Research Article

International Journal of Tea Science
Vol. 10 (3&4), 2014

Inheritance of catechin and caffeine in tea [*Camellia sinensis* (L.) O. Ktze.]

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Abstract: Diallel designs are used in many breeding programmes because of the important genetic information they offer to plant breeders. Eight biochemical traits of Tea (*Camellia sinensis* (L.) O. Kuntze) were studied to investigate the underlying gene action, and 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. The general combining ability (GCA) effects were significant for six of the traits, namely GA, EGC, Caffeine, ECG, EGCG and total catechin implying that these traits are governed by additive gene effects. SCA on the other hand was significant for EGC, Caffeine, EC, EGCG, and total catechin. Maternal effects were significant for EGC, EGCG and total catechin signifying importance of the choice of female parents in breeding programmes targeting these traits. Non-maternal effects were present in EGCG and total catechin. The study revealed that parents which would produce above average progenies for total catechins are AHP S15/10 and EPK TN14-3. The best combiners for total catechins were EPK TN14-3 x TRFK 6/8 and AHP S15/10 self. This information, which has hitherto been lacking will be very valuable for tea breeding programmes targeting high black/green tea qualities.

Keywords Diallel analysis; Tea (*Camellia sinensis* (L.) O. Kuntze); Additive; GCA; SCA

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.[1] 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.[2]

Tea is produced in 52 countries in the world which comprise of mainly tropical and sub-tropical countries.[3] Kenya is the third largest producer of tea in the world after China and India and it is also the world's largest exporter of black tea.[4] Tea cultivation and manufacturing is present in 15 of Kenya's 47 counties and impacts a large proportion of Kenya's 44 million people.[5] In Kenya, tea is the largest employer in the private sector, with more than 3 million people working in the tea sector[6].Over 60% of Kenyan tea is grown by smallholders. In 2013for example, Kenya exported 494.4 million kilograms of made tea, which resulted to over $ 1.4 billion foreign earnings.[6]

Moreover, tea contributes approximately 26% of the export earnings and 4% of the Gross Domestic Product (GDP) to the Kenyan economy.[6] 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 Kenya 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 arising from rationalised tea improvement efforts.[7]

Tea has different chemical composition which includes polyphenols, alkaloids, amino acids, carbohydrates, vitamins, proteins, chlorophyll, volatile compounds, minerals and trace elements.[8,9] Polyphenols, however, are the main bioactive molecules in tea.[10,11] 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.[12] EGCG is usually of high concentration followed

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ISSN: 0972-544X (print)

by EGC, ECG and EC in decreasing order.[13] Catechin and gallocatechin are present in trace amounts.[14] These polyphenols are usually of high concentration in the bud of tea and continue decreasing as the leaves age.[15] 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.[16]

Experimental studies have recognized that tea exhibits a significant health protecting activity due to its high polyphenol content.[17] There is already evidence that tea polyphenols have anti-heartdisease and anticancer activities in human beings.[11,18] Tea has also been shown to have anti-allergic action,[19] anti-inflammatory and antimicrobial properties,[20,21] potential anti-helmintic properties,[22] anti-diarrhoeal properties,[23] anti-diabetic activity[24] and also anti-hyperglycaemic activity.[25]

Total catechin content is also used as an indicator of the quality potential in tea. High catechin teas are usually of high black tea quality.[26] 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 epicatechingallate (ECG), epigallocatechingallate (EGCG) and epicatechin (EC).[27]

For a breeding program to be successful, prior awareness on the mode of gene action, combining ability and heritability is very important.[28] 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.[29] 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 cross based on four popular commercial tea clones in Kenya.

Methodology

Plant Materials

The plant material used in this study consisted of four parents that were involved in the 4 x 4 full diallel cross. These parents are among the most popular Kenyan commercial tea clones that were selected based on their 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.[29]

Site Description

The study was carried out at Timbilil estate in Kericho County (0^0 22' S and 35^0 21' E). It is located at 2180 meters above sea level, with long-term annual average amount of rainfall at 2043mm and average temperature at 16.2^0C.

