6/8	AHP S15/10	TRFCA SFS150	EPK TN14-3
TRFK 6/8	**0.005**	0.00625	-0.00375	-0.0025
AHP S15/10	0	**-0.002**	0.0075	-0.00625
TRFCA SFS150	0.01	0	**-0.006**	0.00375
EPK TN14-3	-0.01	0.01	-0.04	**0.003**

Table 14h. General combining ability effects (diagonal values), specific combining ability effects (above diagonal values) and reciprocal effects (below diagonal) for PUB

	Male parents			
Female parents	TRFK 6/8	AHP S15/10	TRFCA SFS150	EPK TN14-3
TRFK 6/8	**-0.25**	0.348125	-0.610625	-0.335625
AHP S15/10	1.4	**0.3**	-0.560625	0.514375
TRFCA SFS150	0	-1.6	**-0.25**	-0.009375
EPK TN14-3	0.8	-0.4	-0.93	**0.20**

Table 14i. General combining ability effects (diagonal values), specific combining ability effects (above diagonal values) and reciprocal effects (below diagonal) for bud wt (g)

Female parents	Male parents			
	TRFK 6/8	AHP S15/10	TRFCA SFS150	EPK TN14-3
TRFK 6/8	**-0.01**	-0.0125	-0.01	0.015
AHP S15/10	0.01	**0.04**	-0.005	-0.01
TRFCA SFS150	-0.01	-0.03	**0.01**	-0.0175
EPK TN14-3	0.06	-0.02	-0.06	**-0.04**

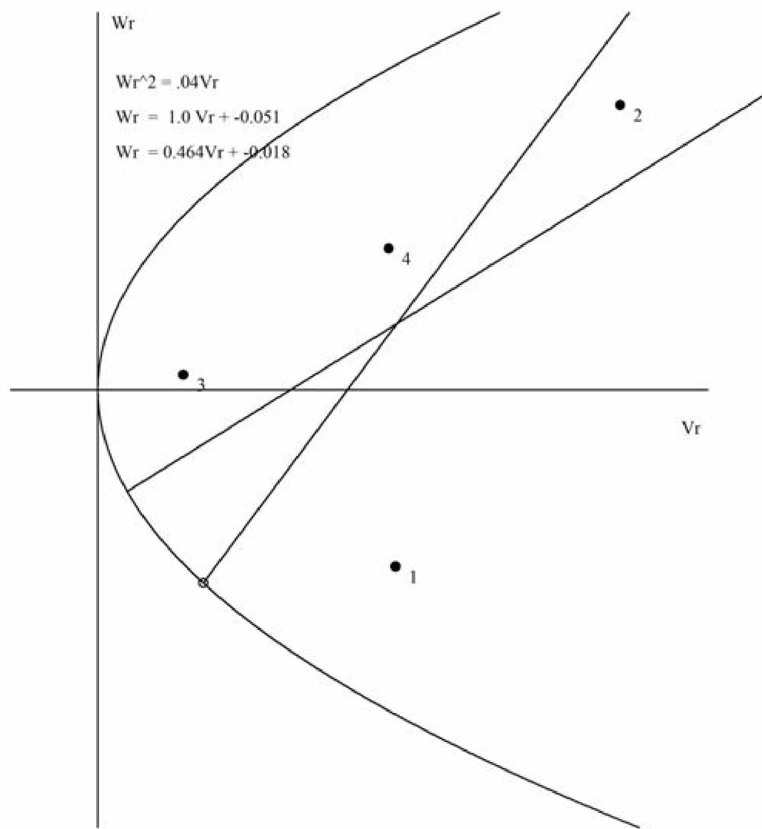

Figure 7. Relationship between Wr (covariance between parents and their progeny) and Vr (variance of each cross) for FERM.

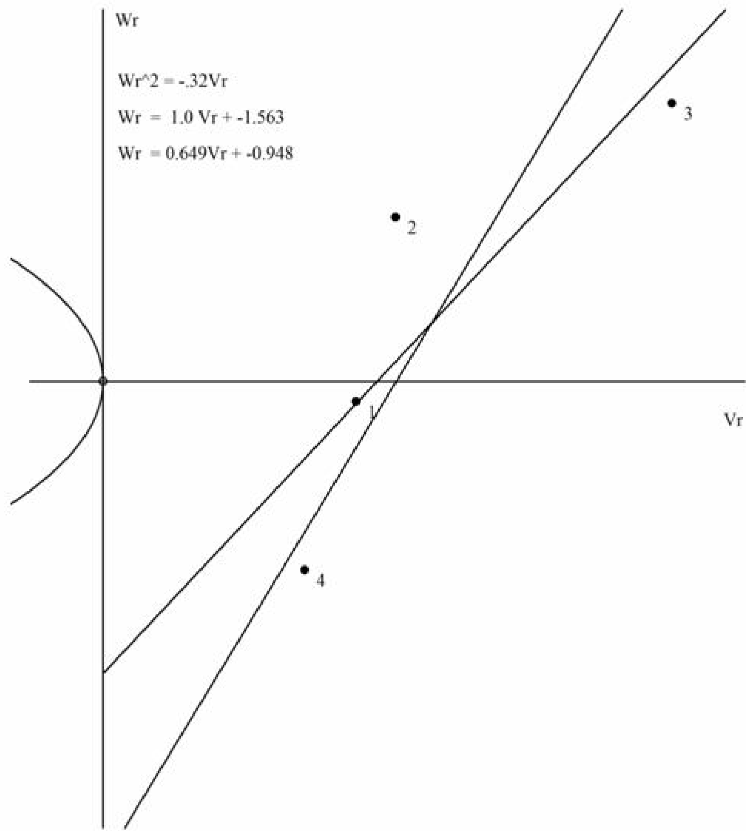

Figure 8. Relationship between Wr (covariance between parents and their progeny) and Vr (variance of each cross) for TF.

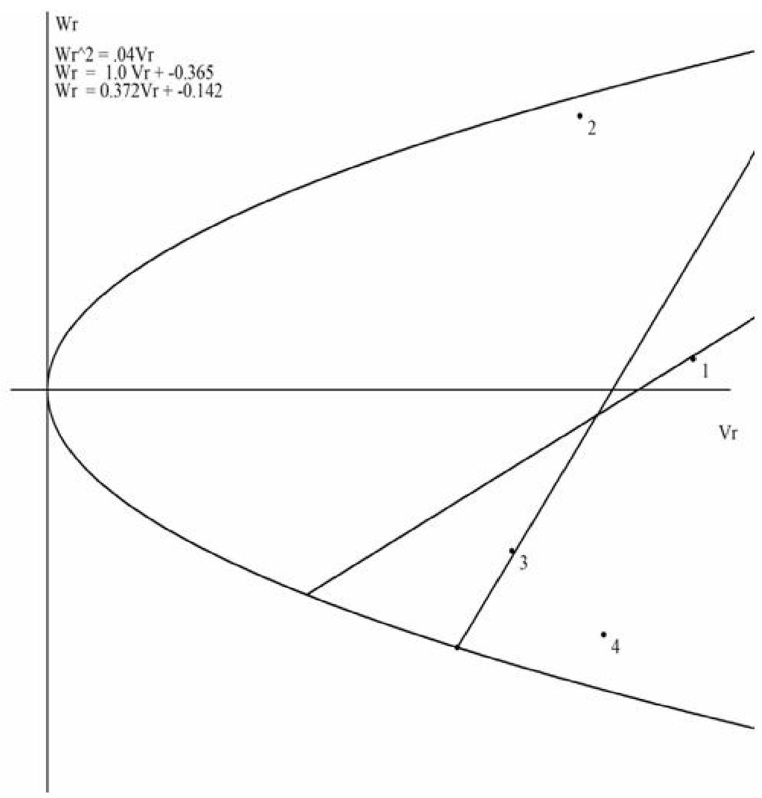

Figure 9. Relationship between Wr (covariance between parents and their progeny) and Vr (variance of each cross) for PUB.

4.3 Discussion

The diallel mating design employed gave good indicators of the type of genes governing the traits measured in the crosses. The present study revealed that observed variation could predominantly be explained by genetic effects. The considerable variation revealed in the test for the measured traits combined with high additive and non-additive gene effects serve to emphasize the great room for selection and significant improvements if judicious breeding and clonal selection efforts are to be instituted.

The analysis by the Griffing's approach revealed significant GCA effects for all traits except TF:TR, while significant SCA (dominant and epistatic genetic effects) effects were detected for the %TP, FERM, PUB and bud weight. Significant reciprocal effects were demonstrated for all traits except TR and bud weight. The use of Hayman's approach provided means of dissecting SCA effects and maternal/non-maternal effects into smaller entities so as to reveal which components contributed most variation. Thus, while significant *b1* for drought tolerance, TF and PUB points to unidirectional dominance, the significance of *b2* for %TP, FERM and PUB indicated that extent of directional dominance varies among the four parents reflecting the fact that they carry different numbers of dominant alleles for the traits (Kearsey and Pooni, 1996). Clones TRFK 6/8 and EPK TN14-3, which the current study showed expressed overdominance for %TP and FERM, are popular high quality cultivars, with TRFK 6/8 accounting for over 60% of the tea produced by smallholder sector (Wachira, 2002).

The significant variation in yield, DT and TF presented herein could be attributed to both additive gene and maternal effects. Maternal effects are attributed to non-nuclear genetic factors inherited through the cytoplasm (Pakkasmaa *et al*., 2003; Barth *et al*., 2003). All the other traits save for TR and bud weight similarly showed significant maternal effects. Significant SCA and reciprocal effects on the other hand were revealed for %TP, FERM, TF, TF:TR and PUB, indicating influence by a combination of non-additive and non-nuclear genetic effects. Significant reciprocal effects have also been observed for height and stem straightness in maritime pine (Harfouche and Kremer, 2000) and for yield in pink stem borer infested and non-infested maize (Butron *et al*., 1999). The successful growth of F_1 hybrids depends largely on the nuclear and cytoplasmic genome with the latter being maternally inherited as well as the nutritional status of the seed parent, which the marked reciprocal effects appear to confirm (Nasrallah *et al*., 2000; Barth *et al*., 2003). In the present study, significant maternal effects for the various traits emphasize the importance of maternal parents in tea improvement programme. Although it is hard to predict the continuity of this

effect in subsequent breeding efforts, in advanced tea improvement programmes, the choice of the female parent in hybridization programmes must be rationalized based on the breeding objectives.

In this study, significant GCA effects were revealed for all traits. However, the significance of additive effects relative to non-additive effects for yield, FERM, DT, TF, TR, pubescence and bud weight suggest that predominant additive genetic effects have stronger influence. The significance of both GCA and SCA implies the applicability of both natural and artificial hybridization process although the predominance of additive genetic effects for majority of the traits studied could easily be improved by leaving elite parents in seed orchards to inter-mate freely with the following generation expected to be much superior. Owing to cost implications associated with artificial hybridization for perennial crops such as tea, this option would attract bigger attention (Anon., 2005). Faster progress may even be realized owing to ease of propagation by vegetative means (Green, 1964) that is preceded by judicious clonal selection in advanced stages of tea improvement. Non-additive genetic variance, however, can easily be captured through clonal propagation of selected biclonal progeny showing superior performance for a given trait or group of traits (Zobel and Talbert, 1984; Kamaluddin *et al.*, 2007). Combining ability studies done for other perennial crops such as cocoa (*Theobroma cacao* L.) gave varied results. While Berry and Cilas (1994) showed GCA to be more important than SCA for yield, Dias and Kageyama (1995) found the contrary for the same trait. From the diallel analysis, AHP S15/10 and TRFK 6/8 had positive GCA effects for yield and total polyphenols implying that more often than not, they are likely to produce progeny with above average performance for the traits irrespective of which other parents are involved in the cross. Positive GCA posted by TRFK 6/8 and EPK TN14-3 for black tea quality traits (i.e. fermentability, TF, TR and TF:TR) emphasizes the importance of using these clones while targeting to improve or select for the two traits. Their inheritance pattern also indicates that the traits are either tightly linked or pleiotropic. Since GCA refers to additive gene effects, collection of open-pollinated seed alone from these parents is likely to lead to improved populations for the respective traits. This observation is apparent in the TRFK tea improvement programme where 27 of 47 TRFK released clones had TRFK 6/8 as a common parent (Kamunya, 2003). Where GCA effect is more important, its utilization in seed gardens that are composed of many parents like in the polyclonal seed orchard of tea at TRFK becomes paramount. This may lead to accumulation of favourable alleles that have additive genetic effects in the phenotypes of the improved generations.

The apparent dominant nature of the fermenting character was also observed by Toyao (1982) in a study of inheritance of non-fermenters in tea plant and the corroboration of the results obtained in the present study confirms that this trait is largely governed by non-additive genes. Since SCA refers to non-additive gene effects, its utilization in polyclonal seed gardens is impracticable as open pollination results in many different combinations of alleles across gene loci. Thus, where SCA is more important, it can be utilized through vegetative propagation to produce commercial quantities of planting stock that are genetically identical to the plants or hybrids from which they were derived. Besides, biclonal specific crosses involving parents with positive SCA effects for yield, fermentability and pubescence followed by prudent clonal selection may predictably result in marked progress in these traits. Although efforts to utilize both GCA and SCA in tea improvement programme have been made, the composition of the various parents entered in the already designed and established seed orchards did not take cognizance of the combining abilities for all traits targeted for improvement. However, the results of the current study, small size of the diallel mating notwithstanding, have demonstrated the need to redesign these orchards with improved ones earmarked for establishment by adopting progenitor clones with known combining abilities. The results show that the assessed traits are highly heritable and guided breeding and judicious clonal selection would lead to further tea improvement, and hence economic upturn of tea farmers, majority of whom, are smallholders. Generally, the current study shows that basic information on combing abilities is instrumental for breeding of elite cultivars.

CHAPTER FIVE

HETEROSIS, HERITABILITY AND GENETIC GAIN OF YIELD, DROUGHT TOLERANCE AND QUALITY TRAITS IN TEA

5.1 Introduction

The successful development of the tea industry in Kenya has in part been ascribed to the tremendous efforts applied in tea improvement research. Although knowledge of genetic parameters such as heritability and heterosis is crucial in estimation and realization of marked gain in tea improvement, tea breeding and clonal selections have generally been continuing even though with minimal progress. The creation of uniform population, through vegetative propagation techniques (Green, 1964; 1966), might have contributed to marked gains in yield and quality of black tea that would otherwise have not been achieved in the pioneer tea plantations that comprised seedling tea populations. Besides, the initial challenge on management and quality maintenance arising from the heterogeneity in seedling populations were also circumvented (Green, 1964). Although early research activities emphasized more on propagating materials and minimizing heterogeneity in quality and productivity, no attention was given to the nature and magnitude of genetic parameters influencing the traits of interest. Early tea improvement activities led to release of better yielding but poor to moderate black tea quality or high quality but low yielding clonal teas compared to the existing seedling teas for commercial use (Green, 1969; Banerjee, 1992; Wachira, 2002).

Conventional breeding using inbreds is untenable in tea, though not outrightly impossible if such breeding (Singh, 1995) is integrated with appropriate biotechnological tools such as doubled haploidization. Knowledge on quantitative genetics such as heterosis and heritability as well as expected gain upon selection is prerequisite as it influences the choice of progenitors, size of breeding population and the design of seed orchards (Dias and Kegayama, 1995; Falconer, 1989). Hence, there is every need to understand the inheritance pattern of the most important attributes so as to inform the breeding programme.

In this study, a diallel cross was used to study genetic variation, heritability, heterosis and genetic gains for three important agronomic traits; yield, bud weight and drought tolerance, four traits related to the quality of tea products; fermentability, total polyphenols, theaflavins and thearubigins, and pubescence. Genetic correlations among the nine attributes and response to their selection were also estimated. The estimated genetic parameters were derived from a diallel cross established at the Timbilil estate, Kericho. The implications of

the relative magnitudes of the genetic parameters and potential gains on breeding strategies and clonal selection are discussed.

5.2 Results

5.2.1 Range in phenotypic variation for yield, total polyphenols, drought tolerance and fermentability

Results on the range of means for the various characters measured in the diallel cross are presented in Table 15. There was a wide range in performance of nearly all the crosses for all the traits assessed. For yield, the widest and narrowest ranges are indicated by crosses TRFCA SFS150 x AHP S15/10 and TRFK 6/8 and AHP S15/10, respectively. The widest range for total polyphenols was depicted by EPK TN14-3 x TRFK 6/8, while the narrowest resulted from the self of EPK TN14-3. Fermentability was the least variable trait for almost all crosses. The widest range was shown by the cross TRFK 6/8 x EPK TN14-3, while the narrowest arose from cross EPK TN14-3 x TRFCA SFS150. Drought tolerance exhibited the greatest variability of the nine traits. In this case self of TRFK 6/8 and AHP S15/10 x TRFCA SFS150 produced the most variable progeny, while crosses TRFCA SFS150 x EPK TN14-3 and a self of EPK TN14-3 gave the least variable progeny. It is also apparent that TF, TR, TF:TR, pubescence and bud weight registered high level of variability.

5.2.2 Heterosis

Table 16 present data on MPVs for the nine traits. Significant differences ($P<0.05$) were observed for all traits. However, no significant differences ($P > 0.05$) in MPH for yield, total polyphenols, TF and pubescence were observed. The converse was true for FERM, drought tolerance, TR, TF:TR and bud weight (Table 17). Similarly, there were no significant differences for BPH in yield, fermentability, drought tolerance, TF and pubescence, while significant differences were observed for %TP (Table 18). The MPH means for fermentability, drought tolerance, TR and bud weight were significantly high at 108%, 16%, -5.13% and -9.56%, respectively. The mean BPH for yield (-5.09%), total polyphenols (-5.7%), fermentability (-12.82%) and pubescence (-10.4%) indicated that the progeny values were markedly lower than those for the best parent. BPH was however significantly higher for TF:TR (14.32%). The clones TRFK 6/8 and AHP S15/10, whether used as female or male parents gave progeny with high BPH for most of the traits but were inconsistent for TF, TR and pubescence. The highest BPH for fermentability, unexpectedly coincided with a self of AHP S15/10. The availability of transgressive segregants among the various hybrids as

exhibited by high BPH was notable for the yield in the crosses TRFK 6/8 x TRFK 6/8 (37.41%), AHP S15/10 x TRFK 6/8 (2.07%); %TP, FERM and DT for crosses AHP S15/10 x AHP S15/10 (14.71%, 40.44% and 28.57%, respectively), TF (14.22%) and TF:TR (33.33%) (Table 18).

5.2.3 Heritability estimates

The heritability estimates presented in Table 19 were quite high for most of the nine traits measured as revealed by the mating analysis method employed. The narrow sense heritabilities (h^2) ranged from 0.09 to 0.74 for total polyphenols and bud dry weight, respectively. The broad sense heritabilities (H^2) ranged from 0.56 for yield to 0.98 for fermentability and pubescence. The narrow-sense heritability estimate for total polyphenols was however quite low, owing to high levels of dominance and environmental variances. Generally, the high heritabilities for the other traits measured indicate that they are highly heritable and significant strides are likely to be achieved if careful breeding and clonal selection were to be undertaken.

5.2.4 Genetic gains

Genetic gains estimated from h^2 and H^2 for the traits measured are presented in Table 20. Generally, genetic gains that would arise from family selection were markedly lower (1.2 to 38.2%) compared to those that would be realised from within family (clonal) selection (2.5 to 68.1%). Additionally, the magnitude of response to selection would be higher under broad sense heritability (3.5 to 68.1%) than narrow-sense heritability (1.2 to 30.0%). Lower magnitudes of genetic gain would be achieved for traits that have hitherto been receiving attention such as yield and fermentability than those that have recently began receiving attention such as %TP, TF:TR, and pubescent teas ("silvery tips"). The lower magnitude of genetic gain got for fermentability under h^2 may be attributed to narrow genetic variability which gives very little room for selection. Generally, higher genetic gains are tenable owing to the high H^2 estimates shown by the nine characters coupled with high clonal selection intensity (2%) that can be imposed when an adequate effective population size is employed.

5.2.5 Correlation coefficients among the nine traits

Significant correlation coefficients among some traits were realized (Table 21). Correlation coefficients (r) among the important traits were significant; yield and %TP (0.61, $P < 0.01$), %TP and FERM (0.54, $P < 0.05$), %TP and %TR (0.66, $P < 0.01$), FERM and DT

(0.67, $P < 0.01$), FERM and %TR (0.67, $P < 0.01$) and TF and TF:TR (0.81, $P < 0.001$), while the rest were weakly correlated (e.g. yield and DT: 0.23, yield and FERM: 0.31, and TF and pubescence: 0.24).

Table 15. Ranges of clonal means within families for the various traits measured

Family code	Pedigree ♀ ♂	Range of traits within family								
		Yield	%TP	Ferm	DT	TF(umol/g)	TR(%)	TF:TR	Pubescence	Bud wt(gm)
467	TRFK 6/8 self	1483-2654	20.10-22.80	1.0-2.0	1.0-2.6	16.56-25.80	16.56-25.80	0.08-0.15	3.0-3.0	0.22-0.33
475	TRFK 6/8 X AHP S15/10	1834-2790	18.60-26.00	1.0-2.0	1.3-2.0	14.45-27.24	14.45-27.24	0.08-0.14	3.0-5.0	0.18-0.42
482	TRFK 6/8 X TRFCA SFS150	1516-3050	19.10-24.20	1.0-1.1	1.0-1.7	15.83-30.67	15.83-30.67	0.07-0.16	3.0-3.0	0.18-0.37
476	TRFK 6/8 X EPK TN14-3	1340-3086	19.40-22.00	1.0-3.0	1.2-2.0	16.80-29.65	16.80-29.65	0.07-0.13	1.0-3.0	0.17-0.50
456	AHP S15/10 X TRFK 6/8	1794-3458	18.10-24.20	1.0-2.0	1.4-2.2	17.06-30.20	17.06-30.20	0.09-0.14	3.0-5.0	0.11-0.51
478	AHP S15/10 self	1631-3002	20.10-26.20	1.0-1.4	1.8-2.2	13.36-26.02	13.36-26.02	0.06-0.17	1.0-3.0	0.20-0.42
485	AHP S15/10 X TRFCA SFS150	1407-3268	17.90-24.50	1.0-1.3	1.2-2.6	12.15-24.72	12.15-24.72	0.05-0.13	3.0-5.0	0.11-0.40
474	AHP S15/10 X EPK TN 14-3	2006-3104	18.80-25.80	1.0-2.5	1.0-2.3	15.95-24-23	15.95-24.23	0.07-0.17	1.0-5.0	0.17-0.43
420	TRFCA SFS150 X TRFK 6/8	1691-3041	18.70-24.90	1.1-1.6	1.1-1.7	15.19-26.67	15.19-26.67	0.06-0.14	1.0-5.0	0.19-0.32
463	TRFCA SFS150 X AHP S15/10	502-3528	18.90-23.20	1.0-2.0	1.4-2.0	14.40-21.57	14.40-21.57	0.07-0.18	5.0-5.0	0.24-0.55
471	TRFCA SFS150 self	1120-2415	19.90-24.60	1.0-2.0	1.6-1.9	13.93-25.18	13.93-25.18	0.07-0.15	1.0-5.0	0.19-0.38
430	TRFCA SFS150 X EPK TN14-3	759-3044	18.00-26.50	1.0-1.6	1.0-1.3	14.47-21.90	14.47-21.90	0.06-0.10	1.0-5.0	0.16-0.29
443	EPK TN14-3 X TRFK 6/8	1634-3023	18.60-28.50	1.4-2.3	1.3-2.0	18.09-32.66	18.09-32.66	0.06-0.20	1.0-3.0	0.15-0.35
447	EPK TN14-3 X AHP S15/10	1885-2986	17.20-25.20	1.0-1.5	1.2-1.7	13.68-30.52	13.68-30.52	0.03-0.15	3.0-5.0	0.17-0.32
488	EPK TN14-3 X TFFCA SFS150	1450-2398	17.00-23.80	1.0-1.0	1.2-2.0	17.06-31.14	17.06-31.14	0.08-0.14	3.0-3.0	0.22-0.41
490	EPK TN14-3 self	796-2212	19.80-22.10	1.0-2.0	1.5-1.7	14.32-25.15	14.32-25.15	0.08-0.14	1.0-3.0	0.14-0.33

***NB**. Traits are as described in the legend for Table 10*

Table 16. Mid-parent value (MPV = P) for the measured traits across the various full-sibs

Family code	Pedigree ♀ ♂	MPV								
		Yield	%TP	Ferm	DS	TF(umol/g)	TR(%)	TF:TR	Pubescence	Bud wt(gm)
467	TRFK 6/8 self	1708	24.30	1.50	2.00	23.03	17.74	0.090	1.00	0.38
475	TRFK 6/8 X AHP S15/10	2132	22.45	1.50	2.40	20.88	16.66	0.085	3.00	0.44
482	TRFK 6/8 X TRFCA SFS150	2204	23.35	1.05	1.65	20.95	16.64	0.085	2.00	0.37
476	TRFK 6/8 X EPK TN14-3	2093	23.30	1.53	2.00	22.74	17.38	0.090	2.00	0.41
456	AHP S15/10 X TRFK 6/8	2132	22.45	1.21	2.40	20.88	16.66	0.085	3.00	0.44
478	AHP S15/10 self	2556	20.60	1.09	2.80	18.73	15.58	0.080	5.00	0.49
485	AHP S15/10 X TRFCA SFS150	2628	21.50	1.11	2.05	18.80	15.56	0.080	4.00	0.43
474	AHP S15/10 X EPK TN 14-3	2517	21.45	1.30	2.40	20.58	16.30	0.085	4.00	0.47
420	TRFCA SFS150 X TRFK 6/8	2204	23.35	1.39	1.65	20.95	16.64	0.085	2.00	0.37
463	TRFCA SFS150 X AHP S15/10	2628	21.50	1.20	2.05	18.80	15.56	0.080	4.00	0.43
471	TRFCA SFS150 self	2699	22.40	1.40	1.30	18.86	15.53	0.080	3.00	0.36
430	TRFCA SFS150 X EPK TN14-3	2589	22.35	1.15	1.65	20.65	16.27	0.085	3.00	0.40
443	EPK TN14-3 X TRFK 6/8	2093	23.30	1.77	2.00	22.74	17.38	0.090	2.00	0.41
447	EPK TN14-3 X AHP S15/10	2517	21.45	1.32	2.40	20.58	16.30	0.085	4.00	0.47
488	EPK TN14-3 X TFFCA SFS150	2589	22.35	1.00	1.65	20.65	16.27	0.085	3.00	0.40
490	EPK TN14-3 self	2478	22.30	1.50	2.00	22.44	17.01	0.090	3.00	0.44
	Overall mean	2360	22.40	1.31	2.03	20.77	16.47	0.085	3.00	0.42
	Significance of t-test ($p < 0.05$)	S	S	S	S	S	S	S	S	S

***Note**. Traits are as described in the legend for Table 10. S denotes significance level at $P < 0.05$*

Table 17. Mid-parent heterosis (MPH) for the nine measured traits

Family code	Pedigree ♀ ♂	MPH								
		Yield	%TP	FERM	DT	TF(umol/g)	TR(%)	TF:TR	Pubescence	Bud wt(gm)
467	TRFK 6/8 self	37.41	-13.29	84.67	0.00	-13.20	-10.15	22.22	200.00	-2.63
475	TRFK 6/8 X AHP S15/10	16.60	-0.53	103.33	25.00	6.37	-8.58	29.41	33.33	-9.09
482	TRFK 6/8 X TRFCA SFS150	10.71	-7.79	127.62	21.21	3.68	-4.93	17.65	-10.00	-2.70
476	TRFK 6/8 X EPK TN14-3	13.76	2.58	74.51	25.00	0.48	-2.24	11.11	50.00	-7.32
456	AHP S15/10 X TRFK 6/8	22.37	-7.80	128.10	17.92	7.90	-12.85	29.41	20.00	-11.36
478	AHP S15/10 self	-2.23	14.71	167.89	28.57	5.98	-2.76	25.00	-48.00	-10.20
485	AHP S15/10 X TRFCA SFS150	-9.63	0.47	145.95	31.71	3.19	-2.83	12.50	-45.00	2.33
474	AHP S15/10 X EPK TN 14-3	0.64	3.50	109.23	32.08	-2.58	-4.79	17.65	-5.00	-23.40
420	TRFCA SFS150 X TRFK 6/8	14.56	-4.80	96.40	11.52	7.26	-4.27	5.88	-10.00	0.00
463	TRFCA SFS150 X AHP S15/10	-6.74	-4.19	135.00	23.41	-1.22	-3.73	12.50	-5.00	9.30
471	TRFCA SFS150 self	-19.56	-2.10	100.00	-38.46	-0.95	-3.73	12.50	0.00	5.56
430	TRFCA SFS150 X EPK TN14-3	-4.60	-4.70	104.35	31.52	-8.81	-3.20	-5.88	-17.67	-25.00
443	EPK TN14-3 X TRFK 6/8	19.92	-2.88	64.41	15.00	4.05	-4.89	22.22	10.00	-21.95
447	EPK TN14-3 X AHP S15/10	-3.30	-1.17	107.58	32.08	5.49	1.84	5.88	5.00	-19.15
488	EPK TN14-3 X TFFCA SFS150	-24.06	-8.14	120.00	23.03	24.12	-4.73	41.18	13.33	-10.00
490	EPK TN14-3 self	-15.17	-8.83	66.67	-16.50	-8.82	-10.23	22.22	10.00	-27.27
	Overall mean	3.17	-2.81	108.48	16.44	2.06	-5.13	17.59	12.56	-9.56
	Significance of t-test (p = 0.05)	NS	NS	S	S	NS	S	S	NS	S

***Note**. NS, S designate not significant (P >0.05) and significant (P<0.05), respectively. Traits are as described in the legend for Table 10*

Table 18. Better-parent heterosis (BPH) for the nine measured traits

Family code	Pedigree ♀ ♂	BPH								
		Yield	%TP	Ferm	DT	TF(umol/g)	TR(%)	TF:TR	Pubescence	Bud wt(gm)
467	TRFK 6/8 self	37.41	-13.29	-18.11	0.00	-13.20	-10.15	22.22	200.00	-2.63
475	TRFK 6/8 X AHP S15/10	-2.74	-8.11	-18.11	10.00	-3.56	-14.15	22.22	-20.00	-18.37
482	TRFK 6/8 X TRFCA SFS150	-9.60	-11.40	17.32	0.00	-5.69	-10.82	11.11	-40.00	-5.26
476	TRFK 6/8 X EPK TN14-3	-3.91	-1.65	-53.00	25.00	-0.78	-4.23	11.11	0.00	-13.64
456	AHP S15/10 X TRFK 6/8	2.07	-14.81	4.72	1.50	-2.17	-18.15	22.22	-48.00	-20.41
478	AHP S15/10 self	-2.23	14.71	40.44	28.57	5.98	-2.76	25.00	-48.00	-10.20
485	AHP S15/10 X TRFCA SFS150	-12.00	-3.57	20.71	-7.69	2.86	-2.95	12.50	-56.00	-10.20
474	AHP S15/10 X EPK TN 14-3	-0.90	-0.45	-30.00	18.50	-10.65	-8.76	11.11	-24.00	-26.53
420	TRFCA SFS150 X TRFK 6/8	-6.45	-8.52	-9.45	-12.31	-2.43	-10.20	0.00	-40.00	-2.63
463	TRFCA SFS150 X AHP S15/10	-9.19	-8.04	14.29	-20.77	-1.54	-3.85	12.50	-24.00	-4.08
471	TRFCA SFS150 self	-19.56	-2.10	0.00	-38.46	-0.95	-3.73	12.50	0.00	5.56
430	TRFCA SFS150 X EPK TN14-3	-8.48	-4.91	-15.00	13.08	-16.09	-7.41	-11.11	-17.67	-31.82
443	EPK TN14-3 X TRFK 6/8	1.29	-6.87	-77.00	15.00	2.74	-6.82	22.22	-56.00	-27.27
447	EPK TN14-3 X AHP S15/10	-4.77	-4.93	-32.00	18.50	-3.25	-2.41	0.00	-16.00	-22.45
488	EPK TN14-3 X TFFCA SFS150	-27.16	-8.35	0.00	2.31	14.22	-8.88	33.33	13.33	-18.18
490	EPK TN14-3 self	-15.17	-8.83	-50.00	-16.50	-8.82	-10.23	22.22	10.00	-27.27
	Overall mean	-5.09	-5.70	-12.82	2.30	-2.71	-7.84	14.32	-10.40	-14.71
		NS	S	NS	NS	NS	S	S	NS	S

Note. *NS, S designate not significant (P >0.0) and significant (P<0.05), respectively. Traits are as described in the legend for Table 10.*

Table 19. Genetic parameters and their standard errors for all the measured traits

Parameter	Yield Estimate	Yield S.E.	Total polyphenols Estimate	Total polyphenols S.E.	Fermentability Estimate	Fermentability S.E.	Drought tolerance Estimate	Drought tolerance S.E.	Theaflavin Estimate	Theaflavin S.E.	Thearubigins Estimate	Thearubigins S.E.	TF:TR ratio Estimate	TF:TR ratio S.E.	Pubescence Estimate	Pubescence S.E.	Bud Dry weight Estimate	Bud Dry weight S.E.
D (Additive variance)	10764.71	16035.96	1.88	0.64	0.04	0.01	0.12	0.03	-0.32	1.12	-0.02	0.29	-0.02	0.26	0.04	0.02	0.00	0.00
H1 (Dominance variance 1)	10491.04	24598.87	5.01	1.33	0.24	0.03	0.16	0.03	5.93	3.63	0.81	0.76	0.81	0.74	*1.50*	0.15	0.00	0.00
H2 (Dominance variance 2)	9498.81	19339.78	3.52	0.94	0.17	0.02	0.13	0.03	5.27	2.99	0.69	0.61	0.69	0.59	1.03	0.10	0.00	0.00
F (Product of add. by dom.)	-4691.39	15878.67	3.17	0.94	0.04	0.02	0.04	0.02	-1.40	1.51	-0.21	0.35	-0.21	0.36	0.12	0.08	0.00	0.00
hh (Square of difference P vs.All)	8004.80	20369.78	-0.09	0.14	0.00	0.00	0.12	0.04	7.29	4.69	0.19	0.54	0.19	0.49	0.03	0.03	0.00	0.00
E (Environmental variance, whole)	8232.78	2223.35	0.14	0.04	0.00	0.00	0.00	0.00	0.90	0.22	0.20	0.05	0.20	0.05	0.01	0.00	0.00	0.00
sqr(H1/D) (Average degree of dominance)	0.99	2.40	1.63	0.21	2.46	0.32	1.18	0.16	0.00	6.37	0.00	3.84	0.00	3.02	6.29	2.51	0.45	0.57
kd/(kd+kr) (Proportion of dominant genes)	0.39	0.34	0.76	0.02	0.60	0.03	0.58	0.04	0.00	0.29	0.00	0.37	0.00	0.45	0.62	0.06	0.14	0.20
hhfH2 (Number of effective factors)	1.12	2.83	-0.03	0.05	-0.01	0.02	1.30	0.30	1.85	0.70	0.37	1.05	0.37	2.63	0.04	0.04	-0.62	0.69
h (Average direction of dominance)	115.79	77.50	-0.0 17	0.32	0.00	0.04	-0.35	0.05	2.81	0.80	0.57	0.40	0.57	0.38	0.18	0.07	0.00	0.02
uv (Balance of positive and negative alleles)	0.23	0.70	0.18	0.01	0.18	0.00	0.20	0.01	0.22	0.02	0.21	0.50	0.21	0.21	0.17	0.00	0.35	0.63
D/(D+E) (Heritability by parents)	0.56	0.86	0.93	0.03	0.96	0.02	0.97	0.01	-0.56	3.48	-0.10	3.18	-0.10	3.28	0.83	0.12	0.83	0.14
H^2 (Heritability in a broad sense)	0.56	0.15	0.88	0.04	0.98	0.01	0.96	0.01	0.71	0.09	0.62	0.13	0.62	0.12	0.98	0.00	0.81	0.06
h^2 (Heritability in a narrow sense)	0.44	0.16	0.09	0.07	0.45	0.04	0.61	0.06	0.28	0.12	0.29	0.15	0.29	0.15	0.43	0.04	0.74	0.09
Mp (Mean of Parents)	2354.60	44.70	21.74	0.18	1.37	0.02	1.77	0.03	19.75	0.47	15.33	0.23	15.33	0.22	2.82	0.04	0.38	0.01
Mfl (Mean of F_1s)	2431.80	25.20	21.73	0.11	1.37	0.01	1.53	0.02	21.62	0.28	15.71	0.13	15.71	0.12	2.94	0.03	0.38	0.01
Vp (Var. of Parents)	18997.50	15886.39	2.02	0.64	0.04	0.01	0.12	0.03	0.57	1.09	0.18	0.28	0.18	0.26	0.05	0.02	0.00	0.00
Vfl (Var. of F_1s)	11772.68	6555.41	0.63	0.19	0.08	0.01	0.05	0.01	2.04	0.83	0.44	0.19	0.44	0.19	0.49	0.04	0.00	0.00
Ep (Env. Var. from Parents)	21290.56	4662.75	0.11	0.07	0.00	0.00	0.00	0.00	0.74	0.50	0.06	0.11	0.06	0.11	0.03	0.00	0.00	0.00
Efi (Env. Var. from F_1s)	3695.18	2556.40	0.16	0.04	0.00	0.00	0.00	0.00	0.86	0.27	0.20	0.06	0.20	0.06	0.00	0.00	0.00	0.00

Table 20. Percent genetic gains of the measured traits based on narrow (h^2) and broad-sense (H^2) heritabilities

Trait	$h^2 \pm$ s.e.	% genetic gain family mean	% genetic gain clonal mean	$H^2 \pm$ s.e.	% genetic gain family mean	% genetic gain clonal mean
Yield	0.44 ± 0.16	2.8	14.1	0.56 ± 0.15	3.5	17.9
%TP	0.09 ± 0.07	1.2	2.5	0.88 ± 0.04	11.3	24.0
Ferm	0.45 ± 0.04	6.7	8.9	0.98 ± 0.01	14.2	19.5
DT	0.61 ± 0.06	11.6	20.6	0.96 ± 0.01	18.5	32.4
TF(umol/g)	0.28 ± 0.12	4.6	13.1	0.71 ± 0.09	11.7	33.2
TR(%)	0.29 ± 0.15	2.0	29.5	0.62 ± 0.13	4.4	61.3
TF:TR	0.29 ± 0.15	3.4	22.0	0.62 ± 0.13	6.2	49.6
Pubescence	0.43 ± 0.04	16.8	30.0	0.98 ± 0.00	38.2	68.1
Bud Dry wt	0.74 ± 0.09	11.0	27.3	0.81 ± 0.06	13.9	29.8

NB. s.e. *designates standard error; traits are described in the legend for Table 10.*

Table 21. Correlation coefficients among the nine measured traits

	Yield	%TP	FERM	DT	TF(umol/g)	TR(%)	TF:TR	Pubescence	Bud wt(gm)
Yield	1	0.612*	0.313	0.225	0.392	0.253	0.566*	0.462	0.441
%TP		1	0.541*	0.215	0.276	0.664**	0.344	0.460	0.356
FERM			1	0.667**	0.373	0.67**	0.466	0.329	0.585*
DT				1	0.399	0.545*	0.651**	0.502*	0.276
TF(umol/g)					1	0.616**	0.809***	0.237	0.531*
TR(%)						1	0.23	0.212	0.637**
TF:TR							1	0.396	0.401
Pubescence								1	0.436
Bud wt(gm)									1

*NB. *, ** and *** denote significance levels at P < 0.05, P < 0.01 and P < 0.001, respectively; traits are described in the legend for Table 10.*

5.3 Discussion

The diallel analysis method employed gave good indicators of the genetic parameters governing the traits measured. Certain progenitors were more frequently associated with superior hybrids than others. For example, clones TRFK 6/8 and AHP S15/10 gave superior progeny for high yields, FERM, drought tolerance and TF:TR, while the progeny with best BPH for total polyphenols resulted from AHP S15/10 either as female or male parent. This implies that efforts to improve on these traits should involve these progenitors in the rationalized hybridization programme. The considerable variation revealed in the test for the nine traits combined with high heritability serves to demonstrate the great potential for progress through selection.

