Biosci. Biotechnol. Biochem., **71**, 70156-1–8, 2007

Polyphenols as Potential Indicators for Drought Tolerance in Tea (*Camellia sinensis* L.)

Erick K. Cheruiyot,[1,†] Louis M. Mumera,[1] Wilson K. Ng'etich,[2] Ahmed Hassanali,[3] and Francis Wachira[4]

[1]*Egerton University, Department of Crop, Horticulture and Soil Sciences, P.O. Box 536, Njoro, Kenya*
[2]*Tea Research Foundation of Kenya, P.O. Box 820, Kericho, Kenya*
[3]*International Centre for Insect Physiology and Ecology, P.O. Box 30772-00100, Nairobi, Kenya*
[4]*Egerton University, Department of Biochemistry and Molecular Biology, P.O. Box 536, Njoro, Kenya*

Received March 19, 2007; Accepted May 8, 2007; Online Publication, September 7, 2007
[doi:10.1271/bbb.70156]

Plant polyphenols have gained prominence in quality of plant products and in human health. An experiment was conducted to determine the association of tea polyphenols with water stress and their suitability as indicators for drought tolerance. The experiment was conducted in a 'rain-out' shelter, and consisted of six tea clones (BBK 35, TRFK 6/8, TRFK 76/1, TRFK 395/2, TRFK 31/30, and TRFK 311/287) and four levels of soil water contents (38, 30, 22, and 14% v/v), which were maintained for a period of 12 weeks. The treatments were arranged in a completely randomized design and replicated three times. Plant growth was monitored over 6 weeks, and a water stress index was calculated to determine water-stress tolerant clones. Total polyphenols in tea shoots was analyzed and a regression analysis done. The results indicate that declining soil water content (SWC) reduced both growth and content of polyphenols in tea. Tolerant clones maintained a high polyphenol content at low SWC, and also showed less fluctuation in phenolics when subjected to changes in SWC. There was significant ($P < 0.001$) correlation of total polyphenol content with shoot growth and WSI of tea, and a linear relationship ($r^2 = 0.97$) between SWC for tea and both, water stress index and shoot polyphenol content. We report that there is a potential to use polyphenols as indicators for selection of drought-tolerant tea cultivars.

Key words: tea (*Camellia sinensis* L.); polyphenols; soil water content; drought tolerance

Tea (*Camellia sinensis* L.) is rich in polyphenol compounds which have been a subject of study as to their effects on human health.[1–8] The crop is the source of manufactured tea, which is consumed worldwide but its production is constrained by frequent recurrence of drought in major production areas.[9–11] Tea germplasm that can tolerate low soil water content (SWC) can reduce the losses occasioned by drought in production areas. Readily identifiable indicators for drought tolerance would hasten development and selection of tea germplasm for water stress environments. Plant response to stress is often manifested by its physiological and biochemical reactions, which can provide a basis for screening and selection of individual varieties and germplasm resistant to stress factors. For instance, plants are known to accumulate organic osmolytes, such as proline, glycine betaine, non-reducing sugars, and polyols[12,13)] in response to stress factors. Though these organic compounds are species specific, their role is not clearly defined, but it is generally accepted that they contribute to ameliorating stress in plants.[13–15] Most of the stress-related organic compounds are secondary plant metabolites and incidentally, tea contains large amounts of polyphenols, particularly of the flavonol class. Some polyphenol derivatives have been used in quality determination in black tea[16] and in fruits,[17] but the role of polyphenols in drought stress and their suitability as indicators of desiccation tolerance in tea have not been explored.

The objective of this study was to quantify levels of green leaf polyphenols in tea and to define their association with SWC and their suitability as indicators for drought stress. It was hypothesized that tea plants exposed to increasing soil-water deficits show a decline in shoot growth and that the severity of this response can be predicted by tea polyphenols.

Materials and Methods

Set-up of a rain-out shelter. A rain-out shelter measuring 17 m by 6.5 m on the ground and a height of 2.5 m was erected. The roof was raised and curved to give a dome-shape with a radius of 0.5 m above the

† To whom correspondence should be addressed. Fax: +254-51-2217737; E-mail: cheruiyotke@yahoo.com

2.5 m height and an extended eve of 0.3 m all round. The roof design was to facilitate flow of rain water out of the structure and to enhance uniform distribution of solar radiation inside. The roof was covered with an ultra violet-treated 200-micron film clear polythene sheet (Sunselector AD-IR 504) designed to transmit 82% of photosynthetically active radiation (PAR) and 65% of diffused light with 88% thermicity. The sides were covered with the same polythene, but to 1 m in height from ground level on the longer sides and to 2.5 m on the shorter ones. The 1.5 m uncovered space along the longer sides was to allow free air flow in and out of the structure. A door measuring 1 m wide and 2 m high was made in the middle of one of the shorter sides and was covered with chicken wire but no polythene.