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. 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

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.*[29]

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.[30]

Sample Preparation

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 dried for 4 minutes using a microwave oven. This was done so as 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^{0}C for 24 hours. The dried samples were then 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.[31] 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%).

Analysis and Quantification of Catechins and Caffeine

HPLC analysis of catechins and caffeine was done according to the procedure by ISO14502-2-2005E[31]. In this protocol, 1.0 ml of the sample was pipetted into a test tube. 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.[32]

Combining Ability Analysis

Analysis of variance was carried out using the GLM procedure of SAS (SAS, 2003)37. 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 α=0.05 level of significance. Combining ability analysis was carried out following Griffing's (1956) Method I, model 1 to estimate the GCA and SCA effects.

Method 1 Assuming Random Effects

The statistical model for Griffing (1956) analysis is;

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

i, j = 1,2, …, n
k = 1, 2… b
l = 1, 2… 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
$1/bc\Sigma\Sigma eijkl$ is the mean error effect.

Results

Variation of Catechins among Parents and Progenies

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, indicating the presence of variability.

Table 2: Means of F_1 sand parents for GA, EGC, C, CAFF, EC, EGCG, ECG and TC at Timbilil

Family	Cross	GA	EGC	C	CAFF	EC	EGCG	ECG	TC
474	AHP S15/10 x EPK TN 14-3	0.75	8.00	0.37	3.55	1.35	11.34	3.06	24.13
447	EPK TN14-3 x AHP S15/10	0.59	7.77	0.35	3.65	1.68	10.87	2.90	23.57
488	EPK TN14-3 x TRFCA SFS150	0.60	8.36	0.37	3.58	2.08	10.21	3.34	24.36
430	TRFCA SFS150 x EPK TN14-3	0.61	7.61	0.32	3.33	1.37	10.25	2.96	22.51
443	EPK TN14-3 x TRFK 6/8	0.61	8.33	0.34	3.38	1.51	11.17	2.96	24.84
476	TRFK 6/8 x EPK TN14-3	0.72	7.66	0.34	3.30	1.52	11.02	2.99	23.53
482	TRFK 6/8 x TRFCA SFS150	0.65	8.08	0.39	3.39	1.65	11.08	2.95	24.15
420	TRFCA SFS150 x TRFK 6/8	0.55	8.13	0.38	3.21	1.88	9.87	3.06	23.31
475	TRFK 6/8 x AHP S15/10	0.83	7.52	0.35	3.14	1.41	11.05	2.91	23.24
456	AHP S15/10 x TRFK 6/8	0.68	7.68	0.34	3.52	1.56	10.27	2.69	22.57
485	AHP S15/10 x TRFCA SFS 150	0.64	7.26	0.41	3.36	1.47	10.78	2.98	22.86
463	TRFCA SFS150 x AHP S15/10	0.73	7.16	0.33	3.31	1.28	10.79	2.86	22.42
467	TRFK 6/8 x TRFK 6/8	0.65	7.96	0.27	3.22	1.04	9.46	2.25	20.97
478	AHP S15/10 x AHP S15/10	0.78	7.91	0.47	3.43	1.41	11.73	3.14	24.65
471	TRFCA SFS150 x TRFCA SFS150	0.64	7.53	0.37	3.44	1.43	10.83	2.94	23.07
490	EPK TN14-3 x EPK TN14-3	0.74	7.22	0.35	3.23	1.48	11.08	2.86	22.99
	Parents performance								
	TRFK 6/8	0.72	8.74	0.41	2.96	1.43	10.45	2.83	23.85
	AHP S15/10	0.75	7.16	0.39	3.58	1.04	11.58	1.87	22.05
	TRFCA SFS150	0.58	8.00	0.28	3.39	1.44	10.48	3.32	23.56
	EPK TN14-3	0.77	7.59	0.23	3.52	1.35	12.17	2.84	24.18
	Mean	0.68	7.78	0.35	3.37	1.47	10.82	2.88	23.34
	LSD ($p<0.05$)	0.16	0.61	0.09	0.30	0.53	0.69	0.56	0.54
	CV (%)	14.50	4.70	17.10	5.30	21.80	3.90	11.70	1.40

NB GA=Gallic acid; EGC=Epigallocatechin; C=Catechin; CAFF=Caffeine; EC=Epicatechin; EGCG=Epigallocatechin3-gallate; ECG=Epicatechin-3-gallate; TC=Total Catechins; mean=mean of all the 20 entries that were used in the experiment.