The significance of heterosis is best exhibited by BPH, which directly illustrates the superiority of a hybrid over the best performing variety under cultivation. The analysis adopted for derivation of various parameters of heterosis and their significance by t-test, has also been used by other workers (Moll *et al.*, 1965; Barth *et al.*, 2003). However, the importance of MPH has been emphasized by Barth *et al.* (2003), especially as regards the expected quadratic relationship to the parental genetic distance under a simple genetic model. In the present study, especially where family means were used to derive heterosis, it was revealed that the best progeny for yield was only marginally worse (BPH of -5.09%) than the best parent TRFCA SFS150, hence the shift of focus to MPH. The highest BPH was recorded for traits TF:TR and drought tolerance at 14.32% and 2.30%, respectively. Black tea quality improvement in Kenya has only been relying on sensory evaluation and fermentability (Green, 1966; Njuguna, 1989a,b). Only recently have efforts been initiated to develop reliable black tea parameters to assist in selection of elite black tea quality cultivars based on correlation between tea tasters scores, total polyphenols, theaflavins and thearubigins (Obanda *et al.*, 1992). A study undertaken by Owuor *et al* (1986) pointed to the positive contribution of theaflavins (TF) in valuation of Kenyan black teas. Experiments carried out in Malawi (Hilton and Ellis, 1972; Cloughley, 1981), and India (Biswas and Sarkar, 1971, 1973) demonstrated good correlations between prices, tea tasters and TF or TF and TR. Liquor characteristics such as brightness and briskness have also been associated with theaflavins and caffeine, respectively. Such characteristics are used by tasters to define tea quality and in appraising the market value of commercial teas. Thus, when a tea product is termed as bright and brisk, usually such a product fetches high market value (Deb and Ullah, 1968; Hazarika *et al.*, 1984). Thus, a high BPH for TF:TR is an indication of a potential to avail a high value black tea quality specialty product using promising clones in the current study

Negative values of BPHs for total polyphenols, fermentability, pubescence and bud weight of -5.70%, -12.82%, -10.40% and -14.71%, respectively, were low when family mean criteria was used. It must however be emphasized that these results represent the overall direction of heterosis for the traits in question. The significant negative heterosis for majority of the characters does not exclude the possibility that some families or individuals within families may show significant positive heterosis or no heterosis. Indeed examination of the results shows that such families and/or individuals exist, although less frequently than families/individuals that show negative heterosis. For example, a self of AHP S15/10 had a BPH of 14.71% and 40.44% for %TP and fermentability, respectively. This indicates the potential for processing of different tea products from different heterotic crosses; those with negative BPH being suitable for green tea, while those with positive BPH passing for black tea manufacture. Thus, caution needs to be observed while judging the performance of the progeny based solely on family means.

The significant MPH values of 108.5%, 17.6% and 16.4% for FERM, TF:TR and DT, respectively, and to a lesser extent 12.6% for pubescence emphasize the availability of elite black tea quality cultivars which should be exploited to process high value tea products. Despite the allogamous nature of the tea crop, yield only scored an MPH of 3.17% which is an indication of a trait which is approaching the potential maxima in its improvement unless approaches such as mutation breeding or genetic modification are employed to further enhance yield trait expression. It is generally held that the amount of heterosis is much smaller in autogamous than in allogamous crops (Melchinger and Gumber, 1998). However, there has been gross lack of knowledge on heterosis in *Camellia* sp with the only reported work being that of Bezbaruah (1974). In that study, the heterotic response for tea yield ranged from 21% to 85% over the mid-parental values, while the quality of made tea approached the mid-parent value for F_1 hybrids for most seedling populations of tea. While four hybrids in this study gave data comparable to that of Bezbaruah (1974) for yield, majority of the crosses had over 25% MPH for fermentability and drought tolerance, respectively. It is worth noting that many of the crosses involving TRFK 6/8 either as female or male parent had significantly higher MPH than others. Such crosses also scored high MPH for quality traits, TF and TF:TR. The fact that all parents involved in this study are among the best performing clones in commercial plantations and coupled with the overemphasis on improving yields and fermentability as the traditional primary traits, it is probable that further progress of these traits might be approaching the ceiling. The use of disparate parental materials in the current study notwithstanding, heterotic hybrids could only be observed within families but not

between families. The ease of propagation of tea using vegetative propagation, however, allows for considerable improvement in the assessed traits if careful clonal selection within families is imposed. Results of the current study also indicate that drought tolerance was the most variable character. This is not surprising as breeding for drought tolerance was not undertaken until recently. The observation that clone TRFK 6/8 harbours some drought tolerant genes can be confirmed in part by one of its commercialized progeny, TRFK 303/577, which apart from being one of the leading high yielders and producer of high black tea quality, its drought and cold tolerance status have now been confirmed by its performance in hot spot areas (Anon. 2004). A positive BPH for this character points to the existence of elite drought tolerant cultivars, which if accompanied by other favourable attributes could be made available to farmers.

The heritability estimates for yield indicated that the character is largely controlled by additive genes and further progress is likely to be realized if high selection intensity is conducted. Yield is a polygenic character, although non-additive gene effects are occasionally observed. Comparative results have been observed in Cacao (Dias and Kageyama, 1995) where the effects of SCA (non-additive effects) were greater than GCA (additive effects) for yield. Thus, in seed gardens that are composed of many parents like in the polyclonal seed orchard of tea at TRFK, accumulation of favourable alleles with additive genetic effects on yield in the improved generations is likely to be successful. The low narrow-sense heritability estimates for total polyphenols, fermentability, drought tolerance, TF, TR, TF:TR, pubescence and bud weights revealed non-additive gene action to be more predominant in the expression of the traits. The apparent dominant nature of the fermenting character was also observed by Toyao (1982) in a study of inheritance of the non-fermenting trait in the tea plant. The consistency of the results obtained in the present study confirms that fermentability is governed predominantly by non-additive genes.

The strong non-additive genetic effects that are also characterized by high broad-sense heritabilities for majority of the traits, coupled with considerable clonal variation and moderate MPH, may imply that the straight forward procedure of selecting suitable clones and allowing them to pollinate randomly even in properly designed polyclonal seed orchards (baries) might predictably not result in improved performance in the traits. Thus, improvement efforts for traits showing strong influence by non-additive gene effects might call for a different breeding approach such as investing more in controlled crosses or establishment of biclonal seed orchards. The improved biclonal seedlings can then be multiplied by vegetative means following progeny testing and clonal selection and used to

establish new plantations. The broadened genetic base of commercialized clones would then mitigate attendant risks normally associated with monocropping.

The most important significant correlation coefficients were those between yield and %TP ($r = 0.61$, $P < 0.01$), %TP and FERM ($r = 0.54$, $P < 0.05$), %TP and %TR ($r = 0.66$, $P < 0.01$), FERM and DT ($r = 0.67$, $P < 0.01$) and TF and TF:TR ($r = 0.81$, $P < 0.001$). These significant correlations indicate that the traits in question are either controlled by linked genes or genes with pleiotropic effects, and therefore improving on one trait may lead to improvement of the others. Incidentally, these are the most important economic traits currently being addressed in the rationalized breeding programme in Kenya, and possibility of simultaneous improvement, points to development of novel varieties for specialty tea products. The weakly correlated traits like yield and drought, yield and FERM attest to lack of significant genetic relationship between any two of the measured traits. This would imply that efforts to select for all or any such two traits in one clone simultaneously may be difficult to achieve, although more studies are needed to confirm this conclusion. Besides, selection for one trait may adversely affect or might fail to enhance the performance of the other. This kind of relationship nevertheless may allow for selection of traits in tandem beginning with the most important attributes. However, further studies in different environments are required to confirm this proposition. Genetic correlation studies involving even more traits would reveal which characters are synergistic (positively linked) and can be packaged into a single genotype and the ones that are antagonistic and hence cannot be recombined easily.

The calculation of genetic gains particularly when considering clonal means showed that moderate gains would be registered for all the traits if high selection intensity was instituted particularly within families based on heritability estimates obtained from this study. The values compare favorably with those reported by Lin and Zsuffa (1993) for biomass based on individual ramets and clonal means (3.2% and 7.4%, respectively) in *Salix eriocephala*. Greater gains are obtainable through manipulating the selection differential and selecting only individuals that meet set minimum criteria for all traits of interest. Faster progress could be realised if more precise selection tools are developed and by employing appropriate methods of indirect selection (Wright 1976; Falconer, 1989) and DNA marker-assisted selection (MAS) as has been proposed by other workers (Hackett *et al.*, 2000; Wachira *et al.*, 1995, 1997, 2001, 2002).

As estimated genetic variances vary with age, season and environment in which genetic tests are conducted (Zhang *et al.*, 2004), their applicability is limited to the

environment in which the study was undertaken. There is need, therefore, to replicate the experiment carried out in this study in other sites with different environmental components in order to strengthen the reliability of the derived genetic variances (Falconer, 1989).

CHAPTER 6

MAPPING QUANTITATIVE TRAIT LOCI FOR YIELD, DROUGHT TOLERANCE, QUALITY ATTRIBUTES AND RESISTANCE TO ROOT KNOT NEMATODE IN TEA

6.1 Introduction

The demand for new varieties (clones) with improved and novel quality traits call for sustained search for methods and strategies to improve on efficiency and reduce time in which such varieties can be availed. The long generation cycle, self-incompatibility and allogamous characteristic make tea breeding a very promising field for marker-aided selection and breeding (MAS and MAB). Interest has increasingly focused on the use of molecular markers in plant breeding programmes owing to their independence of environmental fluctuations. Markers linked to loci (genes) of desired traits can be utilized at an early (seedling) stage to select for individuals with favourable combinations of several traits of interest. Although examples on the use of marker-assisted selection (MAS) can be given for some tree crops (Brown *et al*., 2003; Yang *et al*., 1997; Missiaggia *et al*., 2005; Grattapaglia and Sederoff, 1994; Zhang *et al*., 2004), the effect of genotype by environmental interactions, varying biotic and abiotic factors on a seasonal or yearly basis affect markedly detection of QTLs. In tea, a linkage map exists (Hackett *et al*., 2000), but there is no report on comprehensive QTL analysis in the crop to date. Wachira (1996b) identified markers and genomic regions that influenced the expression of several quantitative traits. MAS holds particular promise in tea breeding, where long juvenile period of this crop in which the polygenic nature of yield and quality traits are major bottlenecks in conventional breeding programmes. The long period utilized in progeny testing and clonal field trials in order to allow reliable quality data to be gathered further exacerbate this problem. Coupled to this is the crop's genetic characteristics such as high heterozygosity and allogamous nature implying that large number of progeny needs to be screened to identify promising genotypes. Thus, any technology upon which substantial economic benefits could be realized needs to be given due weight.

The current study was carried out to detect and map the various QTLs controlling yield, DT and quality traits for black tea and resistance to root knot nematode with the aim of initiating marker-assisted selection and breeding in the tea.

6.2 Results

6.2.1 Variation of phenotypic traits

Data on various phenotypic traits measured both at Timbilil and Kangaita experimental sites are presented in Figures 10 to 27. The normality of distribution for all the traits measured was subjected to chi-square goodness of fit test. Except for bud pubescence (Figure 17) and root knot nematode infection (Figure 27) in Kangaita site, all the other traits showed continuous distribution, an observation that was confirmed by goodness of fit test. Data on annual mean yield (2001 to 2006 denoted as ANYLD06) assessed both at the Timbilil (T) and Kangaita (K) experimental sites are presented in Figures 10 and 11, respectively. The ANYLD06-T among the progeny ranged from 1670 to 2412 Kg made tea (mt)/ha, while ANYLD06-K ranged from 672 to 2428 Kg mt/ha. The F_1 means for the two sites were 2180 and 1504 Kg mt/ha for ANYLD06-T and ANYLD06-K, respectively. The Mid-Parent Values (MPV) were 2163 and 2013 Kg mt/ha for ANYLD06-T and ANYLD06-K, respectively. The mid-parent heterosis (MPH) for ANYLD06-T was marginal (0.8%), while the one for ANYLD06-K was low (-25.3%). The best progeny at Timbilil gave 11.5% more yields over the MPV, while the best progeny in Kangaita outperformed MPV by 20.6%. From the figures, it is apparent that the yield trait as assessed in Timbilil and Kangaita sites (Figure 10 and 11) did not behave the same way as the F_1 means were higher and lower than the parental means for Timbilil and Kangaita, respectively. In this case the average "heterozygotic" value did not match the "homozygotic" values nor was it midway between the two parental values. While this seems to suggest non-additive and non-dominance gene effects, marked unfavourable micro-habitat conditions in the heterogeneous edaphic environment at Kangaita site might have contributed to poor performance of some otherwise superior clones at the site. A number of traits namely total polyphenols (Figures 12 and 13), drought tolerance (Figures 14 and 15) and bud pubescence (Figures 16 and 17) as measured in both the Timbilil and Kangaita sites had their F_1 means falling in between the two parental means for respective traits. However, the F_1 means for bud length (Figures 18 and 19), bud width (Figures 20 and 21), shoot dry weight (Figures 22 and 23) and bud dry weight (Figure 25) were marginally lower than the parental means. Percent total polyphenols at Kangaita (Figure 26) as measured on the family St 526 (TRFCA SFS150 x TRFK 303/577) progeny presents an interesting scenario as the F_1 mean was significantly lower than the two parental means. In this case, a large number of progeny values fell outside those of the parent values pointing to high transgressive segregation. Overall, the confirmation of normal distribution

for majority of the traits gives an indication of involvement of several to many genes in the genetic control of the traits.

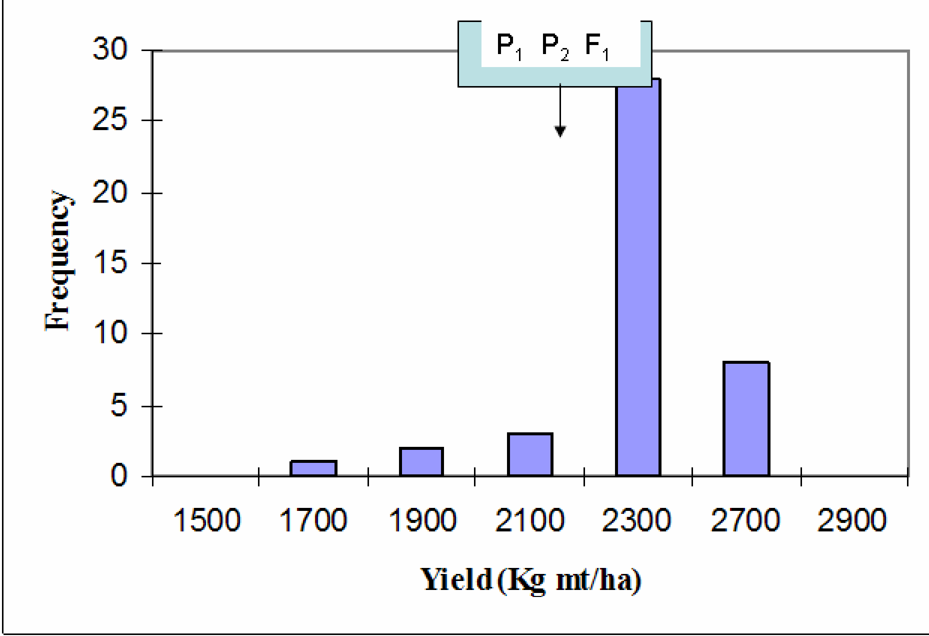

Figure 10. Frequency distribution of annual mean yield (ANYLD06-T) in St 463 progeny at Timbilil site.

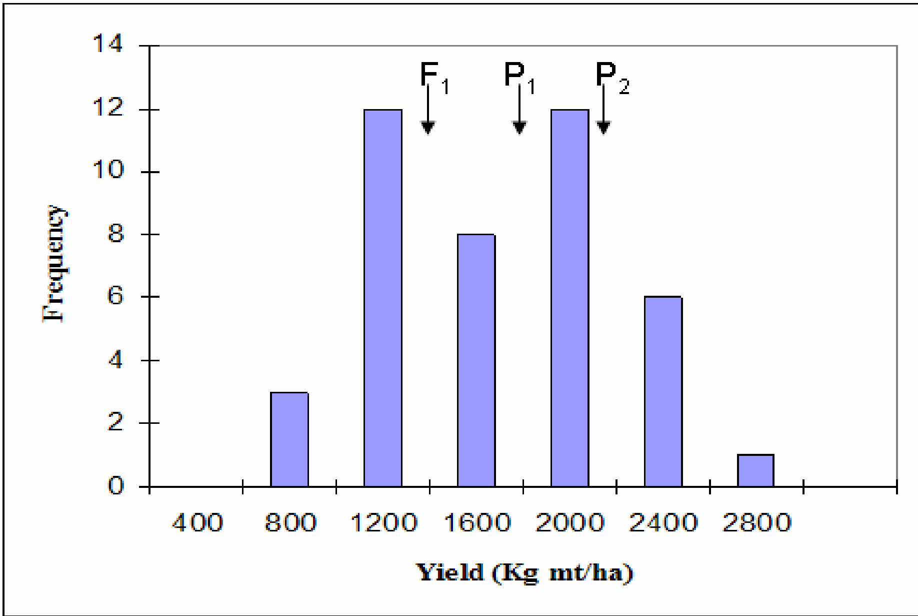

Figure 11. Frequency distribution of annual mean yield (ANYLD06-K) in St 463 progeny at Kangaita site.

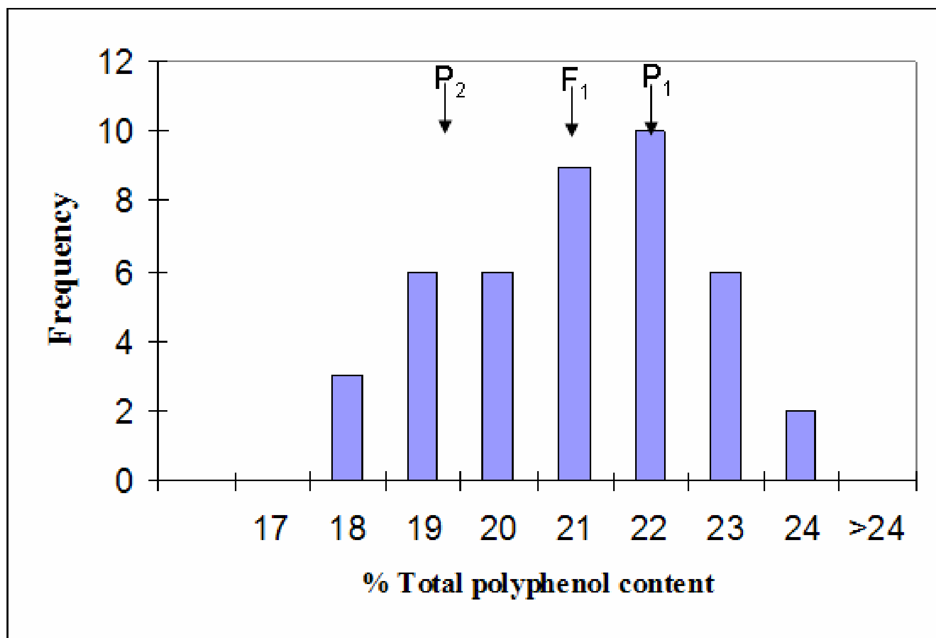

Figure 12. Frequency distribution of % total polyphenols (%TP) in St 463 progeny at Timbilil site.

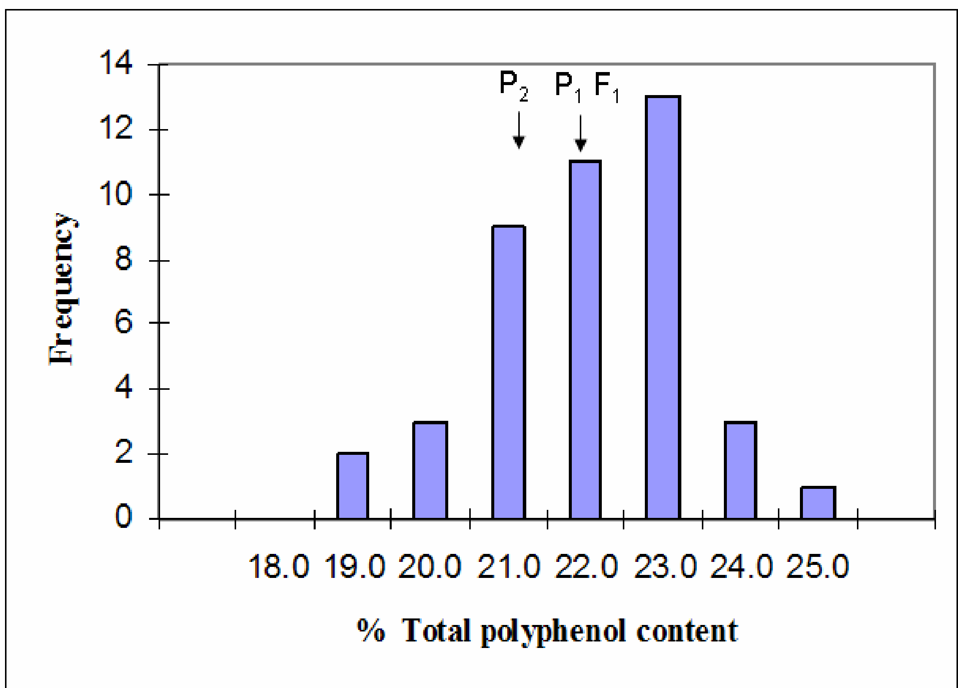

Figure 13. Frequency distribution of % total polyphenols (%TP) in St 463 progeny at Kangaita site.

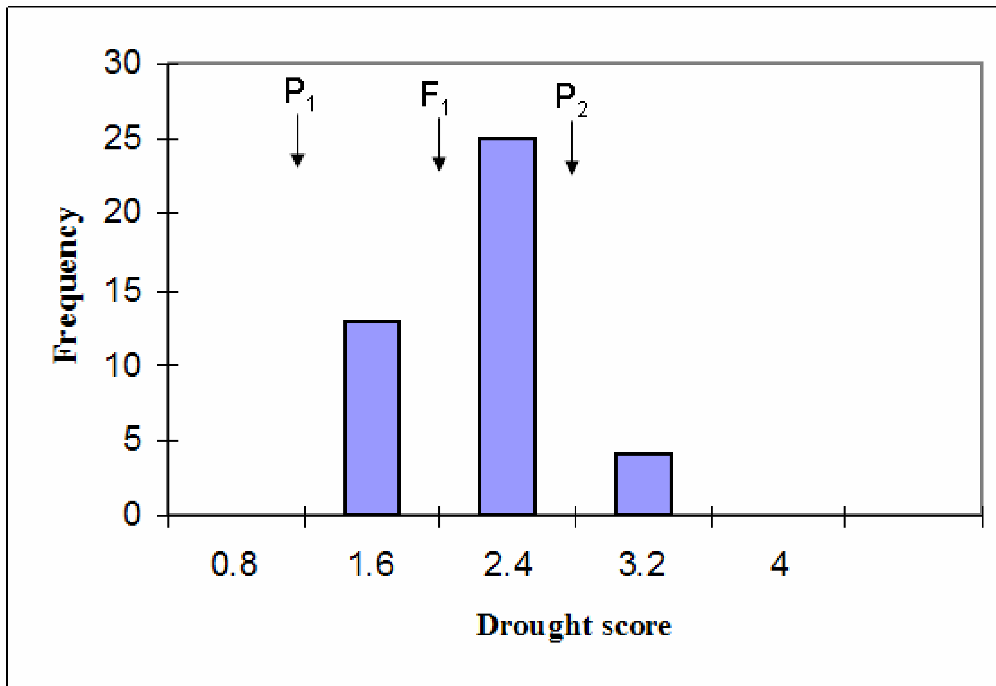

Figure 14. Frequency distribution of drought tolerance (DT) in St 463 progeny at Timbilil site.

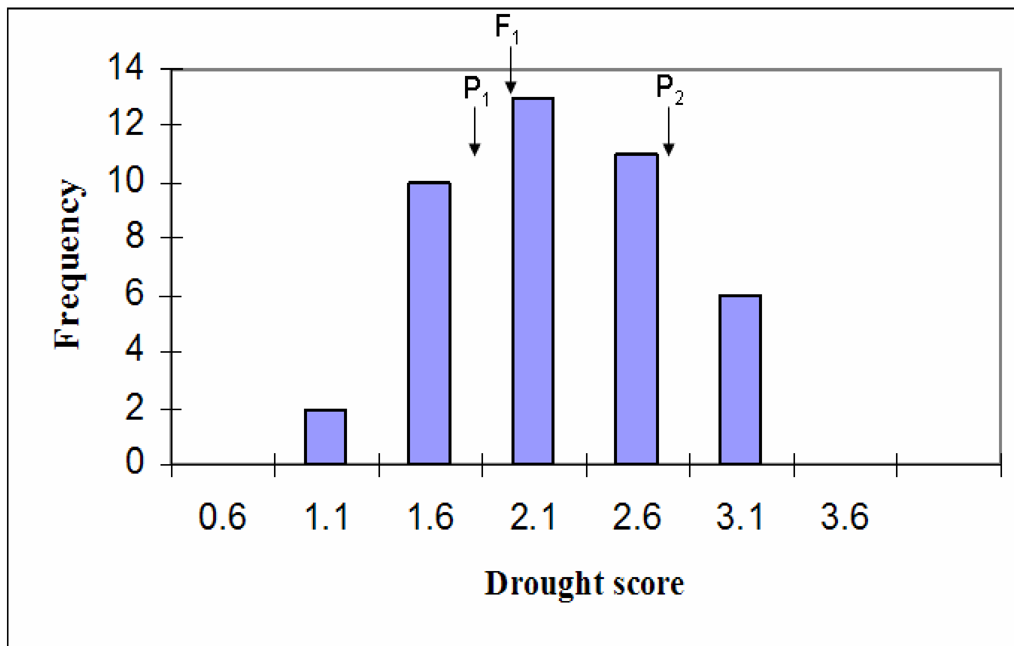

Figure 15. Frequency distribution of drought tolerance (DT) in St 463 progeny at Kangaita site.

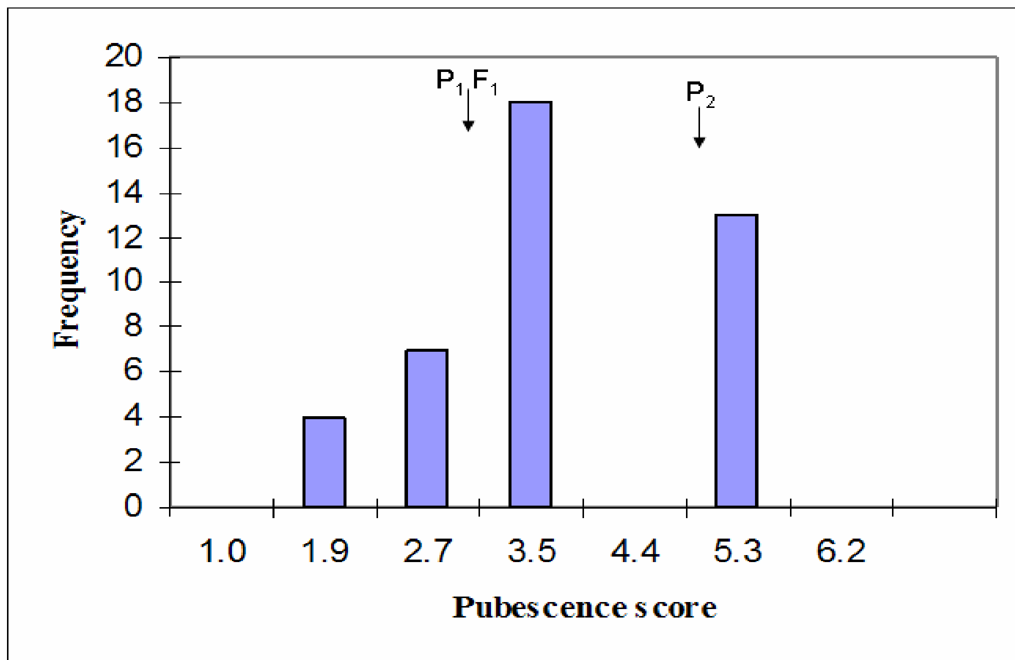

Figure 16. Frequency distribution of pubescence (PUB) in St 463 progeny at Timbilil site.

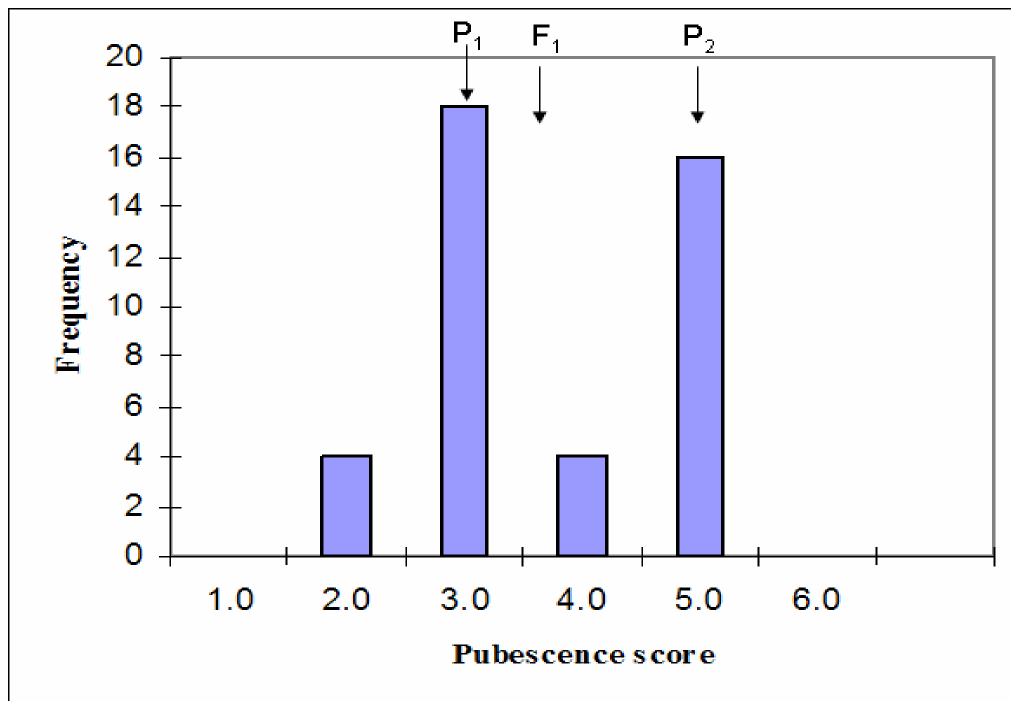

Figure 17. Frequency distribution of pubescence (PUB) in St 463 progeny at Kangaita site.

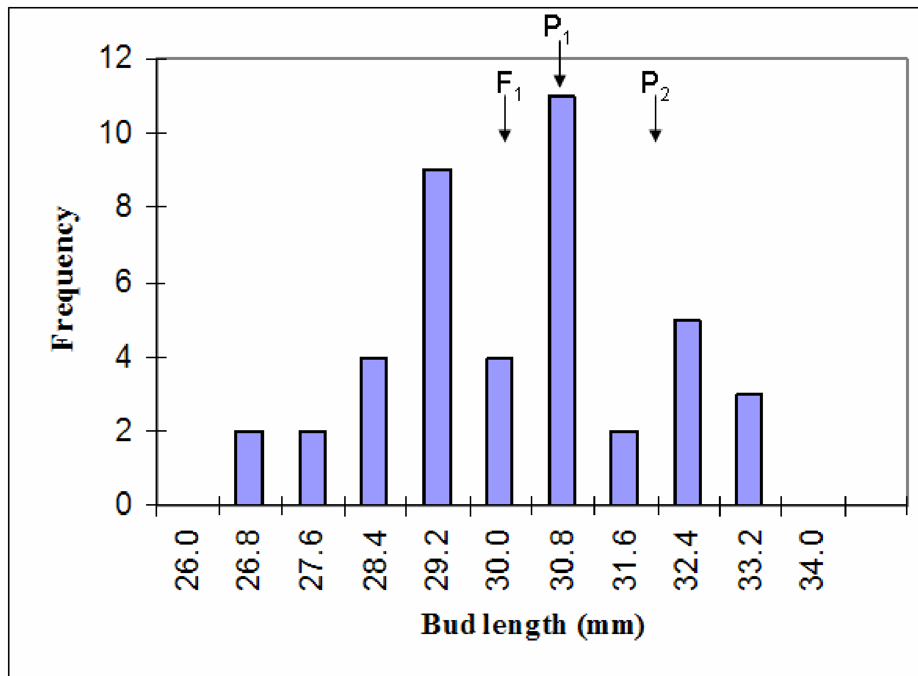

Figure 18. Frequency distribution of bud length in St 463 progeny at Timbilil site.

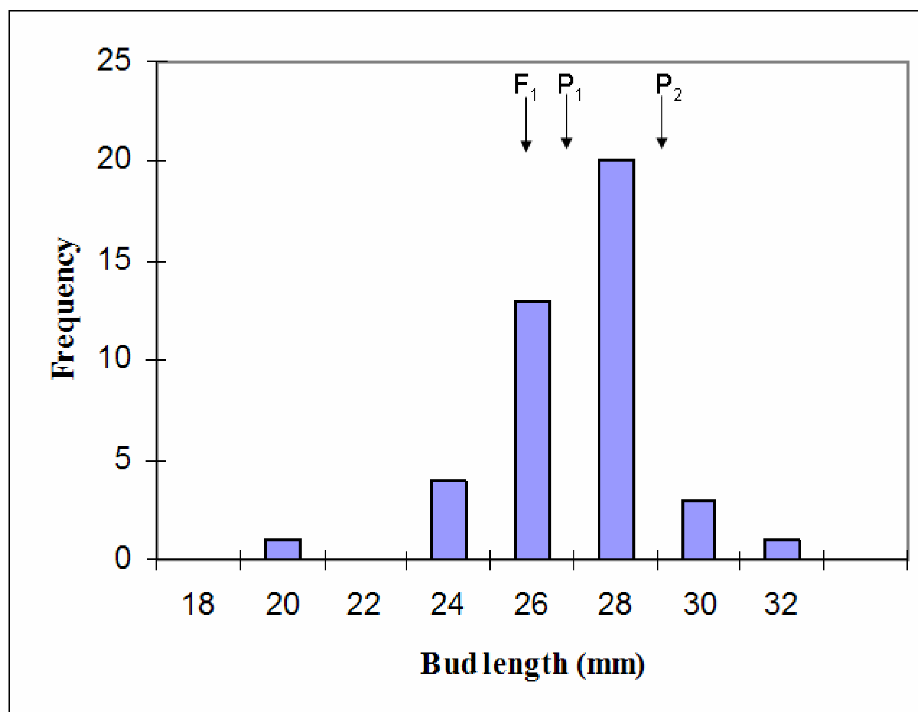

Figure 19. Frequency distribution of bud length in St 463 progeny at Kangaita site.

