INHERITANCE OF CATECHIN AND CAFFEINE CONTENT IN KENYAN TEA (*Camellia sinensis* (L.) O. Kuntze)

BY

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DECLARATION

DECLARATION BY THE STUDENT

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DEDICATION

This work is dedicated to my parents Mr. Henry Lubanga, Mrs Jane Lubanga and my siblings Christine, Dickens, Virginia and Gilbert for their support.

ABSTRACT

Diallel designs are used in many breeding programmes because of the important genetic information they offer to plant breeders. Eight biochemical traits were studied to estimate the general combining abilities (GCA), specific combining abilities (SCA), heterosis and stability of 16 F_1 crosses arising from a 4 x 4 diallel cross of the 4 tea clones evaluated at Timbilil and Kangaita TRFK experimental stations. Data analysis was carried out using GLM procedure from SAS at each of the site. There were significant ($p<0.05$) differences between the genotypes for all the traits under study at both sites. At Timbilil, general combining ability (GCA) effects were significant ($p<0.05$) for GA, EGC, CAFF, ECG, EGCG and TC, while at Kangaita, all traits had significant ($p<0.05$) GCA effects except C, implying that these traits are governed by additive gene effects. EPK TN14-3 had the best general combining ability at both Timbilil and Kangaita for nearly all the assessed traits. Specific combining ability at Timbilil was significant ($p<0.05$) for EGC, CAFF, EC, EGCG, TC, while at Kangaita, significant ($p<0.05$) SCA effects was exhibited in all the traits except CAFF. Maternal effects were significant ($p<0.05$) for EGC, EGCG and TC at Timbilil. At Kangaita maternal effects were significant ($p<0.05$) for EC signifying importance of the choice of female parents in breeding programmes targeting these traits. Both GGE biplot and the AMMI stability methods revealed that cross TRFK 6/8 x EPK TN14-3 (476) was the most stable cross for TC, EGCG and caffeine. The study demonstrated that quantitative genetic parameters such as additive, non-additive gene and maternal effects have considerable influence on the inheritance of catechins and caffeine, and consequently on tea quality targeting high value diversified tea products as well as advanced tea breeding programmes.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of variance
GA	Gallic acid
+C	(+) Catechin
EC	(-) Epicatechin
ECG	(-) Epicatechingallate
EGC	(-) Epigallocatechin
EGCG	(-) Epigallocatechin gallate
TC	Total catechins
HPLC	High performance liquid chromatography
TRFK	Tea Research Foundation of Kenya
AHP	African Highland Produce
UTK	Unilever Tea, Kenya
JFK	James Finlay, Kenya
EPK	East African produce, Kenya

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CHAPTER ONE

INTRODUCTION

1.1 Background information

Tea (*Camellia sinensis* (L.) O. Kuntze) is an evergreen perennial beverage crop that belongs to the family *Theaceae* and genus *Camellia* that has over 200 reported species (Chang and Bartholomew, 1984). It is a highly outcrossing and strongly but not absolutely self-incompatible tree species (Wachira and Kamunya, 2005). The processed tea can either be green, or black. Green tea differs from black tea in the way it is processed. Black tea goes through oxidation processing characterized by the enzyme polyphenol oxidase. Tea plays a very important role in the economies of tea-growing countries (Kamunya *et al.,* 2008). Tea is grown in areas which differ widely in elevation, climate and edaphic conditions. These differences have profound effect on the growth, productivity and quality of tea. Due to global and climatic changes, the frequency and the longevity of drought have increased especially in the traditional tea growing areas.

Different tea clones have different numbers, relative amounts and diversity in catechins (Schijlen *et al.,* 2004). This variation is used as biochemical markers to show diversity in tea. An understanding of the biochemical composition in the plant system might yield information for plant genetic manipulation and crop management strategies that might improve crop value in future (Cheruiyot *et al.,* 2008).

Tea contains different chemical composition which includes polyphenols, alkaloids, amino acids, carbohydrates, vitamins, proteins, chlorophyll, volatile compounds, minerals and trace elements (Hilton, 1973; Mondal *et al*, 2004). Polyphenols are the

main bioactive molecules in tea (Balentine *et al*, 1999; Cabrera *et al*, 2003). The major polyphenols found in green tea are catechins which include: Gallic acid, (-) -epigallocatechin (EGC), (-) -epicatechin (EC), epigallocatechin-3-gallate (EGCG), epicatechin-3-gallate (ECG), (+) catechin and (+) gallocatechin (Ferruzzi and Green, 2005). EGCG is usually of high concentration followed by EGC, ECG and EC in decreasing order (Nakabayashi, 1991). Catechins and gallocatechin are present in trace amounts (Chu and Juneja, 1997). Kenyan tea is renowned for its high quality and safety from harmful pesticide residues. The high quality of black tea is attributed to high levels of biochemical constituents (Singh *et al.*, 1999). For example, high catechin teas have been observed to harbour high black tea quality (Owuor and Obanda, 2007; Wachira and Kamunya, 2005). These polyphenols are usually of high concentration in the bud of tea and continue decreasing as the leaves age (Willson, 1999). The oxidation products of these catechins are theaflavins and thearubigins which are the main components responsible for briskness, brightness, taste, strength and colour of black tea (Woods and Roberts, 1964). Catechins can also be used as biochemical markers in diversity studies of tea (Magoma *et al.*, 2000). The total green leaf catechin concentration as well as the ratio of dihydroxylated to trihydroxylated catechins can be used to elucidate genetic difference in tea clones. The determination of the number, diversity and the relative amounts of catechins can be used as biochemical markers to indicate the diversity in the tea clones.

1.2 Statement of the problem

Tea breeding generally takes a long time compared to other crops. It takes about 21-26 years to obtain an improved seedling population. In addition to that, superior clonal plants may take between 8 to 10 years to be extracted from such a population.

Information on the inheritance patterns of quantitative traits in tea is scanty. Only a few studies on combining ability and inheritance of important quantitative traits in tea have been conducted. Kamunya (2010) for example, studied the inheritance of some quantitative traits in tea such as drought tolerance, yield, thearubigins, theaflavins and bud weight. The major constrain of poor quality in tea can partially be overcome through breeding of high quality varieties. Catechins and caffeine are important biochemical indicators of quality in tea (Wright *et al*., 2000). Breeding of tea with high quality targets the selection of populations with high functional components such as catechins and caffeine (Zongmao, 1995). In addition to that, diversification of tea products through value addition is the most promising approach for tea companies to mitigate the impacts of low world market prices and high domestic production costs (Kamunya and Wachira, 2005). Studies have shown that tea has great health benefits (Friedman, 2007). The majority of beneficial effects of tea have been attributed to primary polyphenol constituents of green tea which consists of mainly catechins and caffeine (Perva-Uzunalic *et al*., 2006). Therefore, breeding and selection of tea with high levels of catechins will open an avenue for a special market and also add value for tea crop in the industry.

Information on the combining ability, heterosis and mode of gene action of catechins and caffeine in tea is largely lacking in the current tea breeding programs. By estimating the degree of heterosis and combining ability, information on the nature of gene action can be known and desirable parents identified and other important traits quantified and selected (Can *et a1*., 1997).

A plant's phenotype is the sum of its genetic constitution and the environment in which it is grown. Catechins and caffeine are important quantitative characters in tea which are influenced by the environment. A study on the genetic stability of caffeine and catechins

has so far not been conducted and there is a need to carry out the study in different environments.

1.3 Justification

Tea (*Camellia sinensis* (L) O. Kuntze) is very important cash crop in Kenya since it is the main foreign exchange earner and also a source of employment to many people (Mbadi and Owuor, 2008). Scientific investigations have indicated that catechins and caffeine have a great potential to be developed as therapeutic agents because of the strong antibiotic properties they possess (Kohri, *et al*., 2001). Besides, catechins and caffeine are attributes used by plant breeders as a basis in selection of tea with high quality. This study is very important as it will provide the information that is currently missing to plant breeders.

Studies conducted previously have shown great variation in the expression of these important biochemicals in different tea genotypes (Magoma *et al*., 2001). Therefore large-scale efforts in tea product diversification demand that the inheritance patterns of the native biochemicals be properly understood to enable the development of superior varieties that are not only high yielding but also of high pharmacological value.

World black tea production has been higher than world demand, while cost of production has continued to rise (Herath and Weersink, 2007). As a result, only producers of high quality black tea sell at good prices. The use of superior quality clones (Kamunya, 2003) can improve the profitability of a tea enterprise, provided other agronomic practices are optimized (Owuor *et al.*, 2009). Tea is an agricultural commodity and hence it is vulnerable to the forces of supply and demand. The

development of tea with high catechins and caffeine content requires precise information on the diversity available.

A successful breeding program is the one that is designed with a view of utilizing the mode of gene action controlling each trait a breeder intends to improve. Knowledge of the genetic control of characters and the role of non-allelic interaction is essential to the breeder when deciding on the selection method and breeding procedure to follow (Esmail, 2007). The likelihood of obtaining superior recombinants is low when parents of unknown heritable traits are crossed. Therefore this study contributes to the addition of information on the combining ability, heterosis and stability of catechins and caffeine in tea which is largely lacking in the current tea breeding programs.

1.4 OBJECTIVES

1.4.1 Broad objective

To study the inheritance and stability of catechins and caffeine in Kenyan tea.

1.4.2 Specific objectives

1. To estimate general and specific combining abilities for catechins and caffeine composition in four parents and their progenies using diallel crosses.
2. To determine the level of heterosis in F_1 progenies for catechins and caffeine.
3. To estimate genetic stability of catechins and caffeine in Kenyan tea.

1.5 HYPOTHESIS

1. There is no significant difference in the estimated general and specific combining abilities for catechins and caffeine composition among the four parents and their progenies.
2. There is no heterosis for catechins and caffeine in the F1 progenies.
3. There is no genetic stability for catechins and caffeine in tea.

CHAPTER TWO

LITERATURE REVIEW

2.1 Botanical classification, distribution and origin of tea

Tea (Camellia sinensis (L.) O. Kuntze) is an evergreen perennial beverage crop which belongs to the genus *Camellia* and family *Theaceae*. The genus *Camellia* is valued due to the presence of caffeine, a purine alkaloid, which acts as a stimulus for the central nervous system in human beings. Tea is diploid with a chromosomal number of 30 (2n=30). Some triploid cultivars have also been reported (Chen *et al.,* 2008). The two main varieties of tea are *Camellia sinensis* var. *assamica* which has relatively large leaves and *Camellia sinensis* var. *sinensis* with small semi-erect leaves. The *Assamica* tea is believed to have originated from the forests of Assam in north-eastern India while the *sinensis* tea from Sichuan province, south-western China (Van der Vossen and Wessel, 2000). The two cultivated taxa can be distinguished based on their morphological, biochemical and molecular properties (Owuor *et al*., 1987; Magoma *et al.,* 2003). *Camellia* is the largest genus of the family *Theaceae* (Sealy, 1958). The genus *Camellia* had 40 species in 1920. However, the number of species increased to 87 in 1958 (Sealy, 1958) and about 267 species were registered in 1982 (Chang and Bartholomew 1984). Taxonomy of the genus *Camellia* has been complicated by the free hybridization between different species, which has led to the formation of many other tea hybrids (Chuangxing, 1988). However, most species are unavailable to scientists for study. Genetic relationships and taxonomy has consequently remained controversial and interest among scientists has seen the discovery of many new species and a revision of taxonomic relationships (Lu and Yang, 1987; Chuangxing, 1988). Currently, *Camellia* is believed to consist of more than 300 species (Mondal *et al*. 2004). Tea (*Camellia sinensis* (L.) O. Kuntze) was first described taxonomically in 1753 by Carl Linnaeus in

Species Plantarum. He referred to tea as *Thea* and then later on, he refined the species into black tea (*Thea bohea*) and green tea (*Thea viridis*). Taxonomists, however by the early 1900s recognized that both green and black tea were both from the same species; *Camellia sinensis* (L.) O. Kuntze. Tea is cultivated to different parts of the world and it is able to grow under diverse climatic conditions ranging from its native mediterranean-type climate to hot humid tropical and subtropical climates (Carr and Stephens, 1992). Cultivation of tea commenced in 793 A.D when the *sinensis* variety was established as a commercial crop (Sealy, 1958). In Burma, Thailand, Laos and Assam (North East India), the assamica type was grown for use as vegetable crop rather than as a beverage (Sealy, 1958). Tea cultivation outside its native range was reported as early as 801 A.D. in Yeinsan, Japan (Weatherstone, 1992). In the later years of the 1800s, both the *assamica* and *sinensis* varieties became the main source of tea worldwide.

2.2 Tea cultivation in Kenya

Tea was introduced to Kenya from India by a colonial settler G.W. Caine in 1903 but commercial planting began in the 1930s (Watts, 1999). Currently, Kenya is the third largest producer of tea in the world after China and India (ITC, 2013). In Kenya, tea is produced by both smallholders and large estates operated by companies such as Unilever Tea, Finlay Tea and Eastern Produce Limited. Estate plantations have production units larger than 20 ha while the smallholders' are smaller units of about 0.25 ha per farmer. The large plantations are organized under the Kenya Tea Growers Association and account for about 40 % of the Kenyan tea production while the smallholders' are organized under the Kenya Tea Development Agency (KTDA) and account for 60%. Currently, tea in Kenya is mainly grown in Kericho, Bomet, Nandi, Kiambu, Murang'a, Nyeri, Kirinyaga, Meru, Kisii and Nyamira counties (TBK, 2013).

These areas have favourable weather patterns suitable for tea growing. The small-scale sectors in these areas have managed to achieve high quality resulting in high auction prices as compared to the multinational companies.

2.3 Tea morphology

Different morphological features of tea have been defined, some of which aid in the characterization of different varieties (Wachira, 1990). The structure of the leaf, growth habits and flowers are the main morphological markers used in the classification of the different taxa of tea (Mondal *et al.,* 2004). The chinary *(sinensis)* tea has small leaves ranging between 3-6 cm long, which are relatively erect, dark-green and with a matt surface. The Assam tea has much larger leaves of about 15-20 cm long which are light-green in colour with a polished surface (De Costa *et al.,* 2007). Assam tea can attain a height of up to 15 m while the China tea shrub can grow up to 8 m when left to grow fresh (De Costa *et al.,* 2007). Under cultivation, tea bushes are usually maintained at a height of between 60 and 100 cm for ease of plucking.

Flowers are present as auxiliary, solitary or up to three in a cluster (De Costa *et al.,* 2007).They are 2.5-3.5 cm in diameter and have six to eight petals. Flowers are pollinated by insects and the wind. Tea is mostly self-sterile and almost entirely a cross-pollinated crop (Wachira and Kamunya, 2005). The fruits are 2–3 cm in diameter, brownish-green in colour when mature and contain one to four spherical or flattened, brown seeds. The fruit ripe in 9–12 months after which the seeds fall to the ground. The tea plant starts bearing fruits 5-6 years after planting.

Tea plants arising from seedlings have a strong taproot with a dense network of feeder roots. Most feeder roots are located in the top 30 cm of soil (De Costa *et al.*, 2007).

Taproots reach a depth of 1.5–3 m and provide good anchorage for the plants (Mondal *et al.,* 2004). The taproot is also important because it stores starch from the sugars produced in the leaves. The more the starch stored in the taproot, the faster the plant can recover from pruning and plucking. Tea plants grown from cuttings generally lack a taproot (De Costa *et al.,* 2007).

Bush vigour, pruning weight, period of recovery from prune, plant height, root mass, root-shoot ratio, plucking point density, dry matter production and partitioning are considered as yield indicator of tea (Mondal *et al.,* 2004). On the other hand, the parameters for quality determination include caffeine, volatile compounds, green leaf pigmentation, leaf pubescence, total catechin and total tannin contents (Mondal *et al*., 2004).

2.4 Agronomy and climatic requirements of tea

2.4.1 Climate

Tea is mainly cultivated in tropical and subtropical climates. Tea grows best under high and evenly distributed rainfall which range from 1150 to 1400 mm per year (Carr, 1972). In areas where rainfall is less than 1,150 mm per annum and which experience long and hot dry spells, irrigation is normally recommended (Carr and Stephens, 1992).

Tea grows under a wide range of temperatures which range from 18 to 20 °C (Carr and Stephens, 1992). Favourable conditions in the East African highlands where tea is planted include a temperature of between 15–25^{o}C. Some cultivars like the *sinensis* varieties can however tolerate lower temperatures. Tea also requires high relative

humidity of between 80–90%. When the air is too dry, shoots form dormant buds and the plant stops growing.

2.4.2 Soils

Tea is grown on a wide range of soil types. Tea grows well in deep, well drained soils with good structures which are essential for vigorous production. Most soils used for tea production are highly weathered and leached soils and moist throughout the year. The degree of leaching and hence the character of the resulting soil depends on rainfall, temperature and the age of the soil. The most important soil physical requirement for tea plant production is a deep and well drained soil, with a minimum depth of two metres and an aggregated or crumb soil structure with about 50% pore spaces (Dey, 1969). With shallow soils it is important that soil moisture is maintained throughout the dry season. Tea grows on soils of nearly any texture ranging from sandy loam to clays including silts and loams of all types. However, the lighter sandy soils have a lower field capacity than the heavier clay soils and also they require a good distribution of rainfall and nutrients.

Soil properties which lead to high tea productivity include soil pH of between 4.5 and 5.5 (Goswami *et al*., 2001), soil depth and organic carbon (Anandacoomaraswamy *et al.,* 2001). High organic matter content is also an important factor contributing to the growth of tea. Studies have shown that soils under tea agro-ecosystems have considerably higher organic matter and nutrient contents than those of other land use systems primarily due to differences in management and crop residue recycling (Tchienkoua and Zech, 2004).

Land preparation prior to tea establishment is critical. Good maintenance of the soil physical characteristics during the cultivation of tea avoids problems associated with soil compaction and soil erosion (Illukpitiya *et al*., 2004; Coomaraswamy *et al*., 1988).

2.5 Types of tea

Tea is mainly classified according to the method of fermentation (Takeo, 1992). The three main types of tea which are manufactured are black, green and oolong teas (Wang *et al*., 2000). Black tea is made from leaves that are completely fermented or oxidized after they have been dried. This is achieved by the deliberate aeration of the leaf which leads to fermentation of the flavanols into theaflavins and thearubigins with the help of polyphenol oxidase (Friedman *et al*., 2005). Theaflavins and thearubigins are important compounds which give tea desirable qualities such as brightness, briskness, colour and strength of black tea (Roberts *et al*, 1958). Oolong tea is that which is partially fermented and falls between the black and green tea. Green tea is usually made from unfermented leaves. Therefore green tea has catechins, which are more preserved than in either oolong tea or black tea (Pelillo *et al*., 2002). Japan and China are the major green tea producers in the world (Golding *et al*., 2009). In Kenya, green tea is produced in smaller quantities by companies such as Unilever Tea and James Finlay Tea. It is mainly produced when there is a demand for it in the market. Oxidation of the catechins in green tea is prevented by inactivation of the enzyme polyphenol oxidase. Green tea is known for its natural antioxidant properties since it contains catechins (Chiu, 2006). However, both black and green teas contain similar amount of flavonoids (Punit *et al.*, 2010), however they differ in their chemical structure; green tea contains more catechins (simple flavonoids), while black tea contains oxidized theaflavins and thearubigins.

2.6 Biochemical compounds in tea

Young shoots of tea consist of two leaves and a bud. A variety of non-volatile compounds exist in fresh shoots which include polyphenols, flavanols and flavonol glycosides, flavones, phenolic acids, amino acids, chlorophyll and other pigments, carbohydrates, organic acids, caffeine and other alkaloids, minerals, vitamins, and enzymes (Hara *et al*., 1995).

Polyphenols are usually referred to as catechins and are the main bioactive molecules which make up 20-35 % of the dry weight in tea (Graham, 1992). Tea catechins or flavan3-ols include (+) catechin (C), (–)-epicatechin (EC), (–)-epigallocatechin (EGC), (–)-epicatechin gallate (ECG), (–)-epigallocatechin gallate (EGCG), and (–)-gallocatechin gallate (GCG).

Flavanols which are mainly catechins are the most important group and occupy 60–80 % of the total amount of polyphenols (Hara *et al*., 1995b). Polyphenols have aromatic rings with multiple hydroxyl residues. The green tea contains 30 to 42% polyphenols on dry weight basis (Balentine *et al*., 1997). At present, more than five thousand of such compounds are known to exist. They are photosynthesised by tea and protect it from damage due to strong sun light through their strong anti-oxidant properties (Kohri *et al*., 2001). Three major flavanols in the fresh leaf are kaempferol, quercetin and myricetin. These compounds contribute to bitterness and astringency in green tea (McDowell and Taylor, 1993).

Caffeine is the main alkaloid present in tea (Graham, 1992). Tea leaves contain about 2 – 4 % caffeine (% dry weight) and is mainly found in young tea leaves. Different tea varieties have varying level of caffeine (Magoma *et al*., 2001). Minor alkaloids in tea

include theobromine and theophyline and constitute about 0.1 % of dry weight in tea (Graham, 1992).

2.7 Tea and Health

In traditional Chinese medicine, tea was regarded as a panacea, because it was known have antipyretic, antidotal, anti-diarrheal and diuretic properties. However, until the end of the 1980s, there were limited reports and experimental data describing the antimicrobial properties of tea (Hamilton-Miller, 1995; Hamilton-Miller, 1997). Tea is now recognized as a drink with medicinal properties and most of the research focus on its pharmacological properties and also on the possible components that make it biologically active (Zongmao, 1995). The beneficial effects of tea are attributed to the polyphenolic compounds present, particularly the catechins, which make up 30% of the dry weight of green tea leaves (Graham, 1992).

Consumption of tea is linked to low occurrence of cancer of the stomach, oral cavity, oesophagus and lungs (Hakim and Chow, 2004). Polyphenols are effective chemo-preventive agents (Gosslau and Chen, 2004; Hsuuw and Chen, 2007). Polyphenols also enhances insulin activity (Cabrera *et al*., 2003), have antimicrobial effect (Stapleton, 2004; Almajano *et al*., 2008), antibacterial activity (Mbata *et al*., 2008), immune stimulatory effect (Matsunaga, 2002), anti-inflammatory effects (Sato and Myata, 2000; Karori *et al*., 2008), protective effect against cardiovascular diseases (Sano, 2004; Khan and Mukhtar, 2007) and cerebral ischemic damage (Suzuki, 2004). Epigallocatechin gallate (EGCG) is the main catechin and is associated with anti-HIV effects when bound to CD4 receptor (Kawai, 2003). Studies also show that tea has antioxidant properties (Zhang, 2004; Karori *et al*., 2007; Maurya and Rizvi, 2008).Several

epidemiological studies and animal models have revealed that green tea protects against cancers of the skin, breast, prostate and lung (Mukhtar and Ahmad, 2000; Yang et al., 2002). In addition to the cancer chemo-preventive properties, green tea is an anti-angiogenic (prevention of tumor blood vessel growth) (Cao and Cao, 1999; Pfeffer *et al*., 2003) and anti-mutagenic (Han, 1997). In addition to that catechin compounds have a variety of physiological functions, such as enacting the duodenum, colon, skin, lung, breast, oesophageal, pancreatic and prostate cancer functions (Goodarznia and Abdollahi, 2009). People who take tea regularly have a healthier intestinal bacterial flora than those who take little or no tea at all (Hara, 2001). Catechins also inhibits the growth of food borne pathogenic bacteria, and do not have adverse effects on the beneficial bacteria (Hara, 2006).

Studies have shown that caffeine has prophylactic properties (Weisburger, 2006). It acts as a stimulant to the central nervous system (CNS) and the cardiovascular systems (Marks, 1992). Moreover, caffeine affects the taste of tea with its sharp bitterness and it is also regarded as an important constituent of tea contributing to tea quality (Caffin *et al*., 2004).

2.8 Gene actions: Additive, dominance and epistasis

The value of a quantitative trait can be partitioned into genotype and environment components. The environment component comprise of the environment component itself and the interactions between genotype and environment (G x E). The genotype is the inherent qualities possessed by the individual.

The genotype value is the sum of the mean (m) and the deviations from that mean due to gene effects or gene actions and hence it is expressed as: G=m+∑deviations or

g=m+∑gene effects. The deviations or gene effects can be additive, dominance, epistasis, or a combination of any two or of all three. Additive gene effects (ai) result from intra-allelic interaction while dominance gene effects result from inter-allelic interactions (di) of the same genes or loci. The epistatic interactions results from inter-allelic interactions of different genes or loci. Therefore additive and dominance results from interactions between alleles of the same gene or locus while epistasis results from the interaction between alleles of different genes or loci. The ratio d/a, called dominance ratio, defines the degree or level of dominance as follows: when d/a=0: there is no dominance, 0<d/a<1 or 0<d<a: there is partial dominance, d/a=1 or d=a: there is complete dominance, d/a>1 or d>a: there is over dominance (Bos and Caligari, 1995).

2.9 Variances in a quantitative trait and combining ability

The phenotypic variance is expressed following the relation P = G + E + G x E as follow; VP = VG + VE + VGE where VP is phenotypic variance, VG is genotypic variance, VE is environmental variance and VGE is the variance due to interaction G x E. VE and VGE are estimated experimentally. The genetic variance VG is made of additive variance VA (due to additive effects), dominance variance VD (due to dominance effects) and epistatic variance VI, hence genetic variance is expressed as: VG= VA +VD+VI. Frequently epistasis is considered as negligible or absent and thus genetic variance is expressed as VG = VA +VD. Moreover, VGE is frequently considered as part of VE so that phenotypic variance is expressed as VP= VA + VD + VE (Falconer and Mackay, 1996).

Hayes and Immer (1942) defined combining ability as the relative ability of parents to transmit desirable traits to their crosses. A parent has good combining ability if it

produces superior progenies when crossed with other parents. Therefore, the performance of a hybrid is related to the general (GCA) and specific (SCA) combining abilities of the inbred lines involved in a particular cross (Sprague and Tatum, 1942).

GCA is the average performance of the progeny of an individual when it is crossed to a number of other individuals in the population (Falconer, 1989). Therefore a parent with a GCA of zero has an average general combining ability. A positive GCA indicates a parent that produces above average progenies, 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. Specific combining ability (SCA) is used to confirm the value of superior genotype combinations. It represents the final stage in the selection of inbred lines since it identifies specific inbred combinations to use in hybrid formation. Specific combining ability can also be used when one needs to determine the heterotic groups of different genotypes (Hallauer and Miranda, 1988).
General combining ability is associated with additive effects of the genes, while SCA is related to dominance and epistatic effects (non-additive effects) of the genes. Rojas and Sprague (1952) noted that the variance of SCA also contains deviations due to the interaction between genotypes and environments, in addition to those that come from dominance and epistasis. General and specific combining abilities are dependent on the particular sets of materials that are included in a test making it important for any new germplasm introduced in a breeding programme to be tested for GCA and SCA.

General combining ability tests are used for preliminary classification of varieties from a large number of genotypes in a breeding programme. Parents with negative GCA are discarded. General combining ability and specific combining ability can also be used to determine the type of gene action governing traits of interest whereby a high value of GCA to SCA indicates additive gene effect and while a negative GCA to SCA ratio shows predominance of non-additive gene effects. (Hallauer and Miranda, 1988).

2.10 Diallel mating

A diallel mating design is a set of crosses that involve *n* parents crossed in all possible combinations. The analysis of such combinations is called diallel analysis. It is produced by crossing a set of parents in all possible combinations. The diallel mating design is used to estimate both general and specific combining ability variances and effects (Dabholkar, 1992).

The two main types of diallel mating designs are complete and partial. Complete diallel is also referred to as type 2 and it involves crossing *n* parents in all possible combinations to produce n^2 possible single crosses and selfs. On the other hand, partial diallel analysis only uses a sample of the crosses (Griffing, 1956).

The diallel analysis technique is extensively considered as one of the most powerful tools used to understand the gene actions in the expression of quantitative traits and characters (Baker, 1978). Two approaches are used in diallel analysis are the Hayman's (1954) analysis which is based on the estimation of the components of variation and Griffing's (1956) approach which gives four different methods of analysis depending on whether one involves both parents and reciprocals in analysis or not. The methods

developed by Griffing's are referred to as methods 1-4. Method 1 involves the parents (n), F1's (n (n-1)/2 and the reciprocals. Method 2 involves only the parents and F1's, Method 3 involves F1's and reciprocals and Method 4 involves the F1's only. Griffing's approach is widely used because its analysis is easily performed and interpreted (Singh, 1995).

From diallel analysis, plant breeders are able to obtain information on heterosis and the effects due to reciprocal, maternal, general combining ability (GCA) and the specific combining ability (SCA) of parents in crosses (Yanchuk, 1996; Glover *et al*., 2005). Diallel mating systems have provided genetic understanding for a particular set of parents (Murray *et al*., 2003) and have been used to study various traits in many crops. This has been demonstrated for cassava (*Manihot esculenta Crantz*), chickpea (*Cicer arietinum L*), common bean (*Phaseolus vulgaris L.*), maize (*Zea mays L.*), soya bean (*Glycine max L.*) and tea among others (Derera *et al*., 2007; Dhliwayo *et al*., 2005; Franco *et al*., 2002; Gwata *et al*., 2005; Jaramillo *et al*., 2005; Kamunya *et al*, 2010).
Generally, for all diallel mating designs, a relatively larger GCA/SCA variance ratio demonstrates the significance of additive genetic effects and the lower ratio indicates dominance and/or epistatic gene effects (Christie and Shattuck, 1992). GCA effects are calculated only when mean squares for GCA are significant (Dabholkar, 1992). The same applies to other effects, i.e. SCA and reciprocal combining ability, as well. Parents with larger significant GCA are referred to as a best combiner. Thus, these significant parental lines are chosen for hybridisation. Parents of the F_1s with large significant SCA effects are considered to have high specific combining ability. In this case, a breeder can therefore choose the best crosses. Significance of reciprocal effects shows the presence

of maternal effects. In this case, the choice of which plant to be pollen source or the mother plant matters.

