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Comparative response of catechin levels in drought tolerant and drought susceptible tea clones (*Camellia sinensis*) (L) O. Kuntze under different moisture regimes

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In tea (*Camellia sinensis*) water stress generally affects the content of various plant secondary metabolites including catechins. The objective of the study was to evaluate the effects of different soil moisture content on the catechin levels of various tea clones. We found out that variation of soil water content and accumulation of catechins were strongly correlated. The experiment was conducted in an open field with the drought tolerant clones namely; SFS150, TRFK 303/577, and drought susceptible clones; TRFK 6/8, TRFK 12/9, TRFK 301/4, TRFK 31/11, S15/10, TRFK 7/9, TRFK 31/8, and BBK 35. During the cold and wet periods, the effect of plant water content on catechin level was not clearly expressed. However, significant clone × soil water content interactions (p≤ 0.05) were observed for all clones during the dry and hot periods. This observation indicated that declining plant water content (PWC) due to soil moisture stress reduced catechin levels. It was concluded that variation of catechin in various tea clones over different soil water content could be of great significance in evaluating water stress tolerance ability of tea clones.

Key words: Catechins, clones, water content, dry and hot periods.

INTRODUCTION

Water stress in tea plant due drought effects results in physiological, biochemical and morphological changes such as reduction of leaf water potential, photosynthesis and stomatal conductance (Bota et al., 2004; Zobayed et al., 2007; Damayanthi et al., 2010). Proline and abscisic acid (ABA) accumulate in higher concentrations in response to water stress, which leads to maintenance of turgor potential (Mayer, 2006). That different forms of stress affect the content of various plant secondary metabolites including polyphenols has been reported (Borland et al., 2009; Cherotich et al., 2013) but variation in tea polyphenolics over different seasons has not been quantitatively evaluated.

The study was conducted to check whether the drought

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tolerant and susceptible clones display a distinct pattern of variation in catechin levels when subjected to different water regimes and whether the pattern agrees with the available data on the drought tolerance ability of the studied clones with a view of using the principle in selection of clones.

MATERIALS AND METHODS

Experimental site

The experiment was located in Field 12C at Timbilil estate, Tea Research Foundation of Kenya (0^0 22' S; 35^0 21' E), altitude; 2178 m above mean sea level. The topography of the area is steeply dissected with an average slope of 30 degrees (Callander and Woodhead, 1981).

Physicochemical characteristics of experimental site

The soil in the study area is a fine mixture of clay of kaolinite type (75-85%) and organic matter (30%) (Othieno et al., 1992) hence, it has many properties in common with those of other tea growing areas of Kenya. It is highly weathered, leached and acidic, pH 4.5 soil conditions in which tea grows best (Othieno et al., 1992). The soils are deep and well drained with crumby surface soil structure grading to a moderate aggregate structure in the sub-soil with many pore spaces (50%) making it ideal for tea growth (Watson, 1986). The surface soil colour is dark brown grading to strong brown in the moist sub soil.

Plant materials

Ten contrasting cultivars of tea in terms of drought tolerance and superior beverage quality attributes were selected from the existing tea bushes (average age 28 years). These were; drought tolerant clones: SFS 150 and TRFK 303/577[control], and drought susceptible clones: TRFK 6/8, TRFK 301/4, TRFK 12/19, TRFK 31/11, BBK 35, S15/10, TRFK 7/9, and TRFK 31/8 [control]

Experimental design and treatments

The experiment was superimpossed on Field 12C with mature, fully grown tea bushes established in 1983 in what was a virgin forest land. The exprimental area was arranged in 30 randomized blocks each measuring 225.5 m^2 surrounded by single guard row. The effective plot area consisted of 100 bushes with spacing of 1.2 m between rows and 0.75 m between plants. The effective plot area was subdivided to form three replicates each having 30 plants.

Biometric measurements

For the biometric measurements, mature, fully grown and healthy leaves on the plucking table were selected. The physiological and biochemical parameters that were evaluated were; total catechin content and leaf water potential. Soil moisture content was also measured to study the responses of tea plants to soil water content. All the measurements were made between 9.00 a.m. and 2.00 pm at intervals of 1 h.

Total catechin content (TCC)

Leaves weighing 300 g (the terminal two leaves and bud) were randomly collected from each plot during the cold June to August and wet September to November periods when the conditions are relatively warm and wet. The last sampling was done towards the end of the dry and hot period December-March when tea plants were experiencing severe water stress.