Plant materials. The six tea (*Camellia sinensis*) clones used in the study were: BBK 35 (2n), TRFK 6/8 (2n), TRFK 76/1 (3n), TRFK 395/2 (3n), TRFK 31/30 (4n), and TRFK 311/287 (4n). They were developed from stem cuttings and raised in a seedling nursery as is normally done.[18] The plants were ready for transplanting after 6 months and were transplanted into 1000-gauge black polythene tubes measuring 0.3 m in diameter and 0.3 m in depth. Each clone had 24 potted seedlings, giving a total of 144, which were later arranged into 72 experimental units.

Soil medium and soil water measurement. The soil medium for raising seedlings in the nursery was a mixture of subsoil and topsoil in ratio 3:1. The same soil medium was used for raising transplanted seedlings in polythene tubes. Analysis of the soil was as follows: 3.5% N, 0.16% P, 169 ppm K, 255 ppm Ca, pH 4.3, organic matter 9.3%. Its textural class was clay, with 25% sand, 67% clay, and 8% silt.

Soil water content was determined and maintained using a time-domain reflectometry (TDR) soil moisture meter (Trime FM-2, Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands) with a 110 mm 2-rod probe. Soil water content (% v/v) was maintained within $\pm 2\%$ of the prescribed level. Measurement was done daily at 9:00 and at 14:00 o'clock during the 12-week period of the study.

Arrangement and application of treatment. This study consisted of two factors: varying soil water content subjected to six different tea clones. Four soil water levels (38, 30, 22, and 14%) per volume basis were subjected to the six clones listed above, giving a total of 24 treatments, which were arranged in a completely randomized design and replicated 3 times. Each experimental unit had two potted plants which gave a total of 72 experimental units. Potted tea plants were allowed to establish themselves for 2 months in the tea nursery prior to start of the study. The plants were watered uniformly to field capacity twice a day during the first 2 weeks, after which watering was progressively reduced on a weekly basis. Each experimental unit was maintained at pre-determined soil water content and stabilized for 1 week before study commenced.

Growth measurements. The response of tea to soil water content was largely determined through shoot growth and leaf expansion, which were measured periodically for 4 and 8 weeks, respectively. In both cases, randomly tagged shoots and leaves in each experimental unit were used in the study. Shoot growth was calculated on the basis of increase in shoot length (l) over time (t). Leaf expansion was determined by measuring leaf length from the base of the leaf to the apex (l) and broadest part (w), and product of these two was multiplied by a factor (k), which is given as 0.62 for tea.[19]

Water stress index. The water stress index (WSI) of the clones was calculated based on shoot growth. The ratio of mean sum of growth rate at the soil water content level to the optimum growth attained by the clone in each of the SWC treatments was determined, and the following formular used:$\mathrm{WSI} = (Y_{\mathrm{actual}}/Y_{\mathrm{max}})$. The formular was used by Younis *et al.*[20] to describe drought susceptibility index (DSI) for shoot dry weight and total green leaf area, was also used here (with slight modifications) to describe water stress level of tea in response to soil water content. WSI is calculated water stress index; Y_{actual} is shoot growth at a given soil water content (shoot growth under stress); Y_{max} is maximum shoot growth attained by the clone within the SWC treatments (assumption: optimum growth attained in the absence of soil water stress). Growth measurements were taken every 7 d for a period of 42 d. WSI ranges between 0 and 1, with a value close to 0 being rated as low tolerance to water stress and a value approaching 1 being rated as tolerance to water stress.

Sampling determination of total polyphenols. Plant tissue for determination of total polyphenols was sampled during the 6th week of water treatment. About 500 g of fresh shoots with two leaves and a bud were plucked in each of the experimental units and immediately steamed for 2 min. The samples were then placed in labelled paper bags and dried in an oven at 70 °C for 24 h. The dry samples were ground using a blender, sealed in paper bags, and safely stored in a dark, dry environment until laboratory analysis. Analysis of total polyphenols followed the ISO procedure.[21] Ground shoot samples (0.2 g) were weighed into 10-ml extraction tubes. Hot methanol/water extraction mixture (5.0 ml, 70% v/v methanol) was added to each extraction tube, which was stoppered and shaken on a vortex mixer. The extraction tube was heated in a water bath for 10 min during mixing on the vortex mixer for between 5 and 10 min. The extraction tube was removed from the water bath and allowed to cool to room temperature after which the stopper was removed and

