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Heterosis for Catechins and Caffeine in Kenyan Tea (*Camellia sinensis*) (L.) O. *Kuntze*).

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ABSTRACT

Tea quality is manifested in its aroma, flavour and taste properties, which are generated by volatile and non-volatile compounds, mainly catechins and caffeine. Several studies have revealed positive significant relationships between (flavan-3-ols) catechins and caffeine in green leaf and black tea quality. Additionally, the health benefits of tea in management of cancer, arthritis, cardiovascular diseases, diabetes and obesity have been attributed to catechins and caffeine. Eight biochemical traits of tea were used to investigate mid-parent heterosis (MPH), better parent heterosis (BPH) and standard heterosis (SDH) in a 4x4 diallel mating design. Overall, genotype x environment interaction influenced heterosis. For example, mean GA, EGC, C, EC and TC contents were higher at Timbilil compared to Kangaita while mean Caffeine, EGCG and ECG were higher at Kangaita compared to Timbilil. The results also showed that, 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. 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.

INTRODUCTION

Tea (*Camellia sinensis*) (L.) O. Kuntze) belongs to *Theaceae* family and is the most widely consumed beverage in the world after water (*1, 2*). Tea is a highly out crossing plant that is pseudo self-incompatible (*3*). *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 (*4*). Generally, beverages tea are broadly classified according to the methods of fermentation (*5*). The three main types of tea beverages are black, green and oolong teas (*6*). Green tea is non-fermented, black tea is completely fermented and oolong tea is partially fermented (*7*). Kenya is the world's largest producer of black tea in the world (*8*). The tea industry contributes approximately 26% of the export earnings and 4% of the Gross Domestic Product (GDP) to the Kenyan economy (*9*). Other types of tea produced are white, yellow and reprocessed tea which include flower scented tea, compressed tea, instant tea and herbal teas (*4*). However, white and yellow teas have been considered as subclasses of green tea (*10*).

The main biochemical compounds in tea are polyphenols, alkaloids (mainly caffeine and theobromine) and essential oils (*11*). The major polyphenols in green tea are catechins which include: gallic acid, -epigallocatechin (EGC), (-) -epicatechin (EC), epigallocatechin-3-gallate (EGCG), epicatechin-3-gallate (ECG), (+) catechin (C) and (+) gallocatechins (GC) (*12*). The oxidation products of these polyphenols include theaflavins and thearubigins, which together with amino acids and caffeine are key factors which determine tea quality (*13*). Catechins are also important pharmacologically due to their anticancer, anti-hypertension, anti-vascular disorders and anti-inûammatory properties (*14*).

Heterosis refers to the superiority of F_1 hybrid in one or more characters over its parents and results after crossing (*15*). Knowledge on heterosis and as well as expected gain upon selection is important as it influences the choice of parents in breeding programmes. Heterosis can be exploited to increase both quality and yield of tea. Great efforts have been directed to improve yield and quality properties in tea. For example, previous studies on heterosis have been carried out on yield, bud weight, drought tolerance, fermentability, total polyphenols, theaflavins and thearubigins and leaf pubescence (*16*). The performance of progenies is estimated in terms of the percentage increase or decrease of their performance over the mid-parent, better parent (*17, 18*) and commercial check variety (standard

heterosis). From the perspective of breeders, better parent heterosis is more effective compared to mid-parent heterosis, especially when the objective is to identify superior hybrids (*19*). However, standard heterosis is also important to compare the crosses with the commercial check variety. This study determined mid parent heterosis, better parent heterosis and standard heterosis to provide a broad picture of performance of some clonal tea crosses at Timbilil and Kangaita sites for catechins and caffeine in tea by crossing 4 parents with diverse attributes. The results could be helpful in the selection of suitable parents of potential transgressive segregants, which can be further evaluated for enhanced catechins and caffeine contents in tea.

METHODOLOGY

Site Description

The study was conducted in two sites namely Tea Research Institute (TRI) at Timbilil Estate in Kericho county and TRI sub-station at Kangaita in Kirinyaga county. Timbilil estate (00 22' S and 350 21' E) is located at 2180 meters above sea level (amsl). It experiences long-term annual average rainfall of 2043mm and average temperature at 16.20^0C. Kangaita (00^0 30' S and 37^0 18'E) is located 2100 meters amsl. The annual mean temperature is 15.27^0c, with an average annual rainfall at 2009 mm.