Preparation of extracts

Sampled fresh green leaves from each plot were carefully steamed in a pressure cooker for one minute then placed in the withering bay and left to dry for twelve hours. The dried samples were processed by crush, tear and curl (CTC) then ground using a blender into fine powder, then sealed in paper bags (with aluminium foil lining) and safely stored in dark dry environment of 4°C awaiting the analysis.

Analysis of catechins

Estimation of catechins was determined according to the procedures of Folin Ciocalteu method (Piendl and Biendl, 2000). Fresh leaves (0.5 g) were homogenized in 5 ml of 70% methanol using a chilled pestle and mortar with subsequent centrifugation at 4000 revolutions per minute for 20 min. From the solution 10 ml was pipetted and mixed with Gallic acid standard solutions (0.1 ml) and 50 g anhydrous Gallic acid transferred into the reagent tube. Folin Ciocalteu phenol reagent was then added to each tube. Within five minutes from adding Folin Ciocalteu phenol reagent, 5.0 ml of sodium carbonate solution was added to stabilize the material and allowed to stand for 2 h at room temperature for completion of the reaction. The amount of catechins in the test sample was calculated from a standard curve generated using Gallic acid and then expressed as the amount of Gallic acid equivalent. A best-fit linear calibration graph from the mass of anhydrous gallic acid standards was constructed against the Gallic acid standard optical densities. The contents of catechins in the leaf were then expressed as percentages of the mass of sample dry matter.

Soil moisture content (SMC)

Soil moisture content of the root zone at 60 cm depth was measured along with physiological parameters of tea, leaf water potential and catechin levels. Soil was augured at 60 cm depth and the soil moisture content was determined using time-domain reflectometry (TDR) soil moisture meter (Trime FM-2, Eijkelkamp Agrisearch Equipment Giesbeek, and the Netherlands)

Leaf water potential (LWP)

Leaf water potential was determined periodically in different study periods, that is during wet and cold season (May-August), hot and wet season (October-December), 2011 and then again during the dry and hot period between January and March, 2012. Leaf water potential was measured between 10.00 am and 2.00 pm at an interval of 1 h on the entire leaf by observing the presence of water on the cut surface of the leaf petiole using a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, California) (plate 3.3). Two mature leaves (2^{nd} and 3^{rd}) and a bud were randomly selected from each plot for leaf water potential measurements. The leaf was enclosed in a reflective plastic bag for 1 h to suppress transpiration and allow stem water potential to equilibrate with leaf water potential (Dale, 2006; Kwach, 2011). The leaf was cut (in slanting manner) and enclosed in the pressure chamber with the cut end protruding through a rubber stopper which is used to seal the chamber. The pressure in the chamber was gradually increased