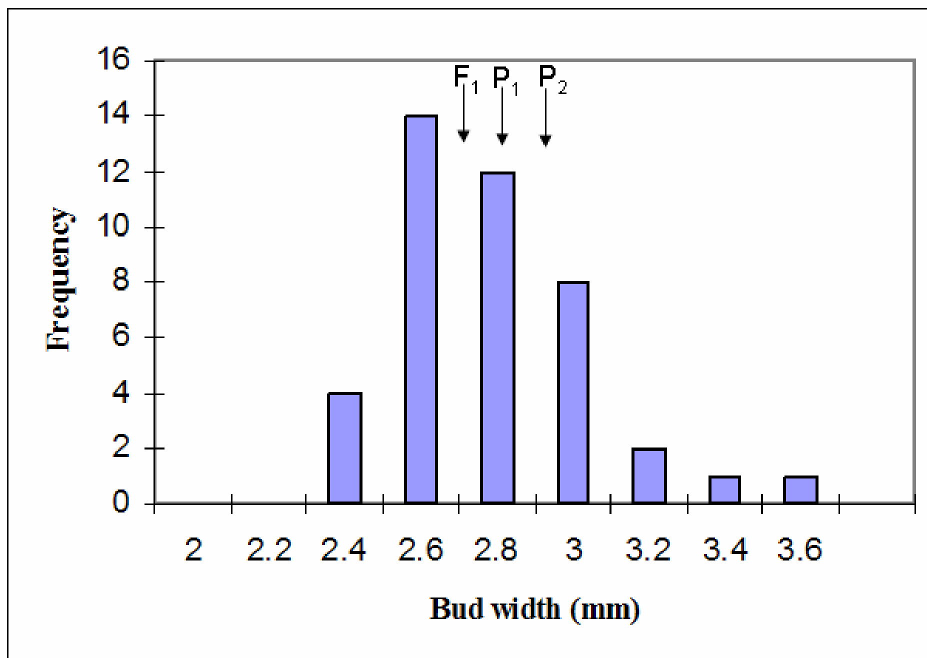

Figure 20. Frequency distribution of bud width in St 463 progeny at Timbilil site.

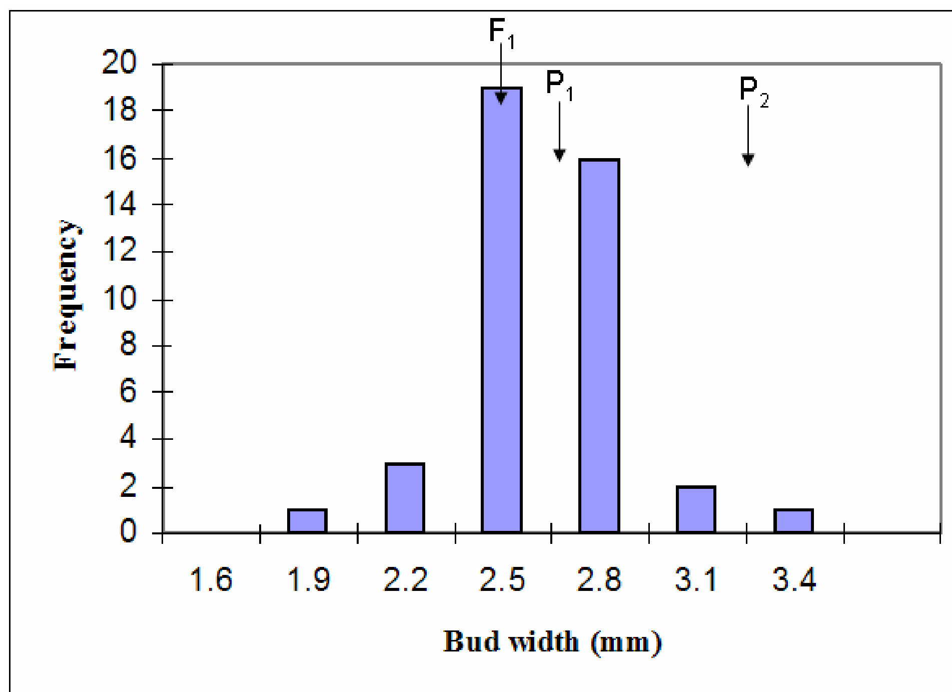

Figure 21. Frequency distribution of bud width in St 463 progeny at Kangaita site.

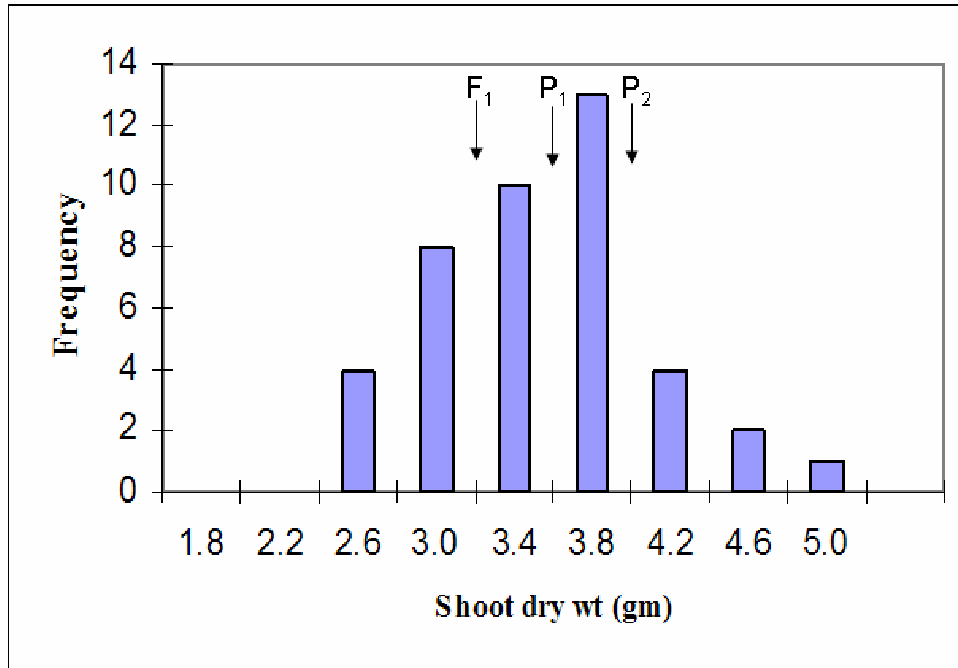

Figure 22. Frequency distribution of shoot dry weight width in St 463 progeny at Timbilil site.

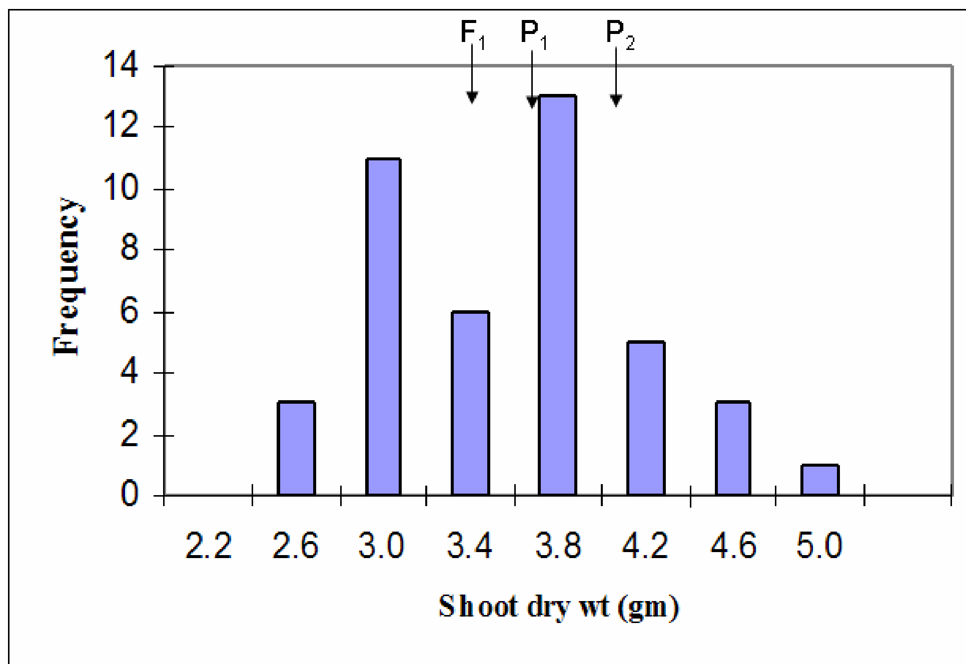

Figure 23. Frequency distribution of shoot dry weight width in St 463 progeny at Kangaita site.

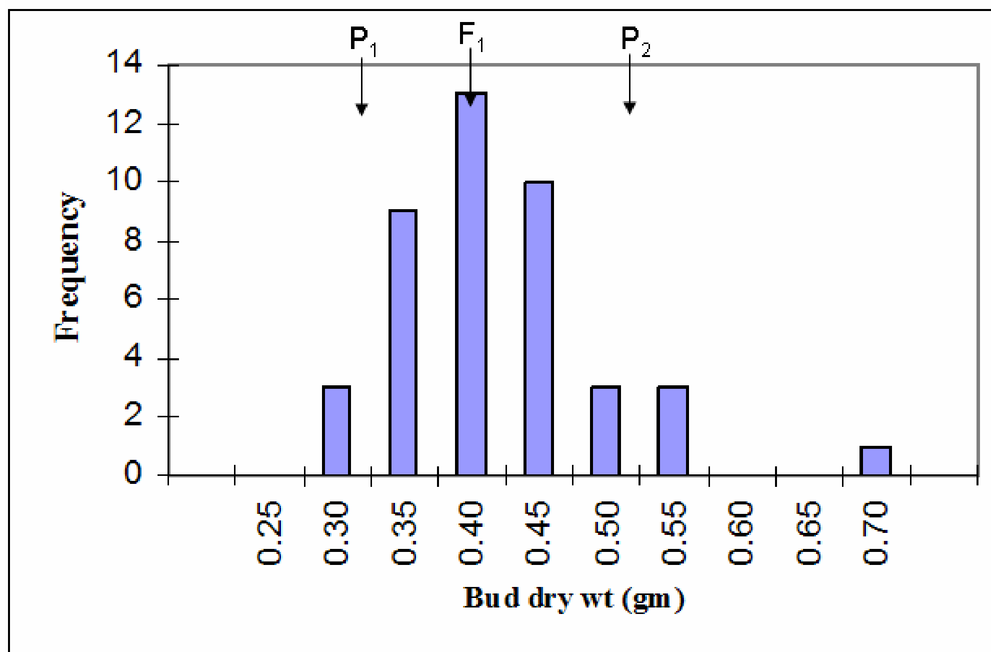

Figure 24. Frequency distribution of bud dry weight width in St 463 progeny at Timbilil site.

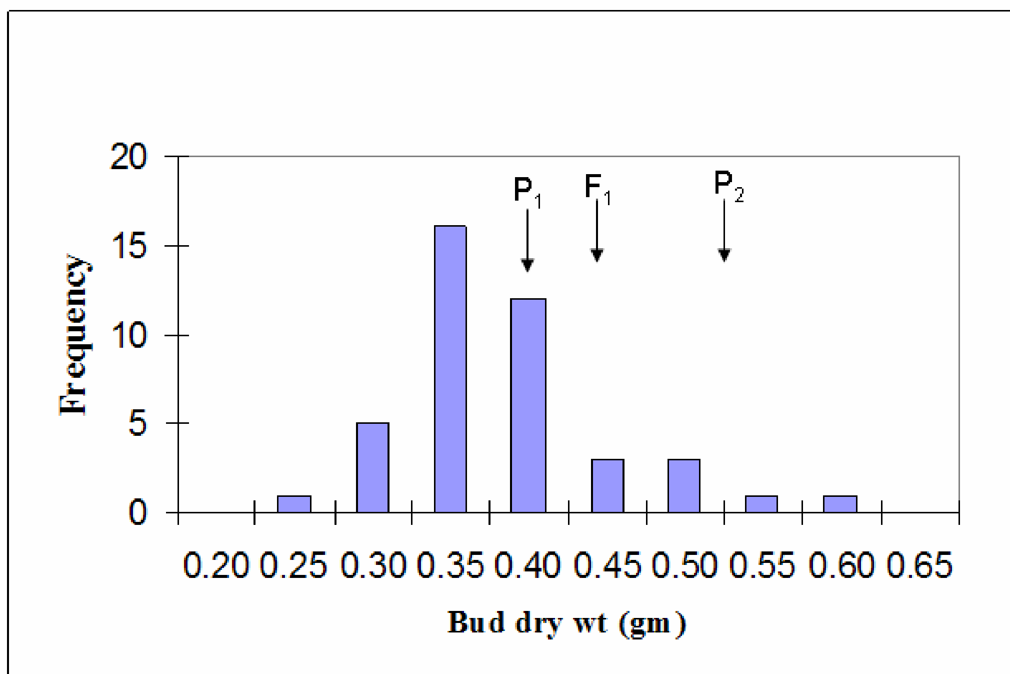

Figure 25. Frequency distribution of bud dry weight width in St 463 progeny at Kangaita site.

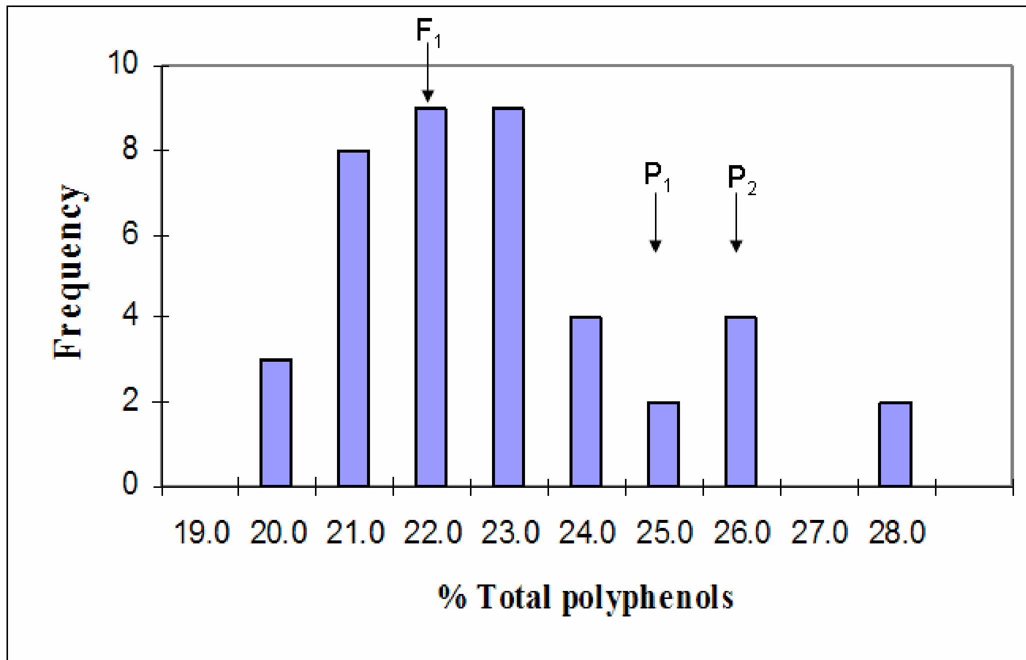

Figure 26. Frequency distribution of % total polyphenols in St 526 progeny at Kangaita site.

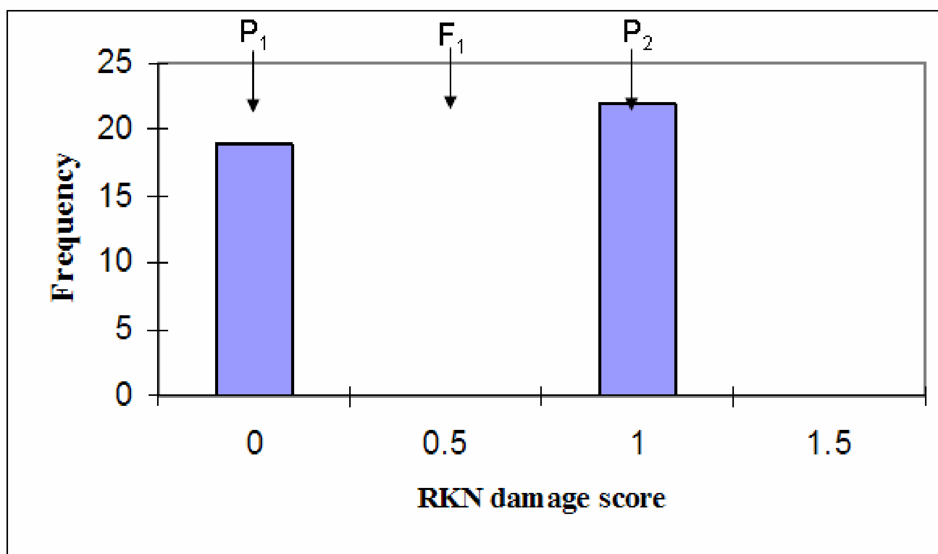

Figure 27. Frequency distribution of root knot nematode damage in St 526 progeny established in a farmer's field.

Where some traits did not pass the goodness of fit test, the population was divided into two groups according to the MPV and subjected to chi-square tests. For pubescence (Figure 17) and root knot nematode infection (Figure 27) at Kangaita, the test indicated that the number of progeny in the two groups agreed with the expected 1:1 Mendelian segregation ratio in accordance with the BC_1F_1 (backcross one into first filial generation) mating type (χ^2 = 0.003, < $\chi^2_{(0.05,\ 1)}$ = 3.84), pointing to influence by a major gene for each of the two traits. Although the results suggest that susceptible male parent (TRFK 303/577) would be in homozygous state for the locus (aa) and female parent (TRFCA SFS150) heterozygous (Aa) suggesting susceptibility to root knot nematode is governed by a single recessive gene and resistance is dominant over susceptibility, the converse would also be true (i.e. the heterozygous susceptible parent TRFK 303/577 (Aa) crossed to resistant parent TRFCA SFS150 (aa) would segregate in 1R:1S in the BC_1F_1 suggesting that resistance to root knot nematode is governed by a single recessive gene and that susceptibility is dominant over resistance).

6.2.2 Phenotypic correlation

Site-wise phenotypic correlations were estimated among the traits studied. At the Timbilil site, significant (*P* < 0.001) negative correlations were observed between PUB-T and TP-T (-0.39), TP-T and SW-T (-0.34) (Table 22). In contrast, significant (*P* < 0.001) positive correlations were recorded between PUB-T and BW-T (0.46), BL-T and BWd-T (0.59), BL-T and BWt-T (0.46) and BWd-T and BW-T (0.45), all being shoot related traits. The same scenario was observed in Kangaita although no two traits were negatively correlated (Table 23).

Table 22. Correlation coefficients (r) between phenotypic traits in the mapping population (St 463) assessed in Timbilil site

	YLD-T	PUB-T	DT-T	TP-T	BL-T	BWd-T	SW-T	BWt-T
YLD-T	1.000	-0.038	0.278	-0.199	-0.153	-0.274	0.019	-0.229
PUB-T		1.000	-0.159	-0.39**	0.129	0.103	0.296	0.46**
DT-T			1.000	-0.178	0.068	0.246	0.106	0.024
TP-T				1.000	-0.069	0.046	-0.34**	-0.247
BL-T					1.000	0.59**	0.274	0.46**
BWd-T						1.000	0.189	0.45**
SW-T							1.000	0.669***
BWt-T								1.000

***Key:** YLD = Yield; PUB = pubescence; DT = drought tolerance; TP = total polyphenols; BL= bud length; BWd = bud width; SW = shoot weight; BWt = bud weight; -T = at Timbilil site; *, ** and, ***denote significance at P < 0.05, P < 0.01 and P < 0.001, respectively.*

Significant correlations were observed mostly between shoot traits such as PUB-K and BWd-K (0.29; $P < 0.05$), DT-K and BL-K (0.29; $P < 0.05$), DT-K and BWd-K (0.35; $P < 0.01$), BL-K and BWd-K (0.73; $P < 0.001$), BWd and SW-K ((0.31; $P < 0.05$) and BWd-K and BWt-K ((0.44; $P < 0.001$). There were no significant ($P > 0.05$) correlations among the four major traits of yield-K, DT-K, TP-K, PUB-K (Table 23).

Table 23. Correlation coefficients (r) between phenotypic traits in the mapping population (St 463) assessed in Kangaita site

	YLD-K	PUB-K	DT-K	TP-K	BL-K	BWd-K	SW-K	BWt-K
YLD-K	1.000	0.099	-0.011	-0.255	0.167	0.207	0.229	0.228
PUB-K		1.000	0.235	-0.258	0.176	0.29*	0.133	0.164
DT-K			1.000	-0.109	0.29*	0.35**	0.087	0.145
TP-K				1.000	0.038	-0.015	-0.133	-0.088
BL-K					1.000	0.73***	0.259	0.31*
BWd-K						1.000	0.31*	0.44**
SW-K							1.000	0.69***
BWt-K								1.000

Key: *as for Timbilil except –K = Kangaita site.*

6.2.3 Genotype x Environment interaction

Rank correlation analysis was applied to determine the effects of genotype x environment interaction on the traits assessed at the two test sites. Rank correlations were obtained by StatistiXL1.8 software. Apart from BL and TP, which were stable across the test sites, all the other traits responded differently to environmental conditions prevailing at the test sites as can be revealed by their insignificant ($P > 0.05$) correlations (Table 24).

Table 24. Rank correlations between traits measured in the different test sites

Trait	r_s	df	P value
BL-T and BL-K	0.53***	46	0.00
BWd-T and BWd-K	0.28	46	0.06
SW-T and SW-K	0.04	46	0.81
BWt-T and BWt-K	0.11	46	0.47
YLD-T and YLD-K	0.08	46	0.59
DT-T and DT-K	0.04	46	0.80
PUB-T and PUB-K	0.24	46	0.10
TP-T and TP-K	0.95***	46	0.00

*NB. *** denotes significance at P < 0.0001*

6.2.4 Bulk Segregant Analysis (BSA)

This study entailed the rapid identification of quantitative trait loci (QTLs) associated with yield, total polyphenols, drought tolerance, pubescence and resistance to root knot nematode. The first four traits (YLD, TP, DT and PUB) were assessed in the mapping population St 463 (TRFCA SFS150 x AHP S15/10), while total polyphenols and resistance to root knot nematodes measurements were also carried out using a different mapping population St 526 (TRFCA SFS150 x TRFK 303/577). Three molecular marker systems namely; RAPD, AFLP and SSR were employed.

A combined total of 252 RAPD primers were screened using BSA initially based on three traits; root knot nematode, yield and total polyphenols (see example in Figure 28) out of which 20 and 59 primers were found polymorphic, at least between parents, for populations St 463 and St 526, respectively. These primers were preferentially utilized to genotype the two sets of ten best and ten poorest performing progeny according to each trait, and later in complete genotyping work using the entire populations. Additionally, 15 SSR and 96 AFLP (Figure 29) primer pairs were screened using the BSA approach. While RAPD analysis utilized the two populations for BSA as well as complete genotyping, SSR and AFLP markers were used to analyse only one population, St. 463, owing to insufficiency of high quality DNA on the part of the second population.

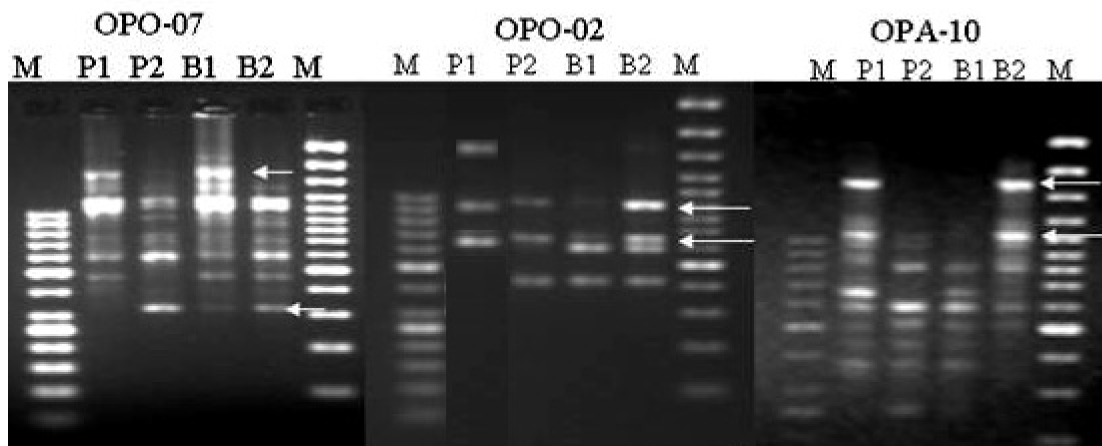

Figure 28. Screening of RAPD primers OPO-07, OPO-02 and OPA-10 using low/high yielding bulks from St. 463 progeny. Markers distinguishing parents and respective bulks are shown by arrows. Left and right M are 50bp & 100 bp DNA ladder standards, respectively, P_1 = TRFCA SFS150, P_2 = AHP S15/10, B1 & B2 are low and high yielding bulks, respectively.

6.2.4.1 Yield

Seventy-nine RAPD primers were screened on DNA bulks constructed from high and low yielding progeny together with parents. Part of the screening profile is presented in

Figure 28 and 29. The fingerprint patterns for individual high yielders and low yielders with their corresponding parents are depicted in Figures 30 and 31.

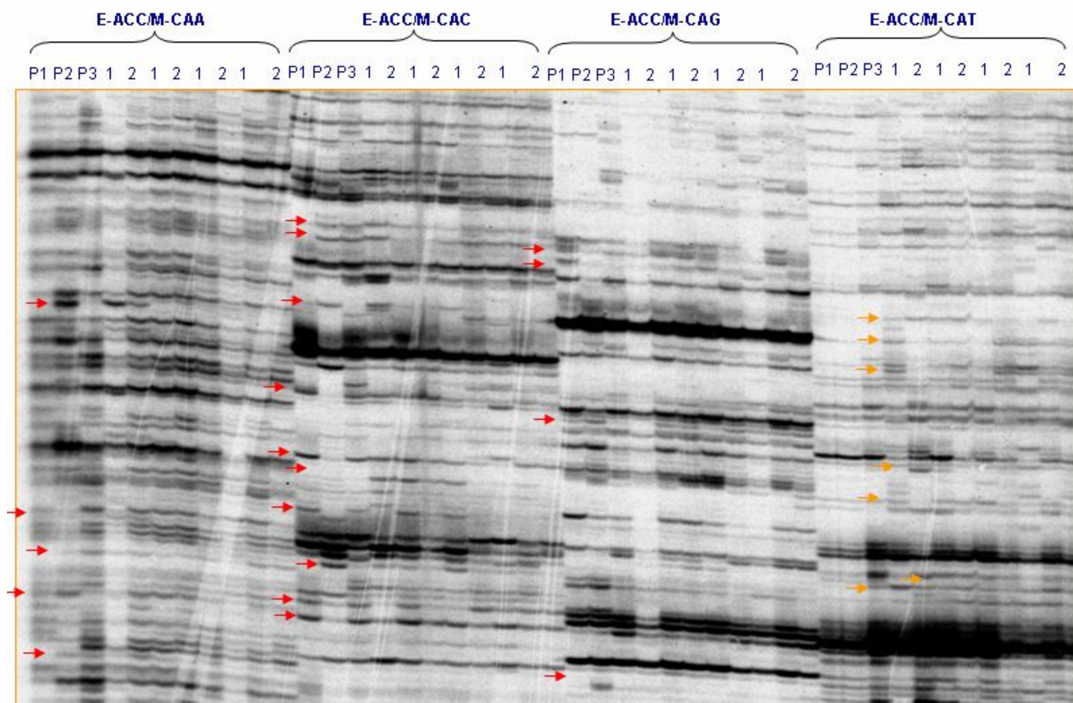

Figure 29. Screening of AFLP primers using BSA comprising, root knot nematode, yield and bud pubescence bulks (1 and 2 for 10 good and poor performers, respectively) alongside three parents; P_1 =TRFCA SFS150, P_2 = AHP S15/10 and P_3 = TRFK 303/577.

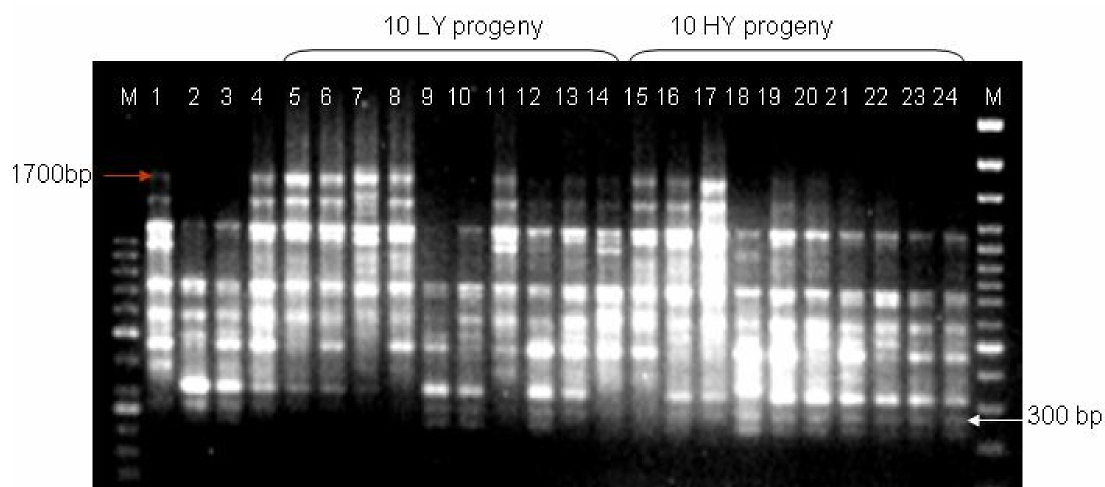

Figure 30. Pattern of RAPD fragments generated by primer OPO-07 on low and high yielding bulks and progeny from St. 463 (TRFCA SFS150 (P_1) x AHP S15/10 (P_2)). Left and right M denote molecular weight ladders at 50bp and 100bp, respectively. 1 = P_1, 2=P_2, 3= HYB (high yield bulk), 4=LYB (low yield bulk).

Although, some distinct fragments discriminating the bulks could be discernible during screening process, in most cases the pattern was not reproducible upon genotyping the individuals forming the bulks. The patterns were mostly confounded by appearance of recombinants in either of the two classes. This is not unexpected because some of the markers are linked to gene alleles that contribute a small proportion to trait expression. The localization of putative QTLs varied depending on the number of recombinants observed in each BSA experiment.

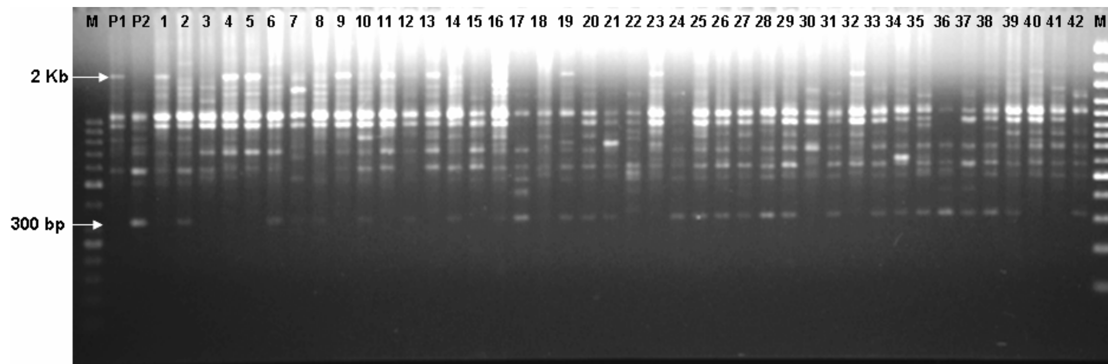

Figure 31. Pattern of RAPD fragments generated by primer OPO-07 on St. 463 (TRFCA SFS150 x AHP S15/10) progeny. Marker OPO-07-300 was associated with high yields as in BSA.

6.2.4.1.1 Co-segregation and statistical analysis

Three markers, two of which cosegregated with high yield (Tables 25 to 28 and Figure 30), while the other one cosegregated with low yield (Tables 29 & 30) were identified while performing BSA experiments using individuals constituting the bulks. However, none of the markers showed a complete distinct molecular profiling that distinguished between the high yielding and low yielding progeny. The two markers namely OPO-07-300 and OPA-10-250 that were significantly associated with high yields (Table 25 and 27), recorded recombination frequencies of 20% and 25% (Table 26 and 28), respectively. The low yielding marker, OPA-1031 also registered significant association with low yields (Table 29) with a recombination frequency of 25% (Table 30). The highly significant regression of the marker on the yield and large coefficient of determination revealed that much of the variation could be attributed to QTL governing the trait. When the three primers were used to genotype the entire population, only marker OPO-07-300 (Figure 31) was found to be significantly ($P < 0.001$) associated with yield, with the QTL accounting for 23% of the phenotypic variance. This marker was subsequently placed on chromosome 16 (Figure 42) upon construction of a linkage map and

the QTL positioned 9 cM from the marker following interval mapping. The markers could be termed high or low yield markers depending on whether the overall mean associated with each marker class was higher or lower for the presence and absence of marker fragments in the progeny. Thus, low yield markers can suitably be used for marker-assisted negative selection, while in contrast the high yield ones could be utilised in marker-assisted positive selection for potential low and high yielding progenies, respectively, in segregating populations.

Table 25. Regression analysis of marker OPO-07-300bp that cosegregated with high yielding progeny

R^2	0.39				
ANOVA	Df	SS	MS	F	Significance F
Regression	1	1.788	1.788	11.65	0.003
Residual	18	2.762	0.153		
Total	19	4.55			

Table 26. Cosegregation of high yield and RAPD marker OPO-07-300bp

Yield	No. progeny	Present	Absent	Recombination frequency
Low	10	3	7	20%
High	10	9	1	

Table 27. Regression analysis of marker OPA-10-250bp that cosegregated with high yielding progeny

R^2	0.35				
ANOVA	Df	SS	MS	F	Significance F
Regression	1	1.715	1.715	9.542	0.006
Residual	18	3.235	0.180		
Total	19	4.95			

Table 28. Cosegregation of high yield and RAPD marker OPA-10-250bp

Yield	No. progeny	Present	Absent	Recombination frequency
Low	10	3	7	25%
High	10	8	2	

Table 29. Regression analysis of marker OPA-10-1031bp that cosegregated with low yielding progeny

R^2	0.30				
ANOVA	df	SS	MS	F	Significance F
Regression	1	1.383	1.383	7.863	0.012
Residual	18	3.167	0.176		
Total	19	4.55			

Table 30. Cosegregation of low yield and RAPD marker OPA-10-1031bp

Yield	No. progeny	Present	absent	Recombination frequency
Low	10	9	1	25%
High	10	4	6	

6.2.4.2 Root knot nematode resistance/susceptibility-preliminary results

Some 66 RAPD primers were subjected to screening using the BSA method based on data recorded for resistance/susceptibility to root knot nematode in St 526 progeny. An example of a gel photograph from the screening process is presented in Figure 32. Of the screened primers, one primer produced a unique marker, OPF-09-600 that discriminated between the two parents and bulks based on susceptibility to the pest. The primer was used to genotype the entire population and the obtained profile is presented in Figure 33. The marker was reproduced, although other polymorphic loci were also revealed. The genotype data was then subjected to single-point genome-wide regression analysis using Map Manager QTX2.0 (Manly *et al.*, 2001). Results in Table 31 indicate that the marker was significantly associated ($P < 0.0000$) with the susceptibility to root knot nematodes with 64% of phenotypic variance explained by the QTL. The results further point to presence of other QTLs affecting the trait elsewhere in the genome but with minor effects. When this marker was used as background loci (cofactor), a second QTL (OPF-14) with minor effect could not be detected, implying that only the QTL tightly linked to OPF-09-600 that had major effects could solely account for the variation observed. Moreover, when QTL analysis was done under high stringent conditions ($P < 0.00001$), only the locus OPF-09-600 could be revealed as significantly associated with susceptibility. The position of the QTL was localised in linkage group 8 (St 526) by interval mapping through Map Manager (Figure 34). Results shown in Figure 34 revealed the QTL to be right on the marker at 0.0 cM with likelihood ratio statistics (LRS) of 41.8, an indication the QTL was lying within the gene(s) locus governing the trait.

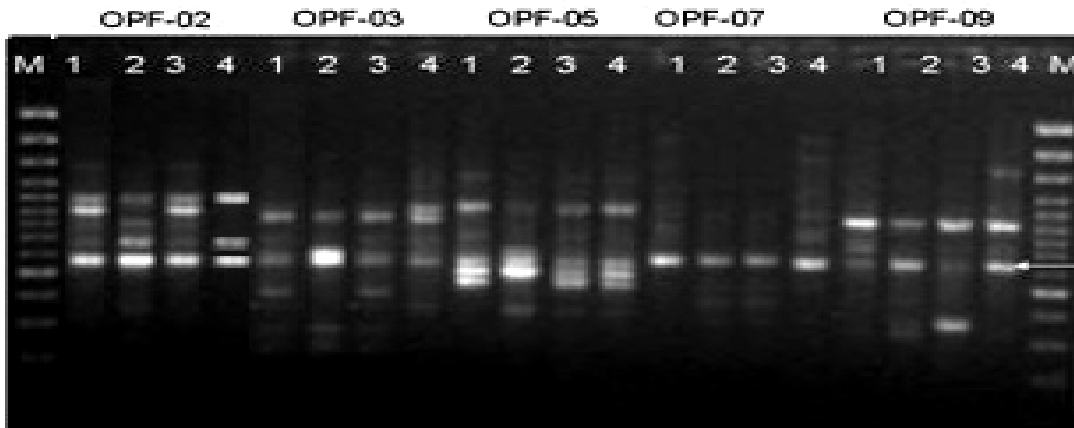

Figure 32. Screening of RAPD primers using root knot nematode tolerant and susceptible bulks from St. 526 (TRFCA SFS150 x TRFK 303/577) progeny. M = 100 bp ladder plus (MBI); arrow shows candidate marker band (OPF-09-600bp) corresponding to nematode susceptibility; 1 = TRFCA SFS150 (Female tolerant parent); 2 = TRFK 303/577 (male susceptible parent); 3 = nematode tolerant bulk; 4 = nematode susceptible bulk.