2.11 Heterosis

The term heterosis was coined by Shull (1952) as the difference between the hybrid value and the mean value of the two parents for the same trait (Falconer and Mackay, 1996). According to Miranda (1999), heterosis is the genetic expression of the superiority of a hybrid in relation to its parents. Generally, heterosis is manifested as an increase in vigour, size, growth rate, yield or some other characteristics. 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). 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).

Heterosis may be positive or negative. Depending upon the breeding objectives, both positive and negative heteroses are useful for crop improvement. In general, positive heterosis is desired for yield and quality traits while negative heterosis for maturity in many crops. Heterosis is expressed in three ways, depending on the criteria used to compare the performance of a hybrid. The three ways are: mid-parent, standard variety

and better parent heterosis. However, from the plant breeders' viewpoint, better parent (Fanseco and Peterson, 1968) and standard variety (standard heterosis) are more useful.

Exploitation of heterosis in agriculture provides enhancing food security and represents a single greatest applied achievement in the discipline of genetics. Tea is a highly out crossing crop and it and it also almost completely self-incompatible (Wachira and Kamunya, 2005), hence expresses strong heterosis in the F1 hybrids. These led to the conclusion of the presence of significant heterosis in tea which could be exploited commercially by developing F_1 hybrids. Kamunya *et al* (2010) too have reported heterosis in tea for yield, drought tolerance and quality.

2.12 Tea improvement in Kenya

Tea improvements in Kenya started with the introduction of seeds from India where they were used to establish the first tea plantations. Since these progenies had not been particularly selected for high yield, quality and drought tolerance, the resultant seedling populations of mixed genotypes were genetically inferior. This heterogeneity resulted in a great variation in yield, quality and suitability for fermentation. The focus thereafter shifted to yield improvement as the main aspect (Green, 1971).

With this population, tea improvement started with the creation of Tea Research Institute of East Africa in 1961, and later becoming the Tea Research Foundation of Kenya in 1980 with the mandate to conduct research on all aspects of tea. The first phase of the tea improvement started with mass selection among the introduced seedlings based on morphological traits. As a result, several cultivars were released to the industry. Since they were heterogeneous genotypes, they formed good breeding

materials for the second phase of mass selection. It was through hybridization of selected parental stocks, that superior varieties for certain desired attributes were selected. The third phase involved selections from bi-clonal full-sib progenies which resulted in the release of better clones. Over the years, tea breeding programmes have resulted in improved varieties that combine high yielding, good quality, drought tolerance and pest and disease resistant traits known as clone cultivars (Banerjee, 1992). Tea breeding for high quality and medicinal teas targets the selection of populations with high functional components such as catechins, flavanols, theanine, b-carotene, 2-amino-5- pentanoic acid and polysaccharides (Zongmao, 1995).

2.13 Genotype x environment (GE) interaction and stability

The performance of a genotype is influenced by its genotype and the environment in which it is grown (Yan and Tinker, 2006; Yan *et al*., 2007). Environmental effects vary from season to season (Sorrells *et al.,* 2000; Yan *et al*., 2007; Mohammadi and Amri, 2013). Thus, genotypes need to be evaluated in various environments and seasons to select the best genotype for a particular environment. Genotype x environment interaction is used to determine whether to select for a wide or specific adaptation, the choice of sites for selection, whether selections in early generations can be conducted under stress or stress free environments, and whether to perform multi environment testing of large numbers of genotypes or subject fewer lines to intensive trait based selections (Yan *et al*., 2007; Bantayehu, 2009). Significant GE interactions indicate that the parental crosses interact with environment, and if not, then the additive gene effect is constant under different environment conditions (Yan and Tinker, 2006; Yan et al., 2007; Bantayehu, 2009; Mohammadi and Amri, 2013). Several methods are used to analyse GE interaction and phenotypic stability (Bantayehu, 2009). These methods

include: analysis of variance, regression analysis, risk assessment, ranking methods, pattern analysis (cluster analysis), principal component analysis (PCA), factor analysis, additive main effects and multiplicative interaction (AMMI) model, and GGE biplot (Lin *et al*., 1986). This review focuses on AMMI and GGE biplots.

CHAPTER THREE

Combining abilities for catechins and caffeine in Kenyan tea (*Camellia sinensis* (L.) O. Kuntze)

Abstract

High catechins and caffeine teas usually confer high black tea quality and also exhibit health benefits. This study was conducted using 16 F_1 crosses generated using a full diallel mating design of four parents. The first experiment was conducted in the field at the Tea Research Foundation of Kenya, Timbilil and Kangaita research stations respectively. The experiment was laid out in a completely randomized block design with three replicates at both sites. Eight biochemical traits of Tea (*Camellia sinensis* (L.) O. Kuntze) were studied to estimate the general combining abilities (GCA) and specific combining abilities (SCA) of parents and crosses using diallel mating system. There were significant ($p<0.05$) differences among the genotypes for all the traits under study at both Timbilil and Kangaita. At Timbilil, general combining ability effects (GCA) was significant ($p<0.05$) for GA, EGC, CAFF, ECG, EGCG and TC while at Kangaita, all traits had significant ($p<0.05$) GCA effects except C, implying that these traits are governed by additive gene effects. EPK TN 14-3 had the best general combining ability at both Timbilil and Kangaita. Specific combining ability at Timbilil was significant ($p<0.05$) for EGC, CAFF, EC, EGCG, TC while at Kangaita, significant ($p<0.05$) SCA effects was exhibited in all the traits except CAFF. Noticeably, inbred EPK TN 14-3 was the best cross for EGCG at both Timbilil and Kangaita. Inbreds AHP S15/10 and TRFK 6/8 were the best crosses for EGC at Timbilil and Kangaita respectively. EPK TN14-3 x AHP S15/10 and TRFCA SFS150 x TRFK 6/8 were the best crosses for CAFF at Timbilil and Kangaita respectively. Cross TRFCA SFS150 x TRFK 6/8 and inbred AHP S15/10 were the best crosses for EC at Timbilil and Kangaita respectively. EPK TN14-3 x TRFK 6/8 and inbred TRFK 6/8 were the best crosses for TC at Timbilil and Kangaita respectively. Maternal effects at Timbilil were significant ($p<0.05$) for EGC, EGCG and TC while at Kangaita maternal effects were significant ($p<0.05$) for EC signifying importance of the choice of female parents in breeding programmes targeting these traits. At Timbilil, significant ($p<0.05$) maternal effects were revealed in TRFK 6/8 for EGCG and GA, while EPK TN14-3 exhibited significant ($p<0.05$) maternal effects for EGC, EC and TC. At Kangaita, EPK TN14-3 exhibited significant maternal effects for EC. This information will be very valuable for tea breeding programmes targeting high black/green tea quality cultivars.

3.1 Introduction

Tea (*Camellia sinensis* (L.) O. Kuntze) is an evergreen perennial beverage crop that belongs to the family *Theaceae* and genus *Camellia* that has over 200 reported species (Chang and Bartholomew, 1984). It is used for the manufacture of the stimulating and the most popular beverage called tea. Tea is consumed mainly as either green (non-fermented), white (silvery tips), yellow/oolong (semi fermented) or black (full-fermented) beverage. Each of these types depends upon the process of manufacture (Takeo, 1992) and type of cultivar (Kamunya, Pers. com, July 2014, TRFK).

Tea is grown in 52 countries in the world all of which fall within tropical and sub-tropical regions (Mukhtar and Ahmad, 2000). Kenya is the third largest producer of tea in the world after China and India but the world's leading exporter of black tea (ITC, 2013). Tea cultivation and manufacturing are present in 15 of Kenya's 47 counties and impacts a large proportion of Kenya's 44 million people (TKB, 2013). It is the largest employer in the private sector, with more than 3 million people working in the tea sector. Over 60% of Kenyan tea is grown by smallholders who produce over 62% of Kenya's total production. In 2013 for example, Kenya exported 494.4 million kilograms of made tea, which resulted to over $ 1.4 billion foreign earnings (TKB, 2013). Moreover, tea contributes approximately 26% of the export earnings and 4% of the Gross Domestic Product (GDP) to the Kenyan economy (TKB, 2013). Since tea is grown in rural areas, it has contributed to the improved living standard of the rural communities. This has led to development of infrastructure such as tea manufacturing factories, better road networks, schools and hospitals. Tea production in the country has improved significantly over the years. This is mainly attributed to the replacement of

low yielding seedling varieties with high yielding and better quality tea clones through rationalised tea improvement efforts (Wachira, 2002).

Tea contains different chemical components which includes polyphenols, alkaloids, amino acids, carbohydrates, vitamins, proteins, chlorophyll, volatile compounds, minerals and trace elements (Hilton, 1973; Mondal *et al*, 2004). Polyphenols are the main bioactive molecules in tea (Balentine *et al*, 1999; Cabrera *et al*, 2003).The major polyphenols found in green tea are catechins which include: Gallic acid, (-) -epigallocatechin (EGC), (-) -epicatechin (EC), epigallocatechin-3-gallate (EGCG), epicatechin-3-gallate (ECG), (+) catechin and (+) gallocatechin (Ferruzzi and Green, 2005). EGCG is usually of high concentration followed by EGC, ECG and EC in decreasing order (Nakabayashi, 1991). Catechins and gallocatechin are present in trace amounts (Chu and Juneja, 1997). Kenya tea is renowned for its high quality and safety from harmful pesticide residues. The high black tea quality is attributed to high levels of biochemical constituents. For example, high catechin teas have been observed to harbour high black tea quality (Owuor and Obanda, 2007; Wachira and Kamunya, 2005). These polyphenols are usually of high concentration in the bud of tea and continue decreasing as the leaves age (Willson, 1999). The oxidation products of these catechins are theaflavins and thearubigins which are the main components responsible for briskness, brightness, taste, strength and colour of black tea (Woods and Roberts, 1964).

Total catechin content is used as an indicator of the quality potential in tea. Furthermore, the individual proportions of the catechins are important in the determination of tea quality. Studies have revealed significant correlation between black

tea quality and polyphenols such as epicatechin gallate (ECG), epigallocatechin gallate (EGCG) and epicatechin (EC) (Obanda and Owuor, 1997).

Experimental studies have recognized that tea exhibits significant health protecting activity due to its high polyphenol content (Manzocco *et al*, 1998). There is already evidence that tea polyphenols have anti-heart disease and anticancer activities in human beings (Carbrera, 2003; Vanessa and Williamson, 2004). Tea has also been shown to possess anti-allergic action (Yamamoto *et al.,* 2004), anti-inflammatory and antimicrobial properties (Paola *et al.,* 2005) potential anti-helmintic properties (Mukai *et al*., 2008), anti-diarrhoeal properties (Besra *et al*., 2003), anti-diabetic activity (Sabu *et al.,* 2002) and also anti-hyperglycaemic activity (Gomes *et al*., 1995). Therefore it is attractive to develop tea clones with specific biochemical characteristics to meet the different user needs. This would require careful choice of parents.

For a breeding program to be successful, prior awareness on the mode of gene action, combining ability and heritability is very important (Chahal and Gosal, 2002). Availability of such information influences the choice of the parents and size of the breeding population. Combining ability studies on tea have previously been carried out in a few studies. For example, Kamunya *et al*. (2010) found out that there were significant maternal effects for yield, theaflavins and drought tolerance. The breeding and selection of tea with high quality requires precise information on the diversity available and also thorough analysis of the biochemicals which contribute towards the black or green quality tea. The present study was carried out to estimate the GCA and SCA for the mentioned biochemical attributes of tea quality using a diallel mating design based on four popular commercial tea clones in Kenya.

3.2 Materials and Methods

3.2.1 Site Description

The study was conducted in two sites, Tea research foundation of Kenya (TRFK) Timbilil estate in Kericho county and Kangaita in Kirinyaga county. Timbilil estate (00 22' S and 350 21' E) is located at 2180 meters above sea level, with long-term annual average amount of rainfall at 2043mm and average temperature at 16.20^0C. Kangaita (00^0 30' S and 37^0 18'E) is located 2100 meters above sea level. The annual mean temperature is 15.27^0 c, with an average annual rainfall at 2009 mm.

3.2.2 Plant materials

The plant material consisted 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 1). 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.

3.2.3 Planting and Field management

The 4 x 4 full diallel cross trial comprising sixteen clonal full-sib families and four parental clones was established in the year 2000 at Timbilil estate, Kericho and in 2010 at TRFK Kangaita sub-station**.** The trial was set up as a completely randomized block design with three replications in plots of 30 plants at Timbilil and 10 plants at Kangaita spaced at 0.61 m within rows and 1.22 m between rows (i.e. 13448 plants per hectare). The trial has 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 1. Attributes of the four diploid parental clones used to generate full-sib families

Clone	Variety type	Special attribute
EPK TN14-3	Kenyan Chinary local selection	Tolerant to high pH and cold, Susceptible to Red crevice mites, Moderate levels of caffeine (2.7%)
TRFCA SFS 150	Malawian Assam selection	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%), Low catechin content
TRFK 6/8	Assam type Kenyan local selection	High black tea quality (fast fermentability and high levels of polyphenols (25%)), Average yielding, susceptibility to water stress, low levels of caffeine (1.7%).

Source: Kamunya *et al*. (2010).

3.2.4 Sample preparation and data collection

3.2.4.1 Leaf Sampling and sample processing

About 500g of fresh leaf in form of two leaves and a bud were plucked from each of the clonal plots and placed in appropriately labelled khaki bags. The samples were then put into a cooler box containing ice packs and then transported to the laboratory. The samples were steamed for 4 minutes using a microwave oven in order to deactivate the enzyme polyphenol oxidase and hence stop the process of oxidation. Finally the

samples were put in an oven which was set at 100^0C for 24 hours. The dried samples were then ground using a coffee miller and stored in aluminium lined bags until analysis.

3.2.4.2 Extraction of catechins and caffeine

Extraction of catechins and caffeine was done according to the procedure of ISO14502-2-2005E, 2005. 0.2g of ground tea samples were weighed into graduated extraction tubes. 5ml of 70% hot methanol/water (MeOH) was added, stoppered and mixed thoroughly by vortexing. Incubation was done in a water bath at 70^0 C for 10 min with vortexing after 5 and 10min, cooling was done at room temperatures and then centrifuged at 3500 rpm for 10min. A second extraction was done on the residue using 5ml of 70% hot methanol and water; the extracts were then combined and made up to 10ml with cold methanol/water (70%).

3.2.4.3 Analysis and quantification of Catechins and caffeine

HPLC analysis of catechins and caffeine was done according to the procedure by ISO14502-2-2005E, 2005. In this protocol, 1.0 ml of the sample was pipetted into a test tube. One ml of ethyl gallate which is an internal standard was added into the test tube and then diluted to 5ml with stabilizing solution (10% v/v acetonitrile with 500µg/ml of EDTA and 500µg/ml ascorbic acid), filtered and loaded into 2ml vials. A Shimadzu LC 20 AT HPLC fitted with a SPD-20 UV-Visible detector and C6, 25cm x 4.6 micron column fitted with a Rheodyne pre-column filter (model 7335) was used at 278nm. Gradient elution was employed using the following solvent systems: Mobile phase A (9:2:89 v/v/v Acetonitrile: Acetic acid: EDTA) and mobile phase B (80:2:18 v/v/v Acetonitrile: acetic acid: EDTA) at a flow rate of 1ml/min. The column temperatures

were set at 35°C± 0.5. The injection volume of 20µl was used. Conditions for the binary gradient were set up as follows; 100% solvent A for 10 minutes then over 15 minutes a linear gradient to 68% mobile phase A, 32% mobile phase B and was held at this composition for 10 minutes. The conditions were reset to 100% mobile phase A and allowed to equilibrate for 10 minutes before the next injection (Zuo *et al.*, 2002).

3.3 Biochemical parameters analysed

Data was generated on percent gallic acid (GA), epigallocatechin (EGC), epicatechin (EC), epigallocatechin-3-gallate (EGCG), epicatechin-3-gallate (ECG), catechins (C), caffeine (CAFF) and total catechins (TC).

3.4 Combining ability analysis

The generated data were entered into excel spread sheets. Analysis of variance was carried out using the GLM procedure of SAS 9.3 (SAS, 2012). The PROC MIXED procedure of SAS was used to calculate adjusted means at individual sites for all the measured traits. Significant differences in treatment means were separated using Duncan's multiple range test at $p<0.05$ level of significance. Combining ability analysis was carried out following Griffing's (1956) Method I, model 1 (assuming random effects) to estimate the GCA and SCA effects as shown in the following model;

$Yij = m + gi + gj + sij + rij + 1/bc\Sigma\Sigma eijkl$

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

$k = 1, 2\ldots b$

$l = 1, 2\ldots. c$

Where,

m is the mean of the experiment

Yij is the mean of i x jth genotype over k and l,

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

gj is the gca effect of the jth parent,

sij is the interaction, i.e. specific combining ability effect,

rij is the reciprocal effect

1/bcΣΣeijkl is the mean error effect.

The relative importance of GCA and SCA were estimated using the general predicted ratio (GPR) for the traits observed (Baker, 1978). Ratios close to one indicate additive effects in the inheritance of the trait are importance while ratios close to zero indicate dominance effects are more important in the inheritance.

3.5 Results

3.5.1 Variation of catechins among parents and progenies at Timbilil and Kangaita

Results presented in Table 2 indicate that there were significant ($p<0.05$) differences among parents and F_1s (including reciprocals) for all the biochemical traits assessed at both Timbilil and Kangaita, indicating the presence of genetic variability.

Table 2. Means of F1s and parents for percentage GA, EGC, C, CAFF, EC, EGCG, ECG and TC at Timbilil and Kangaita

		GA		EGC		C		CAFF		EC		EGCG		ECG		TC	
Family	Cross	Timb	Kang	Timb	Kang	Timb	Kang	Timb	Kang	Timb	Kang	Timb	Kang	Timb	Kang	Timb	Kang
420	TRFCA SFS150 x TRFK 6/8	0.55	0.24	8.13	6.87	0.38	0.32	3.21	3.39	1.88	1.58	9.87	10.61	3.06	3.16	23.31	22.53
430	TRFCA SFS150 x EPK TN14-3	0.61	0.26	7.61	5.41	0.32	0.30	3.33	3.29	1.37	1.33	10.25	9.80	2.96	3.01	22.51	19.84
443	EPK TN14-3 x TRFK 6/8	0.61	0.44	8.33	6.56	0.34	0.33	3.38	3.28	1.51	1.49	11.17	10.69	2.96	3.32	24.84	22.39
447	EPK TN14-3 x AHP S15/10	0.59	0.26	7.77	5.86	0.35	0.41	3.65	3.44	1.68	1.48	10.87	10.32	2.90	3.21	23.57	21.28
456	AHP S15/10 x TRFK 6/8	0.68	0.38	7.68	6.40	0.34	0.27	3.52	3.48	1.56	1.22	10.27	10.42	2.69	2.68	22.57	21.34
463	TRFCA SFS150 x AHP S15/10	0.73	0.27	7.16	5.85	0.33	0.37	3.31	3.59	1.28	1.12	10.79	10.71	2.86	3.18	22.42	21.22
467	TRFK 6/8 x TRFK 6/8	0.65	0.30	7.96	6.34	0.27	0.30	3.22	3.41	1.04	1.36	9.46	10.77	2.25	3.04	20.97	21.81
471	TRFCA SFS150 x TRFCA SFS150	0.64	0.26	7.53	6.20	0.37	0.31	3.44	3.58	1.43	1.17	10.83	10.97	2.94	3.33	23.07	22.19
474	AHP S15/10 x EPK TN 14-3	0.75	0.31	8.00	5.31	0.37	0.52	3.55	3.65	1.35	1.26	11.34	11.25	3.06	3.29	24.13	21.62
475	TRFK 6/8 x AHP S15/10	0.83	0.53	7.52	6.04	0.35	0.34	3.14	3.22	1.41	1.27	11.05	11.11	2.91	2.83	23.24	21.58
476	TRFK 6/8 x EPK TN14-3	0.72	0.40	7.66	6.54	0.34	0.23	3.30	3.40	1.52	1.77	11.02	10.72	2.99	2.95	23.53	22.21
478	AHP S15/10 x AHP S15/10	0.78	0.49	7.91	5.96	0.47	0.34	3.43	3.56	1.41	1.39	11.73	11.13	3.14	3.01	24.65	21.83
482	TRFK 6/8 x TRFCA SFS150	0.65	0.28	8.08	6.07	0.39	0.38	3.39	3.48	1.65	1.41	11.08	10.41	2.95	2.94	24.15	21.22
485	AHP S15/10 x TRFCA SFS 150	0.64	0.32	7.26	5.41	0.41	0.33	3.36	3.33	1.47	1.44	10.78	10.88	2.98	3.57	22.86	21.56
488	EPK TN14-3 x TRFCA SFS150	0.60	0.31	8.36	6.71	0.37	0.45	3.58	3.42	2.08	1.59	10.21	10.31	3.34	3.10	24.36	21.07
490	EPK TN14-3 x EPK TN14-3	0.74	0.54	7.22	7.75	0.35	0.39	3.23	3.08	1.48	1.24	11.08	11.58	2.86	3.40	22.99	24.46
	Parents performance																
	TRFK 6/8	0.72	0.20	8.74	6.55	0.41	0.52	2.96	2.93	1.43	1.75	10.45	9.62	2.83	3.01	23.85	21.45
	AHP S15/10	0.75	0.21	7.16	7.30	0.39	0.29	3.58	3.48	1.04	1.54	11.58	11.26	1.87	3.98	22.05	24.36
	TRFCA SFS150	0.58	0.26	8.00	6.77	0.28	0.19	3.39	3.11	1.44	1.50	10.48	10.27	3.32	2.88	23.56	21.60
	EPK TN14-3	0.77	0.36	7.59	6.17	0.23	0.29	3.52	3.68	1.35	1.83	12.17	11.03	2.84	3.37	24.18	22.69
	Mean	0.68	0.34	7.78	6.30	0.35	0.34	3.37	3.39	1.47	1.44	10.82	10.69	2.88	3.16	23.34	21.91
	LSD ($p<0.05$)	0.16	0.10	0.61	0.91	0.09	0.14	0.30	0.35	0.53	0.25	0.69	0.66	0.56	0.38	0.54	1.15
	CV (%)	14.50	18.60	4.70	8.70	17.10	24.00	5.30	6.20	21.80	10.60	3.90	3.70	11.70	7.30	1.40	3.20

NB GA=Gallic acid; EGC=Epigallocatechin; C=Catechins; CAFF=Caffeine; EC=Epicatechin; EGCG=Epigallocatechin3-gallate; ECG=Epicatechin-3-gallate; TC=Total Catechins; mean=mean of all the 20 entries; Kang=Kangaita; Timb=Timbilil

3.5.1.1 Gallic acid (GA)

Performance based on GA revealed wide spread variability with respect to various crosses, reciprocals and their parents at both sites. At Timbilil, GA content ranged from 0.55% to 0.83% for crosses TRFCA SFS150 x TRFK 6/8 and TRFK 6/8 x AHP S15/10 respectively (Table 2). Interestingly, TRFK 6/8 is the common parent in both crosses although as a parent, it neither had the highest nor the lowest GA content (Table 2). The fact that cross TRFK 6/8 x AHP S15/10 had significantly ($p<0.05$) higher GA content than either parent is an indication of transgressive variation within its family. Genotype TRFK 475/1 which recorded the highest GA value at 0.97% was also derived from TRFK 6/8 x AHP S15/10. The genotype that recorded the lowest GA was TRFK 463/49 at 0.43% from cross TRFCA SFS150 x AHP S15/10 (Appendix 1).

At Kangaita, average GA content for the progenies was 0.35% with a range of 0.24% to 0.54% for TRFCA SFS150 x TRFK 6/8 and EPK TN14-3 self respectively. It is noteworthy that the GA content Kangaita was generally half that of Timbilil for all the crosses and their parents. Performance of the parents ranged from 0.20% to 0.36% for TRFCA SFS150 x TRFK 6/8 and EPK TN14-3 self respectively. The F_1s had a higher mean than the parents (Table 2). Among the genotypes, the range was from 0.11% to 1.16% for TRFK 471/4 and TRFK 475/5 respectively (Appendix 2).

3.5.1.2 Epigallocatechin (EGC)

At Timbilil, the highest EGC value among the crosses was recorded in cross EPK TN14-3 x TRFCA SFS150 at 8.36% while TRFCA SFS150 x AHP S15/10 exhibited the lowest at 7.16 % (Table 2). Among the parents, TRFK 6/8 had the highest EGC value at 8.74% while AHP S15/10 had the lowest at 7.16 % (Table 2). Regarding the

genotypes, TRFK 474/1 derived from AHP S15/10 x EPK TN14-3 had the highest EGC content at 9.08%, while TRFK 476/4 at 6.28% recorded the lowest and was derived from cross TRFK 6/8 x EPK TN14-3 (Appendix 1). Notably, EPK TN14-3 is the common parent in the two crosses. It is also apparent that clone TRFK 474/1 outperformed its' female parent AHP S15/10 by 26.8%, an indication of heterosis.

At Kangaita, mean EGC content for the F1s was 6.20% and the ranged was from 5.31% to 7.75% for AHP S15/10 x EPK TN 14-3 and EPK TN14-3 self respectively. Surprisingly, EPK TN14-3 recorded the lowest EGC content at 6.17% while AHP S15/10 at 7.30% had the highest EGC content among the parents (Table 2). TRFK 456/3 at 9.23% recorded the highest EGC content while TRFK 463/51 at 3.93% had the lowest among the genotypes (Appendix 2). Mean EGC content at Kangaita at 6.30% was lower than at Timbilil which recorded a mean of 7.78% (Table 2).

3.5.1.3 Catechin (C)

At Timbilil, significant differences ($p<0.01$) were observed for C content within and between the families. Performance of the progenies ranged between 0.27% to 0.47% for inbreds TRFK 6/8 and AHP S15/10 respectively (Table 2). By contrast, TRFK 6/8 had the highest percentage C among the parents at 0.41% while EPK TN14-3 had the lowest at 0.23% (Table 2). The genotype which recorded the highest C content was TRFK 478/1 at 0.55% and was derived from TRFK 6/8 self while TRFK 443/1 from cross EPK TN14-3 x TRFK 6/8 had the lowest at 0.16% (Appendix 1).

At Kangaita, progeny mean for C was 0.35% with a range of 0.23% to 0.52% for TRFK 6/8 x EPK TN14-3 and AHP S15/10 x EPK TN 14-3 respectively. The performance of parents ranged from 0.19% to 0.52% for TRFCA SFS 150 and TRFK 6/8 respectively

(Table 2). The genotypic values ranged from 0.25% to 1.06% for TRFK 463/50 and TRFK 488/2. Noticeably, both TRFK 463/50 and TRFK 488/2 are derived from the same parent TRFCA SFS150 (Appendix 2). Mean C content at Kangaita at 0.35% was slightly lower than at Timbilil which recorded a mean of 0.35% (Table 2).

3.5.1.4 Caffeine (CAFF)

At Timbilil, caffeine content ranged from 3.14% to 3.65% for crosses TRFK 6/8 x AHP S15/10 and EPK TN14-3 x AHP S15/10 respectively (Table 2). The performance of the parents ranged from 2.96% to 3.58% for TRFK 6/8 and AHP S15/10 respectively (Table 2). The genotype with the highest caffeine levels at 4.08% namely TRFK 488/3, was derived from cross EPK TN14-3 x AHP S15/10. On the other hand, the genotype namely TRFK 463/49 with the lowest caffeine level at 2.79% resulted from TRFCA SFS150 x TRFK 6/8 (Appendix 1).

Average CAFF content at Kangaita for the progenies was 3.41% with a range of 3.08% to 3.65% for EPK TN14-3 self and AHP S15/10 x EPK TN 14-3 respectively. Among the parents, CAFF range was between 2.93% to 3.687 for TRFK 6/8 and EPK TN 14-3 respectively (Table 2). The genotype with the highest caffeine levels at 4.19% namely TRFK 463/53, was derived from cross TRFCA SFS150 x AHP S15/10 while 456/2 at 2.85% ,which descended from EPK TN14-3 x AHP S15/10 had the lowest CAFF content (Appendix 2). Mean CAFF content at Kangaita at 3.39% was slightly higher than at Timbilil which recorded a mean of 3.39% (Table 2).

3.5.1.5 Epicatechin (EC)

At Timbilil, the EC content ranged from 1.04% to 2.08% for TRFK 6/8 self and EPK TN14-3 x TRFCA SFS150 respectively (Table 2). Among the parents, percentage EC ranged between 1.04% and 1.44% for AHP S15/10 and TRFCA SFS150 respectively (Table 2). The genotype with the highest EC content was TRFK 488/3 and was obtained from EPK TN14-3 x TRFCA SFS150 at 2.54%.The lowest genotype, TRFK 474/2 was obtained from cross AHP S15/10 x EPK TN 14-3 at 0.82% (Appendix 1).

Mean EC content at Kangaita for the progenies was 1.38% with a range of 1.12% to 1.77% for TRFCA SFS150 x AHP S15/10 and TRFK 6/8 x EPK TN14-3 respectively. Among the parents, the range was from 1.50% to 1.83% for TRFCA SFS 150 and EPK TN14/3 respectively (Table 2). The genotype with the highest EC content was TRFK 420/1 and was obtained from TRFCA SFS150 x TRFK 6/8 at 2.19%. The lowest genotype, TRFK 456/4 was obtained from AHP S15/10 x TRFK 6/8 at 0.64% (Appendix 2). Mean EC content at Kangaita at 1.44% was slightly lower than at Timbilil which recorded a mean of 1.47% (Table 2).

3.5.1.6 Epigallocatechin-3-gallate (EGCG)

The performance of EGCG at Timbilil ranged from 9.46% and 11.73% for inbreds TRFK 6/8 and AHP S15/10 respectively (Table 1). The performance of the parents ranged from 10.45% to 12.17% for TRFK 6/8 and EPK TN14-3 respectively (Table 2). Among the genotypes, TRFK 474/2 at 12.54% derived from AHP S15/10 x EPK TN 14-3 and TRFK 443/4 derived from EPK TN14-3 x TRFK 6/8 at 12.53% recorded high EGCG values while TRFK 474/2 from AHP S15/10 x EPK TN 14-3 had the lowest at 8.96% (Appendix 1).