Gallic Acid (GA)

Performance based on GA revealed wide spread variability with respect to various crosses, reciprocals and their parents. The 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. 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. 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. This cross had genotypes with GA ranging from 0.54% to 1.30% (data not presented) indicating a wide room for selection.

Epigallocatechin (EGC)

The highest EGC value among the crosses was recorded by EPK TN14-3 x TRFCA SFS150 at 8.36% while TRFCA SFS150 x AHP S15/10 exhibited the lowest EGC at 7.16%.Among the parents, TRFK 6/8 had the highest EGC value at 8.74%while AHP S15/10 had the lowest at 7.16%.Among the genotypes, the cross AHP S15/10 x EPK TN14-3 had the highest GCA content at 9.08%while the lowest genotype at 6.28 was TRFK 6/8 x EPK TN14-3. (data not presented).

Catechin (C)

Significant differences ($p<0.01$) were observed with regard to percentage catechin content within and between

the families. The performance of the crosses ranged between 0.27% to 0.47% with AHP S15/10 self having the highest and TRFK 6/8 self having the lowest. 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%. The genotype involving AHP S15/10 x TRFCA SFS150 recorded the highest C content at 0.52% while EPK TN14-3 x TRFK 6/8 had the lowest at 0.16%(data not presented).

Caffeine(CAFF)

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. The performance of the parents ranged from 2.96% to 3.58% for TRFK 6/8 and AHP S15/10 respectively. Similarly, the genotype with the highest caffeine levels was derived from a cross between EPK TN14-3 and AHP S15/10 at 4.38%. On the other hand, the genotype with the lowest caffeine level resulted from a cross of TRFCA SFS150 and TRFK 6/8at 2.89% (data not presented), implying that these parents should be used in developing varieties with low caffeine content.

Epicatechin (EC)

The EC content ranged from 1.04% to 2.08% for TRFK 6/8 self and EPK TN14-3 x TRFCA SFS150 respectively. Among the parents, percentage EC ranged between 1.04% and 1.44% for AHP S15/10 and TRFCA SFS150 respectively. Similarly, the genotype with the highest EC content involved EPK TN14-3 x TRFCA SFS150 at 2.54%while AHP S15/10 x EPK TN 14-3 at 0.83% had the lowest (data not presented).

Epigallocatechin-3-gallate (EGCG)

The performance of the crosses ranged between 9.46% and 11.73% for inbreds TRFK 6/8 and AHP S15/10 respectively. The performance of the parents ranged between 10.45-12.17% for TRFK 6/8 and EPK TN14-3 respectively. Among the genotypes, the range was between 8.96% to 12.54% for crosses TRFCA SFS150 x AHP S15/10 and AHP S15/10 x EPK TN 14-3respectively.This indicates the potential of AHP S15/10 and EPK TN 14-3 to be used as parents for developing high EGCG teas.

Epicatechin-3-gallate (ECG)

The ECG content ranged from 2.25 to 3.34% for TRFK 6/8 self and EPK TN14-3 x TRFCA SFS150respectively. The best parent EPK TN14-3 registered 2.84% ECG content while AHP S15/10 registered the lowest performance at 1.87%. The best genotype was derived from a cross between TRFCA SFS150 and TRFK 6/8and it registered 3.69% while the lowest genotype involved cross EPK TN14-3 x AHP S15/10 at 1.87%.