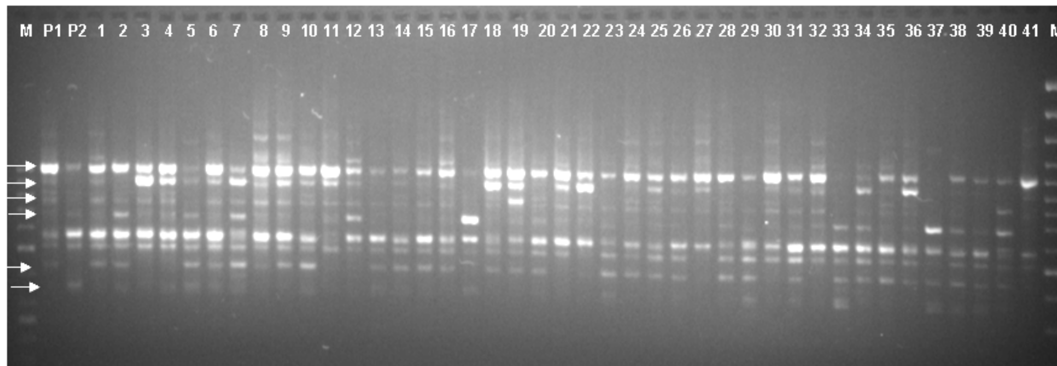

Figure 33. Pattern of RAPD fragments generated by primer OPF-09 on St 526 (TRFCA SFS150 x TRFK 303/577) progeny. P_1= TRFCA SFS150; P_2= TRFK 303/577; 1 to 41 F_1 progeny; left and right Ms are 50 and 100 bp molecular weight ladders, respectively. Note. From top to bottom are informative markers 1031, 950, 850, 600, 450 and 350 bp, respectively, as indicated by arrows.

Table 31. QTL mapping of root knot susceptibility based on single marker regression analysis

Linkage group	Locus	LRS	%	P	CI	Add
Group 8	OPF-9	41.8	64	0.00000	20	0.82
Group 1	OPF-14	11.0	24	0.00092	55	0.54

NB: *The sign of additivity (Add) for each QTL imply which QTL had increasing or decreasing effect on the trait. The LRS (likelihood ratio statistics) indicates significance of potential association; % is the proportion of the total variance attributable to a QTL at a particular locus; P designates significance at probability level given.*

6.2.4.3 Total polyphenols

Of the 219 RAPD primers screened for polymorphism (see Figure 35 for part of the screening profile) based on BSA using total polyphenols, 19 primers distinguished parents and their respective bulks. However, when BSA was extended to 10 individual progeny with high total polyphenols and 10 with low polyphenol contents, none of the primers could co-segregate as expected. When polymorphic primers were used to genotype the entire population one primer gave a unique profile (Figure 36) with one marker showing significant association ($P < 0.01$) between it and total polyphenols (Table 32).

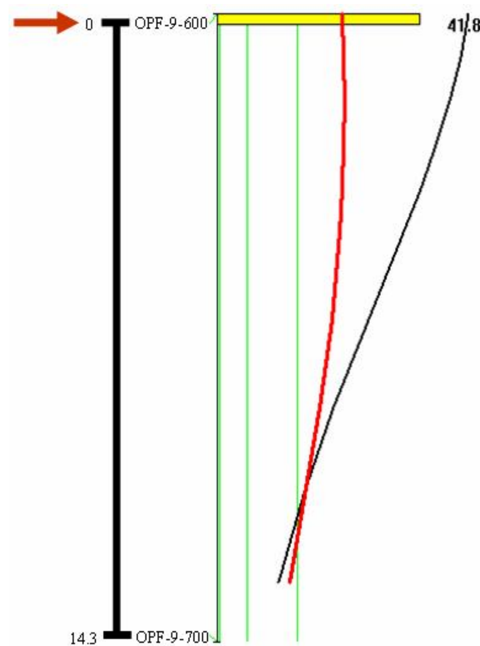

Figure 34. Postulated linkage map of chromosome 8 of St 526 showing position of the root knot susceptibility (RKN-S) QTL on marker OPF-09-600. The likelihood ratio statistic (LRS) black line was obtained by interval mapping RKN-S at regular intervals between marker loci. The width of the bar represents the estimated confidence interval by bootstrap resampling in Map Manager QTX. Fine vertical lines from left to right represent suggestive (1.9), significant (7.6) and highly significant (17.2) threshold LRS values obtained by 10,000 permutation test at P = 0.0001.

Table 32. Single-point regression analysis of association between total polyphenols in St 526 progeny and marker OPT-18-600

Chr	Locus	Stat	%	P	CI	Add
Unlinked	OPT-18-600	7.9	18	0.005	74	1.87

NB: Column headings as described in Table 31

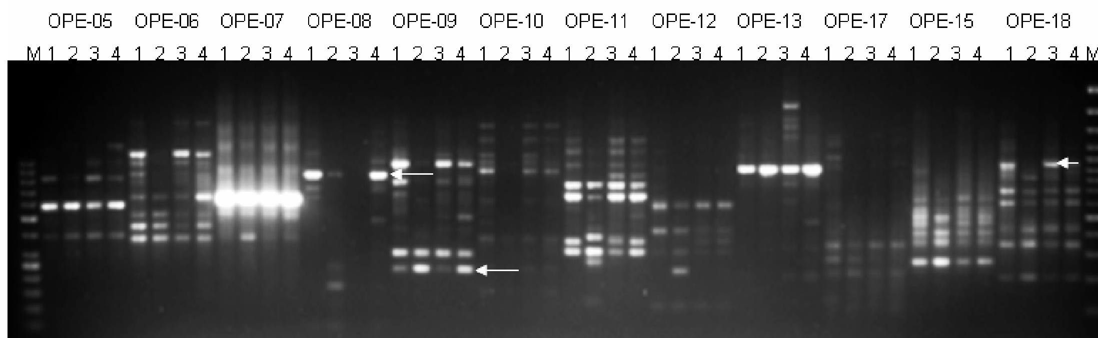

Figure 35. Screening of some 12 RAPD primers using high/low polyphenol bulks from St. 526 (TRFCA SFS150 x TRFK 303/577). 1 = P_1 = SFS 150, 2 = P_2 = 303/577, 3 = high polyphenol bulk (HPB), 4 = low polyphenol bulk (LPB); Left and right M denote molecular weight ladders of 50bp and 100bp, respectively. Some distinguishing markers include OPE-08-900, OPE-09-250 for LPB, OPE-18-1000 for HPB. Primers polymorphic between parents only are OPE-06, and OPE-11.

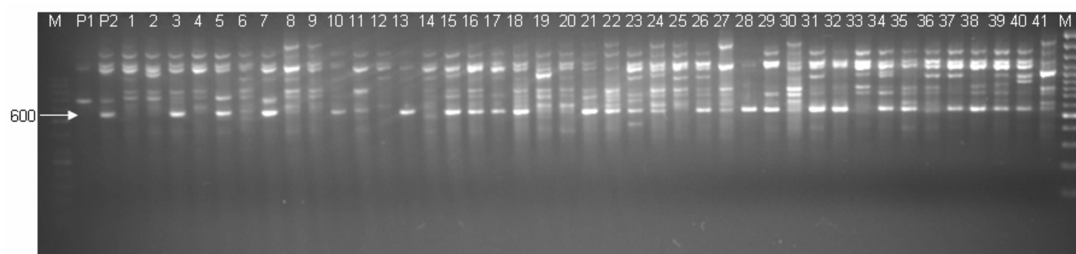

Figure 36. Pattern of RAPD fragments generated on progeny of St. 526 (TRFCA SFS150 x TRFK 303/577) by primer OPT-18. Left and right M denote molecular weight ladders of 50bp and 100bp, respectively.

6.2.4.4 Pubescence

A modified BSA approach was employed to screen primers which had not been utilised during screening of primers based on the yield trait. Part of the profile generated during the screening process is presented in Figure 37. Unlike in yield, polymorphic primers were directly used in complete genotyping of the entire St 463 (TRFCA SFS150 x AHP S15/10) population following lack of distinct profile among the good and poor performers from the cosegregating loci experienced with other traits. One primer produced a marker that was significantly ($P < 0.0001$) associated with a QTL for bud pubescence (Figure 38). 33% of phenotypic variance could be accounted for by the detected QTL (Table 45). The QTL was subsequently localised at 1.4 cM from marker OPO-02-650 mapped on chromosome 10 (Figure 47). The complete genotype data was also amenable to QTL analysis for other traits measured in St 463 population besides pubescence.

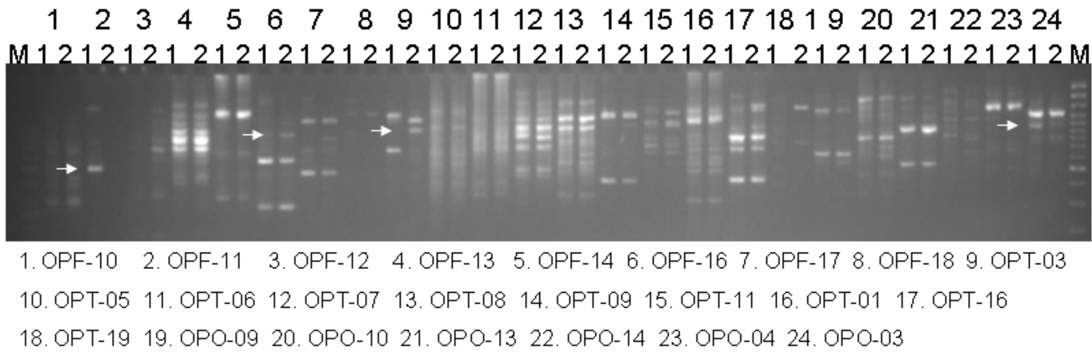

Figure 37. Screening of RAPD primers using high (1) and low (2) pubescent bulks from St. 463 (TRFCA SFS150 x AHP S15/10) progeny. Markers distinguishing bulks are shown by arrows. Left and right M denote molecular weight ladders of 50bp and 100bp, respectively.

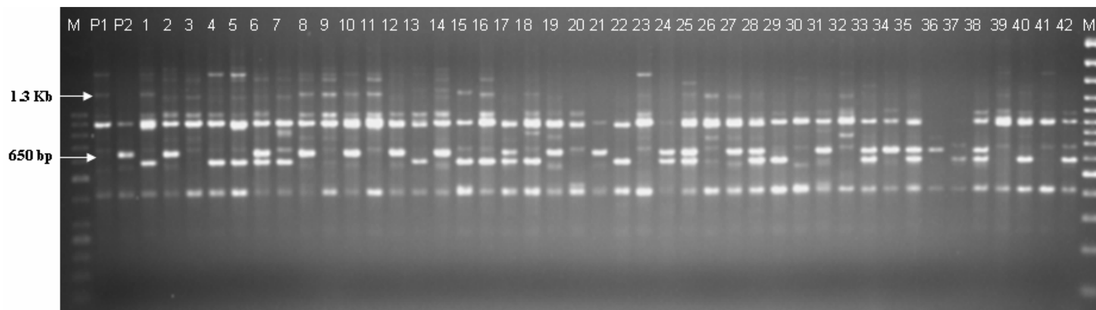

Figure 38. Pattern of RAPD fingerprint generated by primer OPO-02 on St 463 (TRFCA SFS150 x AHP S15/10) progeny. Left and right M denote molecular weight ladders of 50bp and 100bp, respectively.

6.3 Linkage analysis

A total of 42 clonal progeny from St 463 (TRFCA SFS150 x AHP S15/10) for which yield, shoot traits (pubescence, bud length, bud width and bud dry weight), drought tolerance and total polyphenols, and 41 clonal progeny from St 526 (TRFCA SFS150 x TRFK 303/577) for which total polyphenols and root knot nematode data were available were completely genotyped for the various marker systems. However, only St 463 progeny were subjected to the four marker systems (RADP, AFLP, ISSR and SSR), while St 526 was genotyped using 59 RAPD and 5 ISSR primers. The summarized results of informative markers are presented in Tables 33 to 38.

In general, 267 (50 RAPD, 7 ISSR, 11 SSR and 199 AFLP markers) and 115 (105 RAPD and 10 ISSR) informative markers were generated in St 463 and St 526 populations, respectively. Of the 267 markers generated for St 463, 149 (55.8%) markers (RAPD, ISSR and AFLP) showed 1:1 segregation ratio (BC or pseudo testcross), 118 displayed 1:3/3:1 segregation ratio (F_2). Six codominant markers (SSR) segregated according to 1:1 ratio while

another 6 markers that ought to have shown 1:2:1 segregation pattern exhibited distorted segregation when subjected to Mendelian segregation analysis. Similarly, 52 markers of the F_2-type population displayed segregation distortion. All the markers depicting distorted segregation were excluded from linkage analysis as they result in high standard errors for estimation of recombination frequency between markers (Ortiz, 1996b). Of the 149 dominant markers, 93 (62.4%) were maternally inherited, while the rest (i.e. 56 (37.6%)) were paternally inherited. The 155 (149 dominant + 6 codominant) markers were used to construct a linkage map of tea using Map Manager QTX2.0 (Manly *et al.*, 2001). The Kosambi mapping function that assumes cross-over interference (Kosambi, 1944) was employed with the threshold value of $P = 0.0001$ (with LOD score varying from 2.4 to 7.8 with a mean of 3.2 between any two markers) to convert recombination frequencies to map distances. On the other hand, Haldane (1919) ignores the interference of other crossovers, resulting in an overestimation of the distance between markers and the total distance covered.

Table 33. RAPD primers and informative markers generated by each on St 463 (TRFCA SFS150 x TRFK 303/577) population

SN	PRIMER	No. MARKERS
1	OPO-02	3
2	OPO-05	3
3	OPO-07	1
4	OPO-11	2
5	OPV-01	3
6	OPV-06	1
7	OPT-18	3
8	OPM-05	1
9	OPM-07	1
10	OPA-10	1
11	OP-26-08	2
12	OP-26-07	2
13	OP-26-15	2
14	OPG-17	1
15	OPG-07	2
16	OPF-16	2
17	OPT-03	3
18	OPO-03	2
19	OPF-01	1
20	OPF-05	3
21	G-12	1
22	OPW-04	3
23	OPD-18	1
24	OPW-07	2
25	AB4-16	1
26	OPW-11	3
	TOTAL	**50**
	Mean	1.9

Table 34. RAPD primers and number of informative markers generated by each on St 526 (TRFCA SFS150 x TRFK 303/577)

SN	PRIMER	No. of MARKERS
1	OPF-02	3
2	OPF-03	8
3	OPF-05	2
4	OPF-06	2
5	OPF-09	6
6	OPF-15	2
7	OPT-01	3
8	OPT-02	1
9	OPT-04	2
10	OPT-17	1
11	OPT-18	4
12	OPO-10	3
13	OPO-02	1
14	OPO-03	2
15	OPO-05	3
16	OPO-07	2
17	OPO-06	2
18	OP-26-05	2
19	OP-26-08	2
20	OP-26-16	2
21	OP-26-09	2
22	OPE-09	5
23	OPE-19	2
24	OPE-18	4
25	OPE-11	3
26	OPE-06	2
27	OPM-05	1
28	OPA-01	1
29	OPA-10	1
30	OPA-07	3
31	G-8	1
32	G-12	1
33	G-15	3
34	OPG-11	1
35	OPV-01	2
36	OPW-11	1
37	OPW-06	3
38	OPW-03	6
39	OPW-04	4
40	OPW-18	1

Table 34. (ctd)

41	OPU-15	2
42	OPU-20	2
43	OPD-18	1
	TOTAL	**105**
	Mean	2.4

Table 35. ISSR primers used to genotype St 463 (TRFCA SFS150 x AHP S15/10) population and number of informative markers generated

PRIMER	No. MARKERS
ISSR808	1
ISSR849	2
ISSR842	2
ISSR857	2
TOTAL	**7**
Mean	1.8

Table 36. ISSR primers used to genotype St 526 (TRFCA SFS150 x TRFK 303/577) population and number of informative markers generated

PRIMER	No. MARKERS
ISSR808	1
ISSR849	2
ISSR817	2
ISSR857	4
ISSR842	1
TOTAL	**10**
Mean	2.0

Table 37. SSR primers and informative markers generated per primer as used on St 463 (TRFCA SFS150 x AHP S15/10) population

PRIMER	No. MARKERS
CAMSIN2F&R	1
CAMSIN4F&R	1
CAMSIN6F&R	1
CAMSIN8F&R	3
CAMSIN9F&R	2
CAMSIN10F&R	1
CAMSIN11F&R	1
CAMSIN13F&R	1
TOTAL	**11**
Mean	1.4

The "make linkage groups" function was used, followed automatically by "ripple" function to improve the order of the loci in a linkage group by testing alternative orders created by local permutations of the locus order. Under these criteria, 109 markers generated 30 linkage groups, while 40 markers remained unassigned to any linkage group. The linkage groups so constructed were ordered sequentially from longest to the shortest (Figure 39).

Table 38. AFLP primer pairs used to genotype St 463 (TRFCA SFS150 x AHP S15/10) progeny and number of informative markers generated

SN	PRIMER PAIR	No. MARKERS
1	E-ACC/M-CTC	6
2	E-ACC/M-CAGT	4
3	E-ACC/M-CTA	8
4	E-ACT/M-CAC	13
5	E-ACT/M-CTA	8
6	E-ACT/M-CTG	10
7	E-ACT/M-CTC	8
8	E-ACT/M-CTT	8
9	E-AGG/M-CAG	12
10	E-AAC/M-CAGT	8
11	E-AGC/M-CAA	10
12	E-ACA/M-CAG	7
13	E-ACA/M-CTA	10
14	E-AGC/M-CTG	11
15	E-AGC/M-CTT	12
16	E-AGC/M-CAG	16
17	E-AGC/M-CAC	16
18	E-ACC/M-CAC	16
19	E-ACC/M-CAG	13
20	E-ACT/M-CTAG	3
	TOTAL	**199**
	Mean	10.0

However, 3 markers each in groups 2 and 4 and 1 in group 22 were found to be redundant, and had to be temporarily removed with "hide redundant loci" function to allow for QTL mapping of various traits to proceed. The 30 linkage groups comprised 19 maternal linkage groups and 11 paternal linkage groups (Figure 39). The 30 linkage groups of what may now be termed as consensus map spanned 1411.5 cM with mean interval between markers being 14.1 cM. The maternal linkage groups spanned 1012 cM, (without label) while the paternal map covered a total length of 399.5 cM, (labeled M) with mean distance between markers being 14.7 cM and 12.9 cM, respectively (Figure 39). Amongst the 19 maternal linkage groups, the length of individual groups ranged from 18.3 to 213.5 cM for LG 27 and LG 1, respectively.

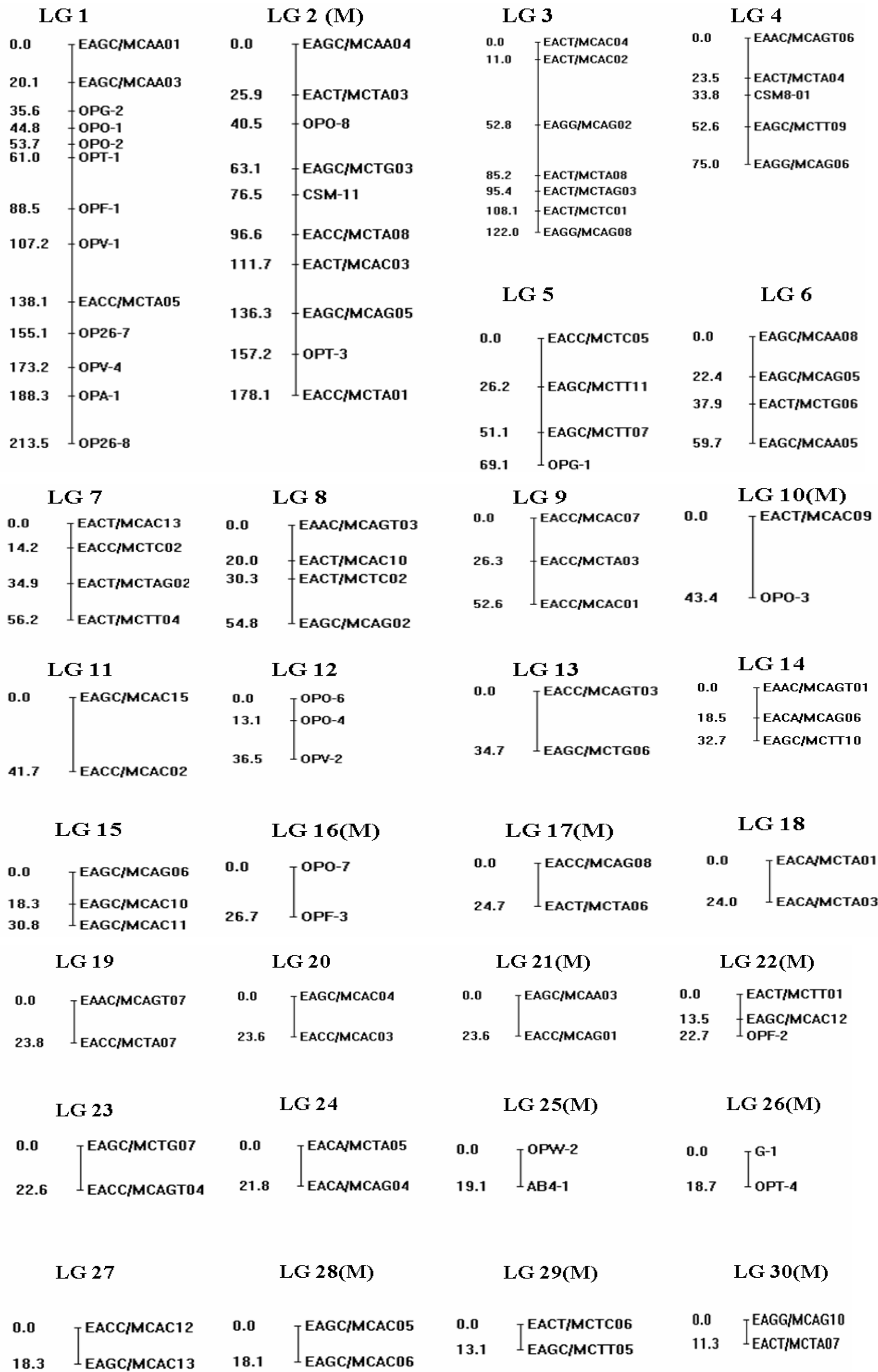

Figure 39. Linkage map of tea constructed using progeny of St 463. Paternal linkage groups are parenthesized as M. LOD thresholds varying between 2.4 and 7.8 with an average of 3.2 were used to construct the map.

On the other hand, for the 11 paternal linkage groups, the length of individual groups ranged from 11.3 to 43.4 cM for LG 30 and LG10, respectively. It could generally be noted that 13 linkage groups had at least 3 markers each, while the other linkage groups had each 2 markers. A saturated map of tea should have 15 linkage groups corresponding to its haploid status. The 30 linkage groups, equivalent to diploid number of tea, were collectively utilized in QTL mapping for all traits assessed in St 463 population.

The St 526 (TRFCA SFS150 x TRFK 303/577) population, however, was treated separately. It generated 115 dominant markers from RAPD and ISSR marker systems. These markers were used to construct a separate partial linkage map, for which 9 linkage groups (See appendix 13) were constructed with 72 markers remaining unlinked. The 9 linkage groups were utilized in QTL mapping of root knot nematode resistance/susceptibility and total polyphenols, the only traits scored in the population.

6.4 Quantitative trait loci detection and mapping

6.4.1 Single marker regression analysis

Single marker regression analysis of trait values for statistical association with genotypes of marker loci in the progeny detected a total of 64 putative QTLs controlling various traits across the two sites. 75% of these QTLs could be mapped into specific chromosomes, while the rest remained unlinked. Out of 32 QTLs detected across traits in Timbilil, 10 were unlinked, while only 6 were unlinked of 32 detected in Kangaita. Generally, the analysis revealed that each trait was controlled by more than one QTL.

6.4.2 Yield

Although yield data recording started in 2001, QTL analysis utilized yield data for 2003, 2006, 2007 and annual mean yields for 2006 (ANYLD06) and 2007(ANYLD07) as the most representative data for the two sites. Yield data collected for the first two years may have been biased against some clones that were slow to establish and therefore might not have been suitable for QTL analysis. Moreover, tea at the Kangaita was subjected to maintenance pruning in 2004, while the same operation was carried out in 2005 at Timbilil. QTL analysis was done separately for each site and period.

Single marker regression analysis of trait values for statistical association with genotypes of marker loci in the progeny detected a total of 23 significant ($P < 0.01$) putative QTLs controlling various traits across the two sites for the five "years" used in the analysis

(Tables 39 to 44). Sixteen of the markers were associated with Timbilil yield traits, while the 7 were detected in Kangaita. Besides, 15 QTLs were inherited from the female parent, while 8 were inherited from the male parent. Further, 5 loci in each of the two sites were not assigned to any linkage group. Surprisingly, none of the markers was mutually detected in the two sites for the entire period under consideration. Another observation worth noting is that at least two loci (EAGC/MCAC02 (790) and OPO-11-400 were consistently identified with significant association ($P < 0.001$) with yield from 2003 to 2007 at the Kangaita site. The amount of variance that each locus could account for also remained more or less the same through out the experimental period for the two loci. The two loci however had opposing main effects but of dissimilar magnitude, although the dominant alleles were paternally inherited. Multiple regression of the two unlinked loci with ANYLD07 showed that QTLs linked to them explained 38% ($P < 0.05$) of the total phenotypic variance.

The pattern was, however different for the Timbilil site. Significant associations ($P < 0.001$) were revealed for different loci in 2003, 2006 and 2007. However, when annual mean yields were taken into account for 2006 and 2007, two unlinked markers (OPW-1 and OPT-18-300) were commonly detected in 2003 and 2006 but not in 2007. Still, 4 of the 10 loci identified in 2006 (ANYLD06) were also significantly associated with ANYLD07 in 2007. In Timbilil, marker OPW-1 was highly significantly associated with yield in 2003 (YLD-2003) as well as overall annual yield means up to 2006 (ANYLD06), accounting for 18% ($P < 0.01$) and 29% ($P < 0.0001$) of the phenotypic variance, respectively, yet it did not fall in any of the linkage groups (Tables 40 and 44). It could also be noted that most of the unlinked markers had stronger effect on the trait than the linked ones.

Seven QTLs associated with long term yield (ANYLD06) whose influence ranged from 15% to 23% in phenotypic variance explained were detected in different linkage groups at the Timbilil site. A cluster of four of these QTLs mapped on linkage group 1 (Table 43). Interestingly, when marker OP26-1 (OP-26-08-380) with the highest LRS was used as a cofactor in composite interval mapping, a new unlinked marker [EAGC/MCAC02 (790)] emerged with significant association with ANYLD06 (14%, $P < 0.01$). This marker had also been detected in Kangaita accounting even for a higher level of phenotypic variance (25%. $P < 0.0001$) at the Timbilil (Table 44). The locus though differing in level of magnitude in affecting the trait in the two sites was consistent in its increasing effect, site differences notwithstanding. Multiple regression involving ANYLD06 and the three loci; OPG-07-2800, OPO-02-900 and OP-26-15-1031 mapped on linkage group 1 that were significantly associated with ANYLD06 and ANYLD07 at the Timbilil site resulted in significant

regression coefficient ($P < 0.05$) with R^2 of 44%. Since the three loci have positive main effects (Tables 44 and 45), their combined effect may have been complementary, which might have inflated the R^2. Addition of OPO-7 loci on linkage 16, which also has a positive effect, in the multiple regression neither changed the significance level of regression coefficient nor the magnitude of R^2.

Some QTLs, however, had small effects on trait expression while others had major effects. For example, yield in Timbilil (YLD-T) was highly significantly ($P < 0.0002$) associated with marker OPW-1, which accounted for 29% of the phenotypic variance, yet it did not fall in any of the linkage groups (Table 42). Seven more QTLs accounting for 15% to 21% in phenotypic variance were detected in different linkage groups. A cluster of four of these QTLs mapped on linkage group 1. Similarly, yield at the Kangaita site (YLD-K) had two major QTLs whose markers (EAGC/MCA02 and OPO-11-400) remained unlinked (Table 42). Perhaps due to GxE interactions, none of the QTLs found with effects on yield at Timbilil were detected in Kangaita site.

Table 39. QTL analysis for yield recorded in 2003 (YLD-2003)

Site	Chr	Locus	LRS	%	QTL Pos. (cM)	Add	Dom. Allele source
Timbilil	Unlinked	OPW-1 (OPW-04)	8.2	18**	-	236.14	P
	Unlinked	EAGC/MCAG01 (940)	7.1	16**	-	-165.19	P
	Unlinked	OPT-2 (OPT-18-1300)	8.0	17**	-	-175.27	M
	Unlinked	EACC/MCAG13 (180)	7.4	16**	-	168.93	M
Kangaita	Unlinked	EAGC/MCAC02 (790)	9.7	21***	-	446.86	P
	Unlinked	EACC/MCAC16 (120	7.0	15**	-	476.36	M
	Unlinked	EAGC/MCAG13 (200)	7.4	16**	-	-483.71	M
	Unlinked	OPO-9 (OPO-11-400)	9.8	21***	-	-418.81	P

NB**. Typically, the sign of additivity (Add) for each QTL imply which QTL had increasing or decreasing effect on the trait depending on the parent contributing the dominant allele. QTL localization was done by interval mapping. The LRS (likelihood ratio statistics) indicates significance of potential association. Logarithm of odds (LOD) can be obtained by dividing LRS by 4.61. % is the proportion of the total variance attributable to a QTL at a particular locus. P and M designate paternal and maternal alleles, respectively. **, ***, * denote significance levels at P < 0.01, 0.001, 0.0001 and 0.00001, respectively.*

Table 40. QTL analysis for yield recorded in 2006 (YLD-2006)

Site	Chr	Locus	LRS	%	QTL Pos. (cM)	Add	Dom. Allele source
Timbilil	Group12	OPO-4 (OPO-05-1100)	10.4	22***	2	195.44	M
	Group16	OPO-7 (OPO-07-350)	7.6	17**	1	172.2	P
Kangaita	Unlinked	EAGC/MCAC02 (790)	16.2	32****	-	492.69	P
	Unlinked	OPO-9 (OPO-11-400)	10.6	23***	-	-386.6	P

***NB**. The various column titles are as described in Table 39*

Ranking on ANYLD06 performance was applied to determine the effects of genotype x environment interaction on the yield assessed at the two test sites. Spearman's rank correlations obtained by StatistiXL1.8 software (www.statistixl.com) revealed considerable G x E interaction ($r_s = 0.08$, $P = 0.59$), further confirming why none of the detected QTLs had common effect on yield in the two sites for the period under consideration.

Table 41. QTL analysis for yield recorded in 2007 (YLD-2007)

Site	Chr	Locus	LRS	%	QTL Pos. (cM)	Add	Dom. Allele source
Timbilil	Group1	EAGC/MCAA03 (400)	8.1	18**	3	197.15	M
	Group11	EACC/MCAC02 (865)	8.7	19**	3	-204.72	M
Kangaita	Group8	EAGC/MCAG02 (910)	9.2	20***	5	-1616.73	M
	Unlinked	EAGC/MCAC02 (790)	17.3	34****	-	1654.44	P
	Unlinked	OPO-9 (OPO-11-400)	11.9	25***	-	1333.11	P

NB. *The various column titles are as described in Table 39*

Table 42. QTL analysis for annual mean yield from 2001 to 2006 (ANYLD06)

Site	Chr	Locus	LRS	%	QTL Pos. (cM)	Add	Dom. Allele source
Timbilil	Unlinked	OPW-1 (OPW-04)	14.4	29****	-	215.26	P
	Unlinked	OPT-2 (OPT-18-1300)	8.4	18**	-	-127.14	M
	Unlinked	OP26-1 (OP-26-08-380)	7.6	17**	-	121.98	P
	Group1	OPG-2 (OPG-07-2800)	7.4	16**	2	125.11	M
	Group1	OPO-2 (OPO-02-900)	9.4	20***	1	139.01	M
	Group1	OPT-1 (OPT-18-2500)	7.1	15**	3	117.62	M
	Group1	OP26-7 (OP-26-15-1031)	10	21***	3	137.27	M
	Group3	EACT/MCTA08 (70)	6.8	15**	13	119.94	M
	Group3	EACT/MCTC01 (355)	7	15**	3	116.85	M
	Group16	OPO-7 (OPO-07-350)	10.6	23***	9	143.71	P
Kangaita	Unlinked	EAGC/MCAC02 (790)	12	25***	-	387.77	P
	Unlinked	EACC/MCAC16 (120	8.2	18**	-	405.13	M
	Unlinked	OPO-9 (OPO-11-400)	12.7	26***	-	-740.08	P
	Group2	EAGC/MCAG05 (725)	8.4	18**	3	411.44	P
	Group8	EAGC/MCAG02 (910)	6.8	15**	9	-386.8	M

NB. *The various column titles are as described in Table 39*

6.4.3 Total polyphenols

Total polyphenols, TP-T and TP-K (Table 44) had four QTLs detected for each, and just like YLD, some mapped on different chromosomes, while others were unlinked.

Table 43. QTL analysis for annual mean yield from 2001 to 2007 (ANYLD07)

Site	Chr	Locus	LRS	%	QTL Pos. (cM)	Add	Dom. Allele source
Timbilil	Group1	OPG-2 (OPG-07-2800)	7.6	17**	2	164.36	M
	Group1	OPO-2 (OPO-02-900)	9.4	20***	1	181.05	M
	Group1	OP26-7 (OP-26-15-1031)	9.2	20***	5	172	M
	Group16	OPO-7 (OPO-07-350)	9.1	20***	9	175.02	P
Kangaita	Group2	EAGC/MCAG05 (725)	8.1	18**	4	1240.26	P
	Group8	EAGC/MCAG02 (910)	7.4	16**	9	-1229.37	M
	Unlinked	EAGC/MCAC02 (790)	13.1	27****	-	1237.72	P
	Unlinked	EACC/MCAC16 (120	7.7	17**	-	1207.31	M
	Unlinked	OPO-9 (OPO-11-400)	12.6	27****	-	-1143.59	P

***NB**. The various column titles are as described in Table 39*

6.4.4 Pubescence

Twelve QTLs associated with PUB were identified at Timbilil site at $P = 0.01$, but when search criterion was made more stringent at $P = 0.0001$, only one QTL, OPO-3 (OPO-02-650), could be detected (Table 45). This QTL accounted for 33% of total phenotypic variance. At the Kangaita site two unlinked QTL were significantly associated ($P < 0.001$) with pubescence. The QTLs explained at least 20% each of total phenotypic variance.

Table 44. QTL analysis for total polyphenols (TP)

Site	Chr	Locus	LRS	%	QTL Pos. (cM)	Add	Source of Dom. allele
Timbilil	Unlinked	857-02	9.6	20***	-	-1.54	M
	Unlinked	OPW-3	9.7	21***	-	-1.15	P
	Group1	EAGC/MCAA01	7.2	16**	7	1.24	M
	Group9	EACC/MCAC07	8.0	18**	1	1.30	M
Kangaita	Unlinked	OPT-03-600	7.3	16**	-	0.84	M
	Group2	OPT-18-1031	5.6	12**	2	-0.95	P
	Group4	CSM8-01	4.5	10*	2	-0.85	M
	Group6	EAGC/MCAA05	4.8	11*	1	-0.84	M

***NB**. The various column titles are as described in Table 39*

6.4.5 Drought tolerance

Three detected QTLs controlling drought tolerance at Timbilil (DT-T) were unlinked (Table 46), while 6 of 10 QTLs at Kangaita (DT-K) were positioned in chromosome 1 (Figure 44).

Table 45. QTL analysis for pubescence (PUB)

Site	Chr	Locus	LRS	%	QTL Pos. (cM)	Add	Source of Dom. Allele
Timbilil	Group2	EAGC/MCTG03	10.6	22***	3	1.06	P
	Group2	EACC/MCTA08	9.4	20***	6	0.99	P
	Group2	EACT/MCAC03	7.8	17**	8	0.88	P
	Group2	OPT-18-1031	7.2	16**	1	0.9	P
	Group2	EACC/MCTA01	13.9	29***	4	1.16	P
	Group5	EAGC/MCTT11	14.1	29***	1	1.16	M
	Group6	EAGC/MCAA05	8.2	18***	5	0.91	M
	Group10	OPO-02-650	16.5	33****	0	1.29	P
	Group14	EACA/MCAG06	6.7	15**	7	0.97	M
	Group29	EACT/MCTC06	7.5	16**	5	-1.06	P
	Group30	EAGG/MCAG10	12.3	25***	4	1.08	P
	Group30	EACT/MCTA07	8.1	18**	4	0.92	P
Kangaita	Unlinked	EAGC/MCAC02	9.2	20***	-	0.86	P
	Unlinked	CSM-10	11.0	23***	-	-0.89	C

***NB**. The various column titles are as described in Table 39*

6.4.6 Bud weight

Bud weight at Timbilil was significantly associated with QTL accounting for up to 25% of total phenotypic variance, while the same character was significantly associated with 7 QTLs at Kangaita with respective phenotypic variance under their influence ranging from 16-24% (Table 47). While the QTL linked to locus EACT/MCAC02, detected in Timbilil and mapped on chromosome 3 was localised at 2 cM from the marker, a QTL linked to locus EACT/MCTC01 as identified at Kangaita site was localized at 8 cM (See chromosome 3, Figure 37 and Table 47).

