

# Genetic mapping and identification of quantitative trait loci for yield and drought tolerance in tea

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## ABSTRACT

Accelerated breeding and selection of improved tea (*Camellia sinensis* (L.) O. Kuntze) cultivars requires sound knowledge and understanding of genetics associated with desirable attributes. Quantitative trait loci (QTL) mapping for yield and drought tolerance in tea was performed using 42 F<sub>1</sub> clonal progeny derived from a cross between tea cultivars TRFCA SFS150 and AHP S15/10 that had been established at two distinct tea growing regions in Kenya. The 100 informative markers identified in the cross that exhibited 1:1 segregation ratio were used to construct a linkage map of tea. The map consisted of 30 (19 maternal and 11 paternal) linkage groups that spanned 1411.5 cM with mean interval of 14.7 cM between loci. QTL analysis was carried out for yield and drought tolerance produced nine putative yield QTLs and 13 QTLs for drought tolerance. None of the QTLs was congruently detected in the two sites owing to G × E interactions. Markers OPT-18-2500 and OPO-02-900 had pleiotropic effects in that they were significantly associated with yield at Timbilil (YLD-T; *P* = 0.007) and drought tolerance at Kangaita (DT-K; *P* = 0.003). The implications of the current study with respect to mapping population, G × E and marker-assisted selection are discussed.

**Key words:** *Camellia sinensis*, Drought tolerance, Kenya, QTL mapping, Yield.

## INTRODUCTION

Tea is one of the most widely consumed soft beverages in the world and plays a significant role on the economy of all the tea producing countries, including Kenya where tea is the leading foreign exchange earner. However, the future of Tea Industry in the light of emerging challenges associated with climate change and the falling global tea prices depend upon the availability of high yielding and drought tolerant tea clones. Kenyan tea has imprinted a place in world position for high black tea quality and yields. However, it has not yet been possible to produce clones with combined optimum leaf yield and tolerance to important biotic and abiotic stress factors such as the ever-increasing incidences of drought occurrences (Kamunya and Wachira, 2004; Kamunya *et al.*, 2004).

Most of the agronomic traits of tea are quantitative in nature and therefore not amenable to easy manipulations in breeding programmes without elaborate and long-term field testing in at least more than one environment in order to, among other aims, determine their adaptability and stability. If molecular markers linked to major QTL for the most desirable traits are identified, they could be utilized for early selection of elite cultivars thereby saving on time, cost of maintenance and land resources. The study was conducted to identify QTL controlling yield

and drought tolerance in tea with the aim of initiating marker-assisted selection and breeding in tea improvement programme.

## **MATERIALS AND METHODS**

### **Mapping population and experimental sites**

A pseudo-test progeny of two heterozygous parental clones TRFCA SFS150 (female) and AHP S15/10 (male) was used for construction of a linkage map and mapping of QTL governing yield and drought tolerance. Clone TRFCA SFS150 is a Malawian Assam type that is a moderate yielder, drought, cold and pest tolerant, sparsely pubescent shoots, with moderate levels of caffeine (2.9%), while clone AHP S15/10 is an Assam type Kenyan local selection that is high yielding, with dense pubescent shoots, but susceptible to water stress and contains moderate levels of caffeine (3.0%). The cross comprising 42 clonal progeny was established in 2000 in two sites, one each at the two research stations of Tea Research Foundation of Kenya (TRFK) in Timbilil (0° 22' S, 35° 21' E, 2180 m a.m.s.l.) (Kericho District) and Kangaita (0.5° S, 37.3° E; 2100 m a.m.s.l.) (Kirinyaga District). The trial was set up in a completely randomized block design with three replications in plots of 30 ramets spaced at 0.61 m within rows and 1.22 m between rows (that is, 13448 plants/ha).

### **Assessment of phenotypic traits**

Yield data entailed plucked two leaves and a bud. Harvesting was carried out at intervals of seven to ten days depending on availability of crop. Drought damage was scored during periods of severe water stress that were accompanied by frost incidences between January and April using a 5-point scale with one and five being least and severest damage, respectively.

### **DNA analysis**

Young fresh leaf (two leaves and a terminal bud) was harvested from the experiments and freeze-dried overnight at -20°C prior to DNA extraction using a modified protocol of Gawel and Jarret (1991). All the DNA samples were checked for quality and quantity and stored at -20°C. 250 RAPD primers (William *et al.*, 1990), 96 AFLP primer combinations (Zabeau and Vos, 1993) and 15 SSR primers pairs (Freeman *et al.*, 2004), were then screened on the parents and all the bulked DNA samples using bulk segregant analysis (BSA) (Michelmore *et al.*, 1991). Only informative primers were used to genotype the entire population.