Total Catechins (TC)

Performance based on TC revealed wide spread variability. Among the crosses, the mean value for TC ranged between 20.97% to 24.84% for crosses TRFK 6/8 self and EPK TN14-3 x TRFK 6/8 respectively. Among the parents, TC content ranged between 22.05% to 24.18% for AHP S15/10 and EPK TN14-3 respectively. Similarly, the best genotype was derived from a cross of EPK TN14-3 x TRFK 6/8 which recorded 26.81% while the lowest genotype involved TRFCA SFS150 x AHP S15/10 at 20.37%.

Combining Abilities

The results presented in Table 3 showed that the GCA effects for GA, EGC, Caffeine, EGCG, ECG and total catechins were significant ($p < 0.05$), an indication of predominant additive effects. GCA effects on the other hand were not significant ($p<0.05$) for C and EC. The specific combining ability was significant ($P < 0.05$) for EGC, Caffeine, EC, EGCG and total catechins suggesting that these traits are predominantly controlled by non-additive genes.

It is noteworthy that the GCA and SCA effects were significant ($p<0.05$) for EGC, Caffeine, EGCG and TC (Table 3) indicating both additive and non-additive gene effects to be playing a role in their expression. To weigh 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, Caffeine, EGCG and total catechins were 2.27, 2.18, 2.81, and 1.39 respectively indicating that these traits are mainly influenced by additive genes. This was in agreement with results of Kamunya et al.[29] for %total polyphenol content, fermentability and pubescence.

Significant maternal effects were revealed for EGC, EGCG and TC. Non maternal effects were significant for EGCG and TC. Significant ($p < 0.05$) reciprocal effects were observed for EGCG and TC.

Comparison between GCA effects associated with each parent is presented in Table 4. GCA effects for GA varied from -0.049 to 0.04. It was observed that EPK TN14-3, TRFK 6/8 and AHP S15/10 showed positive GCA effects for GA, indicating that they would be good parents of high GA content offsprings.

GCA effects for EGC ranged from -0.231 to 0.223. Two parents TRFK 6/8 and EPK TN14-3exhibited pos-

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

		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 catechin titles.

itive GCA effect for EGC providing a potential for improving EGC levels in the crosses.

GCA effects for C ranged from -0.119 to 0.103. Two of the parental linesAHP S15/10 and TRFCA SFS150 had positive GCA values, indicating that they could be potential parents forhigh C progenies.

With regard to caffeine, GCA effects ranged from -0.117 to 0.061. EPK TN14-3 and AHP S15/10 showed positive significant GCA effects while TRFK 6/8 showed negative significant GCA effects for caffeine. This signifies that EPK TN14-3 and AHP S15/10 would be potential parents for high caffeine offsprings while TRFK 6/8 can be used as a parent when developing tea of low caffeine content.

GCA effects for EC ranged between -0.103 to o.069 for AHP S15/10 and TRFCA SFS150 respectively. TRFCA SFS150 and EPK TN14-3 showed positive GCA effects and hence they could be a potential for high EC content in tea breeding programs. AHP S15/10 on the other hand exhibited negative significant GCA effects for EC indicating that it could be avoided when the objective is to develop high EC tea.

GCA effects for EGCG ranged from -0.252 to 0.264. EPK TN14-3 and AHP S15/10 exhibited positive significant GCA effects, providing a potential for improving EGCG content in crosses. TRFCA SFS150 and TRFK 6/8 showed negative significant GCA effects for EGCG.

GCA effects for ECG ranged from -0.088 to 0.133. TRFCA SFS150 and EPK TN14-3had positive GCA effects while AHP S15/10 and TRFK 6/8 had negative GCA effects for ECG.

GCA effects for total TC ranged from -0.414 to 0.434. EPK TN14-3 and AHP S15/10 exhibited positive GCA effects for TC providing a potential for improving TC in tea. TRFK 6/8 exhibited negative significant GCA effects for TC.

Based on the data obtained for GCA, EPK TN14-3 is an above average general combiner for all the studied characters except C. This may indicate the possibility of using it as a source of high GA, C, Caffeine, EGC, ECG, EC, EGCG and total catechins in tea breeding programs. Similarly, AHP S15/10 exhibited positive significant ($p<0.05$) GCA effects for GA, C, Caffeine and EGCG.