Table 46. QTL analysis for drought tolerance (DT)

Site	Chr	Locus	LRS	%	QTL Pos. (cM)	Add	Source of Dom. Allele
Timbilil	Unlinked	EAAC/MCAGT05	6.9	15**	-	-0.42	M
	Unlinked	EAGC/MCAG12	6.7	15**	-	-0.37	P
	Unlinked	EAGC/MCTG04	7.1	16**	-	0.38	M
Kangaita	Group1	OPO-02-900	9.4	20***	2	-0.53	M
	Group1	OPT-18-2500	12.3	25***	2	-0.57	M
	Group1	OPV-01-1500	9	19***	13	-0.5	M
	Group1	OP-26-15-1031	9.4	20***	5	-0.51	M
	Group1	OPV-06-1500	7.2	16**	7	-0.45	M
	Group1	OPA-10-1800	6.7	15**	10	-0.46	M
	Group7	EACT/MCAC13	7.8	17**	1	0.48	M
	Group11	EACC/MCAC02	7.7	17**	2	0.47	M
	Group16	OPO-07-350	7.7	17**	17	-0.47	P
	Group16	OPF-01-580	10.4	22***	4	-0.53	P

***NB**. The various column titles are as described in Table 39*

Table 47. QTL analysis for bud weight (BWt)

Site	Chr	Locus	LRS	%	QTL Pos. (cM)	Add	Source of Dom. allele
Timbilil	Group3	EACT/MCAC02	11.7	25***	2	0.08	M
Kangaita	Unlinked	EAGC/MCTG10	11.3	24***	-	0.07	M
	Group2	CSM-11	7.9	17**	5	-0.06	P
	Group3	EACT/MCTC01	7.4	16**	8	-0.05	M
	Group9	EACC/MCAC07	10.6	23***	10	-0.07	M
	Group9	EACC/MCTA03	7.9	17**	6	-0.06	M
	Group11	EAGC/MCAC15	7.1	16**	6	0.07	M
	Group22	OPF-16-580	0.5	23***	0	-0.08	P

***NB*. The various column titles are as described in Table 39*

6.4.7 Bud length

Two 2 unlinked QTLs associated with bud length at Timbilil (BL-T) were detected, while one QTL influencing the same trait at Kangaita (BL-K) was localised on chromosome 1 (Table 48).

6.4.8 Shoot weight

The same trend of inconsistency in detected QTLs also obtained for shoot weight at Timbilil (SW-T) and Kangaita (SW-K), as different loci were found affecting the trait in the two sites at different levels of significance and influence (Table 49).

Table 48. QTL analysis for bud length (BL)

Site	Chr	Locus	LRS	%	QTL Pos. (cM)	Add	Source of Dom. allele
Timbilil	Unlinked	EACC/MCAG10	12.6	26***	-	1.72	P
	Unlinked	EACC/MCAG13	7.1	16**	-	1.34	M
Kangaita	Group1	OPO-02-900	6.6	15**	2	-1.56	M

***NB*. The various column titles are as described in Table 39*

Table 49. QTL analysis for dry shoot weight (SW)

Site	Chr	Locus	LRS	%	QTL Pos. (cM)	Add	Source of Dom. Allele
SW-T	Unlinked	EAGC/MCTG11	6.9	15**	-	-0.39	P
	Unlinked	EACC/MCAC09	7.4	16**	-	-0.43	M
SW-K	Group2	EAGC/MCTG03	15.9	31****	3	-0.66	P
	Group2	EACC/MCTA01	11.7	25***	2	-0.57	P
	Group10	EACT/MCAC09	12.6	26***	10	-0.64	P
	Group10	OPO-02-650	20	39****	10	-0.73	P

***NB*. The various column titles are as described in Table 39*

6.5 Genotype x environment interaction

The marked effect of genotype by environment interaction could be demonstrated by fact that none of the QTLs had uniform effect across environments for any of the trait measured. This was so even for traits that seemed to be governed by few major genes like pubescence. This observation is also corroborated by rank correlation analysis shown in Table 24.

6.6 Interaction (epistatic) effects

Significant epistatic (interactions) effects between loci EAGC/MCAA01 and EAGC/MCAA03 as well as EAGC/MCAA01 and OPG-2 were detected for yield at Timbilil (YLD-T). For bud pubescence at Timbilil (PUB-T) distinct epistatic effects were detected between markers OPO-3 (OPO-02-650) and EAGC/MCTT01, while significant epistatic effects were detected between loci EACC/MCAC07 and EAGC/MCTG04 for bud pubescence at Kangaita (PUB-K) (Table 50). For YLD, except for OPG-2 which was significantly ($P < 0.01$) associated with the trait, none of the other loci involved in interaction had been individually detected to significantly influence yield. PUB however, had one locus OPO-3 (OPO-02-650), which had been significantly identified to be associated with the trait, but its interaction with a second locus, EAGC/MCTT01, seems to have dampened its overall influence. PUB-K behaved more or less the same ways as with other traits as new interacting loci emerged to influence the expression of the trait. The interaction effects suggest that the effect of each putative QTL for these traits depend highly on the genotypes of other linked or unlinked QTLs even though having only minor effects.

Table 50. Interaction effects for yield and bud pubescence at P = 1.0e-5

Trait	Chr1	Locus1	Chr2	Locus2	LRS	IX	Main1	Main2
YLD-T	Group1	EAGC/MCAA01	Group1	EAGC/MCAA03	26.7	9.1	2.2	3.7
YLD-T	Group1	EAGC/MCAA01	Group1	OPG-2	27.9	10.4	2.2	9.4
PUB-T	Group10	OPO-3	Unlinked	EAGC/MCTT01	28.6	8.1	16.5	4.9
PUB-K	Group9	EACC/MCAC07	Unlinked	EAGC/MCTG04	26.1	20.9	4	0.9

NB. LRS, IX, Main1 and Main2 denote total LRS for association, interaction LRS, LRS for locus 1 main effect and LRS for locus 2 main effect, respectively.

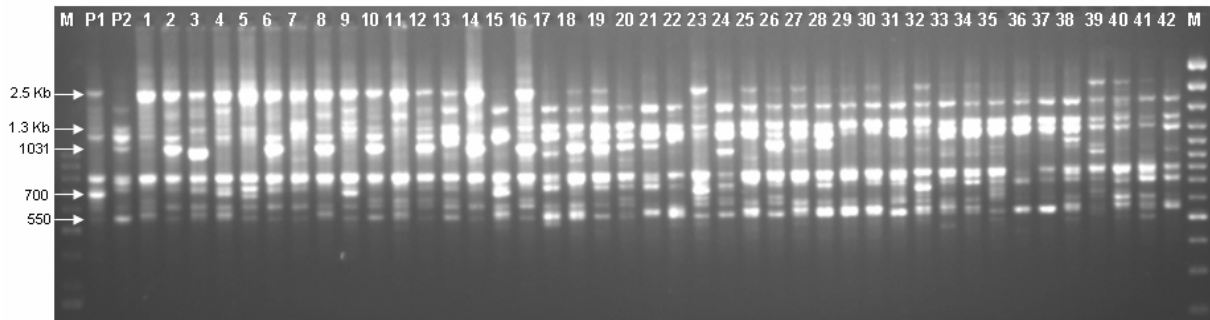

Figure 40. Pattern of RAPD profile generated by primer OPT-18 on St 463 progeny.

6.7 Pleiotropic effects

Some markers displayed pleiotropic effects as they were significantly associated with more than one trait (Table 51). Thus, OPO-02-900 had pleiotropic effect on DT-K (P = 0.002) and BL-K (P = 0.009), while OPT-18-2500 (Figure 32) was significantly associated with four traits; YLD-T (P = 0.007), BWd-T (P = 0.008), DT-K (P = 0.005) and BWd-K (P = 0.003). Further, OPO-02-650 was significantly associated with PUB-T (P = 0.00005) and SWt-K (P = 0.0007), while OP-26-08-900 had marked influence on YLD-T (P = 0.001) and DT-K (P = 0.001).

6.8 Localization of some QTLs

6.8.1 Yield

Some traits with highly significant QTLs merited further attention, and were therefore placed in various linkage groups using interval and composite interval mapping. On the whole, yield appeared to be under the influence of multiple putative QTLs as in Timbilil (YLD-T), two moderate QTLs were detected and localised by composite interval mapping at 2.0 cM away from markers OPG-07-2800 and OPO-02-900, respectively, in Linkage group 1 (Figure 41).

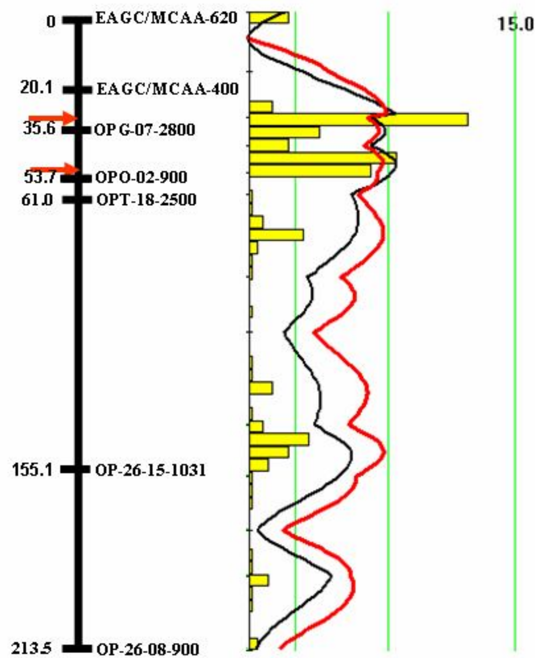

Figure 41. Postulated linkage map of chromosome 1 showing position of the multiple YLD-T QTLs at 2 cM each from markers OPG-07-2800 and OPO-02-900. The likelihood ratio statistic (LRS) black line was obtained by composite interval mapping YLD-T at regular intervals between marker loci. Bars represent the estimated confidence interval by bootstrap resampling in Map Manager QTX. Fine vertical lines from left to right represent suggestive (2.8), significant (8.4) and highly significant (15.0) threshold LRS values obtained by 1000 permutation test at P = 0.01.

The two loci respectively, explained 16% and 15% of the total phenotypic variance. Multilocus model by composite interval mapping indicated that the joint action of mapped QTLs could only account for 21% of phenotypic variance. Additionally, a major QTL influencing yield at Timbilil (YLD-T) which accounted for 23% of phenotypic variance was detected and positioned 9 cM from marker OPO-07-300 (Figure 42). Lack of closely linked markers in chromosome 16 might have affected precise localization of the QTL.

On the other hand at the Kangaita site, one QTL with significant influence on yield was detected and localized at 2.7 cM from marker EAGC/MCAG-725 in linkage group 2 (Figure 43). This QTL accounted for 18% of phenotypic variance.

Table 51. Pleiotropic effects of some of the identified QTLs at various significance levels as detected by single-point genome-wide regression analysis

		Timbilil Trait				Kangaita Trait				
Marker	Linkage group	Yield	TP-T	PUB-T	BWd-T	DT-K	BL-K	BWd-K	SWt-K	BWt-K
OPO-02-900	1					20**	15**			
OPT-18-2500	1	15**			15**	25***		19**		
EACC/MCAC-550	9		18**							23***
OPO-02-650	10			33****					39****	
OPO-02-900	1	20***			19**					
OP-26-08-900	1	21***				20**				

*NB. *, **, ***, and **** denote significance levels at P < 0.05, P < 0.01, P < 0.001 and P < 0.0001, respectively.*

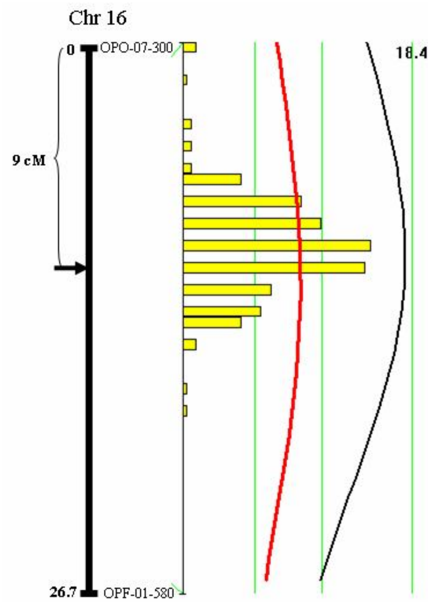

Figure 42. Postulated linkage map of chromosome 16. Arrow shows position of the YLD-T QTL at 9.0 cM from marker OPO-07-300. The likelihood ratio statistic (LRS) black line was obtained by composite interval mapping YLD-T at regular intervals between marker loci. Bars represent the estimated confidence interval by bootstrap resampling in Map Manager QTX. Fine vertical lines from left to right represent suggestive (5.8), significant (11.2) and highly significant (18.4) threshold LRS values obtained by 1000 permutation test at P = 0.01. Red line is additive regression coefficient.

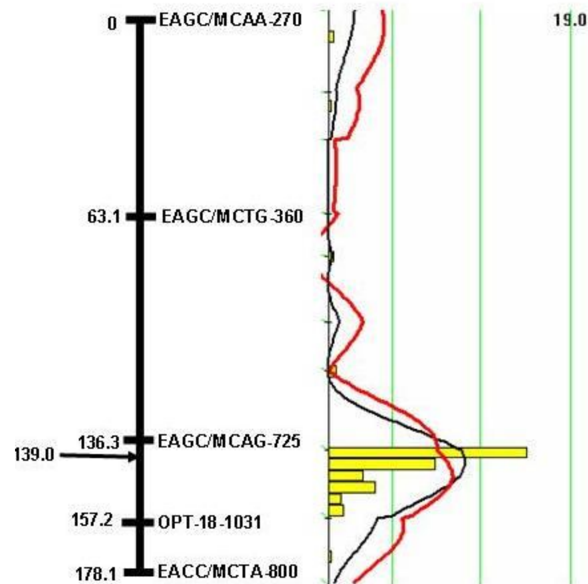

Figure 43. Postulated linkage map of chromosome 2. Arrow shows position of the YLD-K QTL at <3.0 cM from marker EAGC/MCAG-725. The likelihood ratio statistic (LRS) black line was obtained by interval mapping YLD-K at regular intervals between marker loci. Bars represent the estimated confidence interval by bootstrap resampling in Map Manager QTX. Fine vertical lines from left to right represent suggestive (5.9), significant (11.9) and highly significant (19.0) threshold LRS values obtained by 1000 permutation test at P = 0.01. Red line is additive regression coefficient.

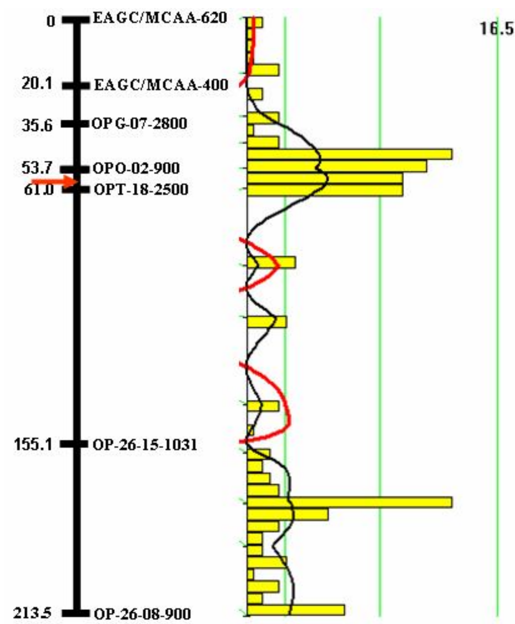

Figure 44. Postulated linkage map of chromosome 1 showing position of the DT-K QTL at 3 cM from marker OPT-18-2500. The likelihood ratio statistic (LRS) black line was obtained by composite interval mapping DT-K at regular intervals between marker loci. Bars represent the estimated confidence interval by bootstrap resampling in Map Manager QTX. Fine vertical lines from left to right represent suggestive (2.5), significant (8.8) and highly significant (16.5) threshold LRS values obtained by 1000 permutation test at P = 0.01.

6.8.2 Drought tolerance

A major QTL affecting drought tolerance at Kangaita (DT-K) was detected and localised in Linkage group 1 at 3 cM from marker OPT-18-2500 (Figure 44). This QTL explained 25% of the total phenotypic variance. It is worth noting that the QTL seemed to be overlapping with one of the QTL affecting YLD-T (see Figure 39), corroborating the pleiotropic effect of locus OPT-18-2500 on YLD-T and DT-K, despite the poor correlation between the two traits.

6.8.3 Total polyphenols

While a moderate QTL influencing total polyphenol content at Kangaita (TP-K) was detected and positioned at 1.2 cM from marker OPT-18-1031 in chromosome 2 (Figure 45), a major QTL affecting the same trait at Timbilil (TP-T) was identified in chromosome 9 at 1.0 cM from marker EACC/MCAC-550. 18% of the phenotypic variance could be accounted for by this QTL (Figure 46).

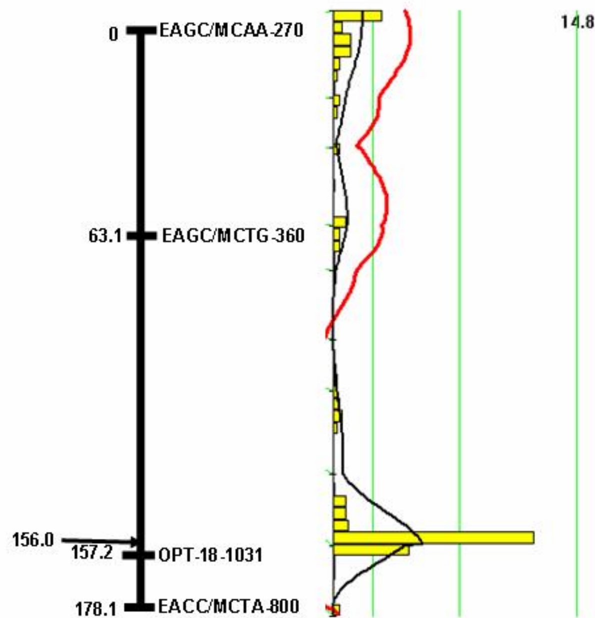

Figure 45. Postulated linkage map of chromosome 2. Arrow shows position of the TP-K QTL (i.e. at 1.2 cM from marker OPT-18-1031. The likelihood ratio statistic (LRS) black line was obtained by interval mapping total TP-K at regular intervals between marker loci. Bars represent the estimated confidence interval by bootstrap resampling in Map Manager QTX. Fine vertical lines from left to right represent suggestive (2.4), significant (7.7) and highly significant (14.8) threshold LRS values obtained by 1000 permutation test at P = 0.05. Red line is additive regression coefficient.

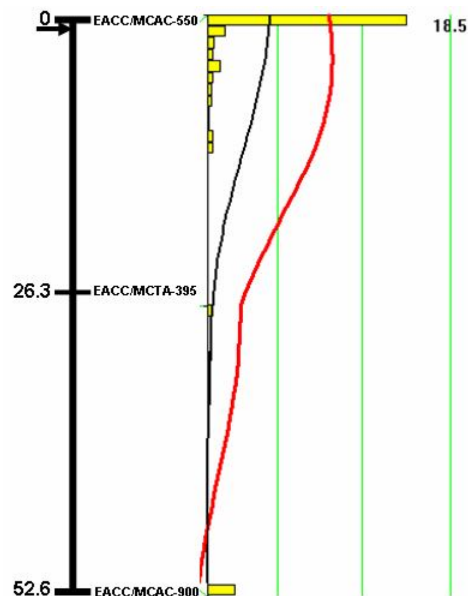

Figure 46. Postulated linkage map of chromosome 9. Arrow shows position of the TP-T QTL (i.e. within at 1.0 cM from marker EACC/MCAC-550. The likelihood ratio statistic (LRS) black line was obtained by composite interval TP-T at regular intervals between marker loci. Bars represent the estimated confidence interval by bootstrap resampling in Map Manager QTX. Fine vertical lines from left to right represent suggestive (6.0), significant (12.2) and highly significant (18.5) threshold LRS values obtained by 1000 permutation test at P = 0.05. Red line is additive regression coefficient.

6.8.4 Pubescence

Although a paucity of markers is apparent in chromosome 10, a major QTL governing PUB was detected and localised at 1.4 cM from marker OPO-02-700 (Figure 47). Only this locus could be significantly associated with PUB, when the search criterion was made very stringent at P = 0.00004. Thus, the QTL with major effects accounting for 33% of phenotypic variance could be localised by interval mapping on chromosome 10.

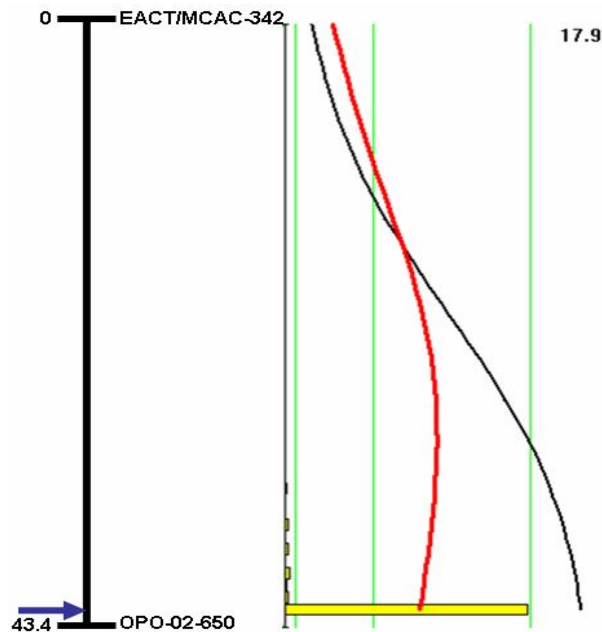

Figure 47. Interval mapping of PUB on chromosome 10. Arrow shows position of the PUB-T QTL at 1.4 cM from marker OPO-02-650. The likelihood ratio statistic (LRS) black line was obtained by interval mapping pubescence at regular intervals between marker loci. Bars represent the estimated confidence interval by bootstrap resampling in Map Manager QTX. Fine vertical lines from left to right represent suggestive, significant and highly significant threshold LRS values obtained by 1000 permutation test. Red line is additive regression coefficient.

6.9 Effects of various alleles on traits

A *t*-test was carried out to determine the level of significance between marker allele means for some of the mapped QTLs and also to establish which markers had an increasing influence and which ones had decreasing effect. From the results presented in Table 52, it is apparent that, the first three putative QTLs associated with yield at Timbilil (YLD-T), which descended from the female parent (TRFCA SFS150) had a net reducing effect ($P < 0.01$). For example maternal locus EAGC/MCAG-910 (Table 43) explained 16% of total phenotypic variance, while the unlinked locus EAGC/MCAC-790 accounted for 27% of the trait at Kangaita. However, one QTL in this category which was mapped in linkage group 16

had a net increasing effect (*P* < 0.001). This marker originated from the male parent (AHP S15/10), which is a popular commercial high yielding clone (Oyamo, 1992). Two markers associated with yield at Kangaita (YLD-K) but mapped in different linkage groups had unexpectedly net increasing effect (P < 0.01) even though they came from the female parent (see also Figure 48), which is a moderate yielder, but performs better than the male parent under water stress conditions. The effect of GxE in triggering certain QTLs to act especially during biotic or abiotic stress conditions is apparently demonstrated here. On scrutinizing drought tolerance at Kangaita (DT-K), one of the markers with reducing effects on YLD-T had similar negative effects on DT-K (P < 0.01). How this QTL OPO-02-900, behaves under different environmental conditions vis a vis its pleiotropic effects, would be a subject of much speculation, where compelling evidence is unavailable. Different loci associated with total polyphenols at the two test sites, which also had net decreasing effects were mapped in different linkage groups (Table 52). However, the significance level of association between TP-K and OPT-18-1031 (P < 0.05), appears too low to consider it as putatively linked to a QTL controlling production of total polyphenols.

Table 52. Results of marker allele means of various traits following complete genotyping

Trait	**Locus**	**Linkage group**	**Marker allele means**		**P value (t test)**
			+	**-**	
YLD-T	OPG-07-2800	1	2102	2225	0.006
	OPO-02-900	1	2084	2232	0.002
	OP-26-15-1031	1	2117	2252	0.002
	*OPO-07-300	16	2252	2104	0.0008
YLD-K	EAGC/MCAG-725	2	1708	1254	0.0008
	EAGC/MCAG-910	8	1693	1306	0.006
DT-K	OPO-02-900	1	2.3	1.8	0.003
	OPT-18-2500	1	2.2	1.7	0.0004
	OP-26-15-1031	1	2.2	1.7	0.001
	*OPF-01-580	16	1.7	2.2	0.001
TP-T	EAGC/MCAA-620	1	19.86	21.09	0.006
	EACC/MCAC-550	9	19.82	21.12	0.005
TP-K	OPT-18-1031	2	20.89	21.85	0.02
PUB-T	*OPO-02-650	10	3.81	2.5	0.00002

NB. Asterisked loci are male-based dominant alleles (i.e. bands associated with the male parent).

6.3 Discussion

The primary aim of QTL mapping is not only to identify and determine the degree of associations between continuous traits and some sets of genetic markers, but also to enhance

development of elite varieties within the shortest time possible through marker-assisted selection and breeding. Conventional breeding programmes not only take long to deliver elite varieties, but also expend large amount of resources. The challenges associated with crop improvement become even more complex for perennial crops like trees, whose longevity, large genome sizes and big sizes pose great and unique difficulties, usually not associated with annual crops. Thus, identification of markers that can be used to select desired genotypes at seedling stage would greatly accelerate varietal development.

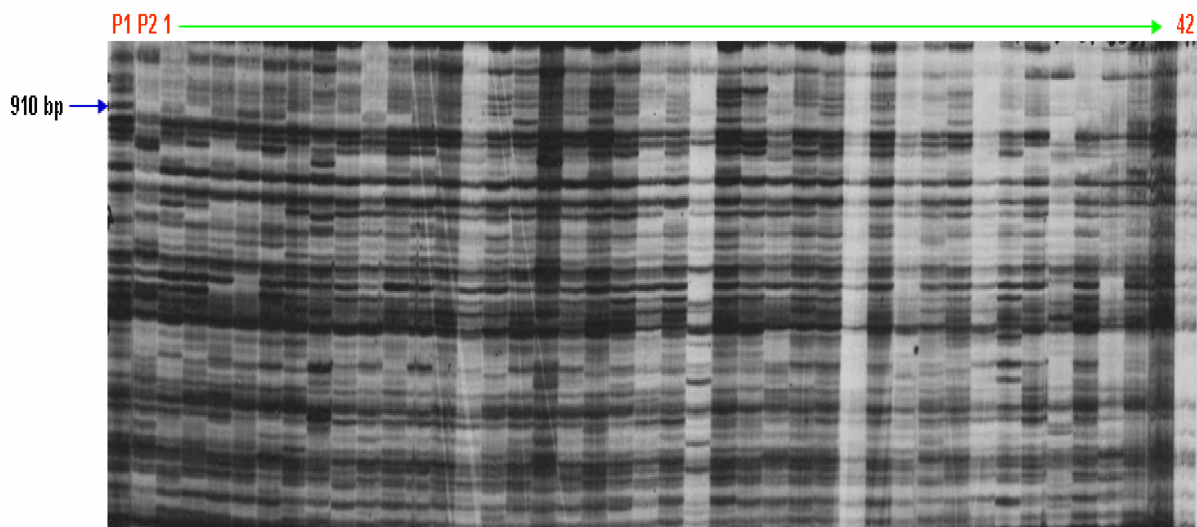

Figure 48. An AFLP profile of 42 F_1 progeny generated with primer pair E-AGC/M-CAG. Arrow shows informative locus EAGC/MCAG-910; P_1 and P_2 are female (TRFCA SFS150) and male (AHP S15/10) parents, respectively.

In addition to selection of advantageous traits, markers linked to complex traits can be used to select against negative characteristics, in a negative selection programme. They can be even used to select parents that would give rise to progeny with desired genotypes. With the foregoing challenges and opportunities in mind, the current study set out to attempt to detect and dissect the various QTLs controlling important attributes in tea with the aim of initiating markers-assisted selection and breeding in the tea improvement programme.

Although a total of 267 markers were generated in the current study, only 100 markers were used to construct a linkage map that comprised 19 maternal and 11 paternal linkage groups, each of which had 69 and 31 markers, respectively. The maternal map spanned 1012 cM, while the paternal one had a total length of 399.5 cM, with mean distance between markers being 14.7 cM and 12.9 cM, respectively. The combined map accumulated a total length of 1411.5 cM, with a mean interval between loci of 14.1 cM. Few markers

notwithstanding, the maternal map was comparable to the one constructed by Hackett *et al.* (2000), which covered 1349.7 cM, with an average distance of 11.7 cM between loci. The map was constructed with 124 markers. In their work, Hackett *et al*. (2000), did not construct a paternal map like in the current study. Their mapping population consisted of 90 genotypes, slightly more than twice used in the present study. The difference between the number of linkage groups got in the present study and the one in Hackett *et al*. (2000) could be attributed to non-saturation of the current map although the size differences between the two populations might not have had any effect (Ortiz, 1996a; Chmielewicz and Manly, 2002). It is likely that if more markers were scored some of the linkage groups with only two markers could coalesce so that only 15 linkage groups that correspond to the haploid numbers for tea remain. Indeed, Ortiz (1996b) inferred that some of the extra linkage groups actually represent distant segments of the same chromosome.

In the current study, of the 155 loci showing BC mating type (test cross configuration), 100 were unambiguously used to construct 30 linkage groups following which 64 QTLs were detected as significantly affecting the various traits studied. By having 30 linkage groups comprising 19 maternal and 11 paternal groups, it is possible to detect QTL significantly associated with either the female or male loci thereby increasing the effectiveness and reliability of QTL analysis. Although none of the markers was common to the parents, all the linkage groups were pooled into one map for the purpose of easing QTL mapping.

The current study used both BSA and complete genotyping experiments to identify QTLs associated with productivity, tea quality and tolerance to abiotic and biotic stress factors using the "pseudo-testcross" strategy in combination with RAPD, AFLP, ISSR and SSR molecular markers. As pointed out by Grattapaglia and Sederoff (1994), the term "pseudo-testcross" is used as the testcross mating configuration is unknown a *priori* as in a conventional testcross where the tester is homozygous recessive for the locus of interest. Rather, the configuration is inferred a *posteriori* after analysing the parental origin and genetic segregation of the marker in the progeny of a cross between highly heterozygous parents with no prior genetic information. When this inference applies for the two parents involved in the cross, the term "two-way pseudo-testcross" is more appropriately used. Based on this approach, the two experiments were able to detect QTLs for all the traits under test although at different levels of sensitivity/significance. The lowest probability level for search of QTL was P = 0.01 for a few traits, which translated into 1% false positives. This less stringent criterion was applied where no QTL could be detected at lower P value. Although

some markers showed distinctive polymorphic pattern between parents and respective bulks for some of the traits considered for BSA experiment, majority of the markers showed either inconsistent or ambiguous pattern involving the individual ten good performers and ten poor performers. None of the markers produced completely distinctive patterns distinguishing the 10 good and 10 poor performers along with their parents for any of the traits. This scenario could be attributed to high heterozygosity of parental clones used in the study. The situation could also be attributed to many QTLs each contributing in a small way to trait expression variance, interaction (epistasis) and pleiotropic effects so that a plant with a low yield phenotype, could have an allele for high yield but because of other interacting alleles, the high yield allele is masked and the plant becomes low yielding. The lowest recombination frequencies of 20% and 25% were recorded for two markers namely OPO-07-350 and OPA-10-250 that were significantly associated with high yields and a low yielding marker, OPA-1031, with a recombination frequency of 20%. The highly significant regression of the marker on the yield and large coefficient of determination, however, revealed that much of the variation could be attributed to presence of putative QTLs governing the trait near the marker.

Much of the work on identification of molecular markers linked to traits of interest has been based on the use of near-isogenic lines (Young *et al*., 1988; Martin *et al*., 1991; Paran *et al*., 1991), double haploids (Somers *et al*., 2001; Shashidar *et al*., 2007) or recombinant inbred lines (Navabi *et al*., 2005). However, with highly self-incompatible crops such as tea (Wachira and Kamunya, 2005a), it is impossible to generate near-isogenic lines or even doubled-haploids. Although self-incompatibility provides a considerable bottle-neck towards identifying markers tightly linked to important traits, some success has been reported in the case of apple. In their work on identification of a molecular marker linked to a scab resistance gene in apple using modified bulk segregant analysis, Yang *et al*. (1997) were able to identify and confirm a marker linked to scab-resistance gene within 4.3% recombination frequency of RAPD marker OPK16/1300. It was however relatively easier for Yang *et al* (1997) to identify the marker as the scab resistance is controlled by a major gene (Baldi *et al*., 2004). The current study nonetheless demonstrates that BSA offers a very useful way of identifying genomic regions controlling quantitative traits of interest in tea.

As the aim of QTL mapping is to undertake marker-assisted selection/breeding, an attempt was made to verify the putative QTLs identified through BSA and three informative primers (OPO-07, OPA-10 and OPF-09-600) were used to genotype the entire population. Of the three markers detected under BSA, only marker OPO-07-300 was found to be

significantly ($P < 0.001$) associated with yield, with the QTL accounting for 23% of the phenotypic variance. This marker was subsequently placed on chromosome 16 (Figure 42) and the QTL positioned 9 cM from the marker by interval mapping. Lack of markers on this linkage group coupled with wide confidence intervals might imply the QTL was not precisely localised. Lack of markers in this linkage group notwithstanding, calculation of marker allele means associated with each trait, made it possible to establish the effect of each marker on the trait. For example, marker OPO-07-300 which was inherited from the male parent, AHP S15/10, a popular high yielding commercial clone, had a net increasing effect on the yield trait. Thus, all the progeny polymorphic for this marker in a segregating population are likely to be high yielders. Such a marker could be used in marker-assisted positive selection. On the converse, a low yield marker can suitably be used for marker-assisted negative selection. In spite of the challenges encountered in the BSA experiment, results obtained in the current study agree with emerging knowledge that pooling DNA strategies for mapping QTL might be successful in tagging QTLs with very large effects on quantitative trait expression (Wang and Paterson, 1994; Grattapaglia *et al.*, 1996).

The current study, being the first comprehensive one of its kind opens windows of opportunities in QTL analysis of tea using larger populations arising from disparate parents. That 64 putative QTLs were identified for the 7 traits that were assessed on St 463 population in two distinct sites is a good starting point. The number, sensitivity (probability of *P* value) and sign of additivity of QTLs for each trait measured appeared to be closely related with the parent's known attributes. Thus, %TP, PUB, DT and SW had over 75% of dominant alleles with increasing net effect coming from either TRFCA SFS150 or AHP S15/10. This serves to emphasize on the rational choice of parent, be it male of female while targeting economic traits, although reciprocal crossing would be the preferable mating option. A previous study undertaken by Wachira (1996) had equally identified some genomic regions associated with the nine characters assessed in his study. The risk that progeny size might bias QTL effects upwards as has been pointed by different workers (Utz *et al.*, 2000) was circumvented in this study by use of cloned population, which increased precision and accuracy while collecting phenotypic data (Ortiz, 1996a). The availability of a larger mapping population that is genotyped with multiplex markers would nonetheless yield a denser linkage map and assist in identification of tightly linked QTLs. That notwithstanding, the findings of the current study forms the basis for works aimed at map-based cloning of genes.

The one putative QTL detected for root knot nematode (RKN) susceptibility associating with OPF-09-600 in St. 526 using BSA and confirmed in the entire population,

indicates considerable hope for breeding for root knot nematode resistance. Studies carried out in plum and peach on location of root-knot nematode resistance genes identified single genes that were localized in different linkage groups (Claverie *et al.*, 2004). The possible existence of RKN resistant genes in different clones in tea can be corroborated by a study carried out by Kamunya *et al.* (2008). Thus, breeding programmes aimed at combining different traits in one variety need to involve all the clones proven to possess those attributes and integrate molecular markers in selection at the earliest stage.

The profound effects of genotype by environment interaction is corroborated by phenotypic variation as displayed in histograms, exploratory rank correlation analysis and QTL analysis of each trait across environments. Tea is a long-lived organism and like other perennial crops, is faced with a wide spectrum of climatic conditions during its lifetime. Modifications in QTL expression over diverse environments have been reported in crop plants (Paterson *et al.*, 1991; Hayes *et al.*, 1993; Crouzillat *et al.*, 2000). QTL analyses performed on fruit trees (Asins *et al.*, 1994) and forest trees such as poplar (Bradshaw and Steller, 1995) or pine (Plomion *et al.*, 1996) and *Eucalyptus* (Verhaegen *et al.*, 1997) indicated an influence of environmental effects on the stability of QTL expression. All the traits were assessed nearly simultaneously in the two sites yet none seems to have been influenced by the same QTL. The complex environmental factors prevailing in the two sites might have exerted predominant effects on different QTLs governing the traits. This implies that multiple QTLs with small to moderate effects exist for each trait and it only requires a significant environmental shift to trigger different QTLs to act or to shut down. It also means that genes which affect each trait are differently expressed with time. According to Beavis (1998), lack of congruency in QTLs among studies was attributed to different sets of polymorphic alleles segregating in the different genetic backgrounds, and these results into large number of QTLs being responsible for variability in the trait. Lack of congruent QTLs may also indicate that the two sites did not depict sufficient environmental pressures as to reveal QTLs with average effects across sites. Kearsy and Pooni (1996), while commenting on nature and causes of G x E, have demonstrated that the genetical variance among a collection of genotypes may alter with the environment, meaning that the effects of given allele substitutions may be quite different in one environment than in another. However, the few loci which could be consistently associated with yield over the period within site, especially at Kangaita, will need to be verified and adopted in marker-assisted selection efforts. For example, markers EAGC/MCAC02 (790) and OPO-11-400 that consistently associated with yield in Kangaita site for the entire period under investigation demonstrated

their potential as candidate markers for site-specific marker assisted selection. According to Ortiz and Crouch (1996), once individual analyses are carried out in each environment, the most informative markers are those that consistently associate with phenotypic variation across environments. In order to overcome the limitations arising from effects of G x E, various workers have come up with recommendations on ways of enhancing phenotypic evaluations. Amongst those recommended include use of large population sizes, use of RIL, DH or clones in replicated experiments across sites and overtime (George *et al.,* 2003; Hackett, 2002; Collard *et al.*, 2005). Except for large population size, all other recommendations were adopted in the current study.