### **Linkage and QTL analysis**

Linkage analysis utilized the backcross mating type with a segregation ratio 1:1 for both dominant and co-dominant markers. A linkage map was constructed using Map Manager QTX2.0 (Manly *et al.*, 2001). QTL mapping was performed using genome-wide single marker regression analysis, interval and where necessary composite interval mapping in Map Manager QTX2.0 (Manly *et al.*, 2001).

## **RESULTS AND DISCUSSIONS**

### **Variation of yield and drought tolerance**

Variations of annual mean yield as assessed at both Timbilil (T) and Kangaita (K) experimental sites showed continuous variation. The Timbilil yield among the progeny ranged from 1670 to 2412 kg made tea (mt)/ha, while the Kangaita progeny ranged from 672 to 2428 kg mt/ha. The  $F_1$  means for the two sites were 2180 and 1504 kg mt/ha for Timbilil and Kangaita, respectively. The mid-parent values (MPV) were 2163 and 2013 kg mt/ha for Timbilil and Kangaita, respectively.

Drought tolerance (DT) as measured in both the Timbilil and Kangaita sites had the  $F_1$  means falling in between the two parental means with variability being continuous implying that additive genes are largely in control of the trait. While in Timbilil, the drought damage score ranged from 0.8 to 3.2; in Kangaita, it ranged from 1.1 to 3.1.

### **Linkage analysis**

The 155 (149 dominant + 6 co-dominant) markers that showed 1:1 segregation ratio were used to construct a linkage map of tea using Map Manager QTX2.0. Of these, 100 markers generated 30 linkage groups, while 55 remained unassigned to any linkage group. The 30 linkage groups comprised 19 maternal linkage groups and 11 paternal linkage groups arising from 69 inherited-female and 31 inherited-male markers, respectively. The maternal linkage groups spanned 1012 cM, while the paternal map covered a total length of 399.5 cM, with mean distance between markers being 14.7 cM and 12.9 cM, respectively (Figure 1). The 30 linkage groups formed a consensus map that spanned 1411.5 cM with mean interval between markers of 14.1 cM. The two parental genomes were treated as a single entity for QTL detection for yield and DT as recorded in the two different sites.

### **QTL analysis for yield and drought tolerance**

QTL analysis was done separately for each site, trait and period. Single marker regression analysis of trait values for statistical association with genotypes of marker loci in the progeny detected a total of 9 significant ( $P < 0.01$ ) putative QTLs controlling various yield across the two sites (Tables 1). Four of the markers were associated with yield at Timbilil, while five were detected in Kangaita site. Besides, five QTLs were inherited from the maternal parent, while eight descended from the paternal parent. Further, three loci in each of the two sites were not assigned to any linkage group. Surprisingly, none the markers was mutually detected in the two sites for the entire period under consideration. The loci had net additive main effects irrespective of whether the dominant alleles were maternally or paternally inherited. Multiple regression of the two unlinked loci associated with ANYLD07 showed that linked QTLs explained 38% ( $P < 0.05$ ) of the total phenotypic variance. Three detected QTLs controlling drought tolerance at Timbilil (DT-T) were unlinked, while six of ten QTLs revealed for drought tolerance at Kangaita (DT-K) were positioned in linkage group 1 (Table 2).

### **Pleiotropic effects**

Some markers displayed pleiotropic effects as they were significantly associated with more than one trait (Table 3). Thus, three loci namely OPT-18-2500, OP-26-08-900 and OPO-02-900 were significantly ( $P = 0.001$ ) associated with yield at Timbilil and drought tolerance at Kangaita. Incidentally, the three loci were placed in linkage group 1.

**Table 1: QTL analysis for annual mean yield from 2001 to 2007 (ANYLD07).**

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

Typically, the sign of additivity (Add) for each QTL imply which QTL had increasing or decreasing effect on the trait depending on the parent contributing the dominant allele. QTL localization was done by interval mapping.

The LRS (Likelihood Ratio Statistics) indicates significance of potential association. Logarithm of Odds (LOD) can be obtained by dividing LRS by 4.61.

**P** and **M** designate paternal and maternal alleles, respectively.

\*, \*\*, \*\*\* denote significance level at  $P < 0.01$ ,  $0.001$  and  $0.0001$ , respectively. This note applies also to Tables 2 to Table 4.