Plant Materials

The plant material consisting of four parental clones used in the 4 x 4 full diallel cross are among the most popular Kenyan commercial tea clones that were selected based on diverse attributes (Table 1). The generated 16 clonal full-sib crosses (F1s) 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 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 (17). Cuttings were collected from selected progeny, rooted and raised in the nursery for one year prior to field transplanting.

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 both sites**.** The trial was set up as a completely randomized block design with three replications in plots of 30 plants spaced at 0.61 m within rows and 1.22 m between rows (i.e. 13448 plants 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 (*20*).

Sample Preparation and Data Collection

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

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 (16)*.

transported to the laboratory. Samples were dried for 4 minutes using a microwave oven. This was done to deactivate the enzyme, polyphenol oxidase and hence stop the process of oxidation. Finally, the samples were put in an oven at 100^0C for 24 hours. The dried samples were ground using a coffee miller and stored in aluminium-lined bags until analysis.

Extraction of Catechins and Caffeine

Extraction of catechins and caffeine was done according to the procedure of ISO14502-2-2005E (*21*). Ground tea samples (0.2g) were weighed into graduated extraction tubes. Five (5) ml of 70% hot methanol/water (MeOH) was added, stoppered and mixed thoroughly by vortexing. Incubation was done in a water bath at 70^0C for 10 min with vortexing after 5 and 10min. The sample was cooled to 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%).

Analysis and Quantification of Catechins and Caffeine

HPLC analysis of catechins and caffeine was done according to the procedure by ISO14502-2-2005E (*21*). In this protocol, 1.0 ml of the sample was pipetted into a test tube. One (1) 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 (*22*). The samples were analysed for percent gallic acid (GA), epigallocatechin (EGC), epicatechin (EC), epigallocatechin-3-gallate (EGCG), epicatechin-3-gallate (ECG), catechin (C), caffeine (CAFF) and total catechins (TC). Heterosis was determined following a procedure of Heiko (*23*), as follows;

Mid parent was calculated as the average of the two parents,

$$\text{Mid parent heterosis} = \frac{\text{F1} - \text{Mid parent}}{\text{Mid parent}} \text{ x } 100$$

$$\text{Better parent heterosis} = \frac{\text{F1} - \text{Better parent}}{\text{Better parent}} \text{ x } 100$$

$$\text{Standard heterosis} = \frac{\text{F1} - \text{Standard variety}}{\text{Standard variety}} \text{ x } 100$$

Significance of relative heterosis was tested by using t-test (*24*).

RESULTS AND DISCUSSIONS

Mean performance of the 16 progenies and 4 parents at Timbilil and Kangaita is presented in Table 2. The analysis of variance revealed significant ($p \leq 0.05$) differences at both Timbilil and Kangaita for all the biochemical parameters investigated which demonstrated existence of variations among the 16 F1s and their parents.. Progenies outperform their parents due to transgressive segregation (*25, 26*). The effects of genotype by environment played a key role. For example, the mean performance of progenies at Timbilil for C, caffeine, EC and ECG was higher than that of the parents. However, the mean performance of the parents at Timbilil was higher than the progenies for GA, EGC, EGCG and TC (Table 2). At Kangaita, the mean performance of the progenies was higher for GA, C, caffeine and EGCG. Meanwhile, the mean performance of the parents was higher than the progenies for EGC, EC, ECG and TC. Notably, the progenies performance for EGC and TC was lower than that of the parents at both sites. This could mean that the parents are superior to the progenies for EGC and TC.

The highest performing crosses for GA were TRFK 6/ 8 x AHP S15/10 and inbred EPK TN14-3 at Timbilil and Kangaita respectively. The best cross for EGC was EPK TN14-3 x TRFCA SFS150 and inbred EPK TN14-3 at Timbilil and Kangaita respectively. The best cross for C was inbred AHP S15/10 and AHP S15/10 x EPK TN 14-3 at Timbilil and Kangaita respectively. The best cross for caffeine was EPK TN14-3 x AHP S15/10 and AHP S15/10 x EPK TN 14-3 at Timbilil and Kangaita respectively. The best cross for EC