CHAPTER SEVEN

CONCLUSIONS AND RECOMMENDATIONS

7.1 CONCLUSIONS

The present study revealed genetic effects rather than environmental factors to be the underlying cause of the observed genetic parameters. The results of 4 x 4 diallel cross demonstrated the importance of both additive (GCA) and non-additive (SCA) gene effects in the expression of yield, fermentability, pubescence and bud weight. Strong maternal effects for all traits was evident for yield, fermentability, theaflavin contents, pubescence and drought tolerance signifying the paramount importance of rationalizing the choice of female parents while breeding for these traits and diversified tea products such as "silvery tips" (white tea). The significant additive effects, coupled with high heritability and moderate genetic gain show that the assessed traits are highly heritable and guided breeding and careful selection would lead to further tea improvement. The GCA for parents like AHP S15/10 and TRFK 6/8 were found to be more important for yield and drought tolerance emphasizing the need to involve these clones in hybridization programmes targeting the two traits. For crosses with significant SCA, breeding programmes will be valuable when hybrid tea varieties development is the principal goal.

The level of heterosis analysed in the same 4 x 4 diallel cross did not however reveal existence of hybrid vigour for all traits. The mid-parent heterosis (MPH) and the better-parent heterosis (BPH) averaged across the families, were high for fermentability, TF:TR and drought tolerance but not for yield and total polyphenols. The use of highly heterozygous parents might have led to a highly heterogeneous F_1 population with contrasting characteristics for all the traits measured thereby masking any heterosis that might have arisen for some genotypes. It is worth noting that several heterotic crosses emerged from this study. For %TP crosses EPK TN14-3 x TRFK 6/8 (TRFK 443/4), TRFK 6/8 x EPK TN14-3 (TRFK 476/4), AHP S15/10 x EPK TN14-3 (TRFK 474/3), AHP S15/10 x TRFCA SFS150 (TRFK 485/5), TRFK 6/8 x AHP S15/10 (475/3), AHP S15/10 x AHP S15/10 (TRFK 478/1, TRFK 478/3) and TRFCA SFS150 x EPK TN14-3 (TRFK 430/125) had progeny that outperformed the best parent in the diallel clone TRFK 6/8 which had 24.3% for the same trait; crosses EPK TN14-3 x TRFK 6/8 (TRFK 443/4) and EPK TN14-3 x TRFCA SFS150 (TRFK 488/4) were outstanding for TF; crosses with over 0.11 TF:TR had superior black tea quality; crosses with a fermentability level of $\geq$ 3.0 could be exploited for green tea processing, while those with $\leq$ 2.0 would be suitable for black tea manufacture; crosses with

over 2700 Kg mt/ha are heterotic for high yields; crosses scoring a pubescence level of 5 would be suitable for a specialty tea product such as “silvery tips” (Appendix 1). As it can be discerned from appendix 1, there is no single genotype with a combination of all the positive attributes measured in the current study.

The QTL analysis detected a total of 64 QTLs controlling various traits across the two sites for St 463, and 2 QTLs associated with root knot nematode susceptibility in St. 526. 75% of these QTLs were mapped into specific linkage groups, while the rest remained unlinked. Of these, QTLs for YLD-T, YLD-K, DT-K and PUB were localised at 2 cM, 2.7 cM, 3 cM and 1.4 cM from markers OPG-07-2800, E-AGC/M-CAG-725, OPT-18-2500 and OPO-02-650, respectively. For St 526, marker OPF-09-600 was found within 0 cM of the QTL controlling the root knot nematode susceptibility. Co-segregation analysis using the entire population confirmed the marker to be useful for MAS with the detected QTL for susceptibility accounting for 64% of phenotypic variance. The effects due to genotype by environment interaction were such that not even one single marker was congruent for the same trait across the two test environments. However, some markers such as OPO-02-900 had pleiotropic effect on DT-K and BL-K, while OPT-18-2500 was significantly associated with four traits; YLD-T, BWd-T, DT-K and BWd-K implying that such markers could be used to select for more than one trait simultaneously. The identified molecular markers linked to some genes of interest would enable prediction of presence or absence of the genes without actually perceiving the phenotype (trait). Thus, traits may be indirectly selected for using DNA markers instead of relying on the phenotype, which is largely influenced by the environment. The identification of molecular markers and their subsequent integration in selection process is promising since early screening could reduce the number of clones to be field-tested. This would ultimately lead to increased genetic gain per unit of time by decreasing the generation interval. Selection for markers without phenotypic evaluation would also result into marked reduction in cost of development of elite varieties. In the commercial sector, these can have a large impact on the profitability of a new trait. Thus the ability to assess complex phenotypes such as yield, quality, drought tolerance and susceptibility to pests and diseases in tea at the seedling stage using genetic markers provides for the first time a technology that can be used to accelerate the development of new elite tea varieties.

7.2 RECOMMENDATIONS

Arising from the findings of this study, the following recommendations are made:

- Since the diallel cross was conducted in one site in Kericho (Timbilil estate), therefore the generated information and knowledge are only applicable to that region. There is need therefore to replicate the same experiment carried out in the study in other sites with varying environmental components in order to strengthen the reliability and wide applicability of the derived genetic variances.
- Although outcrossing species such as tea show severe inbreeding depression, one generation of selfing or 2 to 3 generations of sib-mating may yield parents with greater concentration of desired alleles, which upon crossing would result in more heterotic hybrids.
- Where GCA effect is more important, its utilization in seed gardens that are composed of many parents like in the polyclonal seed orchard of tea at TRFK would be more favourable. This may lead to accumulation of favourable alleles that have additive genetic effects in the phenotypes of the improved generations.
- Since SCA refers to non-additive gene effects, it can be utilized through vegetative propagation to produce commercial quantities of planting stock that are genetically identical to the plants or hybrids from which they were derived. Besides, biclonal specific crosses involving parents with positive SCA effects for example in yield, fermentability and pubescence followed by prudent clonal selection may predictably result in marked progress in these traits.
- Redesign new seed orchards based on the results of the current study using improved progenitor clones with known combining abilities.
- Verify and confirm the effects of detected QTLs using independent and large populations constructed either from the same parental genotypes or closely-related genotypes and established in different environments. Such populations exist in the Tea Research Foundation of Kenya. A number of replicated clonal progeny experiments with varying number of F_1s both within and between sites have been established and may be utilized in such a study.
- Develop a dense linkage map using a larger population based on which detection for more but tightly linked QTLs to traits of special interests such as tea products diversification as well as stress related traits can be executed.

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APPENDICES

Appendix 1. Data for various attributes collected on the 4 x 4 full diallel cross

Cross	Clone	%TP	TF%	TR%	TF:TR	Ferm.	AnYLD06	SHDWt	BudDwt	PUB	BudWd	Bud Lth
TN14-3 X 6/8	443/1	21.8	1.51	16.73	0.09	1.4	2564	1.86	0.22	1.00	2.22	22.36
	443/2	23.0	1.54	16.23	0.10	1.8	2290	2.98	0.35	3.00	2.22	23.16
	443/3	21.2	1.68	16.64	0.10	1.9	2634	2.50	0.36	1.00	2.26	27.19
	443/4	26.1	2.20	17.30	0.13	2.3	2452	2.48	0.32	3.00	2.31	22.70
	443/5	21.0	1.75	15.75	0.11	1.4	2610	2.74	0.37	3.00	2.33	28.47
6/8 X TN14-3	476/1	24.9	1.72	16.82	0.10	1.6	2338	2.81	0.48	3.00	2.84	30.44
	476/2	22.0	1.52	17.22	0.09	1.0	2659	2.47	0.35	3.00	2.22	27.92
	476/3	21.3	1.51	16.03	0.09	3.0	2219	2.36	0.27	3.00	2.16	23.77
	476/4	25.6	1.82	17.70	0.10	1.0	2239	2.44	0.42	3.00	2.53	27.32
	476/5	22.5	1.58	17.18	0.09	1.0	2451	2.67	0.37	3.00	2.41	26.80
TN14-3 X S15/10	447/15	23.2	1.40	16.93	0.09	1.1	2331	2.67	0.35	3.00	2.32	22.68
	447/16	21.0	1.38	15.86	0.08	1.4	2304	2.24	0.34	3.00	2.51	26.90
	447/17	21.5	1.66	15.97	0.10	1.0	2349	3.10	0.38	5.00	2.41	23.91
	447/18	21.5	1.49	18.04	0.09	1.0	2501	2.93	0.37	5.00	2.42	26.46
	447/19	18.9	1.33	16.23	0.08	2.0	2684	3.47	0.43	5.00	2.57	26.20
S15/10 X TN14-3	474/1	21.5	1.09	14.62	0.07	1.0	2558	2.33	0.32	3.00	2.38	25.60
	474/2	22.4	1.57	15.85	0.10	1.0	2497	2.54	0.44	3.00	2.72	27.68
	474/3	24.8	1.69	15.42	0.11	2.5	2201	2.48	0.31	3.00	2.59	28.99
	474/4	20.2	1.34	16.21	0.08	1.0	2810	2.49	0.33	5.00	2.50	24.74
	474/5	22.0	1.78	15.49	0.12	1.0	2566	2.91	0.41	5.00	2.46	26.40
SFS150 X 6/8	420/1	22.1	1.34	16.94	0.08	1.4	2452	2.47	0.28	1.00	2.21	27.62
	420/2	23.1	1.64	15.79	0.10	1.6	2350	2.63	0.34	1.00	2.37	29.27
	420/3	23.1	1.73	15.90	0.11	1.5	2486	2.60	0.40	1.00	2.60	29.32
	420/4	21.5	1.47	15.61	0.09	1.1	2712	2.80	0.39	3.00	2.92	32.34
	420/5	21.4	1.40	15.42	0.09	1.3	2624	3.67	0.45	3.00	2.60	29.47
6/8 X SFS150	482/1	21.0	1.82	15.00	0.12	1.0	2734	2.26	0.27	3.00	2.43	28.07
	482/2	19.5	1.56	15.81	0.10	1.0	2430	2.82	0.36	1.00	2.54	28.79
	482/3	23.0	1.22	15.45	0.08	1.1	2364	2.64	0.38	3.00	2.71	29.13
	482/4	21.9	1.36	15.95	0.09	1.1	2448	2.89	0.38	1.00	2.39	27.22
	482/5	22.3	1.64	16.88	0.10	1.0	2225	2.73	0.40	1.00	2.34	27.11
SFS150 X S15/10	463/49	22.0	1.30	14.93	0.09	2.0	2747	3.49	0.43	3.00	2.74	30.06
	463/50	21.4	1.21	15.75	0.08	1.0	2365	4.28	0.57	5.00	2.67	31.68
	463/51	21.2	1.31	15.54	0.08	1.0	2739	3.65	0.44	3.00	2.80	31.21
	463/52	19.8	1.30	15.18	0.08	1.0	1469	4.12	0.51	5.00	3.07	31.21
	463/53	20.3	1.49	13.74	0.11	1.0	2938	3.24	0.40	3.00	2.61	28.21
S15/10 X SFS150	485/1	21.9	1.47	15.09	0.10	1.0	2413	2.90	0.38	1.00	2.66	27.94
	485/2	19.1	0.86	13.95	0.06	1.0	1802	2.71	0.29	3.00	2.21	25.83
	485/3	23.5	1.48	17.10	0.09	1.0	2677	4.34	0.53	3.00	2.58	29.24
	485/4	19.4	0.99	14.70	0.07	1.3	2391	3.21	0.48	3.00	2.44	25.79
	485/5	24.2	1.68	14.75	0.11	1.2	2593	4.05	0.49	1.00	2.57	33.08
6/8 X S15/10	475/1	21.8	1.53	15.98	0.10	1.0	2523	2.63	0.33	3.00	2.53	26.50
	475/3	25.2	1.44	14.40	0.10	2.0	2360	3.45	0.46	5.00	2.78	30.54
	475/4	19.5	1.69	15.48	0.11	1.0	2451	2.91	0.36	5.00	2.68	30.18
	475/5	22.7	1.75	15.06	0.12	2.0	2609	3.47	0.44	3.00	2.69	27.71
S15/10 X 6/8	456/1	19.0	1.48	14.72	0.10	1.0	2764	3.23	0.33	3.00	2.54	30.92
	456/2	21.6	1.59	15.61	0.11	1.0	2611	2.96	0.37	3.00	2.54	26.73
	456/3	20.4	1.42	13.36	0.11	1.0	2324	2.65	0.35	1.00	2.33	26.64

	456/4	18.9	1.37	13.68	0.10	1.0	2700	3.92	0.46	3.00	2.66	29.32
	456/5	23.7	1.78	15.11	0.12	2.0	2648	3.22	0.45	3.00	2.72	30.19
TN14-3 x TN14-3	490/1	21.7	1.54	13.68	0.11	2.0	2222	2.73	0.32	3.00	2.20	25.98
	490/2	20.3	1.54	16.86	0.09	1.0	1982	1.67	0.32	3.00	2.53	24.52
SFS150 x SFS150	471/1	22.5	1.40	16.37	0.08	2.0	2225	2.71	0.39	5.00	2.76	26.11
	471/2	21.0	1.47	14.45	0.11	1.0	2114	3.31	0.36	3.00	2.41	26.49
	471/3	22.5	1.21	14.62	0.08	1.0	1951	2.39	0.35	3.00	2.39	24.28
	471/4	20.7	1.50	15.14	0.10	1.0	2505	2.82	0.36	3.00	2.29	26.20
	471/5	22.8	1.26	14.17	0.09	2.0	2057	2.96	0.48	1.00	2.20	28.06
6/8 X 6/8	467/1	21.7	1.54	16.09	0.09	2.0	2234	2.94	0.38	3.00	2.68	29.74
	467/2	20.5	1.82	15.78	0.12	1.0	2459	2.94	0.35	3.00	2.53	26.76
S15/10 X S15/10	478/1	25.6	1.58	14.33	0.11	1.0	2419	3.51	0.47	3.00	2.82	31.02
	478/2	22.3	1.28	15.09	0.08	1.0	2662	3.60	0.65	3.00	2.68	27.61
	478/3	24.0	1.59	15.93	0.10	1.0	2611	3.35	0.44	3.00	2.99	30.32
	478/4	20.9	1.36	14.40	0.10	1.0	2474	2.86	0.33	3.00	2.66	28.50
	478/5	22.1	1.94	16.02	0.12	1.4	2328	2.69	0.33	1.00	2.37	27.94
SFS150 x TN14-3	430/121	19.7	1.25	15.72	0.08	1.0	2528	2.42	0.29	3.00	1.84	20.96
	430/122	19.9	1.17	15.62	0.07	1.0	2504	2.64	0.27	3.00	2.57	29.23
	430/123	20.9	1.24	15.31	0.08	1.1	2431	2.33	0.33	3.00	2.52	24.28
	430/124	20.8	1.32	15.01	0.09	1.1	2554	2.34	0.29	3.00	2.48	25.09
	430/125	25.1	1.35	17.10	0.08	1.6	2336	2.87	0.32	1.00	2.32	26.47
TN14-3 X SFS150	488/1	19.4	1.76	15.12	0.12	1.0	2046	2.10	0.33	3.00	2.31	23.30
	488/2	19.2	1.87	14.76	0.13	1.0	2226	2.90	0.34	5.00	2.56	25.87
	488/3	20.4	1.60	15.81	0.10	1.0	2013	2.71	0.39	3.00	2.43	24.66
	488/4	21.6	2.07	14.82	0.14	1.0	1850	2.86	0.31	3.00	2.64	25.59
	488/5	21.9	1.93	17.01	0.11	1.0	1695	2.56	0.41	3.00	2.90	30.30
Controls/parents	6/8	24.3	1.87	17.74	0.09	1.3	1708	2.77	0.38	1.00	2.46	30.03
	303/577	23.2	1.50	16.78	0.09	-	2524	2.13	0.18	1.00	2.31	24.00
	TN14-3	22.3	1.59	17.01	0.09	1.0	2478	2.60	0.44	3.00	2.42	26.50
	S15/10	20.6	1.32	15.58	0.08	2.0	2556	4.04	0.49	5.00	2.83	30.41
	SFS150	22.4	1.29	15.53	0.08	1.4	2699	2.96	0.36	3.00	2.40	27.71
	301/4	20.2	1.05	20.09	0.05	-	1414	3.91	0.50	5.00	2.66	35.18
	TN15-23	20.1				-		2.82	0.39	3.00	2.77	27.23
	TRFK 12/2					4.0						

`Appendix 2. Family and parents' means (F_1), for yield, %TP (percent total polyphenols, Ferm (rate of fermentability), DT (drought tolerance), TF (total theaflavins levels), TR (total thearubigins levels), TF:TR, leaf pubescence and bud weight in the 16 hybrids of *C. sinensis* at Timbilil estate.

		Family mean (F_1)								
Family code	**Pedigree** ♀ ♂	**Yield**	**%TP**	**Ferm**	**DT**	**TF(umol/g)**	**TR(%)**	**TF:TR**	**Pubescence**	**Bud wt(gm)**
467	TRFK 6/8 X TRFK 6/8	2347	21.07	1.50	2.00	19.99	15.94	0.11	3.00	0.37
475	TRFK 6/8 X AHP S15/10	2486	22.33	1.50	1.80	22.21	15.23	0.11	4.00	0.40
482	TRFK 6/8 X TRFCA SFS150	2440	21.53	1.05	1.30	21.72	15.82	0.10	1.80	0.36
476	TRFK 6/8 X EPK TN14-3	2381	23.90	1.53	1.50	22.85	16.99	0.10	3.00	0.38
456	AHP S15/10 X TRFK 6/8	2609	20.70	1.21	1.97	22.53	14.52	0.11	2.60	0.39
478	AHP S15/10 X AHP S15/10	2499	23.63	1.09	2.00	19.85	15.15	0.10	2.60	0.44
485	AHP S15/10 X TRFCA SFS150	2375	21.60	1.11	1.40	19.40	15.12	0.09	2.20	0.44
474	AHP S15/10 X EPK TN 14-3	2533	22.20	1.30	1.63	20.05	15.52	0.10	3.80	0.36
420	TRFCA SFS150 X TRFK 6/8	2525	22.23	1.39	1.46	22.47	15.93	0.09	1.80	0.37
463	TRFCA SFS150 X AHP S15/10	2451	20.60	1.20	1.57	18.57	14.98	0.09	3.80	0.47
471	TRFCA SFS150 X TRFCA SFS150	2171	21.93	1.40	1.80	18.68	14.95	0.09	3.00	0.38
430	TRFCA SFS150 X EPK TN14-3	2470	21.30	1.15	1.13	18.83	15.75	0.08	2.47	0.30
443	EPK TN14-3 X TRFK 6/8	2510	22.63	1.77	1.70	23.66	16.53	0.11	2.20	0.32
447	EPK TN14-3 X AHP S15/10	2434	21.20	1.32	1.63	21.71	16.60	0.09	4.20	0.38
488	EPK TN14-3 X TFFCA SFS150	1966	20.53	1.00	1.27	25.63	15.50	0.12	3.40	0.36
490	EPK TN14-3 X EPK TN14-3	2102	20.33	1.50	2.33	20.46	15.27	0.11	3.30	0.32
	Overall mean	2394	21.06	1.31	1.66	21.16	15.61	0.10	2.95	0.38
	Significance of t-test (p = 0.05)	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	S
Parents' performance										
TRFK 6/8		1708	24.30	1.27	2.00	23.03	17.74	0.09	1.00	0.38
AHP S15/10		2556	20.60	1.83	2.80	18.73	15.58	0.08	5.00	0.49
TRFCA SFS150		2699	22.40	1.40	1.30	18.86	15.53	0.08	3.00	0.36
EPK TN14-3		2478	22.30	1.00	2.00	22.44	17.01	0.09	3.00	0.44

Note. *S denotes significance at $P < 0.05$*

Appendix 3. Mean scores for traits assessed at the Timbilil site for St 463 (TRFCA SFS150 x AHP S15/10)

Genotype$	YLD-T	PUB-T	DT-T	TP-T	BL-T	BWd-T	SW-T	BWt-T
463/1	2167	3.13	2.0	21.3	26.19	2.57	3.48	0.42
463/3	2088	4.77	1.0	23.1	30.58	2.76	3.31	0.52
463/4	2183	4.77	1.8	17.5	27.90	2.49	3.43	0.42
463/5	2155	3.27	1.9	20.5	29.21	2.49	3.18	0.42
463/6	2179	3.27	2.6	20.6	27.74	2.86	2.90	0.28
463/7	1760	4.70	1.0	21.1	28.60	2.54	2.51	0.33
463/8	2061	3.13	2.0	19.2	30.70	2.88	3.67	0.51
463/9	1786	4.87	1.9	20.3	33.14	3.48	4.43	0.68
463/10	1670	1.50	1.4	21.6	32.22	2.91	2.50	0.34
463/12	2201	4.87	1.8	19.9	32.00	2.99	3.82	0.43
463/13	2330	3.10	2.7	18.5	33.10	2.68	3.69	0.38
463/15	2339	4.83	1.8	18.6	27.16	2.81	2.84	0.29
463/16	2230	3.00	1.9	19.3	29.64	2.71	3.68	0.39
463/17	2254	4.87	1.0	20.0	30.47	2.67	4.14	0.49
463/19	2172	4.93	1.9	18.4	28.77	2.36	4.30	0.43
463/20	2209	2.93	1.7	20.2	32.04	3.06	2.49	0.36
463/21	2147	1.33	1.9	21.5	29.19	2.73	3.51	0.35
463/22	2213	3.43	1.9	17.6	30.07	2.52	2.84	0.36
463/23	2151	4.87	1.8	19.2	29.69	2.89	3.15	0.39
463/24	2216	3.00	1.9	19.0	29.19	2.38	3.99	0.49
463/25	2144	4.93	1.5	18.5	29.88	2.58	3.65	0.46
463/26	2403	2.67	1.9	18.7	28.67	2.53	3.48	0.33
463/27	2128	2.93	1.9	20.1	32.26	3.23	4.14	0.53
463/28	2243	4.67	1.8	23.2	31.64	2.90	2.72	0.42
463/29	2168	3.10	1.3	20.4	30.64	2.44	4.62	0.37
463/30	2202	1.33	2.0	22.5	30.80	2.74	2.92	0.35
463/31	2271	3.33	1.8	21.7	29.07	2.56	2.29	0.28
463/32	2412	4.67	1.3	21.1	30.31	2.50	3.01	0.41
463/33	2193	2.67	1.4	20.7	27.80	2.58	3.27	0.33
463/34	2220	4.67	1.1	17.8	30.80	2.47	2.69	0.40
463/35	2316	2.67	2.1	20.2	30.13	3.07	3.74	0.44
463/36	2158	2.60	1.8	21.1	30.94	2.82	3.55	0.42
463/37	2046	3.33	1.0	22.3	28.26	2.59	3.43	0.39
463/38	2167	3.10	3.0	22.8	27.28	2.73	2.74	0.35
463/39	2307	3.33	1.5	21.7	30.81	2.50	3.30	0.39
463/40	2250	3.33	2.9	21.0	30.23	2.72	3.47	0.37
463/41	2370	1.33	2.1	21.8	30.13	2.74	2.79	0.32
463/42	2239	2.33	2.0	21.5	26.72	2.27	3.35	0.34
463/45	2181	2.67	1.4	22.3	28.83	2.36	3.20	0.35
463/46	2114	3.33	1.1	22.2	28.73	2.78	3.46	0.45
463/47	2153	3.33	1.8	22.6	32.41	2.80	3.20	0.34
463/48	2375	2.67	2.0	19.5	29.18	2.74	3.21	0.40
F_1 mean	**2180**	**3.42**	**1.77**	**20.50**	**29.84**	**2.70**	**3.34**	**0.40**
SFS150	2157	3.33	1.3	22.2	30.74	2.84	3.63	0.31
S15/10	2169	4.67	2.8	19.5	31.99	2.90	4.00	0.52
MPV	**2163**	**4.00**	**2.03**	**20.85**	**31.37**	**2.87**	**3.82**	**0.41**
TRFK31/8	2152	1.33	2.0	21.7	30.67	2.67	2.60	0.35
303/577	2113	1.33	1.1	23.0	26.11	2.32	1.98	0.22

Appendix 4. Mean scores for traits assessed at the Kangaita site for St 463 (TRFCA SFS150 x AHP S15/10)

Genotype$	YLD-K	PUB-K	DT-K	TP-K	BL-K	BWd-K	SW-K	BWt-K
463/1	1299	3.33	2.6	20.5	25.82	2.79	3.67	0.38
463/3	1935	3.10	2.8	21.8	27.14	2.76	2.99	0.33
463/4	1426	4.67	2.4	20.7	22.90	2.67	3.65	0.39
463/5	1462	2.67	2.0	22.2	27.28	2.97	3.88	0.49
463/6	1676	4.67	2.0	22.2	24.39	2.49	2.89	0.33
463/7	1029	2.00	2.0	21.1	25.20	2.42	3.03	0.33
463/8	1959	4.67	2.2	21.7	28.70	3.17	4.40	0.50
463/9	1784	2.67	2.0	20.7	24.11	2.29	2.62	0.28
463/10	1393	4.00	2.8	18.7	26.34	2.37	4.22	0.56
463/12	1778	2.67	2.4	22.7	28.14	2.71	2.71	0.26
463/13	2260	4.67	3.0	21.6	27.90	2.52	4.30	0.39
463/15	1951	4.93	1.0	19.6	23.41	2.68	3.87	0.34
463/16	1679	3.00	2.0	21.9	24.67	2.56	2.99	0.36
463/17	1738	4.93	2.0	20.1	26.44	2.51	3.14	0.36
463/19	1993	2.67	1.2	20.8	26.68	2.47	3.82	0.34
463/20	2390	3.00	1.4	21.2	26.03	2.36	3.54	0.38
463/21	1615	2.33	2.0	22.2	24.20	2.30	2.98	0.30
463/22	2145	4.67	1.0	20.0	26.42	2.44	3.48	0.37
463/23	1764	2.67	1.6	21.7	26.01	2.61	2.95	0.34
463/24	2355	4.67	2.0	18.5	24.94	2.42	3.50	0.37
463/25	1690	5.00	1.0	21.5	26.38	2.49	3.51	0.34
463/26	2428	4.93	2.0	20.7	26.57	2.43	3.66	0.36
463/27	1588	4.67	1.2	24.1	31.21	3.02	3.89	0.44
463/28	1558	2.00	1.6	22.8	26.41	2.69	4.92	0.47
463/29	1141	5.00	2.6	21.7	26.08	2.56	3.13	0.44
463/30	2078	2.00	2.0	22.0	25.76	2.33	4.10	0.38
463/31	1112	3.33	2.0	22.3	26.47	2.59	2.82	0.34
463/32	1304	5.00	2.0	20.2	27.34	2.72	2.59	0.32
463/33	1120	5.00	2.0	20.8	24.20	2.30	3.69	0.42
463/34	672	2.67	1.0	19.5	26.50	2.49	3.68	0.37
463/35	2277	2.67	1.0	22.2	26.03	2.48	2.68	0.36
463/36	949	3.00	3.0	22.0	29.44	2.48	3.42	0.32
463/37	800	3.00	1.0	22.6	26.43	2.20	3.71	0.35
463/38	1145	3.00	2.0	22.1	25.07	2.59	2.91	0.31
463/39	1018	5.00	1.4	23.0	23.80	2.13	2.58	0.27
463/40	762	3.00	2.0	20.8	27.93	2.76	3.32	0.35
463/41	874	3.00	2.0	22.8	26.14	2.47	3.26	0.31
463/42	1184	2.00	1.4	22.2	19.37	1.73	3.31	0.35
463/45	846	2.67	1.0	23.3	23.07	2.10	2.69	0.28
463/46	787	5.00	2.0	23.6	25.27	2.62	3.78	0.51
463/47	830	3.00	2.0	22.5	25.60	2.36	2.55	0.25
463/48	1369	2.67	1.0	21.6	24.09	2.26	3.75	0.34
F_1 mean	**1504**	**3.56**	**1.85**	**21.53**	**25.85**	**2.51**	**3.39**	**0.36**
SFS150	1883	3.00	2.0	22.1	27.43	2.72	3.68	0.44
S15/10	2143	5.00	2.8	21.6	29.26	3.12	4.14	0.53
MPV	**2013**	**4.00**	**2.40**	**21.87**	**28.34**	**2.92**	**3.91**	**0.49**
TRFK31/8	2050	2.00	1.2	20.9	28.59	2.70	3.14	0.51
303/577	1996	1.33	1.0	22.9	22.86	2.21	3.43	0.47

Appendix 5. Results on normal distribution goodness of fit for Kangaita data

Frequency Table

YLD	Observed	Expected	Obs - Exp	YLD	Pooled Obs	Pooled Exp	Pooled O-E
< 1000	0	0.965	-0.965	< 1500	8	7.068	0.932
1000-1500	8	6.103	1.897	1500-2000	14	15.169	-1.169
1500-2000	14	15.169	-1.169	2000-2500	14	14.144	-0.144
2000-2500	14	14.144	-0.144	> 2500	6	5.618	0.382
2500-3000	6	4.945	1.055				
> 3000	0	0.673	-0.673				

Test Results

Statistic	Value	DF	P
*Chi-Square	0.240	1	0.624
Yates Correction	0.067	1	0.795
Log-Likelihood	4.118	3	0.249
Yates Correction	0.000	3	
Kolmogorov-Smirnov	0.342	42	0.000
Skewness	0.042	-	0.904
Kurtosis	-0.905	-	0.199

* Chi-Square based on pooled expecteds

Frequency Table

PUB	Observed	Expected	Obs - Exp	Bin	Pooled Obs	Pooled Exp	Pooled O-E
< 1	0	0.232	-0.232	< 2	1	2.243	-1.243
01-02	1	2.010	-1.010	02-03	13	8.119	4.881
02-03	13	8.119	4.881	03-04	7	14.670	-7.670
03-04	7	14.670	-7.670	04-05	17	11.894	5.106
04-05	17	11.894	5.106	> 5	4	5.074	-1.074
05-06	4	4.322	-0.322				
> 6	0	0.752	-0.752				

Test Results

Statistic	Value	DF	P
*Chi-Square	10.053	2	0.007
Log-Likelihood	12.010	4	0.017
Kolmogorov-Smirnov	0.379	42	0.000
Skewness	-0.135	-	0.698
Kurtosis	-1.141	-	0.136

* Chi-Square based on pooled expecteds

Frequency Table

DRT	Observed	Expected	Obs - Exp	DRT	Pooled Obs	Pooled Exp	Pooled O-E
< 1.2	0	0.826	-0.826	< 1.8	8	5.611	2.389
1.2-1.8	8	4.785	3.215	1.8-2.4	7	12.744	-5.744
1.8-2.4	7	12.744	-5.744	2.4-3	19	14.655	4.345
2.4-3	19	14.655	4.345	3-3.6	7	7.283	-0.283
3-3.6	7	7.283	-0.283	> 3.6	1	1.706	-0.706
3.6-4.2	1	1.558	-0.558				
> 4.2	0	0.148	-0.148				

Test Results

Statistic	Value	DF	P
*Chi-Square	5.197	2	0.074
Log-Likelihood	8.259	4	0.083
Kolmogorov-Smirnov	0.429	42	0.000
Skewness	-0.201	-	0.565
Kurtosis	-0.574	-	0.340

* Chi-Square based on pooled expecteds

Frequency Table

TP	Observed	Expected	Obs - Exp	TP	Pooled Obs	Pooled Exp	Pooled O-E
< 18.5	0	0.119	-0.119	< 19.5	2	1.006	0.994
18.5-19	1	0.252	0.748	19.5-20	1	1.369	-0.369
19-19.5	1	0.635	0.365	20-20.5	2	2.535	-0.535
19.5-20	1	1.369	-0.369	20.5-21	3	4.026	-1.026
20-20.5	2	2.535	-0.535	21-21.5	6	5.488	0.512
20.5-21	3	4.026	-1.026	21.5-22	3	6.419	-3.419
21-21.5	6	5.488	0.512	22-22.5	9	6.442	2.558
21.5-22	3	6.419	-3.419	22.5-23	8	5.548	2.452
22-22.5	9	6.442	2.558	23-23.5	5	4.100	0.900
22.5-23	8	5.548	2.452	23.5-24	1	2.600	-1.600
23-23.5	5	4.100	0.900	24-24.5	1	1.415	-0.415
23.5-24	1	2.600	-1.600	> 24.5	1	1.052	-0.052
24-24.5	1	1.415	-0.415				
24.5-25	1	0.661	0.339				
> 25	0	0.391	-0.391				

Test Results

Statistic	Value	DF	P
*Chi-Square	6.729	9	0.665
Log-Likelihood	8.911	12	0.711
Kolmogorov-Smirnov	0.245	42	0.011
Skewness	-0.465	-	0.192
Kurtosis	0.362	-	0.476

* Chi-Square based on pooled expecteds

Frequency Table

Bud Lth	Observed	Expected	Obs - Exp	Bud Lth	Pooled Obs	Pooled Exp	Pooled O-E
< 20	0	0.002	-0.002	< 24	1	1.115	-0.115
20-22	1	0.076	0.924	24-26	5	5.988	-0.988
22-24	0	1.037	-1.037	26-28	13	14.754	-1.754
24-26	5	5.988	-0.988	28-30	21	15.591	5.409
26-28	13	14.754	-1.754	30-32	5	7.068	-2.068
28-30	21	15.591	5.409	> 32	1	1.485	-0.485
30-32	5	7.068	-2.068				
32-34	1	1.368	-0.368				
> 34	0	0.116	-0.116				

Test Results

Statistic	Value	DF	P
*Chi-Square	3.023	3	0.388
Log-Likelihood	8.482	6	0.205
Kolmogorov-Smirnov	0.401	46	0.000
Skewness	-0.727	-	0.041
Kurtosis	2.077	-	0.028

* Chi-Square based on pooled expecteds

Frequency Table

Bud Wdth	Observed	Expected	Obs - Exp	Bud Wdth	Pooled Obs	Pooled Exp	Pooled O-E
< 1.9	0	0.031	-0.031	< 2.5	4	6.574	-2.574
1.9-2.2	1	0.723	0.277	2.5-2.8	20	16.426	3.574
2.2-2.5	3	5.820	-2.820	2.8-3.1	18	16.426	1.574
2.5-2.8	20	16.426	3.574	> 3.1	4	6.574	-2.574
2.8-3.1	18	16.426	1.574				
3.1-3.4	2	5.820	-3.820				
3.4-3.7	2	0.723	1.277				
> 3.7	0	0.031	-0.031				

Test Results

Statistic	Value	DF	P
*Chi-Square	2.944	1	0.086
Yates Correction	1.954	1	0.162
Log-Likelihood	7.641	5	0.177
Yates Correction	0.000	5	
Kolmogorov-Smirnov	0.413	46	0.000
Skewness	0.339	-	0.316
Kurtosis	1.564	-	0.063

* Chi-Square based on pooled expecteds

Frequency Table

SWt	Observed	Expected	Obs - Exp	SWt	Pooled Obs	Pooled Exp	Pooled O-E
< 2.6	0	1.026	-1.026	< 2.6	0	1.026	-1.026
2.6-2.9	3	2.282	0.718	2.6-2.9	3	2.282	0.718
2.9-3.2	6	4.976	1.024	2.9-3.2	6	4.976	1.024
3.2-3.5	9	8.105	0.895	3.2-3.5	9	8.105	0.895
3.5-3.8	7	9.864	-2.864	3.5-3.8	7	9.864	-2.864
3.8-4.1	11	8.970	2.030	3.8-4.1	11	8.970	2.030
4.1-4.4	5	6.095	-1.095	4.1-4.4	5	6.095	-1.095
4.4-4.7	4	3.094	0.906	4.4-4.7	4	3.094	0.906
4.7-5	0	1.173	-1.173	> 4.7	1	1.588	-0.588
5-5.3	1	0.332	0.668				
> 5.3	0	0.083	-0.083				

Test Results

Statistic	Value	DF	P
*Chi-Square	3.532	6	0.740
Log-Likelihood	7.738	8	0.460
Kolmogorov-Smirnov	0.222	46	0.018
Skewness	0.255	-	0.448
Kurtosis	-0.232	-	0.576

* Chi-Square based on pooled expecteds

Frequency Table

Bud Wt	Observed	Expected	Obs - Exp	Bud Wt	Pooled Obs	Pooled Exp	Pooled O-E
< 0.25	0	0.629	-0.629	< 0.3	1	2.731	-1.731
0.25-0.3	1	2.102	-1.102	0.3-0.35	5	5.571	-0.571
0.3-0.35	5	5.571	-0.571	0.35-0.4	16	9.855	6.145
0.35-0.4	16	9.855	6.145	0.4-0.45	12	11.644	0.356
0.4-0.45	12	11.644	0.356	0.45-0.5	4	9.187	-5.187
0.45-0.5	4	9.187	-5.187	0.5-0.55	4	4.841	-0.841
0.5-0.55	4	4.841	-0.841	> 0.55	4	2.171	1.829
0.55-0.6	3	1.703	1.297				
0.6-0.65	1	0.399	0.601				
> 0.65	0	0.069	-0.069				