### Epistatic effects

Significant epistatic effects (interactions) between loci EAGC/MCAA01 and EAGC/MCAA03 as well as between EAGC/MCAA01 and OPG-2 were detected for the annual mean yield at Timbilil (ANYLD06-T) (Table 4). The first two loci were not detected during single point regression analysis although their combined interaction effects were highly significant. Similarly, the interaction effect between loci EAGC/MCAA01 and OPG-2 was higher than that detected for locus OPG-2 alone. When the locus with highest LRS in linkage group 1 was placed in the background, epistatic effects were not detected.

### DISCUSSION

Yield and drought tolerance are among traits that have been found to be under the influence of many genes (QTLs) with the environment largely masking the effects of individual gene action (Crouzillat *et al.*, 2000; Faleiro *et al.*, 2006). When considering perennial crops such as tea, QTL analysis is likely to be confounded not only by the highly varying weather pattern but also the heterogenous edaphic and cultural practices leading to significant and unpredictable genotype X environment (G X E) interactions (Bradshaw, 1998).

**Table 2: QTL analysis for drought tolerance.**

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

**Table 3: Pleiotropic effects of some of the identified QTLs at various significance levels as detected by single-point genome-wide regression analysis.**

Marker	Linkage group	Yield-T	DT-K
OPT-18-2500	1	15**	25***
OP-26-08-900	1	21***	20***
OP-02-900	1	20***	20***

**Table 4: Interaction effects for yield at Timbilil (ANYLD06-T) detected at  $P = 1.0 \text{ e-}5$ .**

Trait	Chr1	Locus1	Chr2	Locus2	LRS	IX	Main1	Main2
ANYLD06-T	Group1	EAGC/MCAA01	Group1	EAGC/MCAA03	26.7	9.1	2.2	3.7
	Group1	EAGC/MCAA01	Group1	OPG-2	27.9	10.4	2.2	9.4

The preponderance of the nine putative yield QTLs and 13 QTLs associated with drought tolerance as assessed across space and time provides a good starting point. It may be possible that progeny size might have slightly biased QTL effects upwards owing to which present findings need to be looked with care especially with regard to marker-assisted selection. Availability of a large mapping population that is genotyped with highly informative markers such as identified in the present case would result in a dense linkage map that would not only lead to identification of tightly linked QTLs but also would be of great importance in works aimed at map-based cloning of genes.

The profound effect of genotype by environment interaction is corroborated by lack of a congruent locus affecting the assessed traits in either of the study sites. Kearsy and Pooni (1996), while commenting on nature and causes of  $G \times E$ , have demonstrated that the genetical variance among a collection of genotypes may alter with the environment, meaning that the effects of given allele substitutions may be quite different in one environment than in another. This, therefore, confirms that for optimal performance of a strain or variety in a particular environment, selection should be carried out in that environment.

The study demonstrated the possibility of using some markers to select simultaneously for more than one trait. Thus, loci with pleiotropic effects such as OPT-18-2500 and OP-26-08-900 that significantly associated with yield in Timbilil site (YLD-T) and drought tolerance at Kangaita site (DT-K), would be of profound importance in selection programmes as their net influence is positive for the two traits even though the two sites are wide apart with unique ecological features. As the pleiotropic loci were localised on the same linkage group, it is possible that they represent a group of linked genes.

The revelation of significant epistatic effects between loci EAGC/MCAA01 and EAGC/MCAA03 as well as between EAGC/MCAA01 and OPG-2 that were detected for ANYLD06-T is an indication that yield is a product not only of individual sparsely localised QTLs but of interactions from other unlinked or linked QTLs. The first two loci were not detected during single point genome-wide regression analysis although their combined interaction effects were highly significant. Similarly, the interaction effect between loci EAGC/MCAA01 and OPG-2 was higher than that detected for locus OPG-2 alone. The interaction effects for yield seems also to be influenced by G  $\times$  E interactions as it was only detected for ANYLD07-T at more or less the same level as for ANYLD06-T, while no such effects were detected for Kangaita yield data at any of the period under consideration.

Considering the long time-span involved in developing elite varieties ( $-20$  years) with majority of desirable attributes, adoption of molecular markers linked to important traits can be of great help in this venture. Where putative QTLs have been tightly linked to markers, there is need to verify and confirm their effects using independent populations constructed from the same parental genotypes or closely-related genotypes used in the primary QTL mapping studies. Integration of molecular markers in breeding and clonal selection would not only reduce the number of clones/seedlings for field testing but also lead to increased genetic gain per unit of time by decreasing developmental interval.

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