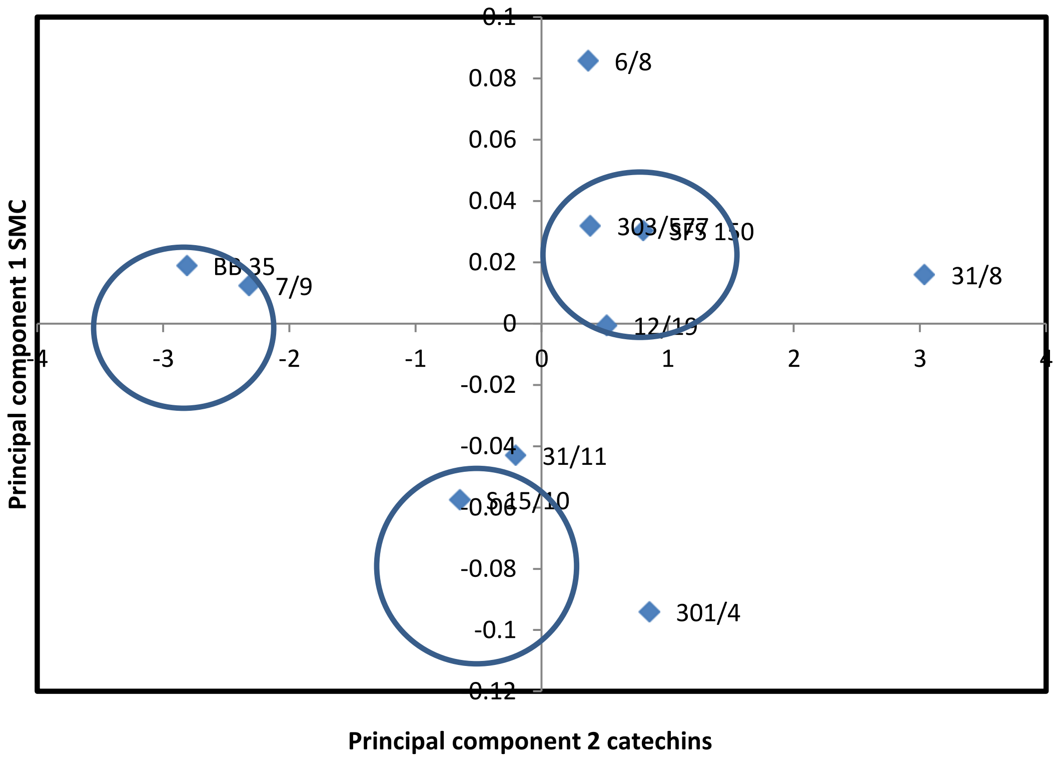

Figure 1. Principal component analysis of catechins for 10 clones across three seasons.

until the sap appeared at the end of the xylem vessels. After the pressure was recorded, the sap was released through an outlet valve and the sample removed (Phene et al., 1990).

Meteorological data

Meteorological data were recorded using instruments installed in Tea Research Foundation of Kenya (TRFK) weather. All instruments were mounted 1 m above the soil. Hourly records, of minimum and maximum temperatures, total radiation and rainfall were taken on a daily basis. The total solar irradiance during the period of study was measured using Gunn Bellani Pyronometer placed at 20 cm above the plucking table. The rate of evaporation was also recorded using Class 'A' pan.

Statistical analysis

Principal components analyses (PCA) were used to draw out the effects and interactions of the different soil moisture content and catechin levels. All one-way ANOVAs were accompanied by mean separation by Duncan Multiple Range Test (DMRT) using the SAS version 8.0 statistical packages (SAS Institute Inc: 1999).

RESULTS AND DISCUSSION

Variation of catechin content across three water regimes

Data were treated with principal component analysis (PCA) (Figure 1) to determine influence of soil water content on catechin accumulation and establish stable clones across the seasons (the different water regimes). The analysis placed the clones into three groups corresponding to stability of catechin content across the three seasons (wet, hot and wet, dry and hot). The first group includes 25% of the clones all known to be possessing moderate to high water stress tolerant characteristics. The rest of the clones (75%) are known to be susceptible to water stress. Clones placed on the side with positive value indicate stable content of catechins across all the seasons. A negative value indicates instability of catechin content in a clone across the three seasons thus indicating low tolerance ability to water stress.

Catechin contents of clones 303/577, 6/8, 31/8 and SFS 15/10 appearing to the right side of vertical ordinate showed little interaction across the three seasons depicting stable characteristic while those to the left are quite unstable across the three periods indicating moderate influence of soil water content on catechin accumulation in these clones whilst those below the horizontal axis are also very unstable suggesting high interaction between soil water content and clonal response.

Clones that have common parental linkage such as 303/577, 31/8 and 6/8 were clustered into the same ordinate. Therefore, it allows the hypothesis that clones that are closely related have almost the same

Table 1. Comparison of leaf water potential (Leaf) and catechins contents in seasons I, II and III at TRFK, Kericho

Clone	Season I[Cool and wet] June-August		Season II[Warm and wet] September-November		Season III [Dry and hot] December-February	
	Ψ_{Leaf} (MPa)	Catechins (%)	Ψ_{Leaf} (MPa)	Catechins (%)	Ψ_{Leaf} (MPa)	Catechins (%)
TRFK 31/8	-4.60	0.18	-6.73	0.16	-16.03	0.20
TRFK301/4	-4.87	0.15	-6.53	0.13	-18.37	0.09
TRFK311/11	-4.40	0.13	-6.53	0.11	-18.93	0.15
TRFK303/577	-4.00	0.23	-6.00	0.22	-17.17	0.24
TRFK 6/8	-3.73	0.17	-6.03	0.18	-18.73	0.21
TRFK 7/9	-4.80	0.11	-6.0	0.07	-17.47	0.17
BBK35	-4.40	0.10	-6.40	0.08	-16.50	0.13
SFS150	-4.67	0.22	-6.20	0.22	-15.80	0.24
S15/10	-5.9	0.11	-6.87	0.14	-18.97	0.16
TRFK 12/19	-4.27	0.12	-6.40	0.09	-16.50	0.15
F	s	s	s	s	s	s
CV (%)	7.54	22.83	4.76	31.23	3.04	34.23
LSD	P≤0.05		P≤0.05		P≤0.05	

s, Significant; MPa. Megapascals; LSD. Least significant difference; cv., coefficient of variation. Measurements were made between 10.00 am and 1.00 pm at intervals of an hour.

response to the environment.