Average EGCG content at Kangaita for the progenies was 10.73 % with a range of 9.80% to 11.58% for TRFCA SFS150 x EPK TN14-3 and EPK TN14-3 self respectively. The best parent AHP S15/10 registered 11.26% EGCG content while the lowest parent was TRFK 6/8 at 9.62% (Table 2). The genotype with the highest EGCG content was TRFK 490/1 at 12.88% and was obtained from EPK TN14-3 self. The lowest genotype, TRFK 447/17 at 9.21% was obtained from EPK TN14-3 x AHP S15/10 (Appendix 2). Mean EGCG content at Kangaita at 10.69% was slightly lower than at Timbilil which recorded a mean of 10.82% (Table 2).

3.5.1.7 Epicatechin-3-gallate (ECG)

At Timbilil, ECG values among the progenies ranged from 2.25% to 3.34% for TRFK 6/8 self and EPK TN14-3 x TRFCA SFS150 respectively (Table 2). The best parent EPK TN14-3 registered 2.84% ECG content while AHP S15/10 recorded the lowest performance at 1.87% (Table 2). The best genotype, TRFK 420/1 was derived from TRFCA SFS150 x TRFK 6/8 and it registered 3.69% while TRFK 447/15 from the cross EPK TN14-3 x AHP S15/10 at 1.87% recorded the lowest performance (Appendix 1).

At Kangaita, ECG values among the progenies ranged from 2.68% to 3.57% for AHP S15/10 x TRFK 6/8 and TRFK 6/8 x TRFCA SFS150 respectively (Table 2). The best parent AHP S15/10 registered 3.98% ECG content while TRFCA SFS 150 recorded the lowest performance at 2.88% (Table 2). The best genotype, 485/2 was derived from TRFK 6/8 x TRFCA SFS150 and it registered 4.77% while TRFK 456/4 from the cross AHP S15/10 x TRFK 6/8 at 2.42% recorded the lowest performance (Appendix 2).

Mean ECG content at Kangaita at 3.16% was higher than at Timbilil which recorded a mean of 2.88% (Table 2).

3.5.1.8 Total catechins (TC)

Performance based on TC revealed wide spread variability at Timbilil. Among the crosses, the mean value for TC ranged between 20.97% and 24.84% for crosses TRFK 6/8 self and EPK TN14-3 x TRFK 6/8 respectively (Table 2). TC content for the parents ranged from 22.05% to 24.18% for AHP S15/10 and EPK TN14-3 respectively (Table 2). The best genotype TRFK 443/4 was derived from EPK TN14-3 x TRFK 6/8 and it recorded 26.81%. The lowest genotype TRFK463/49 was derived from TRFCA SFS150 x AHP S15/10 and it recorded 20.03% (Appendix 1).

At Kangaita, the mean percent TC was 21.76% with a range of 19.84% and 24.46% for TRFCA SFS150 x EPK TN14-3 and EPK TN14-3 self respectively.TC content for the parents ranged from 21.45% to 24.36% for TRFK 6/8 and AHP S15/10 respectively (Table 2). The best genotype TRFK 490/1 was derived from EPK TN14-3 self and it recorded 26.99%. The lowest genotype TRFK 463/51 was derived from TRFCA SFS150 x AHP S15/10 and it recorded 17.80% (Appendix 2). Mean TC content at Kangaita at 21.91% was lower than at Timbilil which recorded a mean of 23.34% (Table 2).

3.5.2 Combining abilities

3.5.2.1 General and specific combining ability effects for agronomic traits at individual sites

At Timbilil, the results presented in Table 3 showed that the GCA effects for GA, EGC, Caffeine, EGCG, ECG and TC were significant ($p < 0.05$), an indication of significant additive gene action. GCA effects on the other hand were not significant ($p<0.05$) for C and EC. As for SCAs there were significant ($P < 0.05$) EGC, CAFF, EC, EGCG and TC suggesting considerable non-additive gene effects for these traits.

At Kangaita, GCAs were significant ($p < 0.05$) for all the traits except for C, also confirming additive gene action for these traits. Also, the SCAs were significant ($P < 0.05$) for all the traits except CAFF further confirming non-additive gene effects for these traits (Table 4).

Table 3. General combining ability, Specific combining ability, Reciprocal, Maternal effects and Non maternal effects mean squares for GA, EGC, C, CAFF, EC, EGCG, ECG and TC at Timbilil.

		Mean squares							
SOURCE	**DF**	**GA**	**EGC**	**C**	**CAFF**	**EC**	**EGCG**	**ECG**	**TC**
Replication	2	0.00	0.41	0.02	0.05	0.13	0.04	0.88*	0.69
Genotype	19	0.02	0.57***	0.01	0.09**	0.27**	1.22***	0.44*	3.12***
GCA	3	0.12*	1.12***	0.01	0.22***	0.35	2.59***	0.468**	3.87***
SCA	6	0.02	0.49**	0.00	0.10*	0.28*	0.92***	0.31	2.78***
Reciprocal	6	0.03*	0.34*	0.00	0.08*	0.32*	0.58**	0.07	2.61***
Maternal	3	0.01	0.38*	0.01	0.08	0.39	0.62*	0.04	3.81***
Non maternal	3	0.02	0.46	0.00	0.07	0.25	0.60*	0.10	1.40*
Error	38	0.01	0.14	0.00	0.03	0.11	0.54*	0.22	0.33

* Significant at $p < 0.05$; ** Significant at $p < 0.01$; ***Significant at $p<0.001$; See Table 2 for abbreviated catechins titles

It is noteworthy that both GCA and SCA effects for Timbilil were significant ($p<0.05$) for EGC, CAFF, EGCG and TC (Table 3) indicating that both additive and non-additive gene effects to be playing a role in their expression. To determine the relative importance of GCA and SCA in the expression of these traits, the proportion of GCA and SCA variances were calculated. The GCA to SCA variance ratios for EGC, CAFF, EGCG and TC were 2.27, 2.18, 2.81, and 1.39 respectively indicating that these traits

are predominantly influenced by additive genes. A study by Kamunya *et al*. (2010) made similar observations for % total polyphenol content, fermentability and pubescence. Maternal effects were revealed for EGC, EGCG and TC indicating the importance of maternal parents in influencing the traits. Non maternal effects were significant only for EGCG and TC. Significant ($p < 0.05$) reciprocal effects were observed for EGCG and TC (Table 3).

Table 4. General combining ability, specific combining ability, reciprocal, maternal and non-maternal effects mean squares for GA, EGC, C, CAFF, EC, EGCG, ECG and TC at Kangaita.

		Mean squares							
SOURCE	**DF**	**GA**	**EGC**	**C**	**CAFF**	**EC**	**EGCG**	**ECG**	**TC**
Replication	2	0.00	4.12**	0.01	0.14	0.03	1.62*	0.25	2.62
Genotype	19	0.58***	1.14***	0.41**	2.17**	0.12***	13.77***	4.82***	63.64*
GCA	3	0.13***	2.20*	0.01	0.33**	0.55***	2.99**	0.35**	1.84*
SCA	6	0.20***	8.70***	0.03**	0.18	0.43**	0.67**	0.23**	5.84***
Reciprocal	6	0.05	4.41*	0.08	0.33	0.48**	0.42*	0.09	1.77
Maternal	3	0.02	1.96	0.02	0.03	0.10*	0.33	0.06	1.65
Non maternal	3	0.03*	2.45	0.06	0.09	0.40*	0.60*	0.16	1.41
Error	38	0.04	1.01	0.01	0.01	0.11	0.27	0.17	1.59

* Significant at $p < 0.05$; ** Significant at $p < 0.01$; Significant at $p<0.001$; See Table 2 for abbreviated catechins titles

As for Kangaita, both GCA and SCA effects were significant ($p<0.05$) for all the traits except C and CAFF (Table 4). The GCA to SCA variance ratios were 0.65, 0.25, 1.28, 4.46, 1.52 and 0.31 for GA, EGC, EC, EGCG, ECG and TC, respectively. Based on the results, GCA to SCA variance ratios were greater than 1for EC, EGCG and ECG indicating that they are mainly influenced by additive genes. GCA to SCA variance ratios were lower than 1 for GA, EGC and TC indicating that these traits are influenced by non-additive genes. Significant ($p<0.05$) maternal effects were observed in EC, while significant ($p<0.05$) reciprocal effects were observed in EGC, EC and EGCG (Table 4).

Comparison between GCA effects associated with each parent at Timbilil and Kangaita is presented in Table 5 and Table 6 respectively. At Timbilil, it was observed that EPK TN14-3, TRFK 6/8 and AHP S15/10 showed positive GCA effects for GA. However, AHP S15/10 showed positive significant ($P<0.05$) GCA effects while TRFCA SFS150 exhibited negative significant ($p<0.05$) GCA effects for GA (Table 5). At Kangaita, parents EPK TN14-3, AHP S15/10 and TRFK 6/8 showed positive GCA effects for GA. EPK TN14-3 showed significant ($p<0.05$) positive GCA effects while TRFCA SFS150 had significant ($P<0.05$) negative GCA effects for the same trait (Table 6).

Two parents TRFK 6/8 and EPK TN14-3 exhibited positive GCA effect for EGC at Timbilil. However, only TRFK 6/8 had significant ($p<0.05$) positive GCA effects for EGC. On the other hand, AHP S15/10 showed significant ($p<0.05$) negative GCA effects for EGC (Table 5). At Kangaita, positive GCA effects for EGC were observed in EPK TN14-3 and TRFK 6/8. TRFK 6/8 exhibited significant ($p<0.05$) positive GCA effects EGC (Table 6).

Table 5. Estimates of general combining ability, specific combining ability, reciprocal effects, maternal and non-maternal effects for GA, EGC, C, CAFF, EC, EGCG, ECG and TC obtained from the 4 x 4 diallel cross at Timbilil.

	Traits							
Genotype	**GA**	**EGC**	**C**	**CAFF**	**EC**	**EGCG**	**ECG**	**TC**
GCA Effects								
TRFCA SFS150	-0.05**	-0.02	0.00	-0.01	0.07	-0.25**	0.13*	-0.05
EPK TN14-3	0.01	0.03	-0.12	0.06*	0.04	0.23**	0.07	0.43
AHP S15/10	0.040*	-0.23**	0.10*	0.06*	-0.10*	0.26***	-0.12	0.03
TRFK 6/8	0.00	0.22***	0.00	-0.12***	-0.01	-0.24**	-0.09	-0.41*
SCA Effects								
TRFCASFS150xTRFCASFS150	0.04	0.02	-0.03	0.04	-0.21	-0.25**	-0.06	0.45
TRFCA SFS150 x EPK TN14-3	-0.02	0.02	0.01	0.02	0.11	-0.53**	0.03	-0.32
TRFCA SFS150 x AHP S15/10	0.01	-0.33*	-0.10	-0.10	-0.10	-0.02	-0.01	-0.72
TRFCA SFS150 x TRFK 6/8	-0.08	0.10	0.06	-0.01	0.40*	-0.19**	0.10	0.14
EPK TN14-3 x EPK TN14-3	0.08*	-0.39**	-0.02	-0.13	-0.17	0.38*	0.21	-0.40
EPK TN14-3 x AHP S15/10	0.09*	0.29*	-0.04	0.09*	0.08	-0.17	-0.11	0.20
EPK TN14-3 x TRFK 6/8	-0.05	0.39*	-0.03	0.14	0.14	-0.06	-0.29	0.93*
AHP S15/10 x AHP S15/10	0.00	0.64***	0.04	-0.15	0.16	-0.49	-0.18	0.63*
AHP S15/10 x TRFK 6/8	0.04	-0.82**	-0.06	0.01	0.03	-0.17	0.05	-0.74
TRFK 6/8 X TRFK 6/8	0.13	0.10	-0.03	-0.14	-0.71**	0.74	-0.65	-0.33
Reciprocal effects								
TRFCA SFS150 x EPK TN14-3	0.00	-0.38*	-0.02	-0.13	-0.35**	0.02	-0.20	-1.32**
TRFCA SFS150 x AHP S15/10	0.03	-0.05	-0.04	0.00	-0.10	0.09	-0.10	-0.38
TRFCA SFS150 x TRFK 6/8	-0.04	0.03	-0.01	-0.03	0.11	-0.61**	0.06	-0.58*
EPK TN14-3 x AHP S15/10	-0.08*	-0.12	-0.01	0.09	0.16	-0.23	-0.08	-0.33
EPK TN14-3 x TRFK 6/8	-0.06	0.34	0.00	0.04	0.01	0.08	0.02	0.45
AHP S15/10 x TRFK 6/8	-0.09*	0.08	-0.01	0.19*	0.04	-0.39*	-0.11	-0.27
Maternal effects								
TRFCA SFS150	0.00*	-0.10	-0.02	-0.06	-0.09	-0.13	-0.06	-0.57**
EPK TN14-3	-0.04*	0.15*	0.00	0.05	0.13*	-0.03	0.04	0.36**
AHP S15/10	-0.01	0.06	0.01	0.04	0.00	-0.06	0.02	0.11
TRFK 6/8	0.05**	-0.11	0.00	-0.04	-0.05	0.23**	0.01	0.10

Traits are as described in the legend for Table 2.

Table 6. Estimates of general combining ability, specific combining ability, reciprocal effects, maternal and non-maternal effects GA, EGC, C, CAFF, EC, EGCG, ECG and TC obtained from the 4 x 4 diallel cross at Kangaita.

	Traits							
Genotype	**GA**	**EGC**	**C**	**CAFF**	**EC**	**EGCG**	**ECG**	**TC**
GCA Effects								
TRFCA SFS150	-0.05***	-0.06	-0.01	-0.00	-0.04	-0.16*	0.01	-0.35*
EPK TN14-3	0.03*	0.07	0.00	0.01	0.07*	0.05	0.07	0.19
AHP S15/10	0.01	-0.22	0.02	0.07	-0.09*	0.22**	0.07	0.09
TRFK 6/8	0.01	0.20*	-0.01	-0.07	0.05	-0.12	-0.15**	0.06
SCA effects								
TRFCASFS150xTRFCASFS150	0.04	0.38	-0.06	-0.05	-0.02	0.29	-0.05	0.84*
TRFCA SFS150 x EPK TN14-3	-0.02	-0.17	0.03	-0.04	-0.00	-0.49**	-0.16	-0.14**
TRFCA SFS150 x AHP S15/10	0.01	-0.33	0.01	-0.01	-0.02	0.07	0.15	-0.11
TRFCA SFS150 x TRFK 6/8	-0.07	-0.27	0.10	0.16	0.07	-0.16	0.10	-0.43
EPK TN14-3 x EPK TN14-3	0.06	0.61*	-0.04	-0.03	-0.02	0.54**	0.11	1.44***
EPK TN14-3 x AHP S15/10	-0.08**	-0.50*	0.09**	0.07	-0.06	-0.16	-0.03	-0.59*
EPK TN14-3 x TRFK 6/8	-0.02	-0.54	-0.03	0.04	0.11	-0.43	0.20	-1.15*
AHP S15/10 x AHP S15/10	-0.01	0.82**	-0.05	-0.03	0.08**	0.08	0.01	1.15**
AHP S15/10 x TRFK 6/8	0.10*	-0.61	0.01	-0.03	-0.36**	-0.09	-0.34**	-1.61*
TRFK 6/8 X TRFK 6/8	-0.02	1.64**	-0.07	-0.18	0.18	0.68	0.44	3.18***
Reciprocal effects								
TRFCA SFS150 xEPK TN14-3	-0.01	-0.64*	-0.08*	-0.13	-0.24**	-0.12	-0.12	-0.77*
TRFCA SFS150 x AHP S15/10	0.00	0.08	-0.08	0.00	-0.12	-0.04	-0.13	-0.19
TRFCA SFS150 x TRFK 6/8	0.00	0.29	-0.02	-0.03	0.1	-0.25	0.08	0.12
EPK TN14-3 x AHP S15/10	-0.05	0.08	-0.03	0.09	0.14	-0.35	-0.06	-0.23
EPK TN14-3 x TRFK 6/8	-0.02	0.17	0.02	0.04	-0.07	0.03	0.85	0.37
AHP S15/10 x TRFK 6/8	-0.08*	0.13	-0.02	0.19*	0.03	-0.37	-0.09	-0.23
Maternal effects								
TRFCA SFS150	-0.02	-0.05	-0.02	-0.06	-0.07*	-0.1	-0.04	-0.21
EPK TN14-3	-0.01	0.19	0.01	0.05	0.08*	-0.05	0.04	0.23
AHP S15/10	-0.01	-0.01	0.01	0.04	0.00	0.01	0.02	0.05
TRFK 6/8	0.02	-0.13	0.00	-0.04	-0.01	0.06	-0.02	-0.07

Traits are as described in the legend for Table 2.

At Timbilil, two of the parents namely AHP S15/10 and TRFCA SFS150 showed positive GCA values for C. However, only AHP S15/10 showed significant ($p<0.05$) positive GCA effects for C (Table 5). At Kangaita, positive GCA effects were revealed in EPK TN14-3 and AHP S15/10.Two of the parents EPK TN14-3 and AHP S15/10 exhibited positive GCA effects for CAFF (Table 6).

At Timbilil, EPK TN14-3 and AHP S15/10 showed significant ($P<0.05$) positive GCA effects for CAFF while TRFK 6/8 showed significant ($P<0.05$) negative GCA effects for caffeine (Table 5). At Kangaita, EPK TN14-3 and AHP S
15/10 exhibited positive although not significant GCA effects for CAFF (Table 6).
At Timbilil, two parents TRFCA SFS150 and EPK TN14-3 exhibited positive GCA effects for EC. AHP S15/10 exhibited significant ($p<0.05$) negative GCA effects for EC (Table 5). At Kangaita, parents EPK TN14-3and TRFK 6/8 exhibited positive GCA effects for EC (Table 6). However, significant ($p<0.05$) positive GCA effects for EC were observed in EPK TN14-3 whereas significant ($p<0.05$) negative GCA effects were observed for AHP S15/10 (Table 6).

At Timbilil, parents EPK TN14-3 and AHP S15/10 exhibited significant ($p<0.05$) positive GCA effects for EGCG while TRFK 6/8 and TRFCA SFS150 showed significant ($p<0.05$) negative GCA effects for EGCG (Table 5). At Kangaita, significant ($p<0.05$) positive GCA effects for EGCG were recorded in AHP S15/10 while significant ($p<0.05$) negative GCA effects were observed in TRFCA SFS150 (Table 6).
At Timbilil, EPK TN14-3 and TRFCA SFS150 showed positive GCA effects for ECG. However, TRFCA SFS150 exhibited significant ($p<0.05$) positive for ECG. Parents

AHP S15/10 and TRFK 6/8 had negative GCA effects for ECG (Table 5). At Kangaita, all the parents exhibited positive GCA effects for ECG except TRFK 6/8 which showed negative significant ($p<0.05$) GCA effects (Table 6).

At Timbilil, EPK TN14-3 and AHP S15/10 exhibited positive GCA effects for TC while TRFK 6/8 showed negative significant ($p<0.05$) GCA effects the same trait (Table 5). At Kangaita, AHP S15/10 and EPK TN14-3 exhibited a positive GCA effects for TC. Significant ($P<0.05$) negative GCA effects were observed for TRFCA SFS150 (Table 6).

TRFK 6/8 exhibited significant ($p<0.05$) maternal effects for EGCG and GA at Timbilil (Table 5). EPK TN14-3 showed significant ($p<0.05$) maternal effect for EGCG, EC and TC at Timbilil (Table 5). At Kangaita, EPK TN14-3 had significant ($p<0.05$) maternal effects for EC (Table 6). Among the ten cross combinations in F1 generation, 60% of the crosses showed positive SCA effects for GA at Timbilil. However, only two progenies EPK TN14-3 self and EPK TN14-3 x AHP S15/10 exhibited significant ($p<0.05$) positive SCA effects for GA (Table 5). At Kangaita, good specific combiners for GA were revealed in forty percent of the combinations. These crosses were TRFCA SFS150 self, TRFCA SFS150 x AHP S15/10, EPK TN14-3 self and AHP S15/10 x TRFK 6/8. Significant ($p<0.05$) positive SCA effects were observed in cross AHP S15/10 x TRFK 6/8 while significant ($p<0.05$) negative effects were recorded in EPK TN14-3 x AHP S15/10 (Table 6).

SCA analysis for EGC at Timbilil indicated that 70% of the crosses had positive SCA effects. Significant ($p<0.05$) positive SCA effects were revealed for crosses EPK TN14-

3 x TRFK 6/8, EPK TN14-3 x AHP S15/10 and AHP S15/10 self. Crosses which exhibited significant ($P<0.05$) negative SCA effects for EGC were TRFCA SFS150 x AHP S15/10, EPK TN14-3 self and AHP S15/10 x TRFK 6/8 (Table 5). At Kangaita, forty percent of the cross combinations had positive SCA effects for EGC (Table 6). These crosses were inbreds EPK TN14-3, TRFCA SFS150, AHP S15/10 and TRFK 6/8. However, among these crosses only inbreds AHP S15/10 and TRFK 6/8 exhibited positive significant ($p<0.05$) SCA effects for EGC (Table 6).

Although 30% of the crosses exhibited positive SCA effects for C at Timbilil, no cross showed significant ($p<0.05$) positive or negative SCA effects for the same trait (Table 5). However, fifty percent of the crosses had positive SCA effects at Kangaita (Table 6). Two crosses, TRFCA SFS150 x TRFK 6/8 and EPK TN14-3 x AHP S15/10 showed significant ($p<0.05$) positive SCA effects for EC while TRFCA SFS150 self had negative significant ($p<0.05$) SCA effects for EC (Table 6).

In this study, half of the crosses showed positive SCA effects for caffeine at Timbilil. However, only EPK TN14-3 x AHP S15/10 displayed positive significant ($p<0.05$) SCA effects. None of the crosses exhibited negative significant ($p<0.05$) for caffeine (Table 5). At Kangaita, only 30% of the crosses showed positive SCA effects for CAFF. These crosses were TRFCA SFS150 x TRFK 6/8, EPK TN14-3 x AHP S15/10 and EPK TN14-3 x TRFK 6/8. None of the crosses exhibited significant ($p<0.05$) positive or negative SCA effects for CAFF (Table 6).

Analysis of SCA at Timbilil showed that 60% of the crosses had positive SCA effects for EC. Significant ($p<0.05$) positive SCA effects for EC were obtained by TRFCA SFS150 x TRFK 6/8 while TRFK 6/8 self showed significant ($p<0.05$) negative SCA effects for the same trait (Table 5). At Kangaita, forty percent of the crosses exhibited positive SCA effects for EC. These crosses were TRFCA SFS150 x TRFK 6/8, EPK TN14-3 x TRFK 6/8, inbreds AHP S15/10 and TRFK 6/8 (Table 6). Among these crosses, only inbred AHP S15/10 showed significant ($p<0.05$) positive SCA effects for EC (Table 6).

Inbreds EPK TN14-3 and TRFK 6/8 revealed positive SCA effects for EGCG at Timbilil. However, only EPK TN14-3 self had significant ($p<0.05$) positive SCA effects for EGCG (Table 5). It was interesting to note that AHP S15/10 which exhibited significant ($p<0.05$) positive GCA effects for EGCG, showed negative SCA effects when it was crossed with other clones. Similarly, all the crosses involving TRFCA SFS150 had negative SCA effects for EGCG (Table 5). Crosses which had significant ($p<0.05$) negative SCA effects for EGCG were TRFCA SFS150 x TRFCA SFS150, TRFCA SFS150 x EPK TN14-3 and TRFCA SFS150 x TRFK 6/8 (Table 5). Positive maternal effects for EGCG were revealed in TRFK 6/8 (Table 5). At Kangaita, five crosses had positive SCA effects for EGCG. These crosses were TRFCA SFS150 x AHP S15/10 and inbreds TRFCA SFS150, EPK TN14-3, AHP S15/10 and TRFK 6/8. Inbred EPK TN14-3 had significant ($p<0.05$) positive SCA effects for EGCG while TRFCA SFS150 x EPK TN14-3 recorded significant ($p<0.05$) negative SCA for the same trait (Table 6).

Although 40% of the crosses exhibited positive SCA effects for ECG, none of the crosses showed significant ($p<0.05$) positive or negative SCA effects for ECG (Table 5). At Kangaita, six crosses exhibited positive SCA effects for ECG. These crosses were TRFCA SFS150 x AHP S15/10, TRFCA SFS150 x TRFK 6/8, EPK TN14-3 x TRFK 6/8, inbreds EPK TN14-3, TRFK 6/8 and AHP S15/10. Cross AHP S15/10 x TRFK 6/8 had significant ($p<0.05$) negative SCA effects for ECG (Table 6).

Regarding TC, half of the crosses displayed positive SCA effects at Timbilil. However, only two crosses TRFK 6/8 x EPK TN14-3 and AHP S15/10 self exhibited significant ($p<0.05$) positive SCA effects for TC. None of the crosses exhibited negative significant ($p<0.05$) SCA effects for TC. Positive maternal effects ($p<0.05$) were observed in EPK TN14-3 (Table 5). At Kangaita, four crosses TRFCA SFS150 x TRFCA SFS150, EPK TN14-3 x EPK TN14-3, AHP S15/10 x AHP S15/10 and TRFK 6/8 X TRFK 6/8 had significant ($p<0.05$) positive SCA effects for TC (Table 7). Crosses TRFCA SFS150 x EPK TN14-3, EPK TN14-3 x AHP S15/10 and AHP S15/10 x TRFK 6/8 had significant ($p<0.05$) negative SCA effects for TC (Table 6).

3.6 Discussion

In a hybridization program, selection of parents is the most important step in any breeding program in order to get desirable recombinants in crop improvement. Development of good quality black and green tea with medicinal properties targets selection of populations with high functional compounds mainly catechins and caffeine (Zongmao, 1995). The significant mean squares for the crosses on GA, EGC, C, CAFF, EC, ECG, EGCG and TC indicated a high genetic variation among the parents and their

crosses. This significant variation shows that varieties with desired biochemical attributes may be selected. Variability in yield, drought tolerance and quality traits has also been reported by Kamunya *et al*. (2010) on the same clones. In the present study, all the traits varied significantly ($P<0.05$) among the parents and the crosses at both sites with EGCG and EGC levels being the highest and +C, GA, ECG, CAFF and EC being less abundant. Similar results were reported by Ender *et al.* (2004). The significant environmental effect on the traits observed justifies multi-locational trials of varieties prior to their release.

The estimate of GCA effects of a parent in a diallel analysis is an important indicator of its potential for generating superior breeding genotypes. In a diallel analysis, significant GCA effects suggest significance of additive gene action (Betrán *et al.*, 2003) while significant SCA shows the importance of non-additive genes (Derera *et al*., 2007). A high positive significant GCA value for the desired traits means that the parent has high potential for generating superior offspring (Cruz and Regazzi, 1997). In the present study significant positive GCA and SCA effects are desirable for GA, EGC, C, EC, CAFF, EGCG, ECG and TC implying that both additive and non-additive gene action were involved in the expression of these traits.

At Timbilil, the GCA and SCA effects were significant ($p<0.05$) for EGC, CAFF, EGCG and TC and their GCA and SCA variance ratios were 2.27, 2.18, 2.81 and 1.39 respectively indicating that these traits are mainly influenced by additive genes. At Kangaita, both GCA and SCA effects were significant ($p<0.05$) for CAFF and EGCG, and the GCA to SCA variance ratios were 1.2 and 2.18 respectively implying that these

traits are mainly influenced by additive genes. Across the sites, GCA to SCA variance ratios for GA, EGC, EC, EGCG and TC were 2.0, 2.51, 2.33, 2.19 and 0.67 respectively, further affirming additive gene action in these traits. Kamunya *et al.* (2010) using full diallel also reported predominance of additive gene effects for yield, FERM, DT, TF, TR, pubescence and bud weight. Across the sites, the GCA to SCA variance ratio for TC was 0.67 indicating non-additive gene actions (dominance and epistasis). Prevalence of non-additive genetic effects in the inheritance of oil content in sunflower was reported by Marinković (1993) and Škorić *et al.* (2000). Cuauhtemoc Cervantes-Martinez *et al* (2006) also reported that disease resistance in Sunflower was mainly influence by additive gene effects. However, Dias and Kageyama (1995) reported the influence of non additive gene effects for yield in cocao.

The study revealed that EPK TN14-3 is an above average general combiner for all the studied characters at both Kangaita and Timbilil. This suggests that it has superior alleles and therefore has the potential to be used as a source of high GA, C, Caffeine, EGC, ECG, EC, EGCG and TC in tea breeding programs. Similarly, AHP S15/10 could also be used in tea breeding programmes for the improvement of GA, C, CAFF, EGCG and TC. On the other hand, clone TRFCA SFS150 either had negative or insignificant GCA effects at both sites for all the studied traits except EC, indicating its inferiority for tea quality improvement and value addition through breeding.

SCA effects indicate which combinations were better or worse compared with the group as a whole, and these effects are indicative of the dominant gene action or epistasis. EPK TN14-3 x AHP S15/10 and EPK TN14-3 self were the best crosses for GA at

Timbilil. However, at Kangaita, the best crosses for GA was inbred EPK TN14-3. These two crosses may be selected and used in crosses for improving GA at both sites. GA is mainly used in the pharmaceutical industry as a standard for determination of the phenol content in various analytes by the Folin - Ciocalteau assay (Fiuza *et al*., 2004).

For EGC, the best crosses at Timbilil were EPK TN14-3 x TRFK 6/8, EPK TN14-3 x AHP S15/10 while at Kangaita, the best crosses were inbreds AHP S15/10 and TRFK 6/8. At Timbilil and Kangaita, the best crosses had TRFK 6/8, an indication that it positively contributed towards high EGC at both sites.