was EPK TN14-3 x TRFCA SFS150 and TRFK 6/8 x EPK TN14-3 at Timbilil and Kangaita respectively. Inbreds AHP S15/10 and EPK TN14-3 were the best crosses for EGCG at Timbilil and Kangaita respectively. EPK TN14-3 x TRFCA SFS150 and AHP S15/10 x TRFCA SFS 150 were the best crosses for ECG at Timbilil and Kangaita respectively. EPK TN14-3 x TRFK 6/8 and inbred EPK TN14-3 were the best crosses for TC at Timbilil and Kangaita respectively (Table 1). However, from the overall results, parent EPK TN14-3 recorded the highest content of EC, Caffeine at Kangaita and EGGC at Timbilil compared. Similarly, parent TRFK 6/8 recorded the highest overall EGC and Caffeine at Timbilil and Kangaita respectively. In addition, AHP S15/10 had the highest overall ECG content at Kangaita. In addition to that, the parent EPK TN14-3 was involved in all the superior crosses for all the traits at Kangaita either as a female or male parent. It was used five of the superior crosses at Timbilil. Previous studies have shown that green leaf catechins (flavan-3-ols) and caffeine have been used to predict plain black tea quality (*27-30*). In view of this, EPK TN14-3 would be recommended to be used in tea breeding programmes as a parent for crossing with other varieties when targeting high quality in tea.

Results on the range of the various characters measured in the diallel cross are presented in Table 3 and Table 4. There was a wide range in performance for most of the crosses in all the characters assessed. The widest range for GA was in the cross TRFK 6/8 x AHP S15/10 at both sites. Cross AHP S15/10 x TRFCA SFS 150 exhibited the broadest range at Timbilil for EGC (Table 3) while cross AHP S15/10 x TRFK 6/8 recorded the widest range at Kangaita (Table 4). Cross TRFCA SFS150 x EPK TN14-3 had the widest range for C at Timbilil (Table 3) while EPK TN14-3 x TRFCA SFS150 had the widest range at Kangaita (Table 4). Cross EPK TN14-3 x AHP S15/10 (Table 3) and AHP S15/10 x EPK TN 14-3 (Table 4) had the widest ranges for caffeine at Timbilil and Kangaita respectively. Cross EPK TN14-3 x AHP S15/10 (Table 3) and TRFK 6/8 x TRFCA SFS150 (Table 4) had the widest ranges for EC at Timbilil and Kangaita respectively. Cross AHP S15/10 x EPK TN 14-3 at Timbilil (Table 3) and EPK TN14-3 x TRFCA SFS150 at Kangaita (Table 4) had the widest ranges for EGCG. Cross EPK TN14-3 x AHP S15/10 (Table 3) and cross TRFK 6/8 x TRFCA SFS150 (Table 4) recorded the widest ranges for ECG. Cross EPK TN14-3 x TRFK 6/8 at Timbilil (Table 3) and AHP S15/10 x TRFK 6/8 at Kangaita (Table 4) recorded the widest range for TC. The estimates of range revealed that there was a significant amount of variability present in these crosses. This wide range is an indication of the variability present and could be utilised to broaden the genetic bases of tea.

Estimation of heterosis effects

Heteroses were estimated in form of mid-parent, better parent and standard heterosis in two environments where the crosses were evaluated. Mid-parent heterosis, better parent heterosis and standard heterosis in the crosses varied significantly and this could be due to genetic diversity of parents used to generate the crosses and environmental influences. Variations recorded imply existence of potential for exploiting hybrid vigour to develop new hybrids. Heterosis is caused by mutation in hybrid populations, chromosome number variation, expression of rare recessive alleles, complementary gene action, over-dominance or epistasis, which indicate involvement of non-additive gene action (*25*). Additive effects are also involved (*31*). 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 desired attributes. Positive heterosis is beneficial for all the studied traits. Heterosis for improving tea had been reported for yield, bud weight, drought tolerance, fermentability, total polyphenols, theaflavins and thearubigins and leaf pubescence (20).