Test Results

Statistic	Value	DF	P
*Chi-Square	9.612	4	0.048
Log-Likelihood	10.719	7	0.151
Kolmogorov-Smirnov	0.264	46	0.003
Skewness	0.792	-	0.028
Kurtosis	0.263	-	0.548

* Chi-Square based on pooled expecteds

Appendix 6. Results on normal distribution goodness of fit for Timbilil data

Frequency Table

ANYLD	Observed	Expected	Obs - Exp	ANYLD	Pooled Obs	Pooled Exp	Pooled O-E
< 1700	0	0.014	-0.014	< 2100	3	1.954	1.046
1700-1900	2	0.219	1.781	2100-2300	3	6.711	-3.711
1900-2100	1	1.721	-0.721	2300-2700	28	25.618	2.382
2100-2300	3	6.711	-3.711	2700-2900	8	6.076	1.924
2300-2700	28	25.618	2.382	> 2900	0	1.641	-1.641
2700-2900	8	6.076	1.924				
> 2900	0	1.641	-1.641				

Test Results

Statistic	Value	DF	P
*Chi-Square	5.084	2	0.079
Log-Likelihood	12.316	4	0.015
Kolmogorov-Smirnov	0.673	42	0.000
Skewness	-1.248	-	0.002
Kurtosis	2.636	-	0.015

* Chi-Square based on pooled expecteds

Frequency Table

DRT	Observed	Expected	Obs - Exp	DRT	Pooled Obs	Pooled Exp	Pooled O-E
< 1.6	0	0.859	-0.859	< 2.4	14	14.098	-0.098
1.6-2.4	14	13.239	0.761	2.4-3.2	24	23.035	0.965
2.4-3.2	24	23.035	0.965	> 3.2	4	4.867	-0.867
3.2-4	4	4.765	-0.765				
> 4	0	0.102	-0.102				

Test Results

Statistic	Value	DF	P
*Chi-Square	0.195	0	-
Log-Likelihood	2.135	2	0.344
Kolmogorov-Smirnov	0.551	42	0.000
Skewness	0.188	-	0.590
Kurtosis	-0.471	-	0.400

* Chi-Square based on pooled expecteds

Frequency Table

Pub	Observed	Expected	Obs - Exp	Pub	Pooled Obs	Pooled Exp	Pooled O-E
< 1.5	0	0.047	-0.047	< 3	1	2.476	-1.476
1.5-3	1	2.429	-1.429	3-4.5	20	17.334	2.666
3-4.5	20	17.334	2.666	4.5-6	18	18.931	-0.931
4.5-6	18	18.931	-0.931	> 6	3	3.259	-0.259
6-7.5	3	3.184	-0.184				
> 7.5	0	0.075	-0.075				

Test Results

Statistic	Value	DF	P
*Chi-Square	1.356	1	0.244
Yates Correction	0.683	1	0.409
Log-Likelihood	1.775	3	0.620
Yates Correction	0.000	3	
Kolmogorov-Smirnov	0.448	42	0.000
Skewness	0.329	-	0.349
Kurtosis	-0.201	-	0.611

* Chi-Square based on pooled expecteds

Frequency Table

TP	Observed	Expected	Obs - Exp	TP	Pooled Obs	Pooled Exp	Pooled O-E
< 18	0	0.644	-0.644	< 19	3	2.554	0.446
18-19	3	1.910	1.090	19-20	6	4.795	1.205
19-20	6	4.795	1.205	20-21	6	8.359	-2.359
20-21	6	8.359	-2.359	21-22	9	10.118	-1.118
21-22	9	10.118	-1.118	22-23	10	8.505	1.495
22-23	10	8.505	1.495	23-24	6	4.965	1.035
23-24	6	4.965	1.035	> 24	2	2.705	-0.705
24-25	2	2.012	-0.012				
> 25	0	0.693	-0.693				

Test Results

Statistic	Value	DF	P
*Chi-Square	1.832	4	0.767
Log-Likelihood	4.801	6	0.570
Kolmogorov-Smirnov	0.258	42	0.006
Skewness	-0.182	-	0.601
Kurtosis	-0.772	-	0.247

* Chi-Square based on pooled expecteds

Frequency Table

BL-T	Observed	Expected	Obs - Exp	BL-T	Pooled Obs	Pooled Exp	Pooled O-E
< 26.8	0	0.641	-0.641	< 27.6	3	1.893	1.107
26.8-27.6	3	1.253	1.747	27.6-28.4	2	2.756	-0.756
27.6-28.4	2	2.756	-0.756	28.4-29.2	4	4.917	-0.917
28.4-29.2	4	4.917	-0.917	29.2-30	9	7.113	1.887
29.2-30	9	7.113	1.887	30-30.8	4	8.343	-4.343
30-30.8	4	8.343	-4.343	30.8-31.6	13	7.935	5.065
30.8-31.6	13	7.935	5.065	31.6-32.4	2	6.120	-4.120
31.6-32.4	2	6.120	-4.120	32.4-33.2	6	3.827	2.173
32.4-33.2	6	3.827	2.173	33.2-34	3	1.941	1.059
33.2-34	3	1.941	1.059	> 34	0	1.155	-1.155
> 34	0	1.155	-1.155				

Test Results

Statistic	Value	DF	P
*Chi-Square	12.760	7	0.078
Log-Likelihood	17.031	8	0.030
Kolmogorov-Smirnov	0.238	46	0.009
Skewness	-0.160	-	0.632
Kurtosis	-0.564	-	0.333

* Chi-Square based on pooled expecteds

Frequency Table

BWd	Observed	Expected	Obs - Exp	BWd	Pooled Obs	Pooled Exp	Pooled O-E
< 2.4	0	1.434	-1.434	< 2.4	0	1.434	-1.434
2.4-2.6	5	4.824	0.176	2.4-2.6	5	4.824	0.176
2.6-2.8	14	10.745	3.255	2.6-2.8	14	10.745	3.255
2.8-3	13	13.695	-0.695	2.8-3	13	13.695	-0.695
3-3.2	10	9.991	0.009	3-3.2	10	9.991	0.009
3.2-3.4	2	4.170	-2.170	3.2-3.4	2	4.170	-2.170
3.4-3.6	1	0.994	0.006	> 3.4	2	1.140	0.860
3.6-3.8	1	0.135	0.865				
> 3.8	0	0.011	-0.011				

Test Results

Statistic	Value	DF	P
*Chi-Square	4.239	4	0.375
Log-Likelihood	7.505	6	0.277
Kolmogorov-Smirnov	0.261	46	0.003
Skewness	0.813	-	0.025
Kurtosis	1.073	-	0.142

* Chi-Square based on pooled expecteds

Frequency Table

BWt	Observed	Expected	Obs - Exp	BWt	Pooled Obs	Pooled Exp	Pooled O-E
< 0.25	0	0.441	-0.441	< 0.3	1	1.924	-0.924
0.25-0.3	1	1.483	-0.483	0.3-0.35	3	4.140	-1.140
0.3-0.35	3	4.140	-1.140	0.35-0.4	10	8.037	1.963
0.35-0.4	10	8.037	1.963	0.4-0.45	14	10.848	3.152
0.4-0.45	14	10.848	3.152	0.45-0.5	10	10.184	-0.184
0.45-0.5	10	10.184	-0.184	0.5-0.55	3	6.650	-3.650
0.5-0.55	3	6.650	-3.650	0.55-0.6	4	3.019	0.981
0.55-0.6	4	3.019	0.981	> 0.6	1	1.197	-0.197
0.6-0.65	0	0.953	-0.953				
0.65-0.7	0	0.209	-0.209				
0.7-0.75	1	0.032	0.968				
> 0.75	0	0.004	-0.004				

Test Results

Statistic	Value	DF	P
*Chi-Square	4.511	5	0.478
Log-Likelihood	12.797	9	0.172
Kolmogorov-Smirnov	0.238	46	0.009
Skewness	0.948	-	0.011
Kurtosis	2.164	-	0.025

* Chi-Square based on pooled expecteds

Frequency Table

SWt	Observed	Expected	Obs - Exp	SWt	Pooled Obs	Pooled Exp	Pooled O-E
< 2.2	0	0.226	-0.226	< 2.6	1	1.312	-0.312
2.2-2.6	1	1.086	-0.086	2.6-3	4	3.767	0.233
2.6-3	4	3.767	0.233	3-3.4	9	8.381	0.619
3-3.4	9	8.381	0.619	3.4-3.8	10	11.969	-1.969
3.4-3.8	10	11.969	-1.969	3.8-4.2	14	10.975	3.025
3.8-4.2	14	10.975	3.025	4.2-4.6	5	6.461	-1.461
4.2-4.6	5	6.461	-1.461	> 4.6	3	3.134	-0.134
4.6-5	2	2.441	-0.441				
5-5.4	1	0.591	0.409				
> 5.4	0	0.102	-0.102				

Test Results

Statistic	Value	DF	P
*Chi-Square	1.628	4	0.804
Log-Likelihood	2.508	7	0.927
Kolmogorov-Smirnov	0.270	46	0.002
Skewness	0.098	-	0.769
Kurtosis	-0.009	-	0.814

* Chi-Square based on pooled expecteds

Frequency Table

%TP-K	Observed	Expected	Obs - Exp	%TP-K	Pooled Obs	Pooled Exp	Pooled O-E
< 20	0	1.937	-1.937	< 20	0	1.937	-1.937
20-21	3	2.936	0.064	20-21	3	2.936	0.064
21-22	8	5.185	2.815	21-22	8	5.185	2.815
22-23	9	7.227	1.773	22-23	9	7.227	1.773
23-24	9	7.949	1.051	23-24	9	7.949	1.051
24-25	4	6.901	-2.901	24-25	4	6.901	-2.901
25-26	2	4.728	-2.728	25-26	2	4.728	-2.728
26-27	4	2.556	1.444	26-27	4	2.556	1.444
27-28	0	1.091	-1.091	> 27	2	1.580	0.420
28-29	2	0.367	1.633				
> 29	0	0.122	-0.122				

Test Results

Statistic	Value	DF	P
*Chi-Square	7.761	6	0.256
Log-Likelihood	15.808	8	0.045
Kolmogorov-Smirnov	0.172	41	0.156
Skewness	0.850	-	0.026
Kurtosis	0.298	-	0.528

* Chi-Square based on pooled expecteds

Appendix 7. Pearson correlation results for traits measured at the Kangaita site

Descriptive Statistics

Variable	Mean	Std Dev.	Std Err	N
YLD-K	1548.601	507.544	74.833	46
PUB-K	3.498	1.132	0.167	46
DT-K	1.839	0.599	0.088	46
TP-K	21.558	1.206	0.178	46
BL-K	25.957	2.053	0.303	46
BWd-K	2.522	0.266	0.039	46
SW-K	3.412	0.550	0.081	46
BWt-K	0.375	0.075	0.011	46

Correlation Matrix (R)

	YLD-K	PUB-K	DT-K	TP-K	BL-K	BWd-K	SW-K	BWt-K
YLD-K	1.000	0.099	-0.011	-0.255	0.167	0.207	0.229	0.228
PUB-K		1.000	0.235	-0.258	0.176	0.292	0.133	0.164
DT-K			1.000	-0.109	0.290	0.349	0.087	0.145
TP-K				1.000	0.038	-0.015	-0.133	-0.088
BL-K					1.000	0.728	0.259	0.305
BWd-K						1.000	0.313	0.444
SW-K							1.000	0.685
BWt-K								1.000

t Statistic

	YLD-K	PUB-K	DT-K	TP-K	BL-K	BWd-K	SW-K	BWt-K
YLD-K	-	0.659	0.073	1.749	1.124	1.403	1.560	1.551
PUB-K		-	1.603	1.770	1.185	2.028	0.890	1.103
DT-K			-	0.728	2.013	2.470	0.580	0.974
TP-K				-	0.253	0.100	0.893	0.584
BL-K					-	7.052	1.777	2.122
BWd-K						-	2.186	3.282
SW-K							-	6.236
BWt-K								-

Correlation Significance (P)

	YLD-K	PUB-K	DT-K	TP-K	BL-K	BWd-K	SW-K	BWt-K
YLD-K	-	0.513	0.942	0.087	0.267	0.168	0.126	0.128
PUB-K		-	0.116	0.084	0.243	0.049	0.378	0.276
DT-K			-	0.471	0.050	0.017	0.565	0.335
TP-K				-	0.801	0.921	0.377	0.562
BL-K					-	0.000	0.082	0.039
BWd-K						-	0.034	0.002
SW-K							-	0.000
BWt-K								-

Appendix 8. Pearson correlation results for traits measured at the Timbilil site

Descriptive Statistics

Variable	Mean	Std Dev.	Std Err	N
YLD-T	2177.377	144.908	21.366	46
PUB-T	3.353	1.133	0.167	46
DT-T	1.775	0.503	0.074	46
TP-T	20.597	1.571	0.232	46
BL-T	29.840	1.762	0.260	46
BWd-T	2.699	0.243	0.036	46
SW-T	3.311	0.579	0.085	46
BWt-T	0.393	0.081	0.012	46

Correlation Matrix (R)

	YLD-T	PUB-T	DT-T	TP-T	BL-T	BWd-T	SW-T	BWt-T
YLD-T	1.000	-0.038	0.278	-0.199	-0.153	-0.274	0.019	-0.229
PUB-T		1.000	-0.159	-0.393	0.129	0.103	0.296	0.462
DT-T			1.000	-0.178	0.068	0.246	0.106	0.024
TP-T				1.000	-0.069	0.046	-0.342	-0.247
BL-T					1.000	0.588	0.274	0.455
BWd-T						1.000	0.189	0.452
SW-T							1.000	0.669
BWt-T								1.000

t Statistic

	YLD-T	PUB-T	DT-T	TP-T	BL-T	BWd-T	SW-T	BWt-T
YLD-T	-	0.250	1.916	1.349	1.024	1.890	0.127	1.562
PUB-T		-	1.068	2.837	0.866	0.689	2.056	3.460
DT-T			-	1.196	0.449	1.680	0.704	0.159
TP-T				-	0.461	0.303	2.417	1.692
BL-T					-	4.827	1.887	3.391
BWd-T						-	1.277	3.365
SW-T							-	5.965
BWt-T								-

Correlation Significance (P)

	YLD-T	PUB-T	DT-T	TP-T	BL-T	BWd-T	SW-T	BWt-T
YLD-T	-	0.804	0.062	0.184	0.311	0.065	0.899	0.125
PUB-T		-	0.291	0.007	0.391	0.494	0.046	0.001
DT-T			-	0.238	0.656	0.100	0.485	0.875
TP-T				-	0.647	0.763	0.020	0.098
BL-T					-	0.000	0.066	0.001
BWd-T						-	0.208	0.002
SW-T							-	0.000
BWt-T								-

Appendix 9. ISSR Primer sequence screened for genotyping mapping population

Code	Primer sequences (5' - 3')					
801	ATA	TAT	ATA	TAT	ATA	TT
803	TAT	ATA	TAT	ATA	TAT	AC
806	TAT	ATA	TAT	ATA	TAT	AG
816	CAC	ACA	CAC	ACA	CAC	AT
821	GTG	TGT	GTG	TGT	GTG	TT
866	CTC	CTC	CTC	CTC	CTC	CTC
868	GAA	GAA	GAA	GAA	GAA	GAA
869	GTT	GTT	GTT	GTT	GTT	GTT
871	TAT	TAT	TAT	TAT	TAT	TAT
873	GAC	AGA	CAG	ACA	GAC	A
877	TGC	ATG	CAT	GCA	TGC	A
879	CTT	CAC	TTC	ACT	TCA	

Appendix10. Number of markers generated by RAPD Primers on the mapping population St 463 (TRFCA SFS150 x AHP S15/10)

PRIMER	CODE	LOCUS	No. MARKERS
OPO-02	OPO-1	OPO-02-1800	3
	OPO-2	OPO-02-900	
	OPO-3	OPO-02-650	
OPO-05	OPO-4	OPO-05-1100	3
	OPO-5	OPO-05-700	
	OPO-6	OPO-05-900	
OPO-07	OPO-7	OPO-07-350	1
OPO-11	OPO-8	OPO-11-900	2
	OPO-9	OPO-11-450	
OPV-01	OPV-1	OPV-01-1500	3
	OPV-2	OPV-01-900	
	OPV-3	OPV-01-350	
OPV-06	OPV-4	OPV-06-1500	1
OPT-18	OPT-1	OPT-18-2500	3
	OPT-2	OPT-18-1300	
	OPT-3	OPT-18-1031	
OPM-05	OPM-1	OPM-05-1200	1
OPM-07	OPM-2	OPM-07-1800	1
OPA-10	OPA-1	OPA-10-1800	1
OP-26-08	OP26-1	OP-26-08-380	2
	OP26-7	OP-26-08-900	
OP-26-07	OP26-3	OP-26-07-600	2
	OP26-4	OP-26-07-300	
OP-26-15	OP26-5	OP-26-15-450	2
	OP26-6	OP-26-15-1031	
OPG-17	OPG-1	OPG-17-1100	1
OPG-07	OPG-2	OPG-07-2800	2
	OPG-3	OPG-07-1300	
OPF-16	OPF-1	OPF-16-900	2
	OPF-2	OPF-16-580	

OPT-03	OPT-4	OPT-03-1200	3
	OPT-5	OPT-03-950	
	OPT-6	OPT-03-600	
OPO-03	OPO-10	OPO-03-750	2
	OPO-11	OPO-03-300	
OPF-01	OPF-3	OPF-01-580	1
OPF-05	OPF-4	OPF-05-1200	3
	OPF-5	OPF-05-500	
	OPF-6	OPF-05-300	
G-12	G-1	G-12-750	1
OPW-04	OPW-1	OPW-04-1500	3
	OPW-2	OPW-04-600	
	OPW-3	OPW-04-400	
OPD-18	OPD-1	OPD-18-600	1
OPW-07	OPW-4	OPW-07-M1	2
		OPW-07-M2	
AB4-16	AB4-1	AB4-16-M1	1
OPW-11	OPW-5	OPW-11-M1	3
	OPW-6	OPW-11-M2	
	OPW-7	OPW-11-M3	
TOTAL			**49**

Appendix 11. Number of markers generated by RAPD Primers on the root knot nematode mapping population St 526 (TRFCA SFS150 x TRFK 303/577)

PRIMER	CODE	LOCUS	No. MARKERS
OPF-02	OPF-1	OPF-02-900	3
	OPF-2	OPF-02-700	
	OPF-3	OPF-02-450	
OPF-03	OPF-4	OPF-03-2500	8
	OPF-5	OPF-03-1500	
	OPF-6	OPF-03-1200	
	OPF-7	OPF-03-850	
	OPF-8	OPF-03-700	
	OPF-9	OPF-03-500	
	OPF-10	OPF-03-400	
	OPF-11	OPF-03-350	
OPF-05	OPF-12	OPF-05-400	2
	OPF-13	OPF-05-300	
OPF-06	OPF-14	OPF-06-1031	2
	OPF-15	OPF-06-400	
OPF-09	OPF-16	OPF-09-1031	6
	OPF-17	OPF-09-950	
	OPF-18	OPF-09-850	
	OPF-19	OPF-09-700	
	OPF-20	OPF-09-450	
	OPF-21	OPF-09-350	
OPF-15	OPF-22	OPF-15-1500	2
	OPF-23	OPF-15-300	
OPT-01	OPT-1	OPT-01-2000	3
	OPT-2	OPT-01-1100	
	OPT-3	OPT-01-480	
OPT-02	OPT-4	OPT-02-1300	1
OPT-04	OPT-5	OPT-04-880	2

	OPT-6	OPT-04-600	
OPT-17	OPT-7	OPT-17-600	1
OPT-18	OPT-8	OPT-18-550	4
	OPT-9	OPT-18-700	
	OPT-10	OPT-18-1200	
	OPT-11	OPT-18-600	
OPO-10	OPO-1	OPO-10-580	3
	OPO-2	OPO-10-450	
	OPO-3	OPO-10-300	
OPO-02	OPO-4	OPO-02-1500	1
OPO-03	OPO-5	OPO-03-1300	2
	OPO-6	OPO-03-350	
OPO-05	OPO-7	OPO-05-1200	3
	OPO-8	OPO-05-700	
	OPO-9	OPO-05-380	
OPO-07	OPO-10	OPO-07-1800	2
	OPO-11	OPO-07-500	
OPO-06	OPO-12	OPO-06-900	2
	OPO-13	OPO-06-600	
OP-26-05	OP26-1	OP-26-05-1031	2
	OP26-2	OP-26-05-450	
OP-26-08	OP26-3	OP-26-08-950	2
	OP26-4	OP-26-08-250	
OP-26-16	OP26-5	OP-26-16-500	2
	OP26-6	OP-26-16-280	
OP-26-09	OP26-5	OP-26-09-1300	2
	OP26-6	OP-26-09-350	
OPE-09	OPE-1	OPE-09-1031	5
	OPE-2	OPE-09-900	
	OPE-3	OPE-09-850	
	OPE-4	OPE-09-550	
	OPE-5	OPE-09-300	
OPE-19	OPE-6	OPE-19-1500	2
	OPE-7	OPE-19-950	
OPE-18	OPE-8	OPE-18-1031	4
	OPE-9	OPE-18-700	
	OPE-10	OPE-18-350	
	OPE-11	OPE-18-200	
OPE-11	OPE-12	OPE-11-1300	3
	OPE-13	OPE-11-400	
	OPE-14	OPE-11-250	
OPE-06	OPE-15	OPE-06-1800	2
	OPE-16	OPE-06-1300	
OPM-05	OPM-1	OPM-05-900	1
OPA-01	OPA-1	OPA-01-1300	1
OPA-10	OPA-2	OPA-10-1200	1
OPA-07	OPA-3	OPA-07-950	3
	OPA-5	OPA-07-240	
	OPA-4	OPA-07-380	
G-8	G-1	G-8-1200	1
G-12	G-2	G-12-1800	1
G-15	G-3	G-15-980	3
	G-4	G-15-650	
	G-5	G-15-550	

OPG-11	OPG-1	OPG-11-550	1
OPV-01	OPV-1	OPV-01-1300	2
	OPV-2	OPV-01-280	
OPW-11	OPW-1	OPW-11-M1	1
OPW-06	OPW-2	OPW-6-M1	3
	OPW-3	OPW-6-M2	
	OPW-4	OPW-6-M3	
OPW-03	OPW-5	OPW-03-M1	6
	OPW-6	OPW-03-M2	
	OPW-7	OPW-03-M3	
	OPW-8	OPW-03-M4	
	OPW-9	OPW-03-M5	
	OPW-10	OPW-03-M6	
OPW-04	OPW-11	OPW-04-M1	4
	OPW-12	OPW-04-M2	
	OPW-13	OPW-04-M3	
	OPW-14	OPW-04-M4	
OPW-18	OPW-5	OPW-18-M1	1
OPU-15	OPU-1	OPU-15-M1	2
	OPU-2	OPU-15-M2	
OPU-20	OPU-3	OPU-20-M1	2
	OPU-4	OPU-20-M2	
OPD-18	OPD-1	OPD-18-M1	1
TOTAL			**105**

Appendix 12. ISSR markers generated on the mapping population St 463 (TRFCA SFS150 x AHP S15/10).

PRIMER	CODE	LOCUS	No. MARKERS
ISSR808	**808-1**		**1**
ISSR849	**849-1**		**2**
	849-2		
ISSR842	**842-1**		**2**
	842-2		
ISSR857	**857-1**		**2**
	857-2		
TOTAL			**7**

Appendix 13. Microsatellite (SSR) markers generated on the mapping population St 463 (TRFCA SFS150 x AHP S15/10).

PRIMER	CODE	LOCUS	No. MARKERS
CAMSIN2F&R	CSM-02	CAMSIN2-258-245	1
CAMSIN4F&R	CSM-04	CAMSIN4-493-472	1
CAMSIN6F&R	CSM-06	CAMSIN6-430-400	1
CAMSIN8F&R	CSM8-01	CAMSIN8-140-130	3
CAMSIN8F&R	CSM8-02	CAMSIN8-220	
CAMSIN8F&R	CSM8-03	CAMSIN8-174	
CAMSIN9F&R	CSM9-01	CAMSIN9-200-207	2
	CSM9-02	CAMSIN9-232-237	
CAMSIN10F&R	CSM-10	CAMSIN10-190-180	1
CAMSIN11F&R	CSM-11	CAMSIN11-158	1
CAMSIN13F&R	CSM-13	CAMSIN13-192	1
TOTAL			**11**

Appendix 14. Polymorphic AFLP primer pairs used in genotyping St 463 (TRFCA SFS150 x AHP S15/10) mapping population.

PRIMER PAIRS	CODE	LOCUS	No. of MARKERS
E-ACC/M-CTC	EACC/MCTC01	E-ACC/M-CTC-400	6
	EACC/MCTC02	E-ACC/M-CTC-283	
	EACC/MCTC03	E-ACC/M-CTC-183	
	EACC/MCTC04	E-ACC/M-CTC-112	
	EACC/MCTC05	E-ACC/M-CTC-93	
	EACC/MCTC06	E-ACC/M-CTC-61	
E-ACC/M-CAGT	EACC/MCAGT01	E-ACC/M-CAGT-137	4
	EACC/MCAGT02	E-ACC/M-CAGT-115	
	EACC/MCAGT03	E-ACC/M-CAGT-98	
	EACC/MCAGT04	E-ACC/M-CAGT-45	
E-ACC/M-CTA	EACC/MCTA01	E-ACC/M-CTA-800	8
	EACC/MCTA02	E-ACC/M-CTA-425	
	EACC/MCTA03	E-ACC/M-CTA-395	
	EACC/MCTA04	E-ACC/M-CTA-350	
	EACC/MCTA05	E-ACC/M-CTA-250	
	EACC/MCTA06	E-ACC/M-CTA-225	
	EACC/MCTA07	E-ACC/M-CTA-198	
	EACC/MCTA08	E-ACC/M-CTA-175	
E-ACT/M-CAC	EACT/MCAC01	E-ACT/M-CAC-815	13
	EACT/MCAC02	E-ACT/M-CAC-685	
	EACT/MCAC03	E-ACT/M-CAC-486	
	EACT/MCAC04	E-ACT/M-CAC-483	
	EACT/MCAC05	E-ACT/M-CAC-394	
	EACT/MCAC06	E-ACT/M-CAC-380	
	EACT/MCAC07	E-ACT/M-CAC-378	
	EACT/MCAC08	E-ACT/M-CAC-375	
	EACT/MCAC09	E-ACT/M-CAC-342	
	EACT/MCAC10	E-ACT/M-CAC-313	
	EACT/MCAC11	E-ACT/M-CAC-236	
	EACT/MCAC12	E-ACT/M-CAC-231	

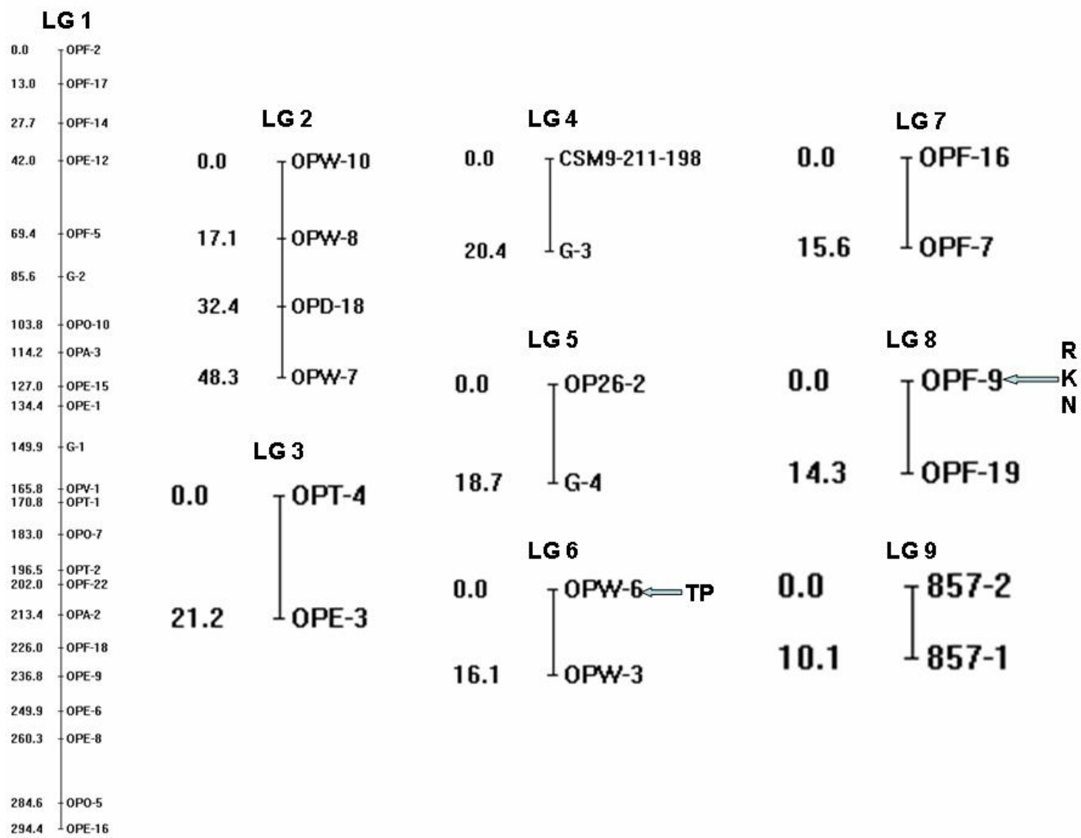
	EACT/MCAC13	E-ACT/M-CAC-179	
E-ACT/M-CTA	EACT/MCTA01	E-ACT/M-CTA-530	8
	EACT/MCTA02	E-ACT/M-CTA-437	
	EACT/MCTA03	E-ACT/M-CTA-273	
	EACT/MCTA04	E-ACT/M-CTA-234	
	EACT/MCTA05	E-ACT/M-CTA-220	
	EACT/MCTA06	E-ACT/M-CTA-185	
	EACT/MCTA07	E-ACT/M-CTA-135	
	EACT/MCTA08	E-ACT/M-CTA-70	
E-ACT/M-CTG	EACT/MCTG01	E-ACT/M-CTG-540	10
	EACT/MCTG02	E-ACT/M-CTG-240	
	EACT/MCTG03	E-ACT/M-CTG-180	
	EACT/MCTG04	E-ACT/M-CTG-125	
	EACT/MCTG05	E-ACT/M-CTG-120	
	EACT/MCTG06	E-ACT/M-CTG-117	
	EACT/MCTG07	E-ACT/M-CTG-96	
	EACT/MCTG08	E-ACT/M-CTG-95	
	EACT/MCTG09	E-ACT/M-CTG-86	
	EACT/MCTG10	E-ACT/M-CTG-75	
E-ACT/M-CTC	EACT/MCTC01	E-ACT/M-CTC-355	8
	EACT/MCTC02	E-ACT/M-CTC-348	
	EACT/MCTC03	E-ACT/M-CTC-275	
	EACT/MCTC04	E-ACT/M-CTC-272	
	EACT/MCTC05	E-ACT/M-CTC-234	
	EACT/MCTC06	E-ACT/M-CTC-156	
	EACT/MCTC07	E-ACT/M-CTC-125	
	EACT/MCTC08	E-ACT/M-CTC-73	
E-ACT/M-CTT	EACT/MCTT01	E-ACT/M-CTT-395	8
	EACT/MCTT02	E-ACT/M-CTT-308	
	EACT/MCTT03	E-ACT/M-CTT-304	
	EACT/MCTT04	E-ACT/M-CTT-262	
	EACT/MCTT05	E-ACT/M-CTT-172	
	EACT/MCTT06	E-ACT/M-CTT-152	
	EACT/MCTT07	E-ACT/M-CTT-75	
	EACT/MCTT08	E-ACT/M-CTT-67	
E-AGG/M-CAG	EAGG/MCAG01	E-AGG/M-CAG-370	12
	EAGG/MCAG02	E-AGG/M-CAG-225	
	EAGG/MCAG03	E-AGG/M-CAG-200	
	EAGG/MCAG04	E-AGG/M-CAG-168	
	EAGG/MCAG05	E-AGG/M-CAG-125	
	EAGG/MCAG06	E-AGG/M-CAG-100	
	EAGG/MCAG07	E-AGG/M-CAG-95	
	EAGG/MCAG08	E-AGG/M-CAG-88	
	EAGG/MCAG09	E-AGG/M-CAG-83	
	EAGG/MCAG10	E-AGG/M-CAG-78	
	EAGG/MCAG11	E-AGG/M-CAG-77	
	EAGG/MCAG12	E-AGG/M-CAG-57	
E-AAC/M-CAGT	EAAC/MCAGT01	E-AAC/M-CAGT-460	8
	EAAC/MCAGT02	E-AAC/M-CAGT-360	
	EAAC/MCAGT03	E-AAC/M-CAGT-325	
	EAAC/MCAGT04	E-AAC/M-CAGT-265	
	EAAC/MCAGT05	E-AAC/M-CAGT-183	
	EAAC/MCAGT06	E-AAC/M-CAGT-158	
	EAAC/MCAGT07	E-AAC/M-CAGT-152	

	EAAC/MCAGT08	E-AAC/M-CAGT-136	
E-AGC/M-CAA	EAGC/MCAA01	E-AGC/M-CAA-425	10
	EAGC/MCAA02	E-AGC/M-CAA-335	
	EAGC/MCAA03	E-AGC/M-CAA-330	
	EAGC/MCAA04	E-AGC/M-CAA-270	
	EAGC/MCAA05	E-AGC/M-CAA-255	
	EAGC/MCAA06	E-AGC/M-CAA-192	
	EAGC/MCAA07	E-AGC/M-CAA-120	
	EAGC/MCAA08	E-AGC/M-CAA-98	
	EAGC/MCAA09	E-AGC/M-CAA-80	
	EAGC/MCAA10	E-AGC/M-CAA-77	
E-ACA/M-CAG	EACA/MCAG01	E-ACA/M-CAG-392	7
	EACA/MCAG02	E-ACA/M-CAG-293	
	EACA/MCAG03	E-ACA/M-CAG-167	
	EACA/MCAG04	E-ACA/M-CAG-152	
	EACA/MCAG05	E-ACA/M-CAG-127	
	EACA/MCAG06	E-ACA/M-CAG-100	
	EACA/MCAG07	E-ACA/M-CAG-87	
E-ACA/M-CTA	EACA/MCTA01	E-ACA/M-CTA-500	10
	EACA/MCTA02	E-ACA/M-CTA-270	
	EACA/MCTA03	E-ACA/M-CTA-173	
	EACA/MCTA04	E-ACA/M-CTA-172	
	EACA/MCTA05	E-ACA/M-CTA-142	
	EACA/MCTA06	E-ACA/M-CTA-141	
	EACA/MCTA07	E-ACA/M-CTA-138	
	EACA/MCTA08	E-ACA/M-CTA-127	
	EACA/MCTA09	E-ACA/M-CTA-116	
	EACA/MCTA10	E-ACA/M-CTA-61	
E-AGC/M-CTG	EAGC/MCTG01	E-AGC/M-CTG-720	11
	EAGC/MCTG02	E-AGC/M-CTG-550	
	EAGC/MCTG03	E-AGC/M-CTG-360	
	EAGC/MCTG04	E-AGC/M-CTG-320	
	EAGC/MCTG05	E-AGC/M-CTG-300	
	EAGC/MCTG06	E-AGC/M-CTG-230	
	EAGC/MCTG07	E-AGC/M-CTG-170	
	EAGC/MCTG08	E-AGC/M-CTG-160	
	EAGC/MCTG09	E-AGC/M-CTG-60	
	EAGC/MCTG10	E-AGC/M-CTG-40	
	EAGC/MCTG11	E-AGC/M-CTG-20	
E-AGC/M-CTT	EAGC/MCTT01	E-AGC/M-CTT-620	12
	EAGC/MCTT02	E-AGC/M-CTT-480	
	EAGC/MCTT03	E-AGC/M-CTT-440	
	EAGC/MCTT04	E-AGC/M-CTT-410	
	EAGC/MCTT05	E-AGC/M-CTT-380	
	EAGC/MCTT06	E-AGC/M-CTT-360	
	EAGC/MCTT07	E-AGC/M-CTT-260	
	EAGC/MCTT08	E-AGC/M-CTT-230	
	EAGC/MCTT09	E-AGC/M-CTT-180	
	EAGC/MCTT10	E-AGC/M-CTT-160	
	EAGC/MCTT11	E-AGC/M-CTT-120	
	EAGC/MCTT12	E-AGC/M-CTT-100	
E-AGC/M-CAG	EAGC/MCAG01	E-AGC/M-CAG-940	16
	EAGC/MCAG02	E-AGC/M-CAG-910	
	EAGC/MCAG03	E-AGC/M-CAG-810	