Regarding CAFF, the best cross at both sites was EPK TN14-3 x AHP S15/10 and both parents also exhibited positive and significant GCA effects. Caffeine is an important biochemical compound in tea as it also contributes to tea quality (Caffin *et al*., 2004). Preference in relation to caffeine content in tea differs between different consumers. Developing teas which satisfy different customer needs in relation to caffeine content is desired. In view of this, when breeding for teas with high caffeine, EPK TN14-3 x AHP S15/10 will be the best cross to use. However, inbred EPK TN14-3 had a negative and significant SCA effects for CAFF. This portrays non-additive gene action towards low caffeine and therefore, it may be used for the development of low caffeine tea varieties.

The best cross for EC at Timbilil was TRFCA SFS150 x TRFK 6/8 while the best cross at Kangaita was inbred AHP S15/10. TRFCA SFS150 x TRFK 6/8 was the best cross across the site an indication that it could be used in the improvement of EC at both sites. The best cross at both Timbilil and Kangaita for EGCG was inbred EPK TN14-3. Experiments have shown that EGCG and EC are indicators of quality in Kenyan black

tea (Owuor and Obanda, 2007). In addition to this, there is sufficient evidence that EGCG has health benefits such as cancer chemoprevention (Hsuuw and Chen, 2007), improving cardiovascular health and it also has antioxidant properties (Fu and Koo, 2006). Analysis across the sites also revealed that inbreds EPK TN14-3 was the best cross for EGCG and thus this crosses could be utilized to develop tea with high EGCG content.

Generally, TC is used as an indicator of the quality potential in tea. Tea with high catechins has good black tea quality (Obanda and Owuor, 1997) and hence teas with high TC content are highly desired. The best crosses for TC at Timbilil were EPK TN14-3 x TRFK 6/8 and inbred AHP S15/10. The best crosses for TC at Kangaita were inbreds TRFCA SFS150, EPK TN14-3, AHP S15/10 and TRFK 6/8.However, analysis across the sites revealed that inbred AHP S15/10 was the best cross signifying its superiority across the two sites.

Considering maternal effects, the clones indicating significant and positive effects for any of the desired traits should be used as mothers while developing cultivars with high levels of those traits. TRFK 6/8 and EPK TN14-3 exhibited significant maternal effects for EGCG and EC respectively. Thus, TRFK 6/8 and EPK TN14-3 should always be used in advanced breeding programmes aimed at enhancing EGCG and EC contents in newly developed cultivars.

CHAPTER FOUR

To determine the level of heterosis for catechins and caffeine in Kenyan tea (*Camellia sinensis*) (L.) O. Kuntze)

Abstract

Tea (*Camellia sinensis*) (L.) O. Kuntze) is important cash in Kenya and it contributes 4% of the Gross Domestic Product to the Kenyan economy**.** Kenya is the world's largest producer of black tea in the world. Selection of tea with high catechins and caffeine is highly desired in tea breeding programmes. Tea with high catechins and caffeine has high black tea quality and also exhibit significant health properties. Positive heterosis is desired for catechins and caffeine in tea. Eight biochemical traits of tea were studied to investigate mid-parent heterosis (MPH), better parent heterosis (BPH) and standard heterosis (SDH) using a 4x4 diallel mating design. Cross TRFK 6/8 x AHP S15/10 had the highest MPH, BPH and SDH for GA in both Timbilil and Kangaita. Inbred AHP S15/10 had the highest MPH and BPH for EGC at both Timbilil, while inbred EPK TN14-3 exhibited the highest SDH for the same trait. Inbred TRFK 6/8 had the highest MPH and BPH for CAFF at both Timbilil and Kangaita. On the other hand, EPK TN14-3 x AHP S15/10 and AHP S15/10 x EPK TN 14-3 had the highest SDH for CAFF at Timbilil and Kangaita respectively. Cross TRFK 6/8 x TRFCA SFS150 exhibited the highest MPH and BPH for EGCG at both Timbilil and Kangaita. Inbreds AHP S15/10 at Timbilil and EPK TN14-3 at Kangaita had the highest SDH for EGCG. For TC, inbreds AHP S15/10 and EPK TN14-3 had the highest MPH, BPH and SDH at Timbilil and Kangaita respectively. Based on the overall results of these experiments inbreds EPK TN14-3, AHP S15/10 and TRFK 6/8 had improved catechins content and could be used in recurrent selection to develop tea with high catechins content.

Key words: Tea (*Camellia sinensis*) (L.) O. Kuntze), Diallel, mid parent heterosis, better parent heterosis, standard heterosis

4.1 Introduction

Tea (*Camellia sinensis*) (L.) O. Kuntze) belongs to *Theaceae* family and is the most widely consumed beverage in the world after water (Wheeler and Wheeler, 2004; Cheng, 2004). Tea is a highly outcrossing plant that is pseudo self-incompatible (Wachira and Kamunya, 2005). *C. sinensis* consists of mainly two varieties, *Camellia sinensis* variety sinensis with small semi-erect leaves and *Camellia sinensis* variety assamica with relatively large leaves (Hara *et al*., 1995).

Generally, beverage tea is broadly classified according to the method of processing (Takeo, 1992). Three main types of tea are black, green and oolong teas (Wang *et al*., 2000). Green tea is non fermented, whereas black tea is completely fermented while oolong tea is partially fermented (Friedman *et al*., 2005). Kenya is the largest producer of black tea in the world (ITC, 2013). The tea industry contributes approximately 26% of the export earnings and 4% of the Gross Domestic Product (GDP) to the Kenyan economy (TBK, 2013). Other types of tea produced are white, yellow and reprocessed tea which include flower scented tea, compressed tea, instant tea and herbal teas (Hara *et al*., 1995). However, white and yellow teas have been considered as subclasses of green tea by Harbowy and Balentine (1997).

Tea was first introduced in Kenya in 1903 and commercial planting began in 1930s (Watts, 1999). Currently, tea in Kenya is mainly grown in Kericho, Bomet, Nandi, Kiambu, Murang'a, Nyeri, Kirinyaga, Meru, Kisii and Nyamira counties (TBK, 2013). Tea production in Kenya is split between smallholders and large estates owned by companies such as Unilever Tea Kenya (UTK), Finlay Tea Kenya (JFK) and Eastern

Produce Limited. The large plantations are organized under the Kenya Tea Growers Association (KTGA) and account for about 40 % of the tea produced in Kenya. Smallholders' are organized under the Kenya Tea Development Agency (KTDA) which was set up in 1964 and they produce about 60% of the tea produced in Kenya (Mbadi and Owuor, 2008).

The main biochemical compounds present in tea are polyphenols, alkaloids (caffeine and theobromine) and essential oils (Mondal *et al*., 2004). The major polyphenols found in green tea are catechins which include: Gallic acid, -epigallocatechin (EGC), (-) - epicatechin (EC), epigallocatechin-3-gallate (EGCG), epicatechin-3-gallate (ECG), (+) catechin and (+) gallocatechin (Ferruzzi and Green, 2005). The oxidation products of these catechins which include theaflavins and thearubigins, the aromatic amino acids and caffeine are key factors which determine tea quality (Takino *et al.,* 1964). Catechins are also important pharmacologically due to their anticancer, anti-hypertension, anti-vascular disorders and anti-inflammatory properties (Adrian and Bolwell, 2000).

Heterosis refers to the superiority of F_1 hybrid in one or more characters over its parents and it results after crossing (Wengui, 2003). Knowledge on heterosis and as well as expected gain upon selection is important as it influences the choice of parents. Heterosis in tea could be exploited to increase yield and quality. Great efforts have been directed to improve yield and quality properties in tea. For example, previous studies on heterosis has been carried out for yield, bud weight, drought tolerance, fermentability, total polyphenols, theaflavins and thearubigins and pubescence (Kamunya *et* al, 2010).

The three types of heterosis used to compare the performance of a hybrid are mid-parent (relative), better parent (heterobeltiosis) and standard heterosis. Application of heterosis in agricultural production yields multi-billion dollar returns and represents a single greatest applied achievement in the discipline of genetics.

The present study was undertaken to estimate the magnitude of genetic variability, mid-parent, better parent and the standard heterosis for catechins and caffeine in tea by crossing 4 parents with diverse attributes. This study would be helpful in the selection of suitable parents of potential transgressive segregants which can be further evaluated for enhanced catechins and caffeine in tea.

4.2 Materials and Methods

4.2.1 Experimental sites

The 4 x 4 full diallel cross trial comprising sixteen clonal full-sib families and four parental clones was established at Timbilil and Kangaita. Descriptions of these experimental sites are as in section 3.2.

4.2.2 Plant materials

The plant material used in this study consisted of four parents that were involved in the 4 x 4 full diallel cross. Details of the plant materials are as provided in section 3.3 of this thesis.

4.2.3 Sample preparation and analysis

Samples preparation, quantification and HPLC analysis was done according to the method described in sections 3.5.1, 3.5.2 and 3.5.3.

4.2.4 Heterosis

Heterosis was determined using procedures by Heiko (2009);

1) Mid parent heterosis = $\frac{\text{F1 – Mid parent}}{\text{Mid parent}} \times 100$

2) Better parent heterosis= $\frac{\text{F1 – Better parent}}{\text{Better parent}} \times 100$

3) Standard heterosis = $\frac{\text{F1 – Standard variety}}{\text{Standard variety}} \times 100$

Significance of relative heterosis was tested by using t-test (Wynne *et al*., 1970).

4.3 Results

4.3.1 Range in phenotypic variation for percent GA, EGC, C, CAFF, EC, EGCG, ECG and TC at Timbilil and Kangaita.

There was a wide range in performance for most of the crosses in all the characters assessed at both Timbilil and Kangaita. Results on the range of means for the various characters measured in the diallel cross are presented in Tables 7 and 8. From the results at Timbilil, TRFK 6/8 self recorded the narrowest range for all the studied traits (Table 7). The widest range for GA at both Timbilil and Kangaita was exhibited in cross TRFK 6/8 x AHP S15/10. Cross AHP S15/10 x TRFCA SFS 150 exhibited the widest range at Timbilil for EGC (Table 7) while the widest range at Kangaita was recorded by AHP S15/10 x TRFK 6/8 (Table 8). Cross TRFCA SFS150 x EPK TN14-3 had the widest

range for C at Timbilil (Table 7) while EPK TN14-3 x TRFCA SFS150 had the widest range at Kangaita (Table 8). Cross EPK TN14-3 x AHP S15/10 (Table 7) and AHP S15/10 x EPK TN 14-3 (Table 8) had the widest ranges for CAFF at Timbilil and Kangaita respectively. Cross EPK TN14-3 x AHP S15/10 (Table 7) and TRFK 6/8 x TRFCA SFS150 (Table 8) had the widest ranges for EC at Timbilil and Kangaita respectively. Cross AHP S15/10 x EPK TN 14-3 at Timbilil (Table 7) and EPK TN14-3 x TRFCA SFS150 at Kangaita (Table 8) had the widest ranges for EGCG. Cross EPK TN14-3 x AHP S15/10 at Timbilil (Table 7) and cross TRFK 6/8 x TRFCA SFS150 at Kangaita (Table 8) recorded the widest ranges for ECG. Cross EPK TN14-3 x TRFK 6/8 at Timbilil (Table 7) and AHP S15/10 x TRFK 6/8 at Kangaita (Table 8) recorded the widest range for TC.

Table 7. Ranges of clonal means within families for the various traits measured at Timbilil.

		Ranges of clonal means within families for the various traits measured							
Family	**Cross**	**GA**	**EGC**	**C**	**CAFFEINE**	**EC**	**EGCG**	**ECG**	**TC**
474	AHP S15/10 x EPK TN 14-3	0.55-0.86	6.95-9.08	0.31-0.43	3.19-3.85	0.82-1.84	9.86-12.54	2.59-3.60	22.04-25.80
447	EPK TN14-3 x AHP S15/10	0.49-0.66	7.07-8.49	0.33-0.40	3.40-3.92	1.30-1.90	10.57-11.30	2.35-3.22	22.84-24.78
488	EPK TN14-3 x TRFCA SFS150	0.51-0.70	7.75-8.80	0.30-0.45	3.25-4.08	1.90-2.54	9.59-10.54	2.85-3.60	23.85-24.84
430	TRFCA SFS150 X EPK TN14-3	0.59-0.71	6.95-8.94	0.23-0.41	3.10-3.51	1.02-1.78	10.30-10.51	2.69-3.28	21.29-23.69
443	EPK TN14-3 x TRFK 6/8	0.54-0.67	7.57-8.73	0.16-0.48	2.90-3.79	1.23-1.80	9.63-12.53	2.44-3.35	21.79-26.81
476	TRFK 6/8 x EPK TN14-3	0.59-0.85	6.28-8.54	0.29-0.42	3.00-3.58	0.92-1.72	10.01-11.56	2.58-3.34	21.07-25.23
482	TRFK 6/8 x TRFCA SFS150	0.49-0.66	6.99-8.55	0.33-0.40	3.08-3.74	1.52-1.90	10.46-11.63	2.81-3.04	23.48-24.61
420	TRFCA SFS150 x TRFK 6/8	0.48-0.71	7.71-8.64	0.31-0.40	3.07-3.50	1.59-1.94	9.25-10.45	2.81-3.69	22.72-24.54
475	TRFK 6/8 x AHP S15/10	0.70-0.90	7.08-7.87	0.25-0.39	2.94-3.42	1.20-1.56	10.32-12.05	2.82-3.10	22.24-24.38
456	AHP S15/10 x TRFK 6/8	0.55-0.75	6.53-8.51	0.26-0.38	3.27-3.65	1.30-1.90	9.01-10.63	2.42-3.06	20.51-23.85
485	AHP S15/10 x TRFCA SFS 150	0.54-0.75	6.42-7.88	0.37-0.50	3.12-3.76	1.43-1.70	9.86-11.36	2.75-3.14	22.37-23.48
463	TRFCA SFS150 x AHP S15/10	0.43-0.89	6.78-7.62	0.31-0.40	2.79-3.61	1.17-1.46	8.96-11.79	2.63-3.06	20.03-23.43
467	TRFK 6/8 x TRFK 6/8	0.54-0.78	7.44-8.91	0.23-0.33	2.98-3.42	0.85-1.18	9.19-9.65	2.14-2.44	20.69-21.18
478	AHP S15/10 x AHP S15/10	0.62-0.90	7.37-8.33	0.41-0.55	3.21-3.57	1.18-1.56	11.10-12.43	2.95-3.42	24.11-25.33
471	TRFCA SFS150 x TRFCA SFS150	0.51-0.77	6.92-8.29	0.31-0.48	2.98-3.94	1.03-1.77	10.13-12.09	2.36-3.63	20.82-26.21
490	EPK TN14-3 x EPK TN14-3	0.69-0.79	6.97-7.32	0.31-0.35	3.20-3.39	1.41-1.52	11.25-11.42	2.77-2.93	22.91-23.34

Traits are as described in the legend for Table 2.

Table 8. Ranges of clonal means within families for the various traits measured at Kangaita.

Family	Cross	GA	EGC	C	CAFF	EC	EGCG	ECG	TC
420	TRFCA SFS150 x TRFK 6/8	0.11-0.48	5.09-8.97	0.18-0.49	2.78-3.82	0.67-2.30	8.50-12.14	2.53-3.67	19.62-26.54
430	TRFCA SFS150 x EPK TN14-3	0.12-0.44	3.69-7.53	0.17-0.49	2.55-3.66	0.95-1.88	8.15-11.39	2.39-3.95	17.82-23.39
443	EPK TN14-3 x TRFK 6/8	0.08-0.76	4.47-9.73	0.13-0.51	3.00-3.67	1.15-1.84	9.32-12.47	2.85-4.02	19.59-26.27
447	EPK TN14-3 x AHP S15/10	0.14-0.52	4.32-7.34	0.14-0.87	2.47-4.06	0.98-1.95	8.68-12.38	2.51-3.79	18.47-23.93
456	AHP S15/10 x TRFK 6/8	0.18-0.70	3.51-9.74	0.16-0.39	2.49-4.07	0.64-2.24	8.09-11.76	2.15-3.38	16.80-26.04
463	TRFCA SFS150 x AHP S15/10	0.13-0.53	3.49-9.63	0.15-0.69	2.91-4.51	0.77-1.56	8.74-13.62	2.27-4.07	17.03-25.80
467	TRFK 6/8 x TRFK 6/8	0.12-0.41	4.72-7.64	0.20-0.36	2.99-3.95	1.32-1.42	10.20-12.22	2.95-3.14	20.32-22.57
471	TRFCA SFS150 x TRFCA SFS150	0.03-0.44	4.09-9.60	0.14-0.62	2.69-4.02	0.20-1.80	9.18-13.04	2.45-4.31	19.94-27.60
474	AHP S15/10 x EPK TN 14-3	0.15-0.58	4.02-6.66	0.17-0.87	2.64-4.24	1.00-1.61	9.34-13.68	2.28-4.68	18.39-24.55
475	TRFK 6/8 x AHP S15/10	0.20-1.30	3.84-8.13	0.20-0.49	2.70-4.16	0.80-1.82	9.47-12.47	2.35-3.13	18.53-24.69
476	TRFK 6/8 x EPK TN14-3	0.23-0.90	4.49-8.71	0.16-0.38	2.99-3.77	1.45-2.17	9.30-12.11	2.30-3.88	19.28-25.05
478	AHP S15/10 x AHP S15/10	0.21-1.30	4.12-8.02	0.26-0.46	2.91-4.24	0.73-1.95	9.53-13.25	2.31-3.29	19.47-24.71
482	TRFK 6/8 x TRFCA SFS150	0.11-0.71	4.40-8.48	0.24-0.83	2.95-3.96	0.97-1.74	8.95-12.54	2.40-3.35	19.08-24.52
485	TRFK 6/8 x TRFCA SFS150	0.08-0.79	3.51-9.23	0.27-0.49	3.02-3.98	0.51-2.25	9.60-13.47	2.59-4.77	18.37-24.19
488	EPK TN14-3 x TRFCA SFS150	0.13-0.70	4.65-9.13	0.10-1.40	2.91-4.06	1.22-2.07	7.86-13.62	2.45-4.32	17.51-24.39
490	EPK TN14-3 x EPK TN14-3	0.20-0.82	6.54-9.79	0.16-0.48	2.95-3.24	1.02-1.56	9.89-13.18	2.90-3.80	20.93-27.38

Traits are as described in the legend for Table 2.

4.3.2 Heterosis values

In the present study, different levels of heterosis were measured as a percentage increase or decrease of the crosses over the mid-parent (Table 10), better parent (Table 11) and standard heterosis (Table 12) for all the studied characters at both Timbilil and Kangaita. MPVs at Timbilil and Kangaita for all the studied traits are presented in Table 9. Significant ($P<0.05$) MPVs were observed for all traits at both Timbilil and Kangaita (Table 9). For each character, the percentage values of the 16 crosses were compared with the mid parent, better parent and the standard variety. Mid-parent heterosis was significant ($P<0.05$) at Timbilil for GA, EGC, Caffeine, EGCG, ECG and TC while C, EC and ECG were not significant ($p<0.05$) (Table 10). At Kangaita, significant ($P<0.05$) mid-parent heterosis were observed for all the traits except GA (Table 10). Heterosis over the better parent at Timbilil was significant ($P<0.05$) for all the studied traits except EC (Table 11). At Kangaita, better parent heterosis was significant ($p<0.05$) for all traits except C (Table 11). Standard heterosis was at Timbilil was significant ($p<0.05$) for C, EGCG, GA, TC and EGC while CAFF, EC and ECG were not significant ($p<0.05$) (Table 12). At Kangaita, Standard heterosis was significant ($p<0.05$) for all the traits except GA and CAFF (Table 12).

Table 9. Mid-parent value (MPV<P) for the measured traits across the various full-sibs at Timbilil and Kangaita.

		MPV															
		GA		EGC		C		CAFF		EC		EGCG		ECG		TC	
Family	Cross	Timb	Kang	Timb	Kang	Timb	Kang	Timb	Kang	Timb	Kang	Timb	Kang	Timb	Kang	Timb	Kang
420	TRFCA SFS150 x TRFK 6/8	0.65	0.23	8.37	6.66	0.35	0.35	3.18	3.02	1.44	1.62	10.47	9.94	3.08	2.94	23.71	21.52
430	TRFCA SFS150 x EPK TN14-3	0.68	0.31	7.80	6.47	0.26	0.24	3.46	3.39	1.40	1.66	11.33	10.65	3.08	3.12	23.87	22.15
443	EPK TN14-3 x TRFK 6/8	0.75	0.28	8.17	6.36	0.32	0.40	3.24	3.31	1.39	1.79	11.31	10.32	2.84	3.19	24.02	22.07
447	EPK TN14-3 x AHP S15/10	0.76	0.28	7.38	6.74	0.31	0.29	3.55	3.58	1.20	1.68	11.88	11.14	2.36	3.67	23.12	23.53
456	AHP S15/10 x TRFK 6/8	0.74	0.20	7.95	6.92	0.4	0.41	3.27	3.21	1.24	1.64	11.02	10.44	2.35	3.49	22.95	22.90
463	TRFCA SFS150 x AHP S15/10	0.67	0.23	7.58	7.03	0.34	0.24	3.49	3.30	1.24	1.52	11.03	10.77	2.60	3.43	22.81	22.98
467	TRFK 6/8 x TRFK 6/8	0.72	0.20	8.74	6.55	0.41	0.52	2.96	2.93	1.43	1.75	10.45	9.62	2.83	3.01	23.85	21.45
471	TRFCA SFS150xTRFCA SFS150	0.58	0.26	8.00	6.77	0.28	0.19	3.39	3.11	1.44	1.5	10.48	10.27	3.32	2.88	23.56	21.60
474	AHP S15/10 x EPK TN 14-	0.76	0.28	7.38	6.74	0.31	0.29	3.55	3.58	1.20	1.68	11.88	11.14	2.36	3.67	23.12	23.53
475	TRFK 6/8 x AHP S15/10	0.74	0.20	7.95	6.92	0.40	0.41	3.27	3.21	1.24	1.64	11.02	10.44	2.35	3.49	22.95	22.90
476	TRFK 6/8 x EPK TN14-3	0.75	0.28	8.17	6.36	0.32	0.4	3.24	3.31	1.39	1.79	11.31	10.32	2.84	3.19	24.02	22.07
478	AHP S15/10 x AHP S15/10	0.75	0.21	7.16	7.30	0.39	0.29	3.58	3.48	1.04	1.54	11.58	11.26	1.87	3.98	22.05	24.36
482	TRFK 6/8 x TRFCA SFS150	0.65	0.23	8.37	6.66	0.35	0.35	3.18	3.02	1.44	1.62	10.47	9.94	3.08	2.94	23.71	21.52
485	AHP S15/10 x TRFCA SFS 150	0.67	0.23	7.58	6.66	0.34	0.35	3.49	3.02	1.24	1.62	11.03	9.94	2.60	2.94	22.81	21.52
488	EPK TN14-3 x TRFCA SFS150	0.68	0.31	7.80	6.47	0.26	0.24	3.46	3.39	1.40	1.66	11.33	10.65	3.08	3.12	23.87	22.15
490	EPK TN14-3 x EPK TN14-3	0.77	0.36	7.59	6.17	0.23	0.29	3.52	3.68	1.35	1.83	12.17	11.03	2.84	3.37	24.18	22.69
	Overall mean	0.71	0.26	7.87	6.67	0.33	0.33	3.36	3.28	1.32	1.66	11.17	10.49	2.72	3.28	23.41	22.43
	Significance of t-test (p = 0.05)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Note*. S designate not significant (P >0.05) and significant (P<0.05), respectively. Timb=Timbilil; Kang=Kangaita. Traits are as described in the legend for Table 2.*

Table 10. Percent mid-parent heterosis (MPH) for GA, EGC, C, CAFF, EC, EGCG, ECG and TC among the 16 progenies at Timbilil and Kangaita.

		GA		EGC		C		CAFF		EC		EGCG		ECG		TC	
Family	Cross	Timb	Kang	Timb	Kang	Timb	Kang	Timb	Kang	Timb	Kang	Timb	Kang	Timb	Kang	Timb	Kang
420	TRFCA SFS150 x TRFK 6/8	-15.63	3.03	-2.82	3.19	9.20	-9.26	0.97	12.16	30.75	-2.92	-5.69	6.66	-0.46	7.27	-1.68	4.69
430	TRFCA SFS150 x EPK TN14-3	-9.49	-16.02	-2.35	-16.40	26.72	26.09	-3.66	-3.05	-1.58	-20.12	-9.50	-7.97	-3.84	-3.76	-5.69	-10.39
443	EPK TN14-3 x TRFK 6/8	-18.13	56.03	1.99	3.04	5.79	-19.37	4.45	-0.75	8.99	-16.52	-1.20	3.56	4.38	4.22	3.42	1.46
447	EPK TN14-3 x AHP S15/10	-22.77	-7.52	5.29	-13.07	11.79	41.75	2.71	-4.03	40.93	-12.26	-8.42	-7.40	22.93	-12.49	1.95	-9.56
456	AHP S15/10 x TRFK 6/8	-11.12	84.87	-3.40	-7.57	-15.75	-32.61	7.65	8.56	26.40	-25.82	-6.74	-0.14	14.38	-23.26	-1.65	-6.85
463	TRFCA SFS150 x AHP S15/10	6.28	16.28	-5.54	-16.87	-2.86	55.54	-4.89	8.86	2.96	-26.14	-2.15	-0.55	10.38	-7.25	-1.69	-7.65
467	TRFK 6/8 x TRFK 6/8	-9.65	47.86	-8.97	-3.27	-33.87	-41.17	8.72	16.32	-27.25	-22.20	-9.51	11.96	-20.56	1.14	-12.07	1.72
471	TRFCA SFS150xTRFCA SFS150	10.23	-0.89	-5.88	-8.34	32.46	68.23	1.39	15.23	-0.95	-21.87	3.33	6.83	-11.55	15.80	-2.07	2.73
474	AHP S15/10 x EPK TN 14-3	-1.56	10.37	8.54	-21.17	20.37	77.15	-0.04	1.86	13.30	-24.93	-4.54	0.91	30.06	-10.46	4.40	-8.09
475	TRFK 6/8 x AHP S15/10	13.76	159.06	-5.41	-12.82	-12.59	-17.06	-3.91	0.28	14.32	-22.86	0.31	6.44	24.02	-19.03	1.28	-5.79
476	TRFK 6/8 x EPK TN14-3	-3.15	42.92	-6.22	2.83	6.20	-43.17	1.83	2.89	9.02	-1.13	-2.52	3.88	5.55	-7.53	-2.02	0.66
478	AHP S15/10 x AHP S15/10	-1.07	139.62	9.49	-18.26	16.50	15.36	-4.52	2.28	26.25	-9.86	1.30	-1.14	40.36	-24.35	10.56	-10.41
482	TRFK 6/8 x TRFCA SFS150	-4.61	22.67	-3.45	-8.81	12.62	8.70	6.73	15.19	15.02	-13.52	5.87	4.73	-4.20	0.04	1.86	-1.41
485	AHP S15/10 x TRFCA SFS 150	-3.85	38.28	-4.25	-18.76	21.01	-7.18	-3.45	10.26	18.95	-11.36	-2.31	9.42	14.83	21.13	0.25	0.15
488	EPK TN14-3 x TRFCA SFS150	-10.69	-0.30	7.30	3.65	45.66	90.20	3.58	0.92	49.11	-4.09	-9.87	-3.15	8.32	-0.71	2.05	-4.85
490	EPK TN14-3 x EPK TN14-3	-4.01	49.62	-4.85	25.50	53.39	32.57	-8.21	-16.36	9.65	-31.85	-8.95	5.05	0.65	0.89	-4.90	7.82
	Overall mean	-5.34	40.37	-1.28	-6.70	12.29	15.36	0.58	4.41	14.74	-16.71	-3.79	2.44	8.45	-3.65	-0.38	-2.86
	Significance of t-test(p = 0.05)	S	NS	S	S	NS	S	S	S	NS	S	S	S	S	S	S	S

***Note**. NS, S designate not significant (P >0.05) and significant (P<0.05), respectively. Timb=Timbilil; Kang=Kangaita. Traits are as described in the legend for Table 2*

Table 11. Percent better parent heterosis for GA, EGC, C, CAFF, EC, EGCG, ECG and TC at Timbilil and Kangaita.