Individual estimates of specific combining ability are also presented in Table 4.Among ten cross combinations in F1 generation, 60% of the crosses had positive SCA effects for GA.AHP S15/10, which exhibited positive significant ($p<0.05$) GCA effects for GA, showed the best SCA when crossed with EPK TN14-3which individually exhibited a positive GCA for the same trait. EPK TN14-3 self also showed significant ($p<0.05$) SCA effects for GA. However, inbred of AHP S15/10 had negative SCA effects for GA. Negative significant reciprocal values were observed for EPK TN14-3 x AHP S15/10 and AHP S15/10 x TRFK 6/8.

GCA analysis for EGC was most favourable for TRFK 6/8 which had positive and significant ($p<0.05$) effects. SCA analysis for the same trait indicated that 70% of the crosses had positive SCA effects. Positive significant ($p<0.05$) 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. Surprisingly, AHP S15/10 had negative significant ($p<0.01$) GCA effects for EGC, this could suggest large variation existing within its progenies. EPK TN14-3 self, AHP S15/10 x TRFK 6/8 and TRFCA SFS150 x AHP S15/10 on the other hand

Table 4: Estimates of general combining ability, specific combining ability, reciprocal effects, maternal effects and non maternal effects for GA, EGC, C, CAFF, EC, EGCG, ECG and TC obtained from a 4 × 4 diallel cross

Genotype	Traits							
	GA	EGC	C	CAFF	EC	EGCG	ECG	TC
GCA Effects								
TRFCA SFS150	-0.049**	-0.021	0.001	-0.005	0.069	-0.252**	0.133*	-0.046
EPK TN14-3	0.006	0.029	-0.119	0.061*	0.039	0.227**	0.071	0.434
AHP S15/10	0.040*	-0.231**	0.103*	0.061*	-0.103*	0.264***	-0.116	0.026
TRFK 6/8	0.002	0.223***	-0.003	-0.117***	-0.005	-0.239**	-0.088	-0.414*
SCA Effects								
TRFCA SFS150 x TRFCA SFS150	0.039	0.020	-0.032	0.044	-0.211	-0.252**	-0.055	0.449
TRFCA SFS150 x EPK TN14-3	-0.017	0.019	0.012	0.016	0.113	-0.533**	0.028	-0.324
TRFCA SFS150 x AHP S15/10	0.014	-0.328*	-0.103	-0.098	-0.095	-0.016	-0.013	-0.716
TRFCA SFS150 x TRFK 6/8	-0.076	0.097	0.064	-0.007	0.402*	-0.190**	0.096	0.141
EPK TN14-3 x EPK TN14-3	0.075*	-0.391**	-0.023	-0.127	-0.167	0.382*	0.210	-0.399
EPK TN14-3 x AHP S15/10	0.091*	0.298*	-0.035	0.094*	0.078	-0.173	-0.106	0.195
EPK TN14-3 x TRFK 6/8	-0.046	0.387*	-0.030	0.143	0.143	-0.057	-0.286	0.928*
AHP S15/10 x AHP S15/10	-0.002	0.642***	0.035	-0.148	0.162	-0.491	-0.182	0.633*
AHP S15/10 x TRFK 6/8	0.037	-0.823**	-0.063	0.007	0.029	-0.172	0.045	-0.744
TRFK 6/8 X TRFK 6/8	0.125	0.096	-0.031	-0.142	-0.711**	0.739	-0.652	-0.325
Reciprocal effects								
TRFCA SFS150 x EPK TN14-3	0.004	-0.376*	-0.024	-0.125	-0.353**	0.021	-0.202	-1.323***
TRFCA SFS150 x AHP S15/10	0.033	-0.049	-0.040	0.004	-0.099	0.086	-0.102	-0.382
TRFCA SFS150 x TRFK 6/8	-0.036	0.026	-0.006	-0.025	0.113	-0.605**	0.057	-0.577*
EPK TN14-3 x AHP S15/10	-0.080*	-0.119	-0.013	0.091	0.164	-0.231	-0.0779	-0.329
EPK TN14-3 x TRFK 6/8	-0.056	0.335	-0.001	0.042	0.014	0.075	0.021	0.445
AHP S15/10 x TRFK 6/8	-0.091*	0.080	-0.006	0.189*	0.037	-0.388*	-0.110	-0.273
Maternal effects								
TRFCA SFS150	0.001*	-0.100	-0.018	-0.060	-0.085	-0.132	-0.061	-0.57***
EPK TN14-3	-0.035*	0.149*	0.003	0.054	0.129*	-0.030	0.0362	0.359**
AHP S15/10	-0.011	0.063	0.012	0.041	-0.002	-0.057	0.017	0.109
TRFK 6/8	0.046**	-0.110	0.003	-0.035	-0.047	0.230**	0.008	0.101
Non maternal effects								
TRFCA SFS150 x EPK TN14-3	-0.032	-0.128	-0.004	-0.066	-0.139	0.100	-0.104	-0.393
TRFCA SFS150 x AHP S15/10	0.022	-0.113	-0.011	0.077	0.012	0.154	-0.023	0.298
TRFCA SFS150 x TRFK 6/8	0.010	0.016	0.015	-0.066	0.203*	-0.255	0.127	0.0945
EPK TN14-3 x AHP S15/10	-0.057*	-0.185	-0.004	0.021	-0.011	-0.254	-0.0967	-0.579**
EPK TN14-3 x TRFK 6/8	0.025	0.051	0.000	-0.046	-0.176	0.349**	-0.007	0.187
AHP S15/10 x TRFK 6/8	-0.035*	-0.111	-0.015	0.113*	-0.002	-0.099	-0.119	-0.281