	EAGC/MCAG04	E-AGC/M-CAG-800	
	EAGC/MCAG05	E-AGC/M-CAG-725	
	EAGC/MCAG06	E-AGC/M-CAG-710	
	EAGC/MCAG07	E-AGC/M-CAG-490	
	EAGC/MCAG08	E-AGC/M-CAG-450	
	EAGC/MCAG09	E-AGC/M-CAG-420	
	EAGC/MCAG10	E-AGC/M-CAG-390	
	EAGC/MCAG11	E-AGC/M-CAG-380	
	EAGC/MCAG12	E-AGC/M-CAG-250	
	EAGC/MCAG13	E-AGC/M-CAG-200	
	EAGC/MCAG14	E-AGC/M-CAG-190	
	EAGC/MCAG15	E-AGC/M-CAG-175	
	EAGC/MCAG16	E-AGC/M-CAG-130	
E-AGC/M-CAC	EAGC/MCAC01	E-AGC/M-CAC-800	16
	EAGC/MCAC02	E-AGC/M-CAC-790	
	EAGC/MCAC03	E-AGC/M-CAC-730	
	EAGC/MCAC04	E-AGC/M-CAC-715	
	EAGC/MCAC05	E-AGC/M-CAC-660	
	EAGC/MCAC06	E-AGC/M-CAC-650	
	EAGC/MCAC07	E-AGC/M-CAC-590	
	EAGC/MCAC08	E-AGC/M-CAC-560	
	EAGC/MCAC09	E-AGC/M-CAC-400	
	EAGC/MCAC10	E-AGC/M-CAC-330	
	EAGC/MCAC11	E-AGC/M-CAC-310	
	EAGC/MCAC12	E-AGC/M-CAC-230	
	EAGC/MCAC13	E-AGC/M-CAC-210	
	EAGC/MCAC14	E-AGC/M-CAC-215	
	EAGC/MCAC15	E-AGC/M-CAC-205	
	EAGC/MCAC16	E-AGC/M-CAC-180	
E-ACC/M-CAC	EACC/MCAC01	E-ACC/M-CAC-900	16
	EACC/MCAC02	E-ACC/M-CAC-865	
	EACC/MCAC03	E-ACC/M-CAC-775	
	EACC/MCAC04	E-ACC/M-CAC-700	
	EACC/MCAC05	E-ACC/M-CAC-650	
	EACC/MCAC06	E-ACC/M-CAC-640	
	EACC/MCAC07	E-ACC/M-CAC-550	
	EACC/MCAC08	E-ACC/M-CAC-420	
	EACC/MCAC09	E-ACC/M-CAC-400	
	EACC/MCAC10	E-ACC/M-CAC-340	
	EACC/MCAC11	E-ACC/M-CAC-320	
	EACC/MCAC12	E-ACC/M-CAC-195	
	EACC/MCAC13	E-ACC/M-CAC-170	
	EACC/MCAC14	E-ACC/M-CAC-140	
	EACC/MCAC15	E-ACC/M-CAC-130	
	EACC/MCAC16	E-ACC/M-CAC-120	
E-ACC/M-CAG	EACC/MCAG01	E-ACC/M-CAG-775	13
	EACC/MCAG02	E-ACC/M-CAG-650	
	EACC/MCAG03	E-ACC/M-CAG-490	
	EACC/MCAG04	E-ACC/M-CAG-480	
	EACC/MCAG05	E-ACC/M-CAG-400	
	EACC/MCAG06	E-ACC/M-CAG-370	
	EACC/MCAG07	E-ACC/M-CAG-310	
	EACC/MCAG08	E-ACC/M-CAG-270	
	EACC/MCAG09	E-ACC/M-CAG-240	

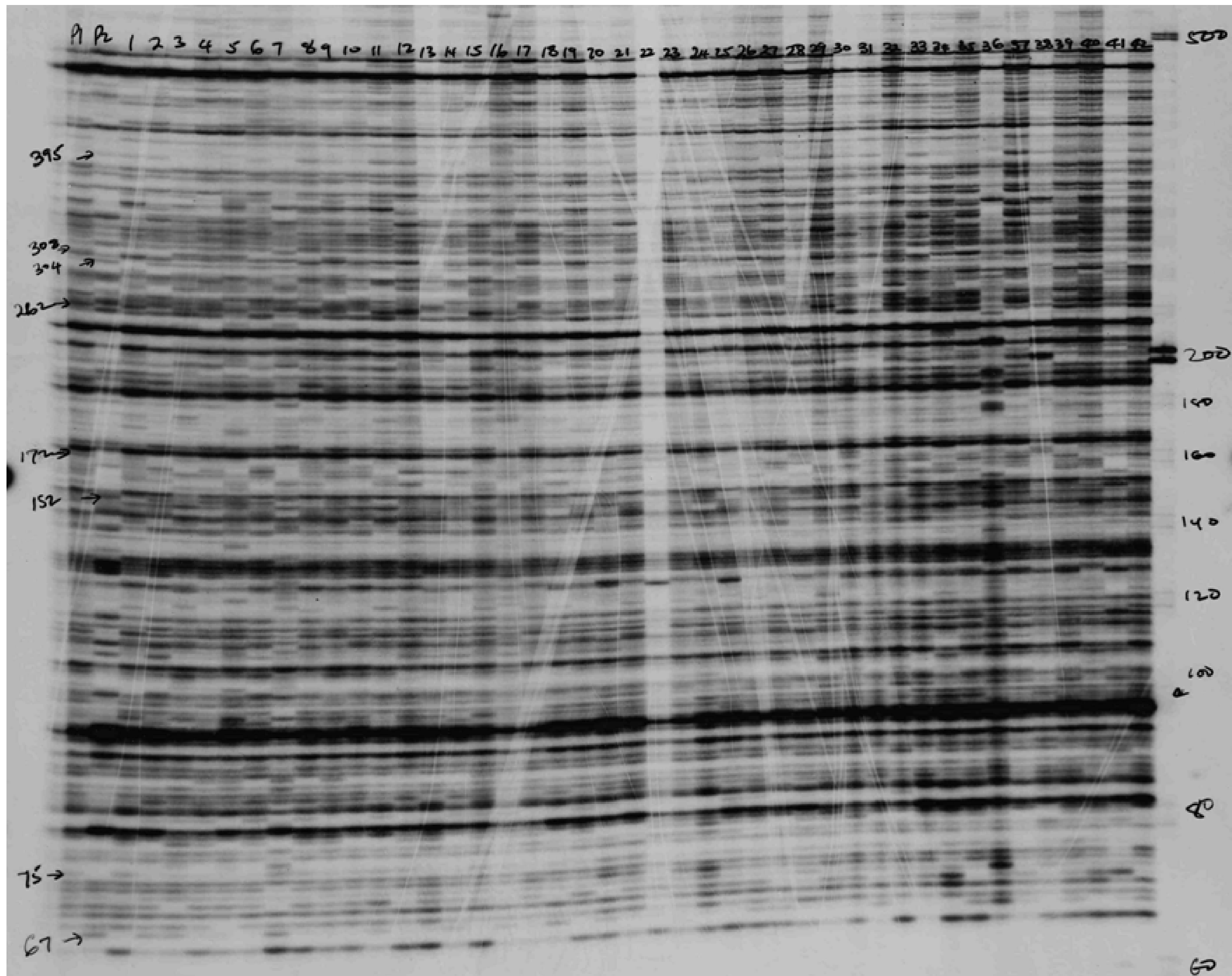
	EACC/MCAG10	E-ACC/M-CAG-230	
	EACC/MCAG11	E-ACC/M-CAG-220	
	EACC/MCAG12	E-ACC/M-CAG-210	
	EACC/MCAG13	E-ACC/M-CAG-180	
TOTAL			**196**

Appendix 15. Incomplete Linkage map of tea constructed using root knot nematode mapping population St 526 (TRFCA SFS150 x TRFK 303/577) progeny based on 39 RAPD and 1 SSR marker. Arrows show position of RKN susceptibility and TP QTLs on LG 8 and LG 6, respectively.

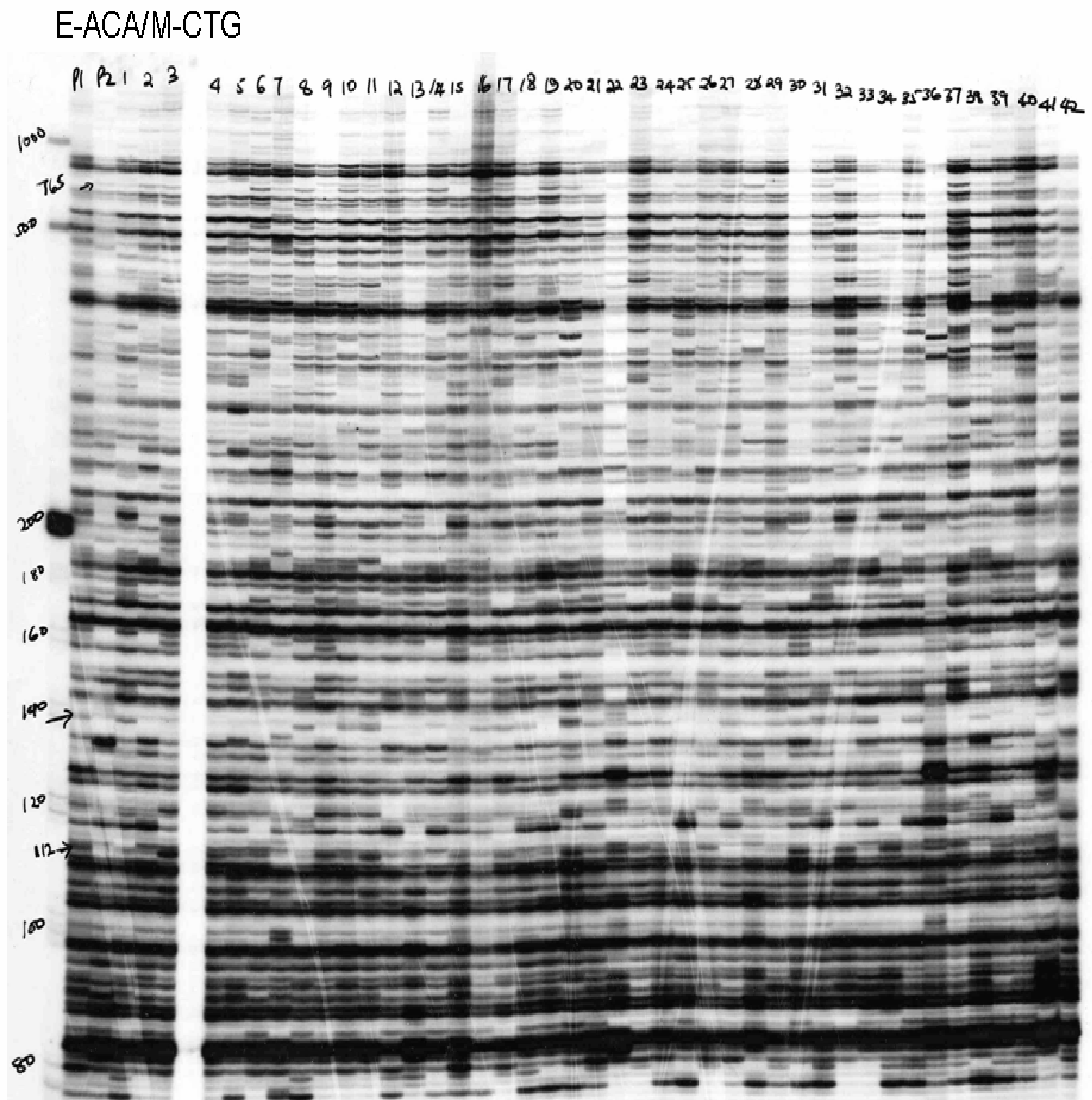


Appendix 16. AFLP fingerprint of St. 463 progeny generated by primer pair

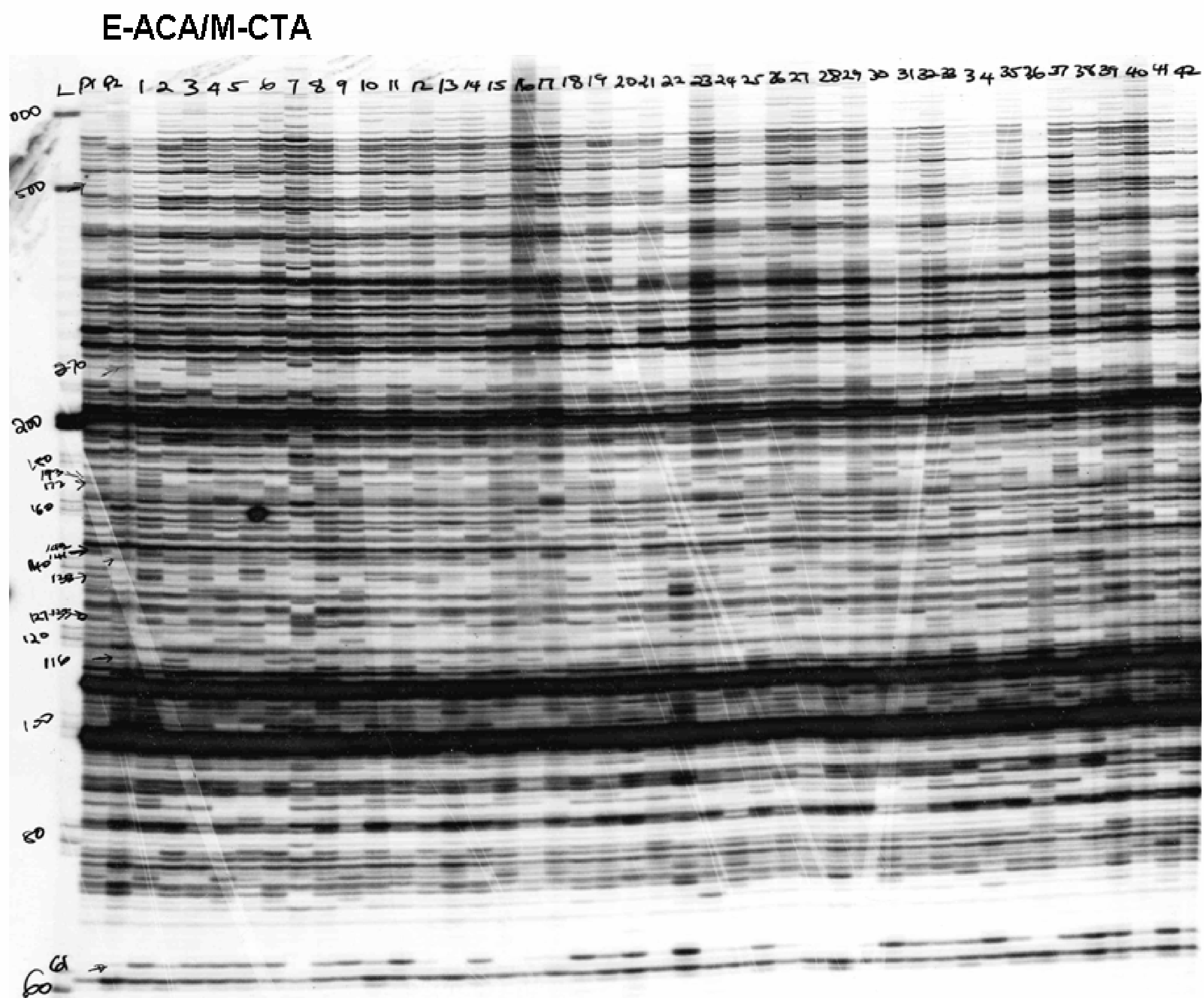
AFLP-1



Appendix 17. AFLP fingerprint of St. 463 progeny generated by primer pair E-ACA/M-CTG

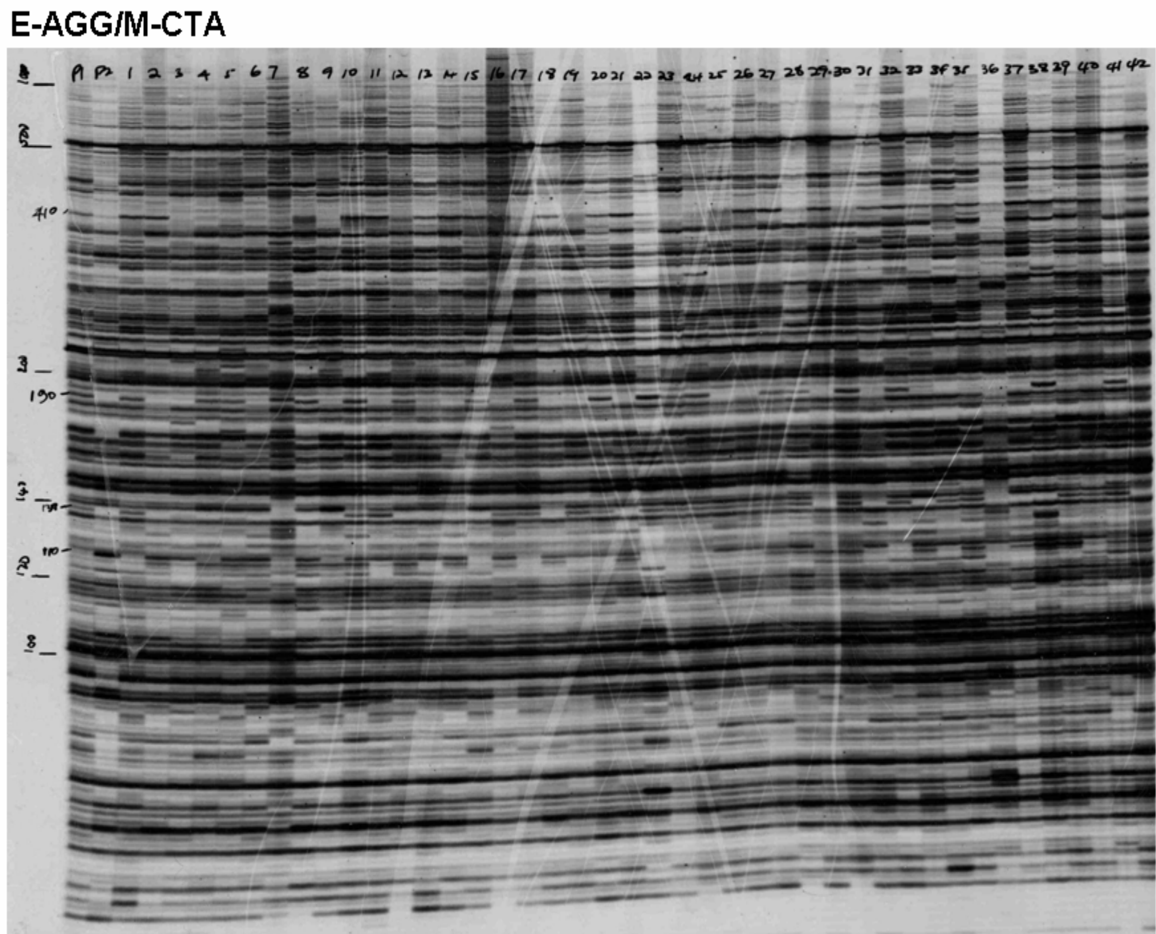


Appendix 18. AFLP fingerprint of St. 463 progeny generated by primer pair E-ACA/M-

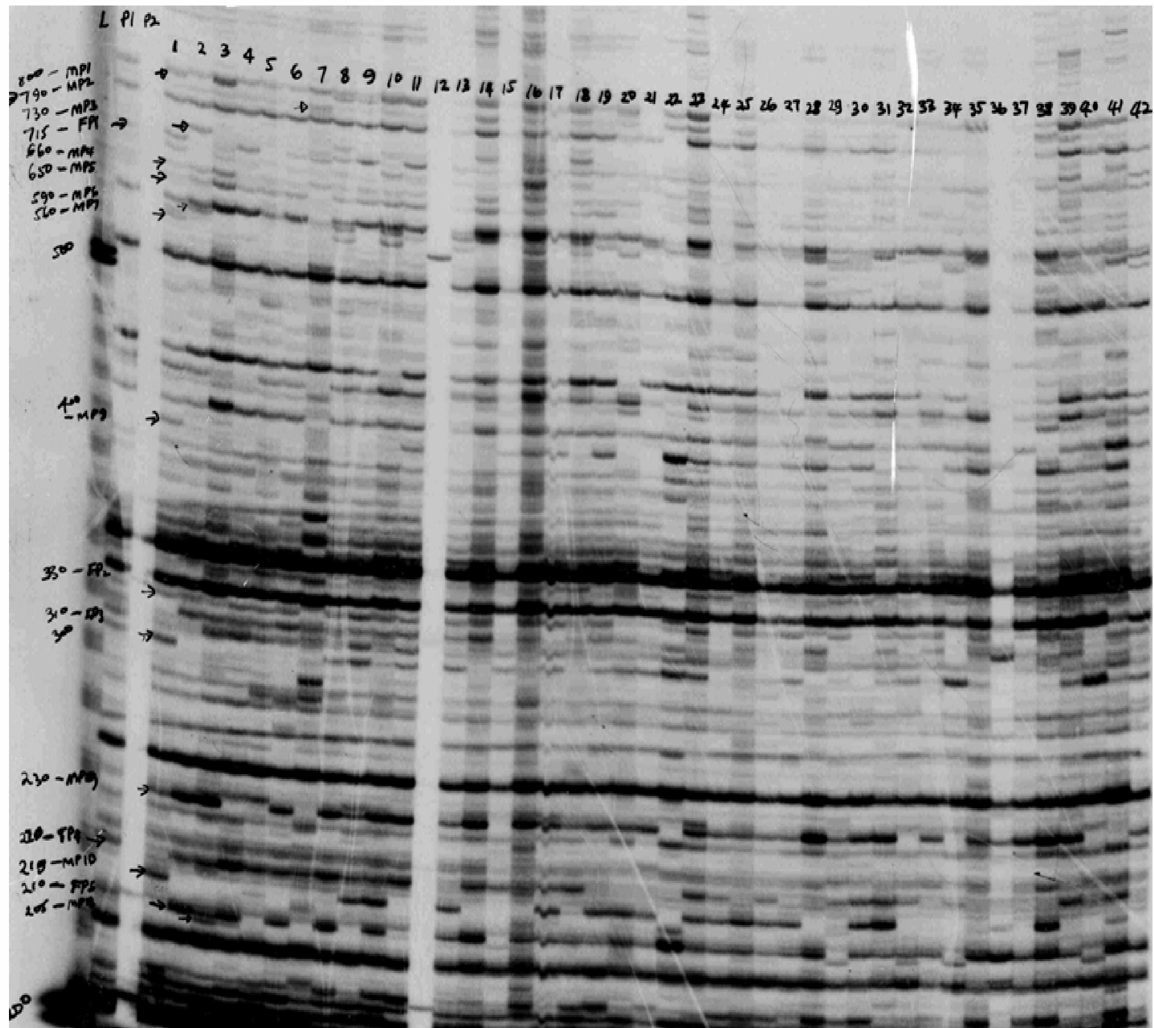


CTA

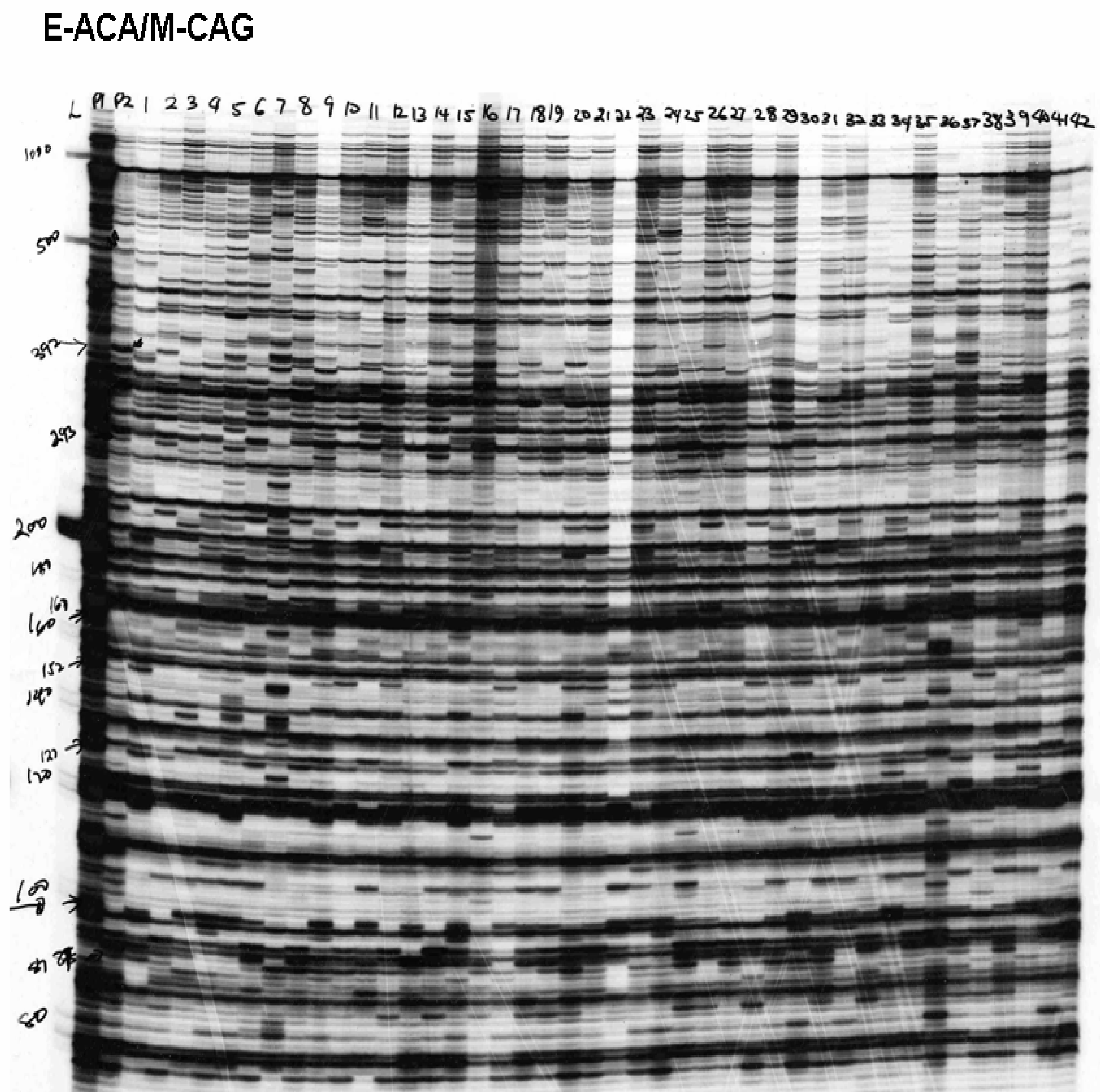
Appendix 19. AFLP fingerprint of St. 463 progeny generated by primer pair E-AGG/M-CTA



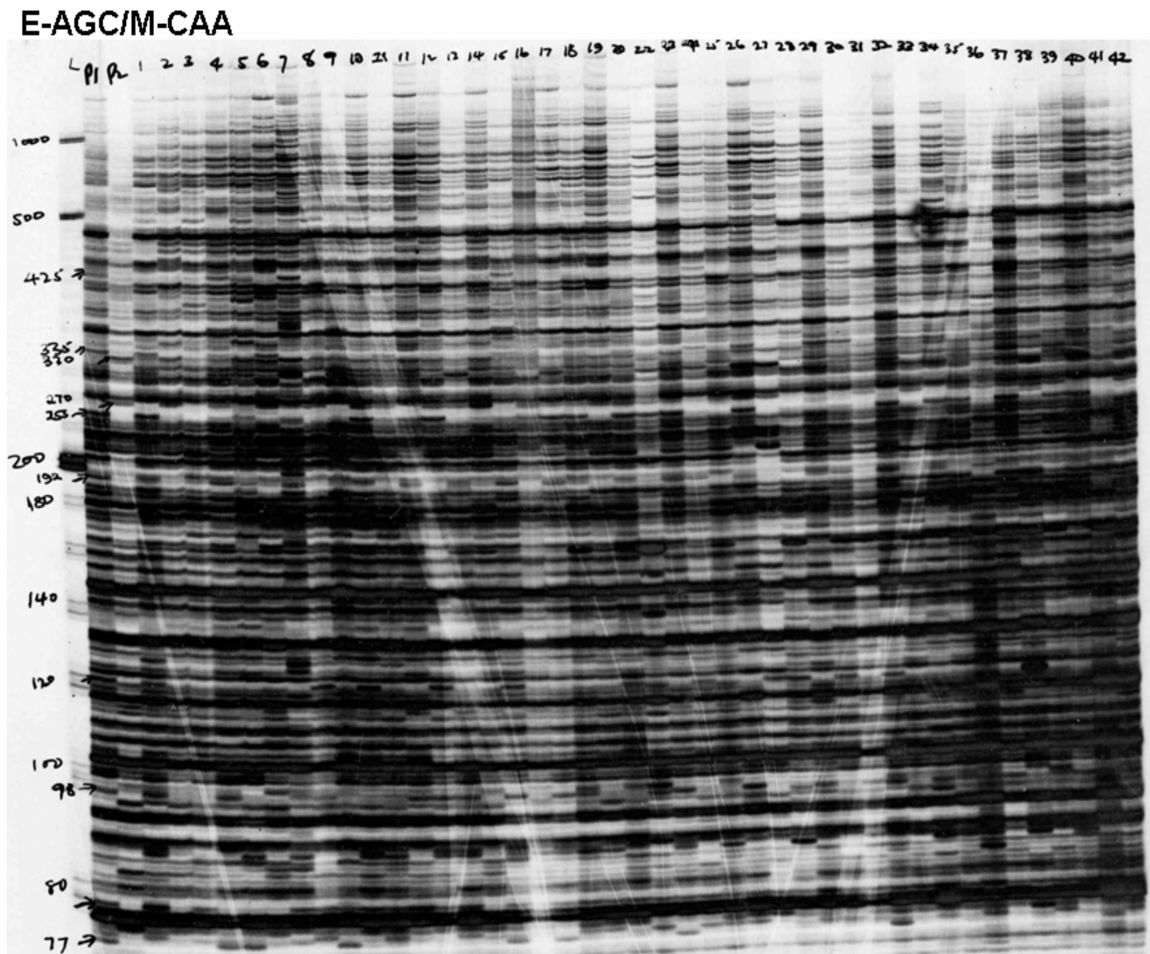
Appendix 20. AFLP fingerprint of St. 463 progeny generated by primer pair E-AGC/M-CAC



Appendix 21. AFLP fingerprint of St. 463 progeny generated by primer pair E-ACA/M-CAG



Appendix 22. AFLP fingerprint of St. 463 progeny generated by primer pair E-AGC/M-CAA



Appendix 23. Pattern of SSR fragments generated on F_1 progeny of St. 463 by primers CAMSINF&R2 and CAMSINF&R8 using 3% Metaphor agarose

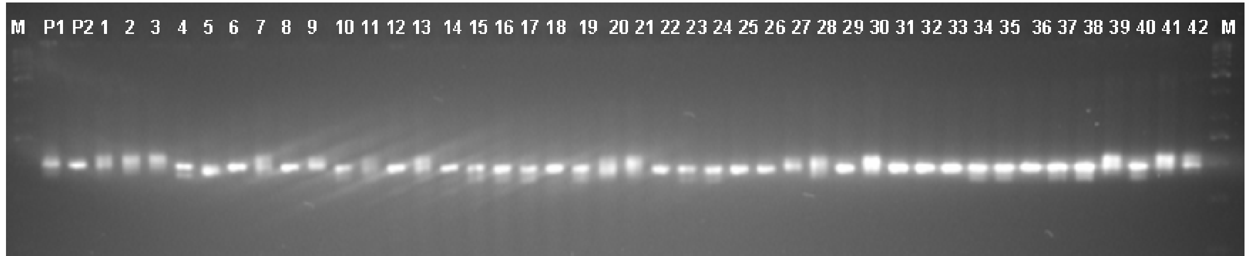
M2F&M2R

M8F&M8R

P1= low pubescent parent; P2= high pubescent parent; 1 to 42 F1 progeny; left and right Ms are 50 and 100 bp molecular weight ladders, respectively.

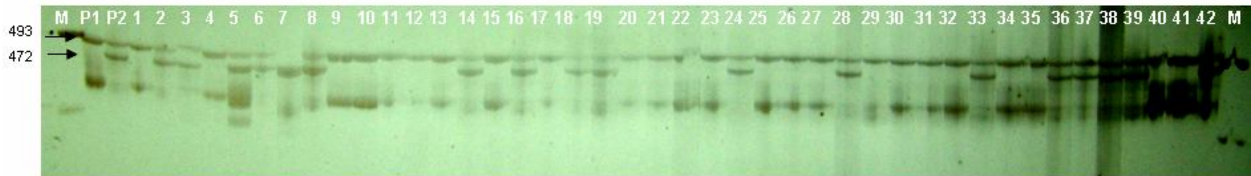
Appendix 24. Pattern of SSR fragments generated on F_1 progeny of St. 463 by primers CAMSINF&R9 and CAMSINF&R10 using 3% Metaphor agarose

M9F&M9R



M10F&M10R

Appendix 25. Pattern of SSR fragments generated on F_1 progeny of St. 463 by primers CAMSINM4 at locus CS-M4-472-493 (A = 493; B = 472) as resolved by 6% PAGE and



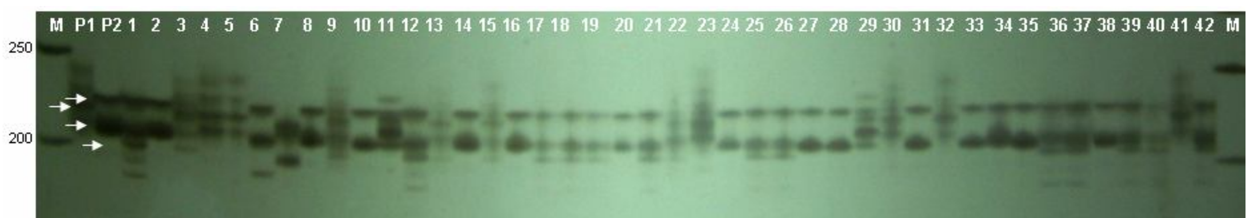
Key: St 463: P1 = SFS150 (F), P2 = S15/10 (M), 1-42 clonal progeny and M is 50 bp molecular wt ladder.

visualized by silver-staining

Appendix 26. Pattern of SSR fragments generated on F_1 progeny of St. 463 by primers CAMSINM8 showing several alleles as resolved by 6% PAGE and visualized by silver-staining

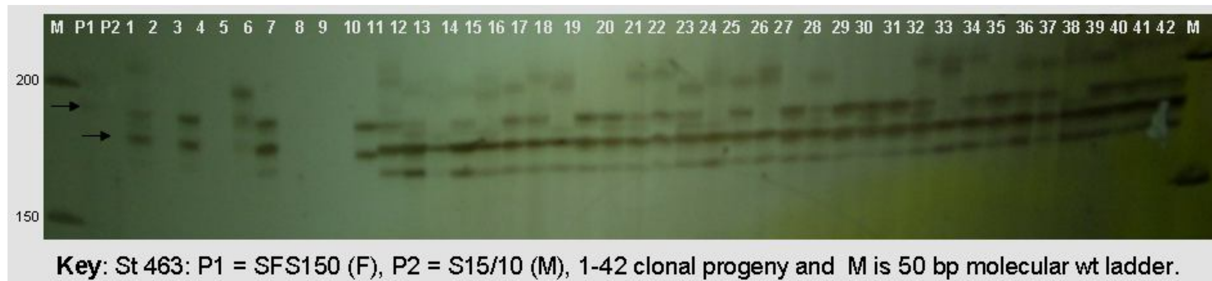
Key: St 463: P1 = SFS150 (F), P2 = S15/10 (M), 1-42 clonal progeny and M is 50 bp molecular wt ladder.

Appendix 27. Pattern of SSR fragments generated on F_1 progeny of St. 463 by primers CAMSINM9 at loci CS-M9-237-232 and CS-M9-207-200 as resolved by 6% PAGE and visualized by silver-staining



Key: St 463: P1 = SFS150 (F), P2 = S15/10 (M), 1-42 clonal progeny and M is 50 bp molecular wt ladder.

Appendix 28. Pattern of SSR fragments generated on F_1 progeny of St. 463 by primers CAMSINM10 at locus CS-M10-190-180 (A = 190bp; B = 180bp) as resolved by 6% PAGE



and visualized by silver-staining

Appendix 29. List of publications from this thesis

1. Kamunya, S.M., R.C. Muoki, F.N. Wachira and R.S Pathak (2007). Inheritance of yield, drought tolerance and quality traits in tea (*Camellia sinensis* (L.) O. Kuntze). ***Tea*** **28(2)**: 20-29.
2. Kamunya, S.M., F.N. Wachira, R.S. Pathak, R.K. Sharma, R. Chalo, R. Korir, V. Sharma, R. Kumar and P.S Ahuja. (2008). Quantitative Trait Loci (QTL) Mapping for Yield in Tea. **Poster** presented during the Annual Research Week and International Conference held at Egerton University on 16-18th September 2008.
3. Kamunya S.M., F.N. Wachira, R.S. Pathak, R.C. Muoki, J.K. Wanyoko, W.K. Ronno and R.K. Sharma. (2009). Quantitative genetic parameters in tea (*Camellia sinensis* (L.) O. Kuntze): I. Combining abilities for Yield, Drought Tolerance and Quality Traits. **African Journal of Plant Science, 3(5):93-101**.
4. Kamunya, S.M., F.N. Wachira, R.S. Pathak, R.K., Sharma, R. Korir, V. Sharma, R. Kumar, P. Bhardwaj, R. Chalo and P.S. Ahuja. (2009). Mapping Quantitative Trait Loci for Yield in Tea, *Camellia sinensis* (L.) O. Kuntze. 5th Moi University international conference held on 4-8th August 2009 in Eldoret, Kenya.
5. Kamunya S.M., F.N. Wachira, P.O. Owuor, R.S. Pathak, J.K. Wanyoko, R.K. Sharma, R.K. Muoki. (2010). Quantitative genetic parameters for yield, drought tolerance and some quality traits in tea (*Camellia sinensis* (L.) O. Kuntze. **Research Journal of Agricultural Sciences, 1(2):53-65**.
6. Kamunya S.M., F.N. Wachira, R.S. Pathak, R.C. Muoki, and W.K. Ronno (2010). **Tea Improvement in Kenya. Book Chapter** entitled **"Tea growing countries of the World".** Being published by Springer.
7. Kamunya S.M., F.N. Wachira, R.S. Pathak, R. Korir, V. Sharma, R. Kumar, P. Bhardwaj, R. Chalo, P.S. Ahuja and R.K. Sharma (2010). Genomic mapping and testing for Quantitative trait loci in tea (*Camellia sinensis* (L.) O. Kuntze)**. Tree Genetics and Genomes DOI10.1007/s11295-010-0301-2**.