		GA		EGC		C		CAFF		EC		EGCG		ECG		TC	
Family	Cross	Timb	Kang	Timb	Kang	Timb	Kang	Timb	Kang	Timb	Kang	Timb	Kang	Timb	Kang	Timb	Kang
420	TRFCA SFS150 x TRFK 6/8	-23.61	-8.92	-6.98	1.52	-7.32	72.36	-5.31	9.02	30.56	5.30	-5.82	3.26	-7.83	9.68	-2.26	4.31
430	TRFCA SFS150 x EPK TN14-3	-20.78	-27.47	-4.88	-12.38	14.29	3.22	-5.40	-10.57	-4.86	-27.31	-15.78	-11.12	-10.84	-10.77	-6.91	-12.53
443	EPK TN14-3 x TRFK 6/8	-20.78	21.58	-4.69	6.18	-17.07	12.22	-3.98	-10.80	5.59	-18.23	-8.22	-3.06	4.23	-1.38	2.73	-1.32
447	EPK TN14-3 x AHP S15/10	-23.38	-27.30	2.37	-5.18	-10.26	42.46	1.96	-6.56	24.44	-19.21	-10.68	-6.42	2.11	-4.61	-2.52	-78.72
456	AHP S15/10 x TRFK 6/8	-9.33	82.66	-12.13	-12.29	-17.07	-6.80	-1.68	-0.02	9.09	-20.65	-11.31	-7.43	-4.95	-32.61	-5.37	-12.43
463	TRFCA SFS150 x AHP S15/10	-2.67	3.88	-10.50	-13.64	-15.38	101.04	-7.54	15.47	-11.11	-25.17	-6.82	4.24	-13.86	10.45	-4.84	-12.88
467	TRFK 6/8 x TRFK 6/8	-9.72	47.86	-8.92	-3.27	-34.15	-41.17	8.78	16.32	-27.27	-22.20	-9.47	11.96	-20.49	1.14	-12.08	1.72
471	TRFCA SFS150 x TRFCA SFS150	10.34	-0.89	-5.88	-8.34	32.14	68.23	1.47	15.23	-0.69	-21.87	3.34	6.83	-11.45	15.80	-2.08	2.73
474	AHP S15/10 x EPK TN 14-3	-2.60	-13.25	5.40	-14.01	-5.13	78.05	-0.84	-0.82	0.00	-30.87	-6.82	1.98	7.75	-2.39	-0.21	-4.69
475	TRFK 6/8 x AHP S15/10	10.67	155.96	-13.96	-17.27	-14.63	14.70	-12.29	-7.64	-1.40	-17.48	-4.58	-1.33	2.83	-28.89	-2.56	-11.43
476	TRFK 6/8 x EPK TN14-3	-6.49	11.37	-12.36	5.96	-17.07	-20.91	-6.25	-7.52	6.29	-3.16	-9.45	-2.76	5.28	-12.50	-2.69	-2.10
478	AHP S15/10 x AHP S15/10	4.00	139.62	10.47	-18.26	20.51	15.36	-4.19	2.28	35.58	-9.86	1.30	-1.14	67.91	-24.35	11.79	-10.41
482	TRFK 6/8 x TRFCA SFS150	-9.72	8.44	-7.55	-10.28	-4.88	106.48	0.00	11.98	14.58	-6.20	5.73	1.39	-11.14	2.29	1.26	-1.77
485	AHP S15/10 x TRFCA SFS 150	-14.67	22.24	-9.25	-20.07	5.13	76.33	-6.15	7.18	2.08	-3.85	-6.91	5.93	-10.24	23.86	-2.97	-0.21
488	EPK TN14-3 x TRFCA SFS150	-22.08	-13.90	4.50	8.64	32.14	55.70	0.00	-6.91	44.44	-12.72	-16.11	-6.47	0.60	-7.95	0.74	-7.12
490	EPK TN14-3 x EPK TN14-3	-3.90	49.62	-4.87	25.50	52.17	32.57	-8.24	-16.36	9.63	-31.85	-8.96	5.05	0.70	0.89	-4.92	7.82
	Overall mean	-9.05	28.22	-4.95	-5.45	0.84	38.12	-3.10	0.64	8.56	-16.58	-6.91	0.06	0.04	-3.83	-2.06	-8.69
	Significance of t-test (p = 0.05)	S	S	S	S	S	NS	S	S	NS	S	S	S	S	S	S	S

***Note**. NS, S designate not significant (P >0.05) and significant (P<0.05), respectively. Timb=Timbilil; Kang=Kangaita. Traits are as described in the legend for Table 2*

Table 12. Percent standard heterosis for GA, EGC, C, CAFF, EC, EGCG, ECG and TC at Kangaita and Timbilil.

		GA		EGC		C		CAFF		EC		EGCG		ECG		TC	
Family	Cross	Timb	Kang	Timb	Kang	Timb	Kang	Timb	Kang	Timb	Kang	Timb	Kang	Timb	Kang	Timb	Kang
420	TRFCA SFS150 x TRFK 6/8	-23.61	18.59	-6.98	4.91	-7.32	-38.42	8.45	15.48	31.47	-9.95	-5.55	10.29	8.13	4.96	-2.26	5.07
430	TRFCA SFS150 x EPK TN14-3	-15.28	29.88	-12.93	-17.41	-21.95	-42.13	12.50	12.11	-4.20	-24.19	-1.91	1.90	4.59	-0.04	-5.62	-7.46
443	EPK TN14-3 x TRFK 6/8	-15.28	117.72	-4.69	0.09	-17.07	-37.09	14.19	11.84	5.59	-14.72	6.89	11.15	4.59	10.48	4.15	4.40
447	EPK TN14-3 x AHP S15/10	-18.06	30.18	-11.10	-10.62	-14.63	-20.13	23.31	17.15	17.48	-15.74	4.02	7.29	2.47	6.86	-1.17	-0.78
456	AHP S15/10 x TRFK 6/8	-5.56	87.14	-12.13	-2.31	-17.07	-47.23	18.92	18.74	9.09	-30.36	-1.72	8.39	-4.95	-10.91	-5.37	-0.51
463	TRFCA SFS150 x AHP S15/10	1.39	35.27	-18.08	-10.76	-19.51	-28.18	11.82	22.30	-10.49	-36.00	3.25	11.33	1.06	5.69	-6.00	-1.03
467	TRFK 6/8 x TRFK 6/8	-9.72	47.86	-8.92	-3.27	-34.15	-41.17	8.78	16.32	-27.27	-22.20	-9.47	11.96	-20.49	1.14	-12.08	1.72
471	TRFCA SFS150 x TRFCA SFS150	-11.11	29.06	-13.84	-5.29	-9.76	-39.90	16.22	22.05	0.00	-33.18	3.64	14.11	3.89	10.82	-3.27	3.48
474	AHP S15/10 x EPK TN 14-3	4.17	55.36	-8.47	-18.95	-9.76	-0.18	19.93	24.34	-5.59	-27.91	8.52	16.93	8.13	9.35	1.17	0.83
475	TRFK 6/8 x AHP S15/10	15.28	162.24	-13.96	-7.86	-14.63	-35.05	6.08	9.69	-1.40	-27.58	5.74	15.53	2.83	-5.99	-2.56	0.62
476	TRFK 6/8 x EPK TN14-3	0.00	99.43	-12.36	-0.12	-17.07	-55.66	11.49	15.95	6.29	1.00	5.45	11.49	5.65	-1.97	-1.34	3.57
478	AHP S15/10 x AHP S15/10	8.33	145.50	-9.50	-8.96	14.63	-34.68	15.88	21.47	-1.40	-20.88	12.25	15.75	10.95	0.02	3.35	1.79
482	TRFK 6/8 x TRFCA SFS150	-9.72	41.20	-7.55	-7.29	-4.88	-26.24	14.53	18.60	15.38	-19.78	6.03	8.30	4.24	-2.11	1.26	-1.05
485	AHP S15/10 x TRFCA SFS 150	-11.11	59.18	-16.93	-17.41	0.00	-37.01	13.51	13.52	2.80	-17.77	3.16	13.14	5.30	18.53	-4.15	0.52
488	EPK TN14-3 x TRFCA SFS150	-16.67	54.18	-4.35	2.40	-9.76	-12.71	20.95	16.71	45.45	-8.97	-2.30	7.24	18.02	3.12	2.14	-1.74
490	EPK TN14-3 x EPK TN14-3	2.78	167.94	-17.39	18.30	-14.63	-25.68	9.12	4.86	3.50	-28.93	6.03	20.45	1.06	13.02	-3.61	14.07
	Overall mean	-6.51	73.80	-11.20	-5.28	-12.35	-32.59	14.11	16.32	5.42	-21.07	2.75	11.58	3.47	3.94	-2.21	1.47
	Significance of t-test (p = 0.05)	S	NS	S	S	S	S	NS	NS	NS	S	S	S	NS	S	S	S

N/B *NS, S designate not significant (P >0.05) and significant (P<0.05), respectively. Timb=Timbilil; Kang=Kangaita. Traits are as described in the legend for Table 2.*

4.3.3 Mid parent, better parent and standard heterosis at Timbilil and Kangaita

4.3.3.1 Gallic acid (GA)

Mid-parent heterosis for GA at Timbilil ranged from -22.77% to 13.76% for EPK TN14-3 x AHP S15/10 and TRFK 6/8 x AHP S15/10 respectively (Table 10). Crosses TRFK 6/8 x AHP S15/10, TRFCA SFS150 x TRFCA SFS150 and TRFCA SFS150 x AHP S15/10 showed higher mid parent heterosis at 13.76%,10.23% and 6.28% respectively (Table 10). On the other hand at Kangaita, MPH mean was 40.37% and ranged from -16.02% to 159.06% for TRFCA SFS150 x EPK TN14-3 and TRFK 6/8 x AHP S15/10 respectively (Table 10). Twelve crosses exhibited positive heterosis over the mid parent for GA.

Better parent heterosis at Timbilil ranged from -23.61% to 10.67% for EPK TN14-3 x AHP S15/10 and TRFK 6/8 x AHP S15/10 respectively (Table 11). Crosses which exhibited positive heterosis over the better parent were TRFK 6/8 x AHP S15/10, TRFCA SFS150 x TRFCA SFS150 and AHP S15/10 self at 10.67%,10.35% and 4% respectively (Table 11). At Kangaita, BPH mean was 28.22% and the range was from -27.47% to 155.96% for crosses TRFCA SFS150 x EPK TN14-3 and TRFK 6/8 x AHP S15/10 respectively (Table 11). Ten crosses recorded positive heterosis over the better parent for GA.

At Timbilil, standard heterosis ranged from -23.61% to 15.28% for TRFCA SFS150 x TRFK 6/8 and TRFK 6/8 x AHP S15/10 respectively (Table 12). The crosses which showed positive heterosis over the standard variety TRFK 6/8, were TRFK 6/8 x AHP S15/10, AHP S15/10 self, AHP S15/10 x EPK TN 14-3, EPK TN14-3 self, TRFCA

SFS150 x AHP S15/10 at 15.28%, 8.33%, 4.17%, 2.78% and1.39% respectively (Table 12). At Kangaita, standard heterosis mean was 73.80% with a range of 18.59% to 167.94% (Table 12). All the crosses exhibited positive heterosis over the standard variety, TRFK 6/8 for GA.

4.3.3.2 Epigallocatechin (EGC)

Mid-parent heterosis at Timbilil ranged from -8.97% to 9.49% for inbreds TRFK 6/8 and AHP S15/10 respectively. Crosses AHP S15/10 self, AHP S15/10 x EPK TN 14-3, EPK TN14-3 x TRFCA SFS150, EPK TN14-3 x AHP S15/10, EPK TN14-3 X TRFK 6/8 showed a higher mid parent heterosis by 9.49% ,8.54%, 7.30%, 5.29% and 1.99% respectively (Table 10). At Kangaita, mean MPH was for EGC was -6.70% and the range was from -21.17% to 25.50% for AHP S15/10 x EPK TN 14-3 and EPK TN14-3 x EPK TN14-3 respectively (Table 10). Five crosses namely EPK TN14-3 x EPK TN14-3, EPK TN14-3 x TRFCA SFS150, TRFCA SFS150 x TRFK 6/8, EPK TN14-3 x TRFK 6/8 and TRFK 6/8 x EPK TN14-3 had positive over the mid-parent.

Better parent heterosis at Timbilil for EGC ranged between -13.96% to 10.47% for TRFK 6/8 x AHP S15/10 and AHP S15/10 self respectively. Crosses AHP S15/10 x AHP S15/10 at 10.47%, AHP S15/10 x EPK TN 14-3 at 5.40%, EPK TN14-3 x TRFCA SFS150 at 4.50% and EPK TN14-3 x AHP S15/10 at 2.37% showed positive heterosis over better parent (Table 11). At Kangaita, mean BPH was -5.45% with a range from -20.07% to 25.50% for TRFK 6/8 x TRFCA SFS150 and EPK TN14-3 self (Table 11). Five crosses namely EPK TN14-3 self, EPK TN14-3 x TRFCA SFS150, EPK TN14-3 x TRFK 6/8, TRFK 6/8 x EPK TN14-3 and TRFCA SFS150 x TRFK 6/8 had positive

heterosis over the better parent at 25.50%, 8.64%, 6.18%, 5.96% and 1.52% respectively.

Standard heterosis at Timbilil ranged between -18.08% to -4.35% for TRFCA SFS150 x AHP S15/10 and EPK TN14-3 x TRFCA SFS150 respectively (Table 12). All the crosses manifested negative heterosis over the standard variety for EGC (Table 12). At Kangaita, standard heterosis mean was -5.28% with a range from 18.95% to 18.30% for AHP S15/10 x EPK TN 14-3 and EPK TN14-3 self respectively (Table 12). Crosses EPK TN14-3 self, TRFCA SFS150 x TRFK 6/8, EPK TN14-3 x TRFCA SFS150 and EPK TN14-3 x TRFK 6/8 at 18.30%, 4.91%, 2.40% and 0.09% exhibited positive standard heterosis.

4.3.3.3 Catechin (C)

Mid-parent heterosis at Timbilil ranged from -33.87% to 53.39% for inbreds TRFK 6/8 and EPK TN 14-3 respectively. Positive heterosis over the mid-parent value was present in 12 crosses (Table 10). At Kangaita, mean MPH for C was 15.36% with a range from -43.17% to 90.20% for cross TRFK 6/8 x EPK TN14-3 and EPK TN14-3 x TRFCA SFS150 respectively (Table 10). Nine crosses had positive heterosis over the mid-parent for C.

At Timbilil, better parent heterosis ranged from -34.15% to 52.17% for inbreds TRFK 6/8 and EPK TN 14-3 respectively. Crosses, EPK TN14-3 self, EPK TN14-3 x TRFCA SFS150, TRFCA SFS150 self, AHP S15/10 self, TRFCA SFS150 x EPK TN14-3, AHP S15/10 x TRFCA SFS 150 showed higher better parent heterosis by 52.17%, 32.14%,

32.14%, 20.51%, 14.29% and 5.13% respectively (Table 11). At Kangaita, mean BPH for C was 38.12% with a range from -41.17% to 106.47% for TRFK 6/8 self and TRFK 6/8 x TRFCA SFS150 respectively (Table 11). Thirteen crosses had positive heterosis over the better parent for C.

Standard heterosis ranged from -34.15% to 14.63% for inbreds TRFK 6/8 and AHP S15/10 respectively. Inbred AHP S15/10 at 14.63% was the only cross which exhibited positive heterosis over the standard variety (Table 12). At Kangaita, mean standard heterosis was -32.59% with a range from -55.66% to -0.18% for TRFK 6/8 x EPK TN14-3 and AHP S15/10 x EPK TN 14-3 respectively. All the crosses exhibited negative standard heterosis for C.

4.3.3.4 Caffeine (CAFF)

Mid-parent heterosis at Timbilil ranged from -8.21% to 8.72% for inbreds EPK TN 14-3 and TRFK 6/8 respectively (Table 10). Positive heterosis was recorded in 9 crosses exhibited higher mid parent (Table 10). At Kangaita, mean MPH for CAFF was 4.41% with a range from -16.36% to 16.32% for inbreds EPK TN14-3 and TRFK 6/8 respectively (Table 10). Twelve crosses exhibited positive heterosis over the mid parent for CAFF.

Better parent heterosis at Timbilil ranged between -12.29% to 8.78% for TRFK 6/8 x AHP S15/10 and TRFK 6/8 self respectively. Crosses TRFK 6/8 self, EPK TN14-3 x AHP S15/10 and TRFCA SFS150 self at 8.78%,1.96% and 1.47% respectively showed a positive heterosis over the better parent (Table 11). At Kangaita, average BPH for

CAFF was 0.64% with a range from -16.36% to 16.32% for inbreds EPK TN14-3 and TRFK 6/8 respectively (Table 11). Seven crosses had positive heterosis over the better parent for CAFF.

Standard heterosis for caffeine ranged between 6.08% and 23.31% for TRFK 6/8 x AHP S15/10 and EPK TN14-3 x AHP S15/10 respectively. However, all the crosses manifested positive heterosis over the standard variety (Table 12). Mean standard heterosis at Kangaita was 16.32% with a range from 4.85% to 24.34 % for inbred EPK TN14-3 and AHP S15/10 x EPK TN 14-3 respectively (Table 12). All the crosses exhibited positive standard heterosis for CAFF.

4.3.3.5 Epicatechin (EC)

Mid-parent heterosis at Timbilil for EC ranged from -27.25% to 49.11% for TRFK 6/8 self and EPK TN14-3 X TRFCA SFS150 respectively. 13 crosses exhibited positive heterosis over the mid parent (Table 10). At Kangaita, mean MPH for EC was -16.71% with a range from -31.85% to -1.13% for inbreds EPK TN14-3 and TRFK 6/8 respectively (Table 10). All the crosses exhibited negative mid-parent heterosis for EC.

Better parent heterosis at Timbilil for EC ranged from -27.27% to 44.44% for inbred TRFK 6/8 and cross EPK TN14-3 x TRFCA SFS150 respectively (Table 11). Eleven crosses showed a positive heterosis over the better parent (Table 11). At Kangaita, average BPH for EC was -16.58% with a range from -31.85% to 5.30% for inbred EPK TN14-3 and TRFCA SFS150 x TRFK 6/8 respectively. All the crosses except TRFCA SFS150 x TRFK 6/8 at 5.30% exhibited negative BPH for EC.

Standard heterosis at Timbilil for EC ranged between -27.27% to 44.45% for inbred TRFK 6/8 and EPK TN14-3 x TRFCA SFS150 respectively. Ten crosses exhibited positive heterosis over the standard variety, TRFK 6/8 (Table 12). At Kangaita, mean standard heterosis for EC was -21.07% with a range from -36.00% to 0.99% for TRFCA SFS150 x AHP S15/10 and TRFK 6/8 x EPK TN14-3 respectively. All the crosses except TRFK 6/8 x EPK TN14-3 at 0.99% exhibited negative standard heterosis for EC.

4.3.3.6 Epigallocatechin-3-gallate (EGCG)

Mid-parent heterosis for EGCG at Timbilil ranged from -9.87% to 5.87% for EPK TN14-3 x TRFCA SFS150 and TRFK 6/8 x TRFCA SFS150 respectively (Table 10).Crosses TRFK 6/8 x TRFCA SFS150, TRFCA SFS150 self, AHP S15/10 self and TRFK 6/8 x AHP S15/10 had higher mid parent heterosis by 5.87%, 3.33%, 1.30% and 0.31% respectively (Table 10). At Kangaita, mean MPH for EGCG was 2.44% with a range from -7.97% to 11.96% for TRFCA SFS150 x EPK TN14-3 and inbred TRFK 6/8 respectively (Table 10). Ten crosses exhibited positive MPH for EGCG.

Better parent heterosis for EGCG at Timbilil ranged from -16.11% to 5.73% for EPK TN14-3 x TRFCA SFS150 and TRFK 6/8 x TRFCA SFS150 respectively (Table 11). Cross TRFK 6/8 x TRFCA SFS150, and inbreds TRFCA SFS150 and AHP S15/10 at 5.73%, 3.34% and 1.30% respectively showed positive better parent heterosis (Table 11). At Kangaita, average BPH for EGCG was 0.06% with a range from -11.12% to 11.96% for TRFCA SFS150 x EPK TN14-3 and inbred TRFK 6/8 respectively (Table 11). Eight crosses exhibited positive BPH for EGCG.

Standard heterosis at Timbilil ranged between -9.47% to 12.25% for inbreds TRFK 6/8 and AHP S15/10 respectively. Eleven crosses had positive heterosis over the standard variety (Table 12). At Kangaita, mean standard heterosis for EGCG was 11.58% with a range from 1.90% to 20.45% for TRFCA SFS150 x EPK TN14-3 and inbred EPK TN14-3 respectively (Table 12). All the crosses exhibited positive standard heterosis for EGCG.

4.3.3.7 Epicatechin-3-gallate (ECG)

Mid-parent heterosis for EGCG at Timbilil ranged from -20.56% to 40.36% for inbreds TRFK 6/8 and AHP S15/10 respectively. Eleven crosses displayed positive heterosis over the mid parent (Table 10). At Kangaita mean MPH for ECG was -3.65% with a range from -24.34% to 21.13% for inbred AHP S15/10 and TRFK 6/8 x TRFCA SFS150 respectively (Table 10). Seven crosses exhibited positive MPH for ECG.

Better parent heterosis at Timbilil ranged from -20.49% to 67.91% for inbreds TRFK 6/8 and AHP S15/10 respectively. Eight crosses showed a positive heterosis over the better parent (Table 11). At Kangaita, average BPH for ECG was -3.83% with a range from -32.60% to 23.85% for AHP S15/10 x TRFK 6/8 and TRFK 6/8 x TRFCA SFS150 respectively. Seven crosses exhibited positive BPH for ECG.

At Timbilil, standard heterosis ranged from -20.49% to 18.02% for inbred TRFK 6/8 and EPK TN14-3 x TRFCA SFS150 respectively. Fourteen crosses had positive heterosis over the standard variety (Table 12). Mean standard heterosis for EC was 3.94% with a range of -10.90% to 18.52% for AHP S15/10 x TRFK 6/8 and TRFK 6/8

x TRFCA SFS150 respectively. Eleven crosses exhibited positive standard heterosis for ECG.

4.3.3.8 Total catechins (TC)

At Timbilil, mid-parent heterosis ranged from -12.07% to 10.56% for inbreds TRFK 6/8 and AHP S15/10 respectively. 8 crosses showed positive heterosis over the mid parent (Table 10). At Kangaita, mean MPH for TC was -2.86% with a range from -10.40% to 7.82% for AHP S15/10 x AHP S15/10 and EPK TN14-3 x EPK TN14-3 respectively (Table 10). Seven crosses exhibited positive MPH at Kangaita.

Heterosis over the better parent at Timbilil ranged between -12.08% to 11.79% for inbreds TRFK 6/8 and AHP S15/10 respectively. Crosses AHP S15/10 self, EPK TN14-3 x TRFK 6/8, TRFK 6/8 x TRFCA SFS150, EPK TN14-3 x TRFCA SFS150 showed higher better parent heterosis by 11.79%, 2.73%, 1.26% and 0.74% respectively (Table 11). At Kangaita, average BPH for TC was -8.69% with a range from -78.72% to 7.82% for EPK TN14-3 x AHP S15/10 and EPK TN14-3 x EPK TN14-3 respectively (Table 11). Four crosses namely EPK TN14-3 x EPK TN14-3, TRFCA SFS150 x TRFK 6/8, inbreds TRFCA SFS150 and TRFK 6/8 at positive BPH at 7.82%, 4.30%, 2.72% and 1.71% respectively.

Standard heterosis at Timbilil ranged from -12.08% to 4.15% for TRFK 6/8 self and EPK TN14-3 x TRFK 6/8 respectively (Table 12). Crosses EPK TN14-3 x TRFK 6/8, AHP S15/10 self, EPK TN14-3 x TRFCA SFS150, TRFK 6/8 x TRFCA SFS150, AHP S15/10 EPK TN 14-3 showed higher standard heterosis by 4.15%,3.35%, 2.14%,

1.26% and 1.17% respectively (Table 12). At Kangaita, mean standard heterosis for TC was 1.47% with a range from -7.46% to 14.07% for TRFCA SFS150 x EPK TN14-3 and EPK TN14-3 x EPK TN14-3 respectively. Ten crosses exhibited positive standard heterosis for TC at Kangaita.

4.4 Discussion

Progenies outperform their parents due to transgressive segregation (Sleper and Poehlman, 2006; Rieseberg *et al.*, 1999). Heteroses were estimated in form of mid-parent, better parent and standard heterosis in two environments where the crosses were evaluated. The present study found great variability among the crosses and a possibility for the exploitation of heterosis for the improvement of catechins and caffeine content in tea. The essence of the superiority of the hybrids over the mid-parent, better parent and the local check can be profitably exploited for commercial production of tea with the desired attributes. Positive heterosis is beneficial for all the studied traits. Exploitation of heterosis for improving quality in tea has been reported (Kamunya *et al*., 2010).

Most of the hybrids in this study had significantly higher and desirable levels of heteroses over the mid parent, better parent and the standard. The best parental cross for GA at both Timbilil and Kangaita was TRFK 6/8 x AHP S15/10 which showed high positive mid-parent, better parent and standard heterosis indicating the presence of exploitable hybrid vigour for GA. This cross also recorded the highest GA at both sites. At Kangaita a high positive mean mid parent, better parent and standard heterosis was exhibited, compared to Timbilil which recorded negative heteroses. This implied that

most of the crosses performed better than the parents at Kangaita than Timbilil and therefore a great potential for exploitation of useful heterosis. GA is an important biochemical compound. It has a wide range of application in the pharmaceutical industry (Fiuza *et al*., 2004). It is also used as a standard for determination of the phenol content in various analytes by the Folin - Ciocalteau assay.

At Timbilil, AHP S15/10 inbred recorded the highest mid parent and better heterosis for EGC. All the crosses had negative standard heterosis for EGC at Timbilil implying that none of the crosses had higher EGC content compared to TRFK 6/8. However at Kangaita, inbred EPK TN14-3 had the highest positive mid parent, better parent and standard heterosis for EGC and it also had the highest EGC content. Cross EPK TN14-3 x TRFCA SFS150 at Timbilil had the highest EGC content. Both crosses contained EPK TN14-3 as one parent, indicating that it could be considered when developing high EGC tea in both Timbilil and Kangaita.

Inbred EPK TN14-3 had the highest mid parent and better parent heterosis for C at Timbilil while inbred AHP S15/10 x AHP S15/10 was the only progeny with positive standard heterosis for the same trait. At Kangaita, highest mid parent and better parent heterosis was exhibited by cross TRFK 6/8 x TRFCA SFS150 while no cross had a positive standard heterosis. This meant that no cross had a higher C content compared to TRFK 6/8.

Inbred TRFK 6/8 showed the highest positive mid parent and better parent heterosis for CAFF at both Timbilil and Kangaita. Similarly at both sites, all the crosses had positive

standard heterosis for CAFF, implying that TRFK 6/8 contained low caffeine levels. Caffeine is a very important biochemical constituent as it contributes to the quality of tea (Fernandez *et al*., 2002; Caffin *et al.,* 2004). Medically, caffeine acts as a diuretic, cardiac muscle stimulant, central nervous system stimulant, smooth muscle relaxant, gastric acid secretion stimulant, elevates plasma free fatty acids and glucose (Harbowy and Balentine, 1997). Consumer preference with regard to caffeine content in tea differs between different individuals. In view of this, when developing teas with low caffeine levels, TRFK 6/8 will be the best clone to use. On the other hand, if high caffeine tea is desired, cross EPK TN14-3 or AHP S15/10 would be the preferred parent.

Development of varieties with high EC is considered important in tea breeding programs. Cross EPK TN14-3 x TRFCA SFS150 had the highest and positive mid parent, better parent and standard heterosis for EC at Timbilil. At Kangaita, EPK TN14-3 x TRFCA SFS150 had the highest positive better parent heterosis for EC and thus it is the most desirable for developing high EC tea varieties because it exhibits useful and positive heterosis for EC.

Development of varieties with high EGCG content is highly desired in tea improvement. EGCG has several health benefits including cancer chemoprevention (Hsuuw and Chen, 2007), improving cardiovascular health (Hirai et al., 2007), and antioxidant properties (Fu and Koo, 2006). At Timbilil, cross TRFK 6/8 x TRFCA SFS150 had the highest positive mid parent and better parent heterosis for EGCG. At Kangaita, inbred TRFK 6/8 exhibited the highest mid parent and better heterosis for EGCG. All the crosses exhibited positive standard heterosis for EGCG at both sites

implying that TRFK 6/8 contained very low EGCG content. In view of this, inbred EPK TN14-3 and AHP S15/10 which had the highest positive standard heterosis are the best crosses for developing high EGCG tea varieties.

Inbred AHP S15/10 and cross TRFK 6/8 x TRFCA SFS150 recorded the highest positive mid parent, better parent and standard heterosis for ECG at Timbilil and Kangaita respectively. This indicates the possibility of these crosses to be used in tea breeding programme to boost up the ECG contents in tea.

TC content is generally used as an indicator of the quality potential in tea with high catechins teas having high black tea quality (Obanda and Owuor 1997). At Timbilil, EPK TN14-3 x TRFK 6/8 and inbred AHP S15/10 recorded the highest and positive significant mid parent, better parent and standard heterosis for TC. At Kangaita, inbred EPK TN14-3 exhibited the highest positive mid parent, better parent and standard heterosis for TC. In addition to this, they recorded high percent TC values and seem to be the most suitable crosses when developing tea with high TC content using the materials in the study.

CHAPTER FIVE

Effects of Genotype by Environment Interaction for TC, EGCG and CAFF in Kenyan Tea (*Camellia sinensis*) (L.) O. Kuntze)

Abstract

Genotype x environment interactions (GEI) indicates the inconsistency in relative performance of genotypes over environments. Assessment of the stability of genotypes across different environments is useful for recommending cultivars for known conditions of optimized cultivation. The objective of this study was to investigate GEI of 16 tea hybrids developed for both the Timbilil and Kangaita environments. Additive main effects and multiplicative interaction (AMMI) and GGE (genotype and genotype by environment interaction) biplot were used to assess the stability of hybrids. There were highly significant ($p<0.05$) differences between genotypes and GEI for all the traits except caffeine. The first interaction principal component axes (IPCA) of the AMMI model accounted for 82.54%, 64.95% and 52.11% of the total G x E interaction sum of squares for TC, EGCG and caffeine. The AMMI biplot clearly depicted the genotypes on the bases of their adaptation patterns. Both models revealed that crosses TRFCA SFS150 x EPK TN14-3, TRFK 6/8 x EPK TN14-3 and TRFK 6/8 x AHP S15/10 were stable for TC. Crosses AHP S15/10 x EPK TN 14-3, TRFK 6/8 x TRFCA SFS150, inbred of TRFCA SFS150 and TRFK 6/8 x AHP S15/10 were more stable with regards to EGCG. Similarly, crosses TRFK 6/8 x EPK TN14-3 and TRFK 6/8 x TRFCA SFS150 were found to be more stable and responsive to favourable environments. These crosses did not necessarily contain genotypes with the highest quantities of TC, EGC and caffeine. These genotypes could therefore be considered for the tea breeding programme to develop or avail cultivars with high TC, EGCG and caffeine contents to meet the highly demanding consumer needs as well as the changing climatic conditions.