Most crosses showed significant and desirable level of heterosis over the mid parent, better parent and standard heterosis. 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 crosses respectively (Table 6). Better parent heterosis ranged from -23.61% to 10.67% for TRFCA SFS150 x TRFK 6/8 and TRFK 6/8 x AHP S15/10 respectively (Table 7). Standard heterosis at Timbilil ranged from -23.61% to 15.28% for TRFCA SFS150 x TRFK 6/8 and TRFK 6/8 x AHP S15/10 respectively (Table 8). Cross TRFK 6/8 x AHP S15/10 showed the highest positive mid-parent heterosis, better parent heterosis and standard heterosis for GA at 13.76%, 10.7%, 15.28% at Timbilil. It also had the highest mid-parent heterosis, better parent heterosis and standard heterosis for GA at 159.06%, 155.96% and 162.24% at Kangaita respectively. TRFK 6/8 x AHP S15/10 could therefore be advanced in the pre-release trials.GA is an important biochemical compound with wide range of application in the pharmaceutical industry (*32*). It is also used as a standard for determination of the phenol content

Table 2. Mean performance of parents and F_1s 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	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

in various analytes by the Folin - Ciocalteau assay.

Mid-parent heterosis at Timbilil for **EGC** ranged from -8.97% to 9.49% for inbreds TRFK 6/8 and AHP S15/10 respectively. At Kangaita, mean MPH for EGC was -6.70% and the range was from -21.17% to 25.50% for crosses AHP S15/10 x EPK TN 14-3 and EPK TN14-3 x EPK TN14-3 respectively (Table 6). Better parent heterosis at Timbilil for EGC ranged between -13.96% to 10.47% for cross TRFK 6/8 x AHP S15/10 and inbred AHP S15/10 respectively. At Kangaita, better parent heterosis for EGC ranged from -20.07% to 25.50% for cross TRFK 6/8 x TRFCA SFS150 and inbred EPK TN14-3 (Table 10). Standard heterosis for EGC at Timbilil ranged from -18.08% to -4.35% for TRFCA SFS150 x AHP S15/10 and EPK TN14-3 x TRFCA SFS150 respectively (Table 8). At Kangaita, standard heterosis ranged from -18.95% to 18.30% for AHP S15/10 x EPK TN 14-3 and inbred EPK TN14-3 self respectively (Table 8). At Kangaita, inbred EPK TN14-3 had the highest positive mid-parent, better parent and standard heterosis for EGC. However, at Timbilil, inbred AHP S15/10 had the highest mid-parent and better parent heterosis. By contrast, all the crosses exhibited negative standard heterosis for EGC at Timbilil implying that no cross was superior to the standard check TRFK 6/8 that was also one of the parents. Therefore, inbred EPK TN14-3 could be considered for release at Kangaita site. However, TRFK 6/8 could be used to boost EGC levels in crosses in tea breeding programmes. EGC is an important compound in tea since it contributes to the formation of theaflavins in black tea and correlates positively with high quality black tea (*33*).

Table 3. 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 4. 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.

Table 5. 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	1.2	1.68	11.88	11.14	2.36	3.67	23.12	23.53
456	AHP S15/10 x TRFK 6/8	0.74	0.2	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	22.9
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	2.6	3.43	22.81	22.98
467	TRFK 6/8 x TRFK 6/8	0.72	0.2	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	21.6
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	0.2	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	22.9
476	TRFK 6/8 x EPK TN14-3	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
478	AHP S15/10 x AHP S15/10	0.75	0.21	7.16	7.3	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	2.6	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**. 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.*

Mid-parent heterosis for catechin (C) at Timbilil ranged from -33.87% to 53.39% for inbreds TRFK 6/8 and EPK TN 14-3 respectively. Mid-parent he<terosis at Kangaita ranged from -43.17 to 90.2 for TRFK 6/8 x EPK TN14-3 and EPK TN14-3 x TRFCA SFS150 respectively (Table 6). At Timbilil, better parent heterosis ranged from -34.15% to 52.17% for inbreds TRFK 6/8 and EPK TN 14-3 respectively. At Kangaita, mean BPH for C ranged from -41.17% to 106.47% for inbred TRFK 6/8 and TRFK 6/8 x TRFCA SFS150 respectively (Table 7). Standard heterosis ranged from -34.15% to 14.63% for inbreds TRFK 6/8 and AHP S15/10 respectively at Timbilil site (Table 8). At Kangaita, standard heterosis ranged from -55.66% to -0.18% for TRFK 6/8 x EPK TN14-3 and AHP S15/10 x EPK TN 14-3 respectively. Standard heterosis for C at Kangaita was negative for all the crosses implying that the standard check TRFK 6/8 that was also used as a parent in this study was superior and could continue to be used in the breeding programmes targeting tea with high catechin content.