exhibited negative significant ($p<0.05$) SCA effects for EGC.

Half of the crosses had positive SCA effects for caffeine. EPK TN14-3 and AHP S15/10 showed positive significant ($p<0.05$) GCA estimates for caffeine. Similarly, the cross involvingEPK TN14-3 and AHP S15/10 also exhibited positive significant ($p<0.05$) SCA effects for caffeine. AHP S15/10 x TRFK 6/8 displayed significant reciprocal effects for caffeine.

30% of the crosses exhibited positive SCA effects for C. The best crosses were those involving either AHP S15/10or TRFCA SFS150, which were also good general combiners for C. In contrast, AHP S15/10 x TRFCA SFS150 cross had negative SCA effects.

TRFCA SFS150 and EPK TN14-3 were good general combiners for EC. Analysis of SCA showed that 60% of the crosses had positive SCA effects for EC. Significant positive SCA effects for EC were obtained by TRFCA SFS150 xTRFK 6/8 while TRFK 6/8 self showed negative significant SCA effects for the same trait.

GCA analysis for EGCG revealed good general combining ability for EPK TN14-3 and AHP S15/10 which had positive and significant ($p<0.05$) effects. However, only two crosses showed positive SCA effects. Consequently, inbreds EPK TN14-3 and TRFK 6/8 revealed positive SCA effects for EGCG. It was interesting to note that AHP S15/10which exhibited positive significant ($p<0.05$) 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.

TRFCA SFS150 and EPK TN14-3 were good general combiners for ECGwhile40% of the crosses had positive SCA effects. The best cross for ECG was TRFCA SFS150 x TRFK 6/8.

EPK TN14-3 and AHP S15/10 showed positive GCA effects for TC. Half of the crosses showed positive SCA effects for TC. TRFK 6/8 x EPK TN14-3 and AHP S15/10 self exhibited significant ($p<0.05$) SCA effects for total catechins indicating the potential for high total polyphenol content in their progenies.