Change in Leaf water potential (Ψ_{Leaf}) in relation to catechins contents

The changes in leaf water potential (LWP) of different clones are presented in (Table 1). Leaf water potential declined over dry period in all cases but the tolerant clones showed the least change. In general, leaf water potential in susceptible clones fell to almost similar low mean values during dry periods. These results suggest that clones with tolerant characteristics compared to the susceptible clones have better mechanism of maintaining of high leaf water status through effective the regulation of catechins levels.

At the lowest moisture levels, the drought tolerant clones TRFK 303/577 and SFS 150 maintained significantly higher (P≤0.05) leaf water potential compared to the drought susceptible clones: TRFK 6/8, TRFK 12/9, TRFK 301/4, TRFK 31/11, S15/10, TRFK 7/9, TRFK 31/8 and BBK 35.

Although there were some variations in catechin levels among the individual clones in the susceptible group during dry period the differences were not statistically significant (P≤0.05). For example the average catechin content for clone TRFK 6/8 and TRFK 31/8 was much higher than that in the rest of the clones (all droughts susceptible) within the same season. The catechin levels at the end of the dry and hot period differed significantly (P≤0.05) between the drought tolerant and drought susceptible clones. The interaction between the soil moisture content and clone were also significant. Catechin content in leaves of tea remained almost constant from June-November period (that period was relatively wet, and the tea plants were not experiencing any stress) and gradually decreasing from December reaching the lowest levels towards the end of January-March season (dry and hot). Low leaf water potential resulted in increased catechin levels content in tea leaves, and there were significant correlations (P≤0.05) between soil water content and catechin content. The catechin content was consistently high in clones 303/577 and SFS 150. Ranking drought tolerance on the basis of stability suggested that clone SFS 150 was the most stable clone, followed by 303/577. The susceptible clones were, with two exceptions (6/8 and 31/8), the most unstable across the seasons.

Catechin concentrations in all the clones' increased during the dry period. These results agree with findings by Ojeda et al. (2002), Flexas and Medrano (2002) and Cheruiyot et al. (2008) who reported that, phenolic biosynthesis in tea is significantly influenced by soil moisture deficit. The results also support the findings of Esteban et al. (2001), Roby et al. (2004) and Salón et al. (2005), who reported that a direct response on phenolic biosynthesis to water deficit by a plant can be positive or negative, depending on the type of phenolic compound, the degree of water deficit and the growth period during which stress is applied.

Clones TRFK 303/577 and TRFK SFS 150 had significant levels of catechin content than the other clones in the study (Table 2). Similarly, the same clones had higher leaf water potential, indicating that they were more tolerant to water stress. Given the close correlation, these results suggest an association of catechin contents with water stress in tea. This observation agrees with results of Khan and Mukhtar (2007) who noted increased polyphenols in light and water-stress resistant safflower and cucumber seedlings as compared to

Table 2. Variation in catechin levels of ten clones over the three seasons.

Clone	Season I	Season II	Season III
	June-August	September-November	December-February
TRFK 31/8	0.4700[a]	0.4767[a]	0.2600[a]
TRFK301/4	0.4267[ab]	0.4033[ab]	0.2433[a]
TRFK311/11	0.3833[ab]	0.4033[ab]	0.2133[ab]
TRFK303/577	0.3233[bc]	0.3300[ab]c	0.2067[abc]
TRFK 6/8	0.3200[bc]	0.3167[bc]	0.1767[abcd]
TRFK 7/9	0.3100[bc]	0.3033[bc]	0.1333[bcd]
BBK35	0.2300[cd]	0.2967[bc]	0.1300[bcd]
SFS150	0.2067[cd]	0.1900[cd]	0.1200[cd]
S15/10	0.1867[d]	0.1867[cd]	0.0966[d]
TRFK 12/19	0.1800[d]	0.1300[d]	0.0900[d]

The interaction means and marginal means followed by a common letter are not significantly different at 5% levels by Duncan's multiple range tests.

those which responded weakly to the stresses.

Clones accounted for 7.5% of the treatments sum of squares while season accounted for 74.3% (data not shown). This showed a great influence that the season had on variations of catechin contents in the study. Interactions between clones and season accounted for 18.2% for mean sum of squares amongst 80% of the clones. This meant that there was substantial influence on catechin accumulation from soil water differences from one season to another.

Conclusion

The study indicate varied fluctuation of catechin content with changes in soil moisture content, and suggest that clones with more stable catechin contents across different water regimes are more tolerant to water stress. This implies that clones that have less fluctuation in catechin content are less affected by changes in soil moisture content and reflect better water stress tolerance ability.

Conflict of Interest

The authors have not declared any conflict of interest.

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