Mid-parent heterosis for caffeine at Timbilil ranged from -8.21% to 8.72% for inbreds EPK TN 14-3 and TRFK 6/8 respectively (Table 6). At Kangaita, MPH for caffeine ranged from -16.36% to 16.32% for inbreds EPK TN14-3 and TRFK 6/8 respectively (Table 6). Better parent heterosis at Timbilil ranged from -12.29% to 8.78% for TRFK 6/8 x AHP S15/10 and inbred TRFK 6/8 respectively. At Kangaita, BPH for caffeine ranged from -16.36% to 16.32% for inbreds EPK TN14-3 and TRFK 6/8 respectively (Table 7). Standard heterosis for caffeine at Timbilil ranged from 6.08% to 23.31% for TRFK 6/8 x AHP S15/10 and EPK TN14-3 x AHP S15/10 respectively. Standard heterosis at Kangaita ranged from 4.85% to 24.34 % for inbred EPK TN14-3 and cross AHP S15/10 x EPK TN 14-3 respectively (Table 8). Inbred TRFK 6/8 had the highest positive mid-parent and better parent heterosis at both sites. Interestingly, all the crosses exhibited positive standard heterosis implying that they were superior to the standard check TRFK 6/8. Caffeine is a very important biochemical constituent as it contributes to the quality of tea (29, 30). Medically, caffeine acts as a diuretic, cardiac muscle stimulant, central nervous system stimulant, smooth

Table 6. Percent mid-parent heterosis (MPH) for GA, EGC, C, CAFF, EC, EGCG, ECG, TC in 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 7. 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 6/8xTRFK 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 8. Percent standard 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	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

***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*

muscle relaxant, gastric acid secretion stimulant, elevates plasma free fatty acids and glucose (*10*). Consumer preference regarding caffeine content in tea differs between different individuals. In view of this, clone TRFK 6/8 could be used when developing teas with low caffeine levels. However, if high caffeine tea is desired, EPK TN14-3 and AHP S15/10 and AHP S15/10 x EPK TN 14-3 would be the most preferred crosses and could be advanced in the pre-released trials for commercialization.

For Epicatechin (EC), MPH at Timbilil ranged from -27.25% to 49.11% for inbred TRFK 6/8 and cross EPK TN14-3 x TRFCA SFS150 respectively (Table 6). At Kangaita, MPH for EC ranged from -31.85% to -1.13% for inbreds EPK TN14-3 and TRFK 6/8 respectively (Table 6). 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 7). At Kangaita BPH for EC ranged from -31.85% to 5.30% for inbred EPK TN14-3 and TRFCA SFS150 x TRFK 6/8 respectively. Standard heterosis at Timbilil for EC ranged from -27.27% to 44.45% for inbred TRFK 6/8 and EPK TN14-3 x TRFCA SFS150 respectively. At Kangaita, standard heterosis ranged from -36% to 0.99% for TRFCA SFS150 x AHP S15/10 and TRFK 6/8 x EPK TN14-3 respectively. Development of varieties with high EC is desired in tea breeding programs. Through oxidative dimerization, it is involved in the formation of theaflavins during the fermentation phase of black tea manufacture, catalysed by polyphenol oxidase as follows:

Epicatechin (EC) + Epigallocatechin (EGC) = simple theaflavin (TF).

EC + Epigallocatechin gallate (EGCG) = Theaflavin-3-gallate (TF-3-g)