5.1 Introduction

Tea (*Camellia sinensis*) (L.) O. Kuntze) is an important crop for millions of people in the world and is the most widely consumed beverage water (Wheeler and Wheeler, 2004; Cheng, 2004). Generally, beverage tea is broadly classified according to the method of fermentation (Takeo, 1992). The three main types of teas are black, green and oolong (Wang *et al*., 2000). Green tea is non-fermented, whereas black tea is completely fermented, while oolong tea is partially fermented (Friedman *et al*., 2005). Kenya is the world's largest producer of black tea in the world (ITC, 2013). The tea industry contributes approximately 26% of the export earnings and 4% of the Gross

Domestic Product (GDP) to the Kenyan economy (TBK, 2013). Other types of tea produced are white, yellow and reprocessed tea which include flower scented tea, compressed tea, instant tea and herbal teas (Hara *et al*., 1995). However, white and yellow teas have been considered as subclasses of green tea by Harbowy and Balentine (1997). The genotype by environment interaction (GEI) makes genotypes to perform differently in different environments especially in the highly variable weather conditions (Sibiya *et al*., 2011). This has complicated the breeding and selection for important traits due to cross interaction among the hybrid ranks in different environments and hence promising genotypes with wide adaptation are barely selected (Beyene *et al*., 2011; Mitrovic *et al*., 2012). The GE component reduces the heritability of the traits affecting breeding progress due to inaccurate selections especially in single environments.

Through genotype by environment interaction (GEI) studies, stable genotypes which are adapted to specific target areas and potential candidates for hybrid combinations are identified (Abay *et al*., 2009). The identification of stable tea varieties could help to enhance the farmers' acceptability or adoption of elite new varieties. The by genotype by environment interaction (GEI) analysis has been performed using different methods in different crops such as additive main effects and multiplicative interaction model (AMMI), principal component analysis (PCA) and linear regression analysis (LRA), analysis of variance (ANOVA) and GGE biplot method of analysis (Akcura *et al*., 2011; Mitrovic *et al*., 2012).

The AMMI method uses the principal component to interpret cultivar performance by integrating the use of ANOVA and PCA. The AMMI analyses combine additive components in a single model for the main effects of genotype and environment as well as multiplicative components for the interaction effect. The graphic analyses bring out phenotypic stability, genotypic behaviour of the cultivars and environments which optimize performance (Miranda *et al.,* 2009). The AMMI model displays main effects of genotypes and environment and their interactions. It also contributes to improved cultivar evaluation, recommendations and selection of test sites (Abay *et al.,* 2009). The GGE biplot analysis integrates the genotype and genotype by environment effects in the evaluation of cultivars. It uses graphic axes and identifies superior cultivars in the mega environments (Akcura *et al*., 2011). Mega environments comprise groups of locations which consistently share the same test cultivars (Abay *et al*., 2009). It also combines ANOVA and PCA by partitioning together sum of squares of genotypes and sum of squares of genotype by environment interaction using the PCA method. It is also used for the presentation and estimation of genotypes in different environments (Miranda *et al*., 2009). The growing of high yielding, high quality and stable tea varieties will help to increase production and lead to improved living standards.

5.2 Materials and methods

5.2.1 Experimental sites

The 4 x 4 full diallel cross trial comprising sixteen clonal full-sib families and four parental clones was established at Timbilil and Kangaita. Descriptions of these experimental sites are presented in section 3.2 of this thesis.

5.2.2 Plant materials

The plant material used in this study consisted of four parents that were involved in the 4 x 4 full diallel cross. Details of the plant materials are as in section 3.3 of this thesis.

5.2.3 Sample preparation and analysis

Samples preparation and HPLC analysis was done according to the method described in sections 3.5.1, 3.5.2 and 3.5.3 of this thesis.

5.3 Statistical analysis

The data on all the biochemical traits studied for the two locations and seasons were combined and analysed using AMMI and GGE biplots. A combined analysis of variance (ANOVA) for the biochemical traits was done using GenStat 12th edition (Payne, 2008), to determine the effects of environment (E) genotype (G), and all possible interactions among the factors. The mean values of genotypes for each experiment were used to analyse the relationships among genotypes.

5.3.1 AMMI stability method

The model used was;

$Y_{ij} = \mu + G_i + E_j + GE_{ij}$

Where Yij is the corresponding variable of the ith genotype in jth environment (location), μ is the total mean, G_i is the main effect of ith genotype, E_j is the main effect of jth environment, GE_{ij} is the effect of genotype x environment interaction.

AMMI model, which combines the standard analysis of variance with principal component analysis, was used to analyze and interpret genotype x environment

interaction (Zobel *et al*., 1988). The AMMI model first fits additive effects for the main effects of genotypes and environments, using the additive analysis of variance procedure. Subsequently the program fits multiplicative effects for GEI by principal component analysis (Zobel *et al.*, 1988). The AMMI model equation is as follows:

$$Y\,ij = \mu + Gi + Ej + \sum_{k=1}^{t} \lambda k\ \alpha ik\ \lambda jk + \varepsilon ij$$

Where; Yij is the cells mean for the ith genotype and jth environment, μ is the overall mean gi and ej are the main effects of genotype and environments respectively, and εij is the experimental error. Residuals of the ijth cell, Zij = Yij – Yi. –Y.j + Y form a matrix Z. The least square estimate of the AMMI parameters λk is the singular value of the nth PCA axis, αik and yjk are the scores for ith genotype and jth environment. The AMMI model was adjusted depending on the number of principal components (PC) considered. In this study two PC's were factored, therefore, the model was adjusted to Yij = μ + gi + ej +λ1αi1yj1+ λ2αi2yj2+ εij which considered the main effects in addition to IPCA1 and IPCA2 for non-additive variation (Gauch, 1992).

5.3.2 GGE biplot

Biplots (GGE - biplot, 2009), were used to illustrate the relationships among genotypes, environments and genotypes and environments.

The GGE biplot analysis used the following model:

$$Y_{ij} - \mu - E_j = \lambda_1\, \varepsilon_1\, \eta_{1j} + \lambda_2\, \varepsilon_2\, \eta_{2j} + e_{ij}$$

Where Yij is the corresponding variable of the ith genotype in jth environment (location), μ is the total mean, Ej is the main effect of jth environment, λ1 and λ2 are singular values of principal components PC1 and PC2; εi1 and εi2 are eigen vectors in jth environment (location) for PC1 and PC2 of i genotype in j environment.

The GGE biplots were constructed by plotting the first two principal components (PC1 and PC2) derived from subjecting environment centred biochemical data to singular value decomposition (Yan *et al*., 2000). In the biplot, the best genotypes in each environment and groups of environments were identified through a polygon view (Yan *et al*., 2000) that was drawn by connecting genotypes that were furthest from the biplot origin such that all genotypes were enclosed within the polygon. Perpendicular lines were then drawn to each side of the polygon starting from the biplot origin.

5.4 Results

5.4.1 Combined AMMI analysis of variance for TC

The AMMI analysis of variance (additive main effects) for TC showed significant effects for genotypes, environment and the genotype by environment interaction, GEI (Table 13). This implies that different crosses could be selected for the different agro ecological zones. The relative magnitudes of the different sources of variation varied greatly as shown by their variance components in Table 13. The treatments (crosses and environment) and genotype by environment interaction (GEI) both accounted for 82.54% of the total variation, with genotype by environment interaction (GEI) accounting for the highest amount of variation at 35.55%, followed by environment (E) at 34.67% and Genotype (G) at 29.78%. The interaction principal component one (IPCA1) accounted for 35.55% while principal component two (IPCA2) accounted for 0% of the genotype by environment (GEI) variation sum of squares (Table 13). The large sum of squares for environments and GEI indicated that the environments included in the study were diverse with large differences among environmental means causing most of the variation in total catechins.

Table 13. Source of variation, mean squares and significance and their contribution to total variation of the genotypes for TC.

Source	df	SS	MS	%SS explained	GxE explained
Treatments	39	176.44	4.5***	82.54	
Genotypes	19	52.54	2.77***	29.78	
Environments	1	61.18	61.18***	34.67	
GEI	19	62.72	3.30**	35.55	
Block	4	14.69	3.67*		
IPCA(1)	19	62.72	3.30**		35.55
IPCA(2)	17	0	ns		
Residuals	-17	0	ns		
Error	76	22.63	0.298		
Total	119	213.76	1.796		

Where; *, ** and *** denote significant differences at $p<0.05$, $p<0.01$ and $P<0.001$, ns = not significant

IPCA= Interaction principal component axis

5.4.2 Combined AMMI analysis of variance for EGCG

The AMMI analysis of variance (additive main effects) for EGCG showed significant effects for genotypes and the genotype by environment interaction, GEI (Table 14). The treatments (G and E) and GEI accounted for 64.95% of the total variation, with the genotype accounting for the highest amount of variation at 48.53%, followed by GEI at

15.54% and E at 0.89%. The interaction principal component one (IPCA1) accounted for 15.54% while principal component two (IPCA2) accounted for 0% of the GEI variation sum of squares (Table 14). The large sum of squares for genotype indicated that the environment was not the main source of variation for EGCG.

Table 14. Source of variation, mean squares and significance, and their contribution to total variation of the genotypes for EGCG

Source	df	SS	MS	%SS Explained	GxE explained
Treatments	39	37.91	0.97***	64.95	
Genotypes	19	28.33	1.49**	48.53	
Environments	1	0.52	0.52	0.89	
Block	4	7.87	1.97		
GE1	19	9.07	0.48***	15.54	
IPCA(1)	19	9.07	0.48***		15.54
IPCA(2)	17	0	NS		
Residuals	-17	0	NS		
Error	76	12.59	0.17		
Total	119	58.37	0.49		

Where; *, ** and *** denote significant differences at $p<0.05$, $p<0.01$ and $P<0.001$, NS = not significant, IPCA= Interaction principal component axis

5.4.3 Combined AMMI analysis of variance for Caffeine (CAFF)

The AMMI analysis of variance (additive main effects) for CAFF showed significant effects for genotypes and the genotype by environment interaction, GEI (Table 15). The treatments (G and E) and GEI accounted for 52.11% of the total variation, with the genotype accounting for the highest amount of variation at 43.11%, followed by GEI at 9.00% and E at 0.14%. The interaction principal component one (IPCA1) accounted for 15.54% while principal component two (IPCA2) accounted for 0% of the GEI variation sum of squares (Table 15). The large sum of squares for genotype indicated that the genotype was the main source of variation for CAFF.

Table 15. Source of variation, mean squares and significance, and their contribution to total variation of the genotypes for CAFF.

Source	df	SS	MS	% SS Explained	G x E explained
Treatments	39	3.82	0.10***	52.11	
Genotypes	19	3.16	0.17***	43.11	
Environments	1	0.01	0.01	0.14	
Block	4	0.57	0.14**		
GEI	19	0.66	0.03	9.00	
IPCA(1)	19	0.66	0.03		9.00
IPCA(2)	17	0	0		
Residuals	-17	0	0		
Error	76	2.93	0.04		
Total	119	7.33	0.06		

Where; *, ** and *** denote significant differences at $p<0.05$, $p<0.01$ and $P<0.001$, ns = not significant, IPCA= Interaction principal component axis

AMMI Analysis has also been done in other crops and the stability of genotypes predicted on the basis of mean performance and the magnitude of IPCA1 scores in sorghum (Zavala-Garcia, *et al*. 1992), barley (Romagossa, *et al*. 1993) and chickpea (Zali, *et al*., 2011).

Table 5 present the AMMI analyses data with the scores for the 16 crosses and the test environments, respectively. The IPCA scores indicate how far the individual genotype or environment deviates from the zero (origin). The more deviation from zero (either negative or positive direction) the more unstable they are.

Table 16. IPCA1 scores and graph IDs for the 16 crosses and their parents sorted based on the mean of TC, EGCG and TC when evaluated in the two environments.

Genotype	Family	Cross	TC		EGCG		CAFF	
			(%)Mean	IPCA1	(%)Mean	IPCA1	(%)Mean	IPCA1
G1	471	TRFCASFS150xTRFCA SFS150	22.58	0.18	10.37	-0.04	3.25	-0.31
G2	430	TRFCA SFS150 x EPK TN14-3	23.43	0.02	11.6	-0.54	3.6	0.15
G3	463	TRFCA SFS150 x AHP S15/10	23.21	-1.24	11.42	-0.1	3.53	-0.11
G4	420	TRFCA SFS150 x TRFK 6/8	22.65	0.32	10.03	-0.38	2.95	-0.05
G5	488	EPK TN14-3 x TRFCA SFS150	22.92	-0.22	10.24	0.47	3.3	0.17
G6	490	EPK TN14-3 x EPK TN14-3	21.18	0.41	10.02	-0.17	3.31	-0.06
G7	447	EPK TN14-3 x AHP S15/10	23.61	0.34	10.93	-0.19	3.33	-0.12
G8	443	EPK TN14-3 x TRFK 6/8	22.42	0.28	10.6	-0.23	3.54	-0.23
G9	485	TRFK 6/8 x TRFCA SFS150	21.95	-0.06	10.35	0.15	3.5	-0.05
G10	474	AHP S15/10 x EPK TN 14-3	21.82	-0.08	10.75	0.02	3.45	0.27
G11	478	AHP S15/10 x AHP S15/10	21.39	-0.75	10.11	0.77	3.32	0.18
G12	456	AHP S15/10 x TRFK 6/8	22.63	-0.18	10.90	0.15	3.51	0.13
G13	482	TRFK 6/8 x TRFCA SFS150	22.88	0.36	11.29	0.02	3.6	0.09
G14	476	TRFK 6/8 x EPK TN14-3	22.41	0.08	11.08	0.1	3.18	0.06
G15	475	TRFK 6/8 x AHP S15/10	22.87	-0.04	10.87	-0.09	3.35	0.09
G16	467	TRFK 6/8 x TRFK 6/8	23.24	0.46	11.43	-0.25	3.49	0.13
G17	1	TRFCA SFS150	22.68	0.5	10.75	-0.29	3.43	0.08
G18	2	EPK TN14-3	22.21	-0.04	10.83	0.13	3.35	-0.05
G19	3	AHP S15/10	22.72	0.61	10.26	0.13	3.5	-0.18
G20	4	TRFK 6/8	23.73	-0.96	11.33	0.34	3.15	-0.18

Table 17. First four AMMI selections per environment

Trait	Environment	Mean	Score	1	2	3	4
CAFF	Kangaita	3.39	0.4836	G2	G13	G10	G12
	Timbilil	3.374	-0.4836	G8	G19	G3	G13
EGCG	Kangaita	10.69	0.9323	G20	G3	G13	G16
	Timbilil	10.82	-0.9323	G2	G16	G3	G13
TC	Kangaita	23.34	1.512	G7	G16	G19	G2
	Timbilil	21.91	-1.512	G20	G3	G2	G5

Crosses are as described in the legend for Table 5.

Table 17 summarizes the first four genotypes considered as best in the 2 environments for all the three traits. G13 (TRFK 6/8 x TRFCA SFS150) was among the best four crosses for CAFF in the two environments. With regards to EGCG, G3 (TRFCA SFS150 x AHP S15/10), G13 (TRFK 6/8 x TRFCA SFS150) and G16 (inbred TRFK 6/8) were the best crosses in the two environments. In the case of TC, G2 (TRFCA SFS150 x EPK TN14-3) was among the top four crosses (Table 17).

5.4.4 AMMI biplot for TC

The IPCA scores of a genotype in the AMMI analysis are an indication of the stability of a genotype over environments. The greater the IPCA scores, either positive or negative, the more specifically adapted a genotype is to certain environments. The more IPCA scores approximate to zero, the more stable the genotype is over all environments sampled. Accordingly, hybrids G2 (TRFCA SFS150 x EPK TN14-3), G14 (TRFK 6/8 x EPK TN14-3) and G15 (TRFK 6/8 x

AHP S15/10) can be considered as the most stable hybrids, as their IPCA scores are closer to zero (Table 16 and Figure 1).

5.4.5 AMMI biplot for EGCG

The crosses G10 (AHP S15/10 x EPK TN 14-3), G13 (TRFK 6/8 x TRFCA SFS150), G1 (inbred TRFCA SFS150) and G15 (TRFK 6/8 x AHP S15/10) were the most stable hybrids for EGCG. Their IPCA scores are closer to zero (Table 16 and Figure 2).

5.4.6 AMMI biplot for CAFF

Crosses G14 (TRFK 6/8 x EPK TN14-3) and G9 (TRFK 6/8 x TRFCA SFS150) were the most stable hybrids for CAFF as their IPCA scores are closer to zero (Table 16 and Figure 3).

Overall, Timbilil had a higher mean CAFF, EGCG and TC than Kangaita, implying that it was the most favourable environment for these traits. The best three crosses for CAFF at Timbilil were EPK TN14-3 x AHP S15/10, EPK TN14-3 x TRFCA SFS150 and AHP S15/10 x EPK TN 14-3 (Figure 3). The best three crosses for EGCG at Timbilil were AHP S15/10 self, AHP S15/10 x EPK TN 14-3, EPK TN14-3 x TRFK 6/8 (Figure 2). The best three crosses for TC at Timbilil were EPK TN14-3 x TRFK 6/8, AHP S15/10 self and EPK TN14-3 x TRFCA SFS150 (Figure 1).

The best three crosses for CAFF at Kangaita were AHP S15/10 x EPK TN 14-3, TRFCA SFS150 x AHP S15/10 and TRFCA SFS150 self (Figure 3) . The best three

crosses for EGCG at Kangaita were EPK TN14-3 self, AHP S15/10 x EPK TN 14-3 and AHP S15/10 self (Figure 2). The best three crosses for TC were EPK TN14-3 self, TRFCA SFS150 x TRFK 6/8 and EPK TN14-3 x TRFK 6/8 (Figure 1).

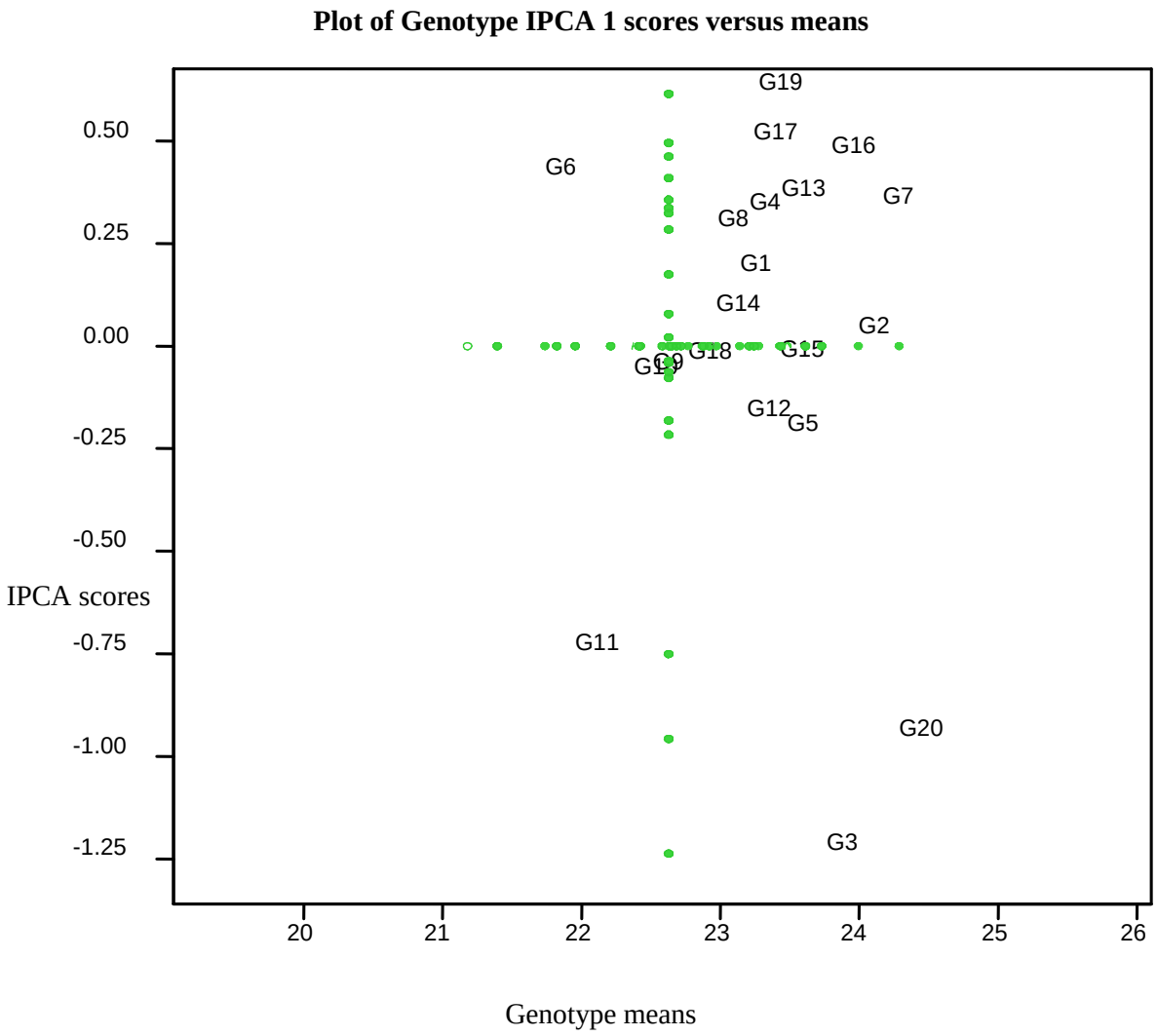


Figure 1. AMMI1 biplot for TC showing genotypes plotted against their IPCA1 scores

The crosses with low stability or associated with one or two sites would have a disadvantage of not adapting to other sites. It is therefore important to release

promising cultivars with average stability that would also not only adapt, but also be productive in unstable environments. According to the AMMI analysis the crosses TRFCA SFS150 x EPK TN14-3 (430), TRFK 6/8 x EPK TN14-3 (476) and TRFK 6/8 x AHP S15/10 (475) can be considered as the most stable hybrids for TC. Hybrids AHP S15/10 x EPK TN 14-3 (474), TRFK 6/8 x TRFCA SFS150 (482), inbred TRFCA SFS150 (471) and TRFK 6/8 x AHP S15/10 (475) were the most stable hybrids for EGCG. On the other hand, crosses TRFK 6/8 x EPK TN14-3 (476) and TRFK 6/8 x TRFCA SFS150 (482) were the most stable for CAFF.

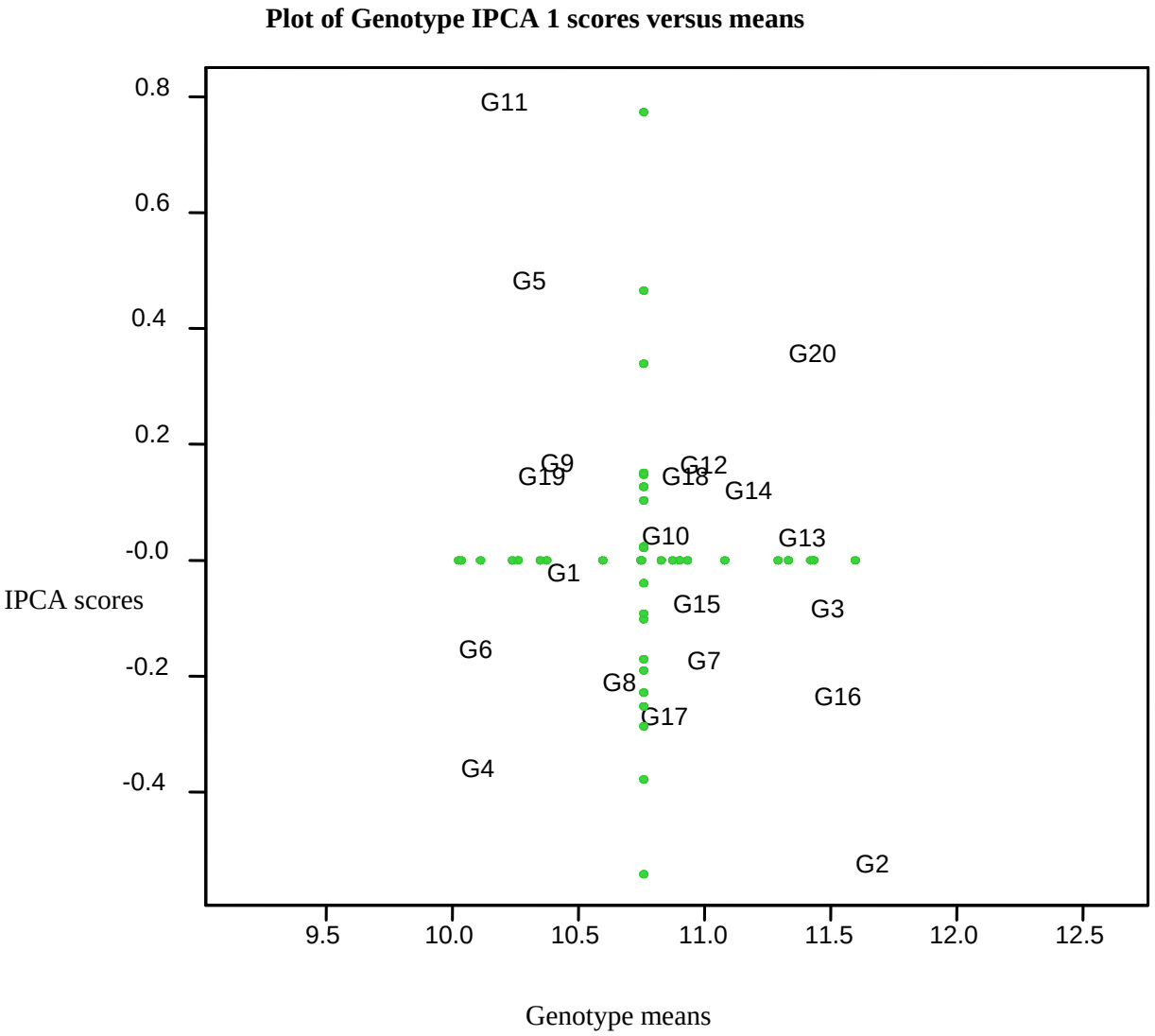


Figure 2: AMMI 1 biplot for EGCG showing genotypes means plotted against their IPCA1 scores

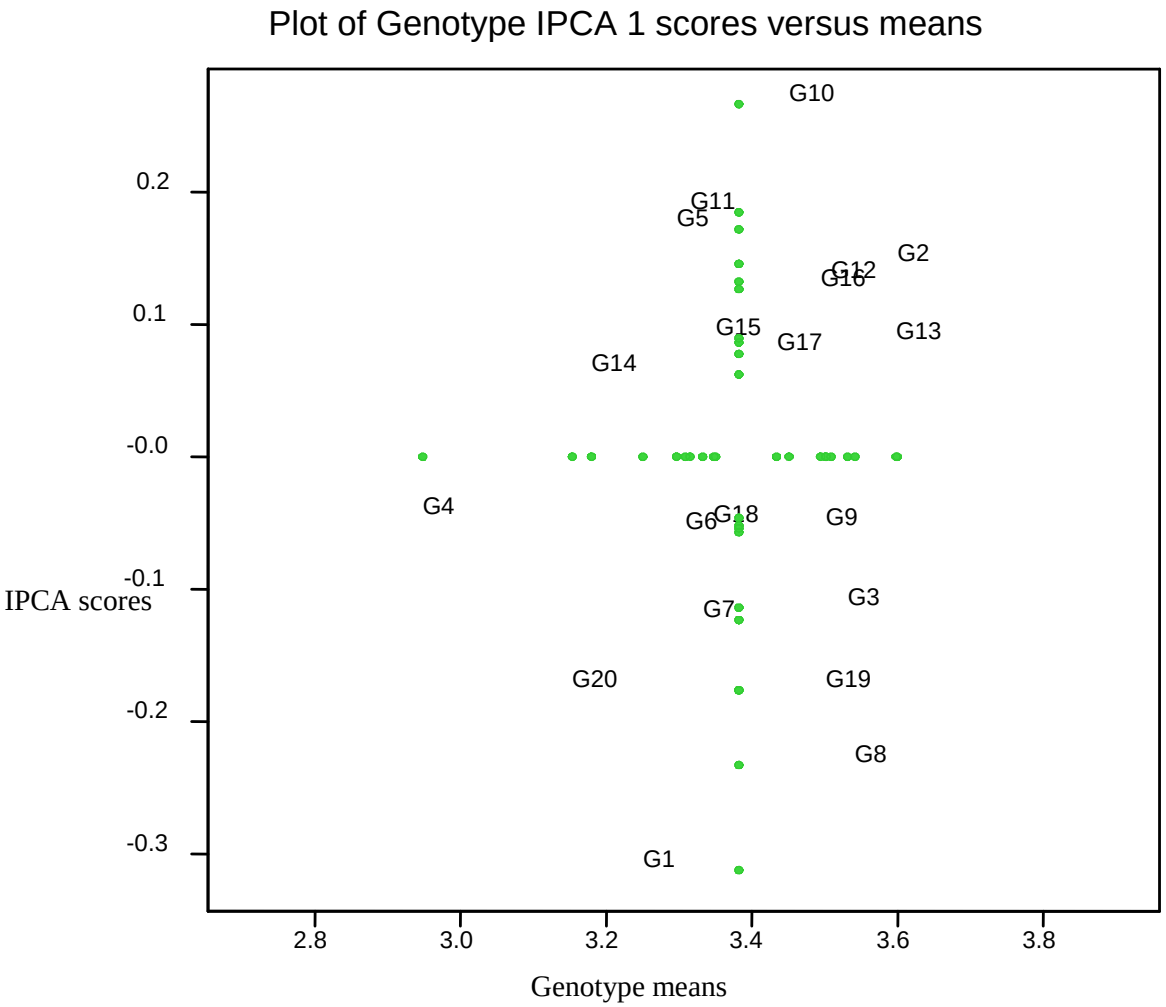


Figure 3: AMMI 1 biplot for CAFF showing genotypes means plotted against their IPCA1 scores

5.5 GGE biplot analysis for TC

The GGE biplot was generated using the first two principal component scores and it showed a clear association between genotypes and environments (Figure 4). The total catechins adaptability and stability of the crosses derived from the four parents were visually assessed using the GGE biplot analysis. In this study, the two principal components explained a total of 100% GGE variation (PC1 56.83%, PC2 43.71%, Figure 1).