Discussion

Significant differences observed among the crosses indicate the existence of a high level of variation for various characters in this study. This makes selection possible for improved black and green quality tea. Variability in the performance for yield, drought tolerance and quality traits in tea has also been reported by Kamunya et al.[29] The present study provides a good understanding of the performance of four tea clones in diallel mating design. Individual catechins varied significantly ($P<0.05$) among the parents and the crosses with EGCG and EGC levels being the highest and +C, GA, ECG, Caffeine and EC being less abundant. These results are similar to those of Ender et al.[40]

Significant positive or negative SCA effects for the traits shows that the crosses performed better or poorer than what would be expected from the GCA effects of their respective parents (Tables 4). The estimate of GCA effects of a parent in a diallel analysis is an important indicator of its potential for generating superior breeding genotypes. For tea significant positive GCA and SCA effects are desirable for GA, EGC, C, EC, EGCG, ECG and TC.

Considering their performance for the desired traits, TRFCA SFS150 x TRFK 6/8 was considered as the best combination showing high positive SCA for C, EC and ECG while EPK TN14-3 x AHP S15/10 were the best combination for GA and caffeine. Therefore, these crosses could be utilized in tea breeding programs for the improvement of these traits

Inbreds EPK TN14-3 and AHP S15/10 were the best crosses for EGCG and EGC respectively whereas EPK TN14-3 x TRFK 6/8 was the best cross for TC. Previous experiments have shown that EGCG and EC are suitable for predicting the quality of Kenyan black tea.[26] Total catechin content is also used as an indicator of the quality potential in tea with high catechin teas having high black tea quality.[27] Therefore, crosses involving EPK TN14-3 x TRFK 6/8 can be exploited in tea breeding programs when targeting tea with high black and green quality.

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 low caffeine levels, clone TRFK 6/8 will be the best parent to use. On the other hand, if high caffeine tea is to be developed, AHP S15/10 and EPK TN14-3 are the best clones to use as parents.

From the study, majority of the crosses which had significant SCA effects involved EPK TN14-3 and TRFK 6/8. This cross can be exploited when breeding for tea with high catechin content. Studies by Kamunya et al.[29] working with similar clones also reported that 6/8 and EPK TN14-3 were superior clones and could be utilized in breeding for high black tea quality.

The study also revealed that TRFK 6/8 had either negative or insignificant GCA effects in all the studied characters except EGC. However when crossed with other clones, most of the best crosses involved TRFK 6/8 as one of the parent. Similarly inbred TRFK 6/8 performed poorly as revealed by negative SCA effects it exhibited (Table 4). In contrast, inbred EPK TN14-3 and AHP S15/10 recorded good performance for EGCG and ECG respectively.

Selection of parents is the most important step in any breeding program in order to get desirable recombinants in crop improvement. Tea breeding targets the selection of populations with high functional compounds such as catechins in tea where high black or green tea quality is targeted.[34] Catechins and caffeine play a very important role in green and black tea quality.[35] Catechins are also important pharmacologically due to their anticancer, anti-hypertension, anti-vascular disorders and anti-inflammatory properties.[36]

In a diallel analysis, significant GCA effects suggest the predominance of additive gene effects[37] while significant SCA shows the importance of non-additive gene effects.[38] A high positive significant GCA value means the parent has high potential for generating superior offspring.[39]

Conclusion

From this study, it was evident that additive and non-additive gene effects were all involved in the inheritance of catechins and caffeine, therefore they should always be considered when developing new breeding schemes to select superior tea. The magnitude of GCA mean squares was higher than the SCA mean squares 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 total catechin signifying that the choice of the female parent is important for these traits.

EPK TN14-3 was the best parent to use in quality improvement as revealed by the combining ability effects. TRFK 6/8 performed well when crossed with other clones while it performs poorly when selfed. Inbreds EPK TN14-3 and AHP S15/10 were the best crosses for EGCG and EGC respectively.

The present study clearly indicated that tea breeders must give proper attention to a systematic breeding approach by using the selected promising crosses having significant high SCA values as well as high performance for catechins. Moreover, inclusion of F_1s of high SCA and using parents with positive significant GCA in multiple crosses will be a worthwhile approach for the breeding of high catechin teas.

Acknowledgement

This study was supported by the Tea Research Foundation of Kenya (TRFK).

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