TRFK 6/8 x EPK TN14-3 was the only cross with a positive standard heterosis at Kangaita and therefore it could be selected and exploited for breeding high quality tea. 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 6). At Kangaita, MPH for EGCG ranged from -7.97% to 11.96% for TRFCA SFS150 x EPK TN14-3 and inbred TRFK 6/8 respectively (Table 6). 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 7). At Kangaita, better parent heterosis for EGCG ranged from -11.12% to 11.96% for TRFCA SFS150 x EPK TN14-3 and inbred TRFK 6/8 respectively (Table 7). Standard heterosis at Timbilil ranged from -9.47% to 12.25% for inbreds TRFK 6/8 and AHP S15/10 respectively. At Kangaita, standard heterosis for EGCG ranged from 1.90% to 20.45% for TRFCA SFS150 x EPK TN14-3 and inbred EPK TN14-3 respectively (Table 8). All the crosses exhibited positive standard heterosis for EGCG at Kangaita site implying that they are more superior to TRFK 6/8. However, inbred TRFK 6/8 had the highest positive mid-parent and better parent heterosis at Kangaita. This could mean that inbred TRFK is more superior to the parent TRFK 6/8. Development of varieties with high EGCG content is highly desired in tea improvement. EGCG is the most abundant compound in tea and has also been widely studied (*34*). EGCG contributes to astringency and give tea the characteristic bitter taste (*35*). EGCG also has several health benefits, which include cancer chemoprevention (31), improving cardiovascular health (32) and it also has antioxidant properties (33). Therefore TRFK 6/8 x TRFCA SFS150 and inbred TRFK 6/8 could be considered for release as varieties with high EGCG content.

Mid-parent heterosis for ECG at Timbilil ranged from -20.56% to 40.36% for inbreds TRFK 6/8 and AHP S15/10 respectively (Table 6). At Kangaita MPH for ECG ranged from -24.34% to 21.13% for inbred AHP S15/10 and cross TRFK 6/8 x TRFCA SFS150 respectively (Table 6). Better parent heterosis at Timbilil ranged from -20.49% to 67.91% for inbreds TRFK 6/8 and AHP S15/10 respectively. At Kangaita, BPH for ECG ranged from -32.60% to 23.85% for AHP S15/10 x TRFK 6/8 and TRFK 6/8 x TRFCA SFS150 respectively. At Timbilil, standard heterosis ranged from -20.49% to 18.02% for inbred TRFK 6/8 and EPK TN14-3 x TRFCA SFS150 respectively. Standard heterosis for ECG at Kangaita ranged from -10.90% to.52% for AHP S15/10 x TRFK 6/8 and TRFK 6/8 x TRFCA SFS150 respectively. Therefore, inbred AHP S15/10 and TRFK 6/8 x TRFCA SFS150 could be exploited and recommended as varieties with high ECG content.

For total catechins (TC), mid-parent heterosis at Timbilil ranged from -12.07% to 10.56% for inbreds TRFK 6/8 and AHP S15/10 respectively. At Kangaita, MPH for TC ranged from -10.40% to 7.82% for crosses AHP S15/10 x AHP S15/10 and inbred x EPK TN14-3 respectively (Table 6). Heterosis over the better parent at Timbilil ranged from -12.08% to 11.79% for inbreds TRFK 6/8 and AHP S15/10 respectively (Table 7). At Kangaita, BPH for TC ranged from -78.72% to 7.82% for EPK TN14-3 x AHP S15/10 and inbred EPK TN14-3 respectively (Table 7). Standard heterosis at Timbilil ranged from -12.08% to 4.15% for inbred TRFK 6/8 and EPK TN14-3 x TRFK 6/8 respectively (Table 8). At Kangaita, standard heterosis for TC ranged from -7.46% to 14.07% for TRFCA SFS150 x EPK TN14-3 and inbred EPK TN14-3 respectively. Inbreds EPK TN14-3 and AHP S15/10 were the most important for TC. Inbred EPK TN14-3 had the highest positive mid-parent, better parent and standard heterosis at Kangaita. At Timbilil, inbred AHP S15/10 had the highest mid-parent and better parent heterosis at Timbilil. This implies that the effect of environment is very important when breeding for quality in tea. Total catechins content is generally used as an indicator of the quality potential in tea since high total catechins teas have high black tea quality (34).

In conclusion, genetic variability was observed in the parents and progenies for all the eight traits that were studied. The crosses, which exhibited a high positive mid-parent, better parent and standard heterosis, may be screened in advanced trials and the best clones subsequently released as a variety. Environmental effects were important in this study since some crosses performed better in Kangaita than in Timbilil. The inbreds (EPK TN14-3, AHP S15/10 and TRFK 6/8) were important in this study as they had the highest mid parent, better parent and standard heterosis in most of the traits. This is interesting considering they are inbreds and it would be expected that inbreeding depression would come to effect. Therefore, further research is recommended.

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