The first principal component (PC1) represented on the x axis and across its value was used to estimate total catechins whereby the genotypes with higher PC1 values were considered to be more productive. The second principal component which was represented on the y axis explained the stability of genotypes (Abay, et al., 2009; Muhammadi and Amri, 2009).

Percent total catechins and stability of genotypes was estimated using the average environment coordinates (AEC) method (Yan, 2001; Yan and Hunt, 2001). From the joint biplot, the highest total catechins were registered by cross EPK TN14-3 x TRFK 6/8 (443), inbreds EPK TN14-3 (490) and AHP S15/10 (478) (Figure 1). The poorest performers included TRFCA SFS150 x EPK TN14-3 (430), inbred TRFK 6/8 (467) and TRFCA SFS150 x AHP S15/10 (463) (Figure 4).

From this study, inbred EPK TN14-3 (490) and cross EPK TN14-3 x TRFK 6/8 (443) showed the best performance in Kangaita and Timbilil respectively (Figure. 4). They were also the most responsive genotypes which also showed specific adaptation to the environments where by they performed better. They also exhibited lower or decreased stability (Sharma *et al*., 2010).

Crosses which were scattered next to the origin, indicating minimal interaction with the environment were inbred TRFCA SFS150, TRFK 6/8 x EPK TN14-3 (476) and TRFCA SFS150 x TRFK 6/8 (420) (Figure 4).

AHP S15/10 (478), EPK TN14-3 x TRFCA SFS150 (488) and TRFK 6/8 x TRFCA SFS150 (482) exhibited high TC content while TRFCA SFS150 x EPK TN14-3

(430) and inbred TRFK 6/8 (467) had low TC (Figure 5). The environments were divided into two mega environments (Figure 5).

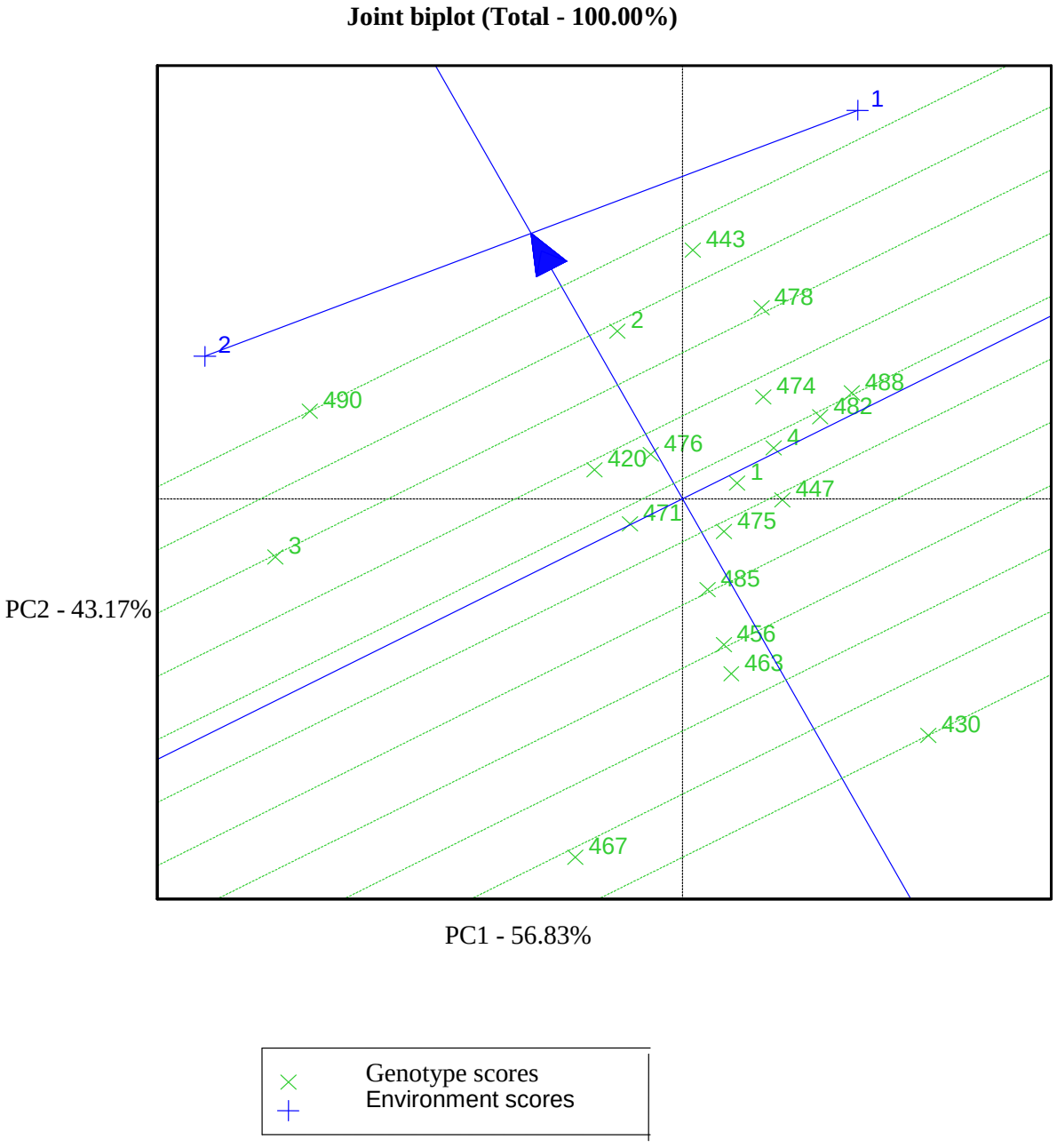


Figure 4: GGE biplot showing ranking of the crosses based on the percent TC and stability across the two environments; Timbilil and Kangaita.

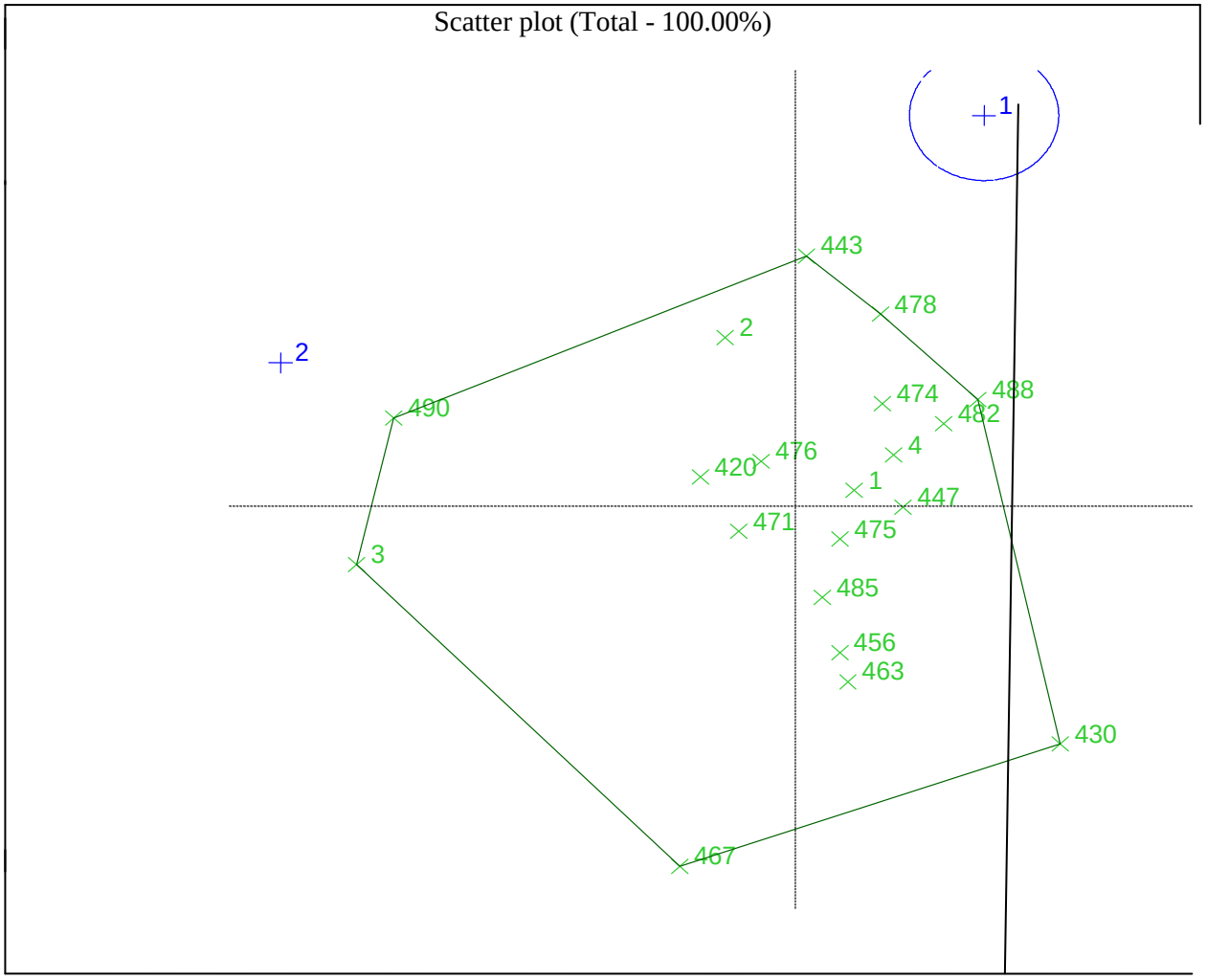


Figure 5: Polygon view of the GGE biplot showing the mega-environments and their respective highest percent TC and stable crosses. N/B 1= Timbilil, 2= Kangaita x= Genotype scores, + = Environmental scores.

Distribution of the crosses and parents in the biplot revealed that crosses TRFK 6/8 x EPK TN14-3 (476), TRFCA SFS150 x TRFK 6/8 (420), inbred TRFCA SFS150 (471) and TRFK 6/8 x AHP S15/10 (475) was scattered close to the origin, indicating minimal interaction of these crosses with the environments (Figure 5).

5.5.1 Ranking GGE biplot for TC

GGE biplot analysis also enabled visual assessment of the total catechins performance of PC1 and PC2 of the crosses for the two environments as presented in a circle (Figure 6). From the circular view, genotypes with the highest TC were inbreds EPK TN14-3 (490) and AHP S15/10 (478), crosses EPK TN14-3 x TRFK 6/8 (443) and EPK TN14-3 x TRFCA SFS150 (488) while the lowest were TRFCA SFS150 x AHP S15/10 (463), TRFCA SFS150 x EPK TN14-3 (430) and inbred TRFK 6/8 (467) (Figure 6).

The ideal genotype can be used as a reference for genotype evaluation. In this study, TRFK 6/8 x AHP S15/10 (475) and EPK TN14-3 x AHP S15/10 (447) were ideal genotypes (the center of concentric circles) and genotypes located closer to the ideal genotypes are more desirable than the others (Figure 6). Crosses grouped in the concentric circle next to ideal genotype were more desirable and these were AHP S15/10 x EPK TN 14-3 (474) and TRFK 6/8 x EPK TN14-3 (476) (Figure 6).

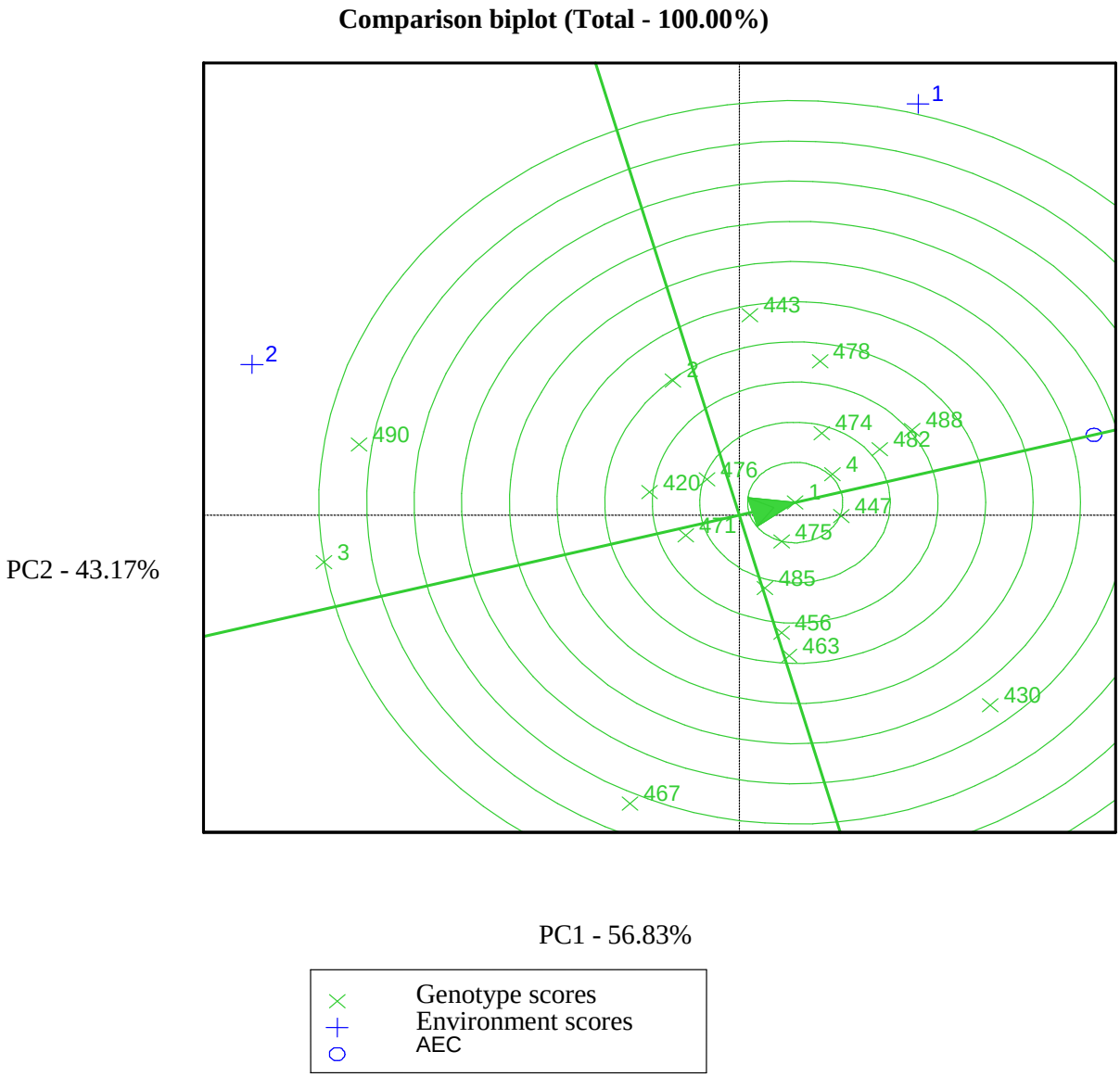


Figure 6: Ranking of genotypes relative to an ideal genotype. The ideal genotype can be used as a reference for genotype evaluation.

5.6 GGE biplot analysis for EGCG

The two principal components explained a total of 100% GGE variation for EGCG (PC1 78.92%, PC2 21.08%, Figure 7). From the joint biplot, the highest EGCG were registered by inbred AHP S15/10 (478) and EPK TN14-3 x TRFCA SFS150 (488)

(Figure 7). The poorest performers for EGCG included TRFCA SFS150 x EPK TN14-3 (430) and TRFCA SFS150 x TRFK 6/8 (420). Inbred AHP S15/10 (478) and cross AHP S15/10 x EPK TN 14-3 (474) showed the best performance in Kangaita and Timbilil respectively (Figure 7). Crosses which were scattered close to the origin, indicating minimal interaction with the environments were TRFK 6/8 x EPK TN14-3(476) and AHP S15/10 x TRFCA SFS 150 (485) and are the most stable (Figure 7).

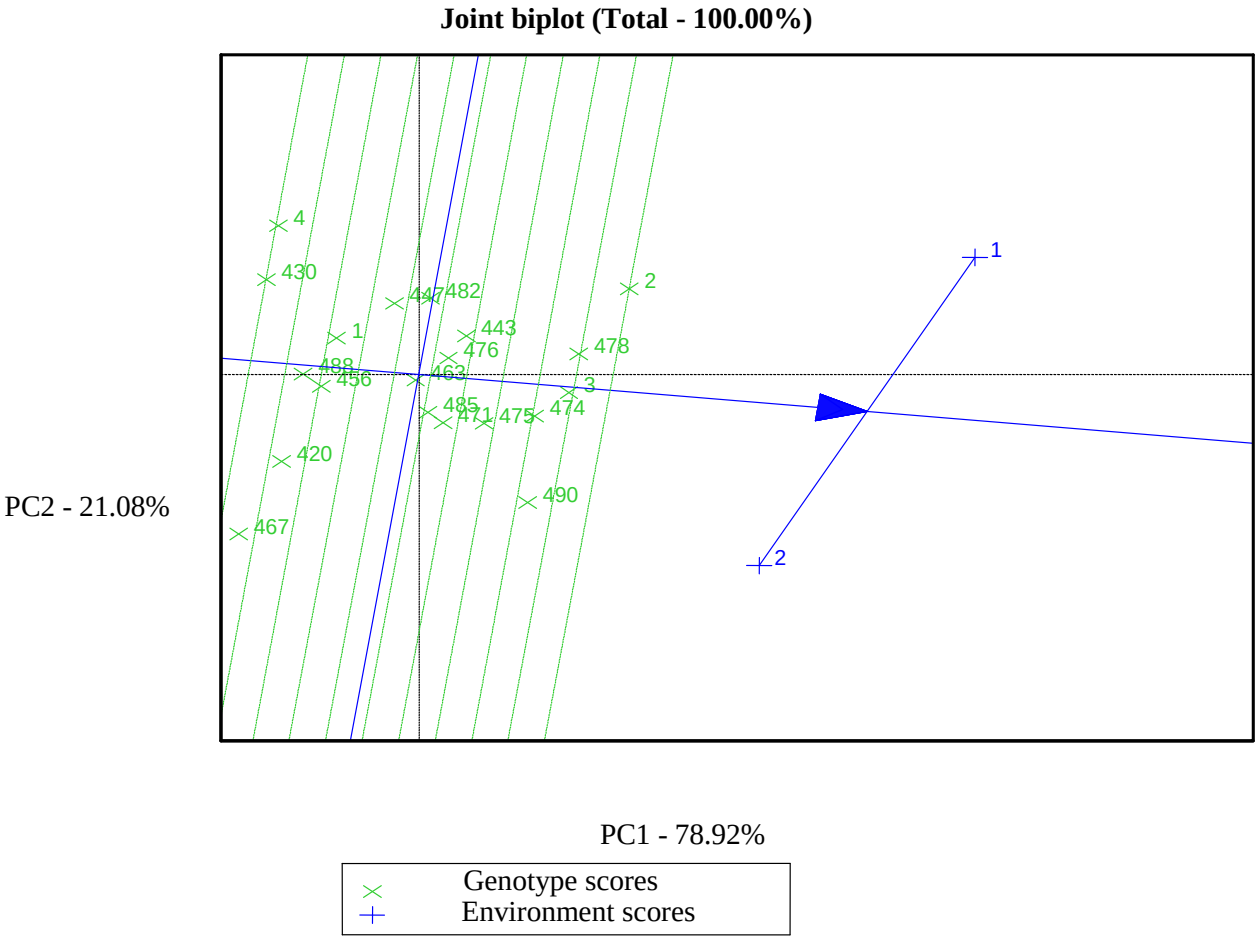
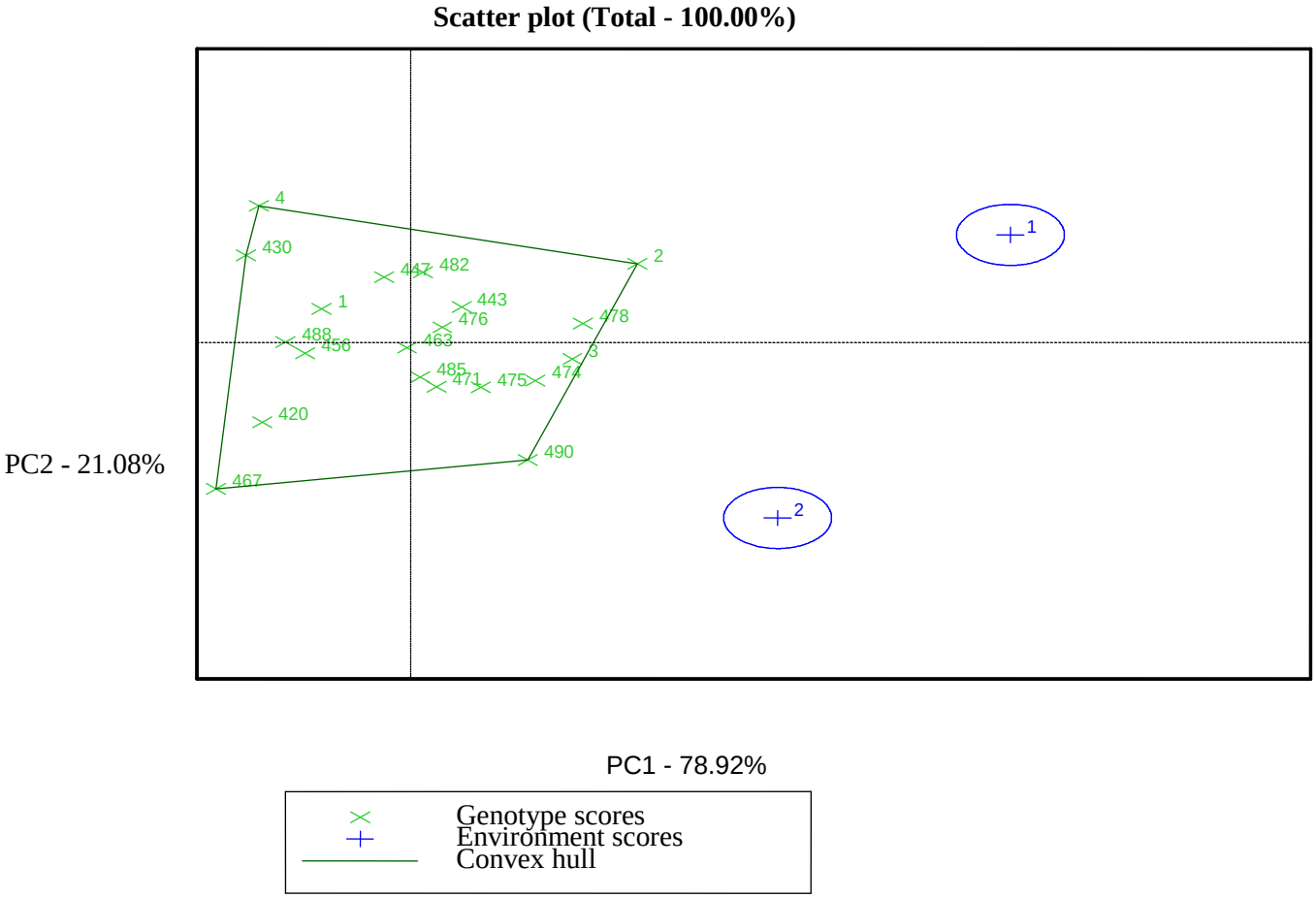


Figure 7: GGE biplot showing ranking of 16 crosses based on the percent EGCG and stability performance across the two environments; Timbilil and Kangaita.

5.6.1 Mega environment classification of the genotypes for EGCG

Figure 8 presents schematic view of which cross performed well with respect to percent EGCG content in the two environments. Inbred AHP S15/10 (478), AHP S15/10 x EPK TN 14-3 (474) and EPK TN14-3 x TRFK 6/8 (443) were the top performers in Timbilil for EGCG (Figure 8). The best crosses at Kangaita for EGCG were AHP S15/10 x EPK TN 14-3(474), inbreds EPK TN14-3 (490) and AHP S15/10 (478) (Figure 8). Cross AHP S15/10 x EPK TN 14-3 (447) performed well.

Figure 8: Polygon view of the GGE-biplot showing the mega environments and their respective highest percent EGCG and stable crosses. N/B 1=



5.7 GGE biplot analysis for CAFF

The two principal components explained a total of 100% GGE variation for EGCG (PC1 83.56%, PC2 16.44%, Figure 9). From the joint biplot, the highest CAFF were registered by AHP S15/10 x EPK TN 14-3 (474), EPK TN14-3 x AHP S15/10 (447), inbred TRFCA SFS150 (471), AHP S15/10 x TRFK 6/8 (456) and EPK TN14-3 x TRFCA SFS150 (488) (Figure 9). Crosses which had low performance for CAFF were inbred EPK TN 14-3 (490), TRFK 6/8 x AHP S15/10 (475) and TRFCA SFS150 x TRFK 6/8 (420) (Figure 9). Crosses which were scattered close to the origin, indicating minimal interaction with the environments were AHP S15/10 x TRFCA SFS 150 (485) and TRFK 6/8 x EPK TN14-9 (476) and are the most stable (Figure 9).

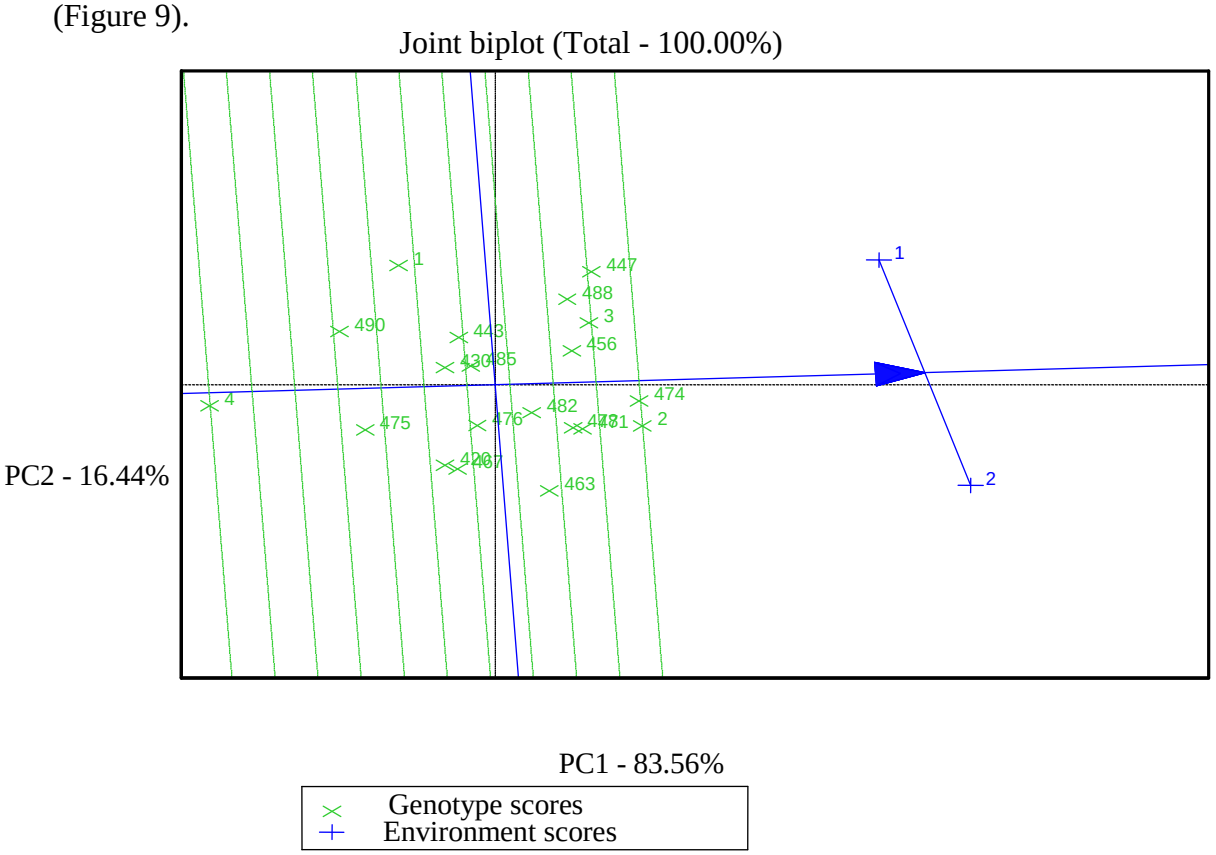


Figure 9: GGE biplot showing ranking of the crosses based on percent CAFF and stability performance across the two environments; Timbilil and Kangaita.

5.7.1 Mega environment classification of the genotypes for CAFF

Figure 10 presents schematic view of which cross performed well in a specific environment. Crosses EPK TN14-3 x AHP S15/10 (447) and EPK TN14-3 x TRFCA SFS150 (488) were the best crosses at Timbilil while AHP S15/10 x EPK TN 14-3 (474) was the best cross in Kangaita (Figure 10).

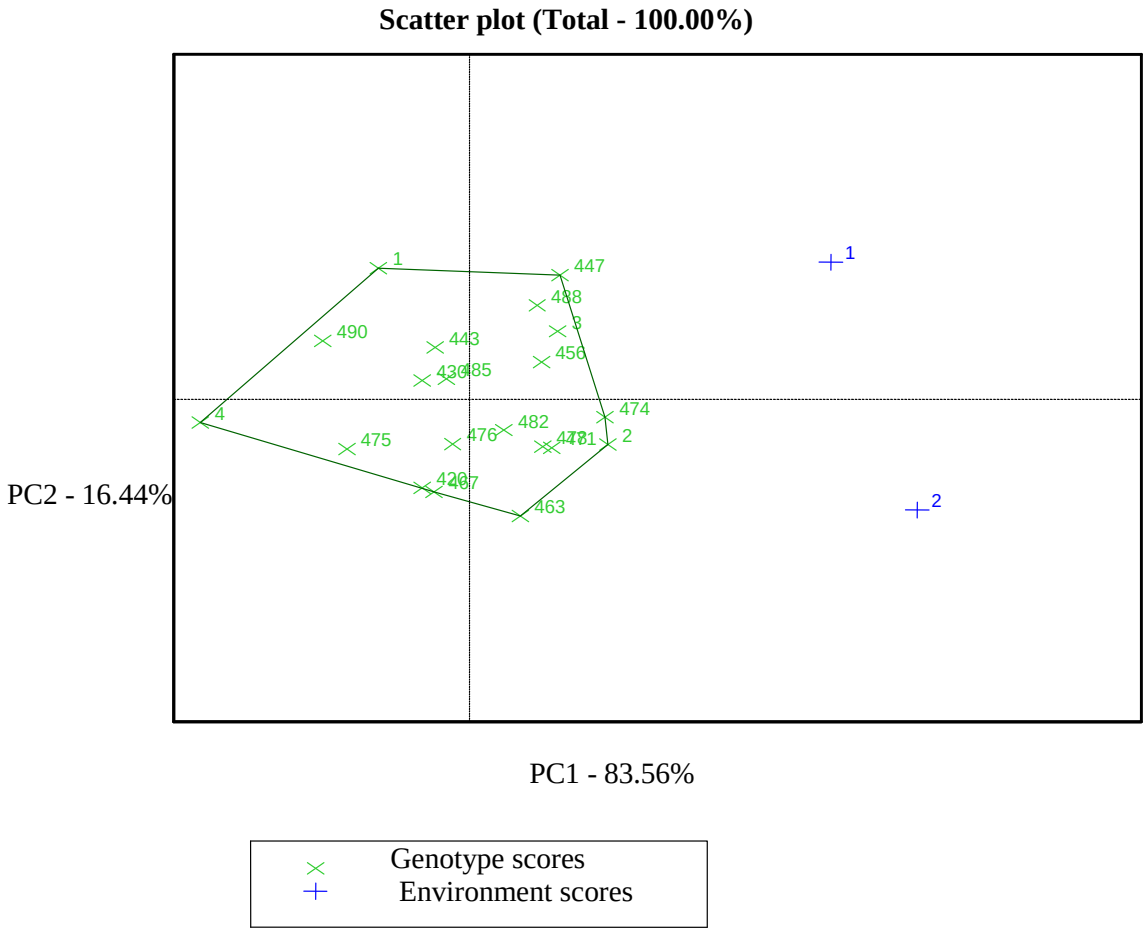


Figure 10: Polygon view of the GGE biplot showing the genotype means and the stable crosses. N/B 1= Timbilil, 2= Kangaita.

5.8 Discussion

Genotypes tested in different locations often have significant fluctuation in agronomic traits due to the response of genotypes to environmental factors such as soil fertility and climatic conditions (Kang, 2004). These fluctuations are known as genotype x environment interaction (GEI). The present study used the AMMI and the GGE models and summarized patterns and relationships of genotypes and environments successfully. These models are useful and they provide a good prediction of stability in varieties (Ezatollah *et al.*, 2012). However, Becker and Léon (1988) stated that multivariate methods are too sophisticated to provide a simple measure of stability which allows a ranking of genotypes. In the present study the models have clearly demarcated the pattern of adaptation of the crosses to environments and can be used to identify the superior genotypes in relation with the environments. The AMMI model (G + E + IPCA1 +IPCA2) accounted for 82.54%, 64.95% and 52.11% of the TC, EGCG and CAFF suggesting that the model fitted well. The AMMI method of stability analysis revealed that crosses TRFCA SFS150 x EPK TN14-3 (430), TRFK 6/8 x EPK TN14-3 (476) and TRFK 6/8 x AHP S15/10 (475) are the most stable crosses for TC while crosses AHP S15/10 x EPK TN 14-3 (474), TRFK 6/8 x TRFCA SFS150 (482), inbred TRFCA SFS150 (471) and TRFK 6/8 x AHP S15/10 (475) are the most stable hybrids for EGCG. Stable crosses as revealed by AMMI for CAFF were TRFK 6/8 x EPK TN14-3 (476) and TRFK 6/8 x TRFCA SFS150 (482). Therefore, these crosses can be recommended for cultivation in Kangaita and Timbilil with regards to stability for TC, EGCG and CAFF contents.

The GGE biplot revealed the GEI of the crosses and the environments. This was done by plotting the most discriminating environment, by revealing the cross that performed well in each environment. An ideal genotype should have both high mean performance and high stability across environments (Kaya *et al.,* 2006; Yan and Tinker, 2006). Furthermore, the ideal genotype is a genotype that is at the average environment coordinate (AEC) on the positive direction. (Kaya *et al.,* 2006; Yan and Tinker, 2006). The line, which is perpendicular to the AEC line and passes through the origin, is called the average ordinate environment (AOE). By projecting the genotypes on AEC axis, the genotypes were ranked by TC, EGCG and CAFF, where these traits increased in the direction of the arrow. From the GGE biplot analysis, the highest TC was obtained in cross EPK TN14-3 x TRFK 6/8 (443), inbreds EPK TN14-3 (490) and AHP S15/10 (478), while the lowest were TRFCA SFS150 x EPK TN14-3 (430), inbred TRFK 6/8 (467) and TRFCA SFS150 x AHP S15/10 (463). Crosses which were scattered next to the origin, indicating minimal interaction with the environment and therefore stable for TC across the two environments were inbred TRFCA SFS150 (471), TRFK 6/8 x EPK TN14-3 (476) and TRFCA SFS150 x TRFK 6/8 (420). These three crosses could be planted at both sites since they showed minimal environmental influence.

Crosses with the highest EGCG as observed by the GGE biplot were registered by inbred AHP S15/10 (478) and EPK TN14-3 x TRFCA SFS150 (488) while the poorest performers were TRFCA SFS150 x EPK TN14-3 (430) and TRFCA SFS150 x TRFK 6/8 (420). Inbred AHP S15/10 (478) and AHP S15/10 x EPK TN 14-3 (474) showed the best performance for EGCG in Kangaita and Timbilil respectively.

Crosses which were spread close to the origin signified minimal interaction with the environments. In view of this, crosses TRFK 6/8 x EPK TN14-3 (476) and TRFCA SFS150 x AHP S15/10 (463) were the most stable for EGCG at both Timbilil and Kangaita. However, these crosses were not among those with the highest quantities of TC, EGC and caffeine.

The genotypes with the highest CAFF as revealed by the GGE biplot analysis were AHP S15/10 x EPK TN 14-3 (474), EPK TN14-3 x AHP S15/10 (447), inbred TRFCA SFS150 (471), AHP S15/10 x TRFK 6/8 (456) and EPK TN14-3 x TRFCA SFS150 (488) (Figure 9). Crosses which had low contents of CAFF were inbred EPK TN 14-3 (490), TRFK 6/8 x AHP S15/10 (475) and TRFCA SFS150 x TRFK 6/8 (420). Stable crosses with regards to percent CAFF content were AHP S15/10 x TRFCA SFS 150 (485) and TRFK 6/8 x EPK TN14-9 (476).

GEI results from a change in the relative rank of genotype performance or a change in the magnitude of differences between genotype performances from one environment to another. GEI affects breeding progress because it complicates the demonstration of superiority of any genotype across environments and the selection of superior genotypes (Kang, 2004).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

From this study, it was evident that quantitative genetic parameters such as additive, non-additive gene and maternal effects have considerable influence on the inheritance of catechins and caffeine, and consequently on tea quality targeting high value diversified tea products products as well as advanced tea breeding programmes. The magnitude of GCA variance was higher than the SCA variance for all traits under both conditions, indicating that additive gene action was more important than non-additive genetic effects for these traits in development of high catechin tea. Maternal effects were significant for EGC, EGCG and total catechin while non-maternal effects were significant for EGCG and TC signifying that the choice of the female parent is important for these traits.

The crosses which showed high positive heterosis over the mid-parent, better parent and the standard variety could be utilized to generate transgressive segregants in the later generations with high catechins and caffeine.

Consistent high performance of tea varieties for quality traits across different production sites is an important attribute for varietal adoption. All the crosses evaluated had significant ($p<0.05$) genotypic differences for TC, EGCG and CAFF indicating the need to select high performing and stable cultivars. All the traits evaluated except CAFF are influenced by GEI indicating the need for multi-location replicated varietal evaluation where variety selection should be based on both mean

performance (productivity) and stability. Both the AMMI and GGE biplot method correlated implying that any of the methods can be used for stability analysis of tea varieties.

Both GGE biplot and the AMMI stability methods revealed that cross TRFK 6/8 x EPK TN14-3 (476) was the most stable cross for TC, EGCG and caffeine and therefore can be recommended for considered to develop cultivars with high TC, EGCG and caffeine at both Timbilil and Kangaita.

6.2 Recommendations

1. Cross EPK TN14-3 x AHP S15/10 (447) is recommended for further performance trials as it ranked the best overall for Caffeine.
2. Inbreds of EPK TN14-3 (490) and AHP S15/10 (478) exhibited the best performance for EGCG and hence they are recommended for development of heterotic hybrids (F_2) in advanced tea breeding programmes targeting high EGCG contents in pharmacological tea products.
3. TRFK 6/8 x EPK TN14-3 (476) was the most stable cross for EGCG, CAFF and TC. It is recommended that either the best genotypes be released for commercial use in a broad range of environments or in advanced tea breeding programmes.

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APPENDICES

Appendix I. Genotype data for the quality attributes collected at Timbilil

Cross	Clone	GA	EGC	C	CAFF	EC	EGCG	ECG	TC
EPKTN14-3x TRFCA SFS150	TRFK 488/1	0.51	8.80	0.30	3.68	1.90	10.26	2.85	24.11
	TRFK 488/2	0.62	8.65	0.37	3.40	1.95	10.34	3.36	24.68
	TRFK 488/3	0.70	7.75	0.41	4.08	2.54	10.54	3.59	24.84
	TRFK 488/4	0.56	8.26	0.32	3.48	2.08	9.59	3.60	23.85
	TRFK 488/5	0.63	8.35	0.45	3.25	1.92	10.31	3.29	24.32
TRFCASFS150xTRFCASFS150	TRFK 471/1	0.51	8.29	0.42	3.94	1.77	12.09	3.63	26.21
	TRFK 471/2	0.68	7.90	0.33	3.37	1.64	10.84	2.87	23.57
	TRFK 471/3	0.66	6.99	0.32	3.34	1.03	10.23	2.61	21.12
	TRFK 471/4	0.58	6.92	0.31	2.98	1.14	10.13	2.36	20.82
	TRFK 471/5	0.77	7.55	0.48	3.55	1.55	10.85	3.21	23.63
EPK TN14-3 x TRFK 6/8	TRFK 443/1	0.63	8.43	0.16	3.35	1.23	11.14	2.92	23.94
	TRFK 443/2	0.56	8.50	0.29	3.54	1.60	11.45	3.21	25.04
	TRFK 443/3	0.67	8.40	0.36	3.34	1.27	11.12	2.88	26.52
	TRFK 443/4	0.65	8.73	0.41	3.79	1.80	12.53	3.35	26.81
	TRFK 443/5	0.54	7.57	0.48	2.90	1.68	9.63	2.44	21.79
TRFK 6/8 x EPK TN14-3	TRFK 476/1	0.75	8.31	0.30	3.10	1.72	10.66	2.89	23.88
	TRFK 476/2	0.85	7.26	0.42	3.36	0.92	11.56	2.58	22.74

	TRFK 476/3	0.59	8.54	0.35	3.58	1.67	11.45	3.22	25.23
	TRFK 476/4	0.64	6.28	0.29	3.00	1.56	10.01	2.94	21.07
	TRFK 476/5	0.77	7.91	0.33	3.46	1.71	11.44	3.34	24.74
AHP S15/10 x EPK TN 14-3	TRFK 474/1	0.55	9.08	0.42	3.57	1.84	11.20	3.09	25.62
	TRFK 474/2	0.86	7.63	0.43	3.85	0.82	12.54	3.60	25.03
	TRFK 474/3	0.81	8.10	0.35	3.52	1.77	12.28	3.29	25.80
	TRFK 474/4	0.73	6.95	0.31	3.60	1.34	10.70	2.74	22.04
	TRFK 474/5	0.80	8.26	0.36	3.19	1.00	9.96	2.59	22.17
AHP S15/10 x TRFCA SFS 150	TRFK 485/1	0.58	7.58	0.41	3.76	1.43	10.94	3.12	23.48
	TRFK 485/2	0.56	7.18	0.38	3.53	1.70	10.75	2.99	22.99
	TRFK 485/3	0.54	7.06	0.42	3.21	1.59	10.88	3.14	23.08
	TRFK 485/4	0.63	7.88	0.50	3.28	1.49	9.86	2.75	22.37
	TRFK 485/5	0.75	6.42	0.37	3.12	1.48	11.36	2.90	22.48
TRFCA SFS150 x TRFK 6/8	TRFK 420/1	0.71	8.13	0.39	3.33	1.88	10.45	3.69	24.54
	TRFK 420/2	0.48	8.27	0.39	3.06	1.94	9.25	2.86	22.72
	TRFK 420/3	0.50	8.64	0.33	3.07	1.84	9.60	3.01	23.37
	TRFK 420/4	0.61	7.71	0.45	3.13	1.79	10.23	2.81	22.98
	TRFK 420/5	0.59	7.85	0.31	3.50	1.59	10.31	3.06	23.12
EPK TN14-3 x AHP S15/10	TRFK 447/15	0.49	7.92	0.35	3.55	1.90	10.22	2.35	22.74
	TRFK 447/16	0.62	8.49	0.40	3.92	1.81	11.02	3.06	24.78
	TRFK 447/17	0.51	7.56	0.30	3.79	1.86	10.57	2.96	23.24

	TRFK 447/18	0.66	8.18	0.33	3.62	1.30	10.81	2.91	23.53
	TRFK 447/19	0.64	7.07	0.36	3.40	1.62	11.30	3.22	23.57
TRFK 6/8 x TRFCA SFS150	TRFK 482/1	0.61	6.99	0.40	3.55	1.71	11.42	2.95	23.48
	TRFK 482/2	0.66	8.00	0.33	3.08	1.70	11.01	3.00	24.05
	TRFK 482/3	0.60	7.91	0.46	3.74	1.52	11.63	2.94	24.46
	TRFK 482/4	0.49	8.55	0.40	3.37	1.90	10.95	2.81	24.61
	TRFK 482/5	0.64	8.43	0.38	3.19	1.67	10.46	3.04	23.98
AHP S15/10 x TRFK 6/8	TRFK 456/1	0.75	7.67	0.38	3.27	1.17	10.20	2.49	21.91
	TRFK 456/2	0.59	8.50	0.34	3.34	1.65	10.63	2.72	23.83
	TRFK 456/3	0.74	7.16	0.38	3.62	1.56	10.62	2.42	22.15
	TRFK 456/4	0.55	6.53	0.33	3.55	1.96	9.01	2.68	20.51
	TRFK 456/5	0.67	8.51	0.26	3.65	1.30	10.56	3.06	23.85
TRFK 6/8 x AHP S15/10	TRFK 475/1	0.97	7.60	0.25	3.42	1.56	12.05	2.92	24.38
	TRFK 475/2	0.70	7.08	0.35	3.25	1.54	10.32	3.10	22.39
	TRFK 475/3	0.82	7.81	0.39	3.22	1.20	11.97	2.94	24.30
	TRFK 475/4	0.82	7.21	0.30	2.94	1.43	10.48	2.82	22.24
	TRFK 475/5	0.90	7.87	0.32	3.18	1.28	11.20	2.87	23.53
AHP S15/10 x AHP S15/10	TRFK 478/1	0.69	7.88	0.55	3.21	1.56	11.10	3.02	24.11
	TRFK 478/2	0.79	7.37	0.49	3.34	1.18	12.43	3.36	24.83
	TRFK 478/3	0.90	7.75	0.46	3.62	1.47	12.23	3.42	25.33
	TRFK 478/4	0.62	8.48	0.48	3.39	1.35	11.62	2.95	24.77

	TRFK 478/5	0.62	8.33	0.41	3.57	1.50	11.33	3.00	24.66
TRFCA SFS150 x EPK TN14-3	TRFK 430/121	0.71	8.94	0.35	3.33	1.33	10.21	2.86	23.69
	TRFK 430/122	0.64	7.69	0.41	3.39	1.46	10.51	2.71	22.74
	TRFK 430/123	0.64	7.31	0.32	3.47	1.78	10.25	3.28	22.94
	TRFK 430/124	0.59	7.65	0.23	3.51	1.34	10.47	3.13	22.82
	TRFK 430/125	0.64	6.95	0.32	3.10	1.02	10.30	2.69	21.29
TRFCA SFS150 x AHP S15/10	TRFK 463/49	0.43	6.78	0.40	2.79	1.27	8.96	2.63	20.03
	TRFK 463/50	0.63	7.16	0.31	3.32	1.17	10.02	2.86	21.51
	TRFK 463/51	0.67	7.62	0.34	3.18	1.46	10.44	2.87	22.74
	TRFK 463/52	0.89	6.90	0.31	3.32	1.32	11.79	3.02	23.35
	TRFK 463/53	0.75	7.18	0.31	3.61	1.26	11.62	3.06	23.43
EPK TN14-3 x EPK TN14-3	TRFK 490/1	0.69	7.32	0.31	3.20	1.52	11.42	2.77	23.34
	TRFK 490/2	0.79	6.97	0.35	3.39	1.41	11.25	2.93	22.91
TRFK 6/8 x TRFK 6/8	TRFK 467/1	0.76	7.95	0.30	3.12	1.07	9.72	2.47	21.50
	TRFCASFS150	0.71	7.77	0.26	3.44	1.49	10.95	3.37	23.90
	EPK TN 14-3	0.66	7.55	0.29	3.39	1.15	11.73	2.61	23.32
	AHP S15/10	0.74	7.17	0.38	3.67	1.28	11.91	1.98	22.72
	TRFK 6/8	0.66	8.31	0.42	3.16	1.09	10.16	2.70	22.68

Appendix II. Genotype data for the quality attributes collected at Kangaita

Cross	Clone	GA	EGC	C	CAFF	EC	EGCG	ECG	TC
TRFCA SFS150 x TRFK 6/8	TRFK 420/1	0.17	6.72	0.35	3.03	2.19	9.69	3.36	22.31
	TRFK 420/2	0.23	8.44	0.43	3.22	1.79	10.32	3.44	24.42
	TRFK 420/3	0.20	5.97	0.29	3.35	1.65	11.40	2.78	22.09
	TRFK 420/4	0.45	7.33	0.28	3.62	1.27	11.20	3.27	23.34
	TRFK 420/5	0.13	5.90	0.24	3.72	0.98	10.43	2.94	20.50
TRFCA SFS150 x EPK TN14-3	TRFK 430/121	0.26	5.13	0.28	3.23	1.00	9.48	2.62	18.52
	TRFK 430/122	0.64	6.85	0.20	3.13	1.49	9.22	2.63	20.38
	TRFK 430/123	0.22	4.83	0.39	3.53	1.17	9.89	2.88	19.16
	TRFK 430/124	0.18	5.63	0.40	3.42	1.34	10.76	3.73	21.86
	TRFK 430/125	0.13	4.61	0.23	3.13	1.63	9.64	3.18	19.30
EPK TN14-3 x TRFK 6/8	TRFK 443/1	0.65	6.77	0.39	3.44	1.50	11.57	3.28	23.51
	TRFK 443/2	0.57	6.85	0.40	3.32	1.41	10.58	3.21	22.45
	TRFK 443/3	0.21	7.35	0.38	3.29	1.51	10.92	3.50	23.67
	TRFK 443/4	0.27	4.82	0.19	3.20	1.41	10.43	3.62	20.47
	TRFK 443/5	0.27	6.99	0.27	3.16	1.64	9.93	3.01	21.85
EPK TN14-3 x AHP S15/10	TRFK 447/15	0.30	6.23	0.69	3.42	1.65	10.30	3.27	22.15
	TRFK 447/16	0.16	4.87	0.46	3.25	1.42	9.99	2.70	19.44
	TRFK 447/17	0.13	5.98	0.36	3.28	1.79	9.21	3.09	20.42
	TRFK 447/18	0.21	5.16	0.19	3.87	1.54	11.58	3.55	22.02
	TRFK 447/19	0.51	7.04	0.37	3.36	0.99	10.50	3.46	22.35
AHP S15/10 x TRFK 6/8	TRFK 456/1	0.60	6.61	0.32	3.21	1.43	9.57	2.68	20.61
	TRFK 456/2	0.18	5.51	0.25	2.85	1.04	10.14	2.87	19.82
	TRFK 456/3	0.52	9.28	0.18	3.73	1.87	10.55	2.52	24.40
	TRFK 456/4	0.29	7.59	0.24	3.85	0.64	11.07	2.42	21.96
	TRFK 456/5	0.34	4.71	0.37	3.78	1.12	10.79	2.91	19.90
TRFCA SFS150 x AHP S15/10	TRFK 463/49	0.50	7.39	0.21	3.43	1.29	10.03	2.47	21.39

	TRFK 463/50	0.21	6.43	0.16	3.34	0.77	9.59	3.17	20.12
	TRFK 463/51	0.24	3.93	0.45	3.13	1.03	9.44	2.95	17.80
	TRFK 463/52	0.16	4.31	0.47	3.84	1.22	12.09	3.97	22.06
	TRFK 463/53	0.25	7.16	0.57	4.19	1.31	12.38	3.34	24.76
TRFK 6/8 x TRFK 6/8	TRFK 467/1	0.18	5.16	0.25	3.71	1.40	11.18	3.10	21.08
	TRFK 467/2	0.41	7.52	0.36	3.11	1.33	10.35	2.99	22.55
TRFCASFS150xTRFCA SFS150	TRFK 471/1	0.40	6.37	0.32	3.72	1.60	10.12	3.26	21.67
	TRFK 471/2	0.17	5.65	0.19	3.23	0.82	11.24	3.33	21.22
	TRFK 471/3	0.35	8.39	0.48	3.96	0.90	11.83	4.01	25.61
	TRFK 471/4	0.11	4.89	0.30	3.35	1.35	10.32	2.75	20.60
	TRFK 471/5	0.27	5.72	0.26	3.64	1.19	11.36	3.32	21.85
AHP S15/10 x EPK TN 14-3	TRFK 474/1	0.31	5.62	0.36	3.74	1.23	11.50	2.78	21.49
	TRFK 474/2	0.35	5.52	0.61	3.91	1.09	11.84	4.12	23.17
	TRFK 474/3	0.45	5.19	0.54	4.03	1.05	10.43	2.81	20.03
	TRFK 474/4	0.19	4.78	0.59	3.53	1.36	10.75	3.38	20.86
	TRFK 474/5	0.27	5.44	0.49	3.02	1.58	11.69	3.36	22.57
TRFK 6/8 x AHP S15/10	TRFK 475/1	0.28	5.10	0.20	3.01	1.33	11.20	3.05	20.88
	TRFK 475/2	0.41	5.50	0.34	3.36	1.31	10.92	2.81	20.88
	TRFK 475/3	0.55	7.56	0.44	3.08	1.10	11.00	2.72	22.82
	TRFK 475/4	0.24	5.84	0.42	3.28	0.85	10.50	2.57	20.18
	TRFK 475/5	1.16	6.17	0.29	3.36	1.76	11.94	2.98	23.14
TRFK 6/8 x EPK TN14-3	TRFK 476/1	0.41	7.10	0.24	3.43	1.75	10.73	2.85	22.66
	TRFK 476/2	0.41	7.53	0.25	3.30	1.49	10.77	2.57	22.61
	TRFK 476/3	0.73	5.75	0.17	3.32	1.66	10.70	3.16	21.44
	TRFK 476/4	0.23	7.63	0.27	3.57	1.85	11.46	3.00	24.20
	TRFK 476/5	0.23	4.71	0.22	3.38	2.09	9.96	3.16	20.14
AHP S15/10 x AHP S15/10	TRFK 478/1	0.24	5.27	0.36	4.04	0.75	12.36	2.88	21.61
	TRFK 478/2	0.37	5.86	0.33	3.72	1.32	10.75	2.89	21.15
	TRFK 478/3	1.03	6.17	0.29	3.59	1.85	11.50	2.98	22.80
	TRFK 478/4	0.27	5.32	0.43	3.31	1.66	10.62	3.09	21.12

	TRFK 478/5	0.56	7.20	0.29	3.14	1.36	10.42	3.20	22.47
TRFK 6/8 x TRFCA SFS150	TRFK 482/1	0.33	7.34	0.38	3.28	1.54	10.32	2.89	22.48
	TRFK 482/2	0.20	5.09	0.44	3.41	1.67	10.82	2.92	20.94
	TRFK 482/3	0.47	6.99	0.33	3.66	1.27	10.39	2.74	21.72
	TRFK 482/4	0.17	5.92	0.33	3.25	1.31	10.11	2.94	20.62
	TRFK 482/5	0.25	5.02	0.44	3.79	1.23	10.42	3.23	20.34
TRFK 6/8 x TRFCA SFS150	TRFK 485/1	0.53	8.22	0.31	3.37	1.42	10.53	3.51	23.99
	TRFK 485/2	0.27	5.15	0.30	3.30	2.18	10.92	4.77	23.33
	TRFK 485/3	0.13	4.35	0.39	3.33	1.30	10.28	3.07	19.40
	TRFK 485/4	0.33	3.95	0.34	3.33	0.94	10.86	3.24	19.32
	TRFK 485/5	0.35	5.38	0.29	3.32	1.03	11.81	3.23	21.74
EPK TN14-3 x TRFCA SFS150	TRFK 488/1	0.30	7.49	0.26	3.72	1.41	10.43	3.10	22.70
	TRFK 488/2	0.21	5.14	1.06	3.66	1.53	10.74	3.11	21.59
	TRFK 488/3	0.46	7.61	0.33	3.08	1.45	9.72	2.77	21.88
	TRFK 488/4	0.24	7.30	0.24	3.66	1.85	11.34	3.76	18.99
	TRFK 488/5	0.34	6.00	0.37	3.00	1.74	9.34	2.76	20.20
EPK TN14-3 x EPK TN14-3	TRFK 490/1	0.30	8.90	0.30	3.15	1.42	12.88	3.50	27.00
	TRFK 490/2	0.78	6.60	0.47	3.00	1.07	10.29	3.30	21.93
	AHP S15/10	0.21	7.30	0.29	3.48	1.54	11.26	3.98	24.36
	TRFCASFS150	0.26	6.77	0.19	3.11	1.50	10.27	2.88	21.60
	EPK TN14/3	0.36	6.17	0.29	3.68	1.83	11.03	3.37	22.69
	TRFK 6/8	0.20	6.55	0.52	2.93	1.75	9.62	3.01	21.45

APPENDIX III. ANOVA TABLES AT KANGAITA

Variate: GA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.03391	0.01695	1.04	
Block.*Units* stratum					
Clone	77	8.54860	0.11102	6.84	<.001
Residual	154	2.49939	0.01623		
Total	233	11.08190			

Variate: EGC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	10.534	5.267	4.99	
Block.*Units* stratum					
Clone	77	325.446	4.227	4.00	<.001
Residual	154	162.604	1.056		
Total	233	498.583			

Variate: C

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.00277	0.00138	0.09	
Block.*Units* stratum					
Clone	77	4.37434	0.05681	3.65	<.001
Residual	154	2.39426	0.01555		
Total	233	6.77137			

Variate: CAFF

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.

Block stratum	2	2.27821	1.13911	15.11	
Block.*Units* stratum					
Clone	77	19.52269	0.25354	3.36	<.001
Residual	154	11.60922	0.07538		
Total	233	33.41013			

Variate: EC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.30857	0.15428	3.59	
Block.*Units* stratum					
Clone	77	25.11640	0.32619	7.59	<.001
Residual	154	6.61402	0.04295		
Total	233	32.03900			

Variate: EGCG

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	36.3999	18.1999	25.39	
Block.*Units* stratum					
Clone	77	140.8620	1.8294	2.55	<.001
Residual	154	110.3862	0.7168		
Total	233	287.6481			

Variate: ECG

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.2184	0.1092	0.97	
Block.*Units* stratum					
Clone	77	38.8583	0.5047	4.49	<.001
Residual	154	17.2953	0.1123		

Total	233	56.3720			

Variate: TC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	66.582	33.291	21.23	
Block.*Units* stratum					
Clone	77	628.927	8.168	5.21	<.001
Residual	154	241.467	1.568		
Total	233	936.975			

Appendix 4. ANOVA tables at Timbilil

Variate: GA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.02382	0.01191	1.13	
Block.*Units* stratum					
Clone	22	0.47004	0.02137	2.03	0.023
Residual	44	0.46297	0.01052		
Total	68	0.95683			

Variate: EGC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.9838	0.4919	2.27	
Block.*Units* stratum					
Clone	22	17.7279	0.8058	3.73	<.001
Residual	44	9.5140	0.2162		
Total	68	28.2257			

Variate: C

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.032365	0.016183	4.28	
Block.*Units* stratum					
Clone	22	0.212736	0.009670	2.56	0.004
Residual	44	0.166182	0.003777		
Total	68	0.411283			

Variate: CAFF

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.30790	0.15395	3.04	
Block.*Units* stratum					
Clone	22	2.12685	0.09668	1.91	0.033
Residual	44	2.22460	0.05056		
Total	68	4.65935			

Variate: EC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.1200	0.0600	0.60	
Block.*Units* stratum					
Clone	22	6.2881	0.2858	2.85	0.002
Residual	44	4.4113	0.1003		
Total	68	10.8194			

Variate: EGCG

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.0095	0.0048	0.02	
Block.*Units* stratum					
Clone	22	33.4058	1.5184	6.67	<.001
Residual	44	10.0167	0.2277		
Total	68	43.4320			

Variate: ECG

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	1.1123	0.5561	4.48	
Block.*Units* stratum					
Clone	22	7.6680	0.3485	2.81	0.002
Residual	44	5.4634	0.1242		
Total	68	14.2436			

Variate: TC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	2	0.1460	0.0730	0.55	
Block.*Units* stratum					
Clone	22	54.8525	2.4933	18.86	<.001
Residual	44	5.8158	0.1322		
Total	68	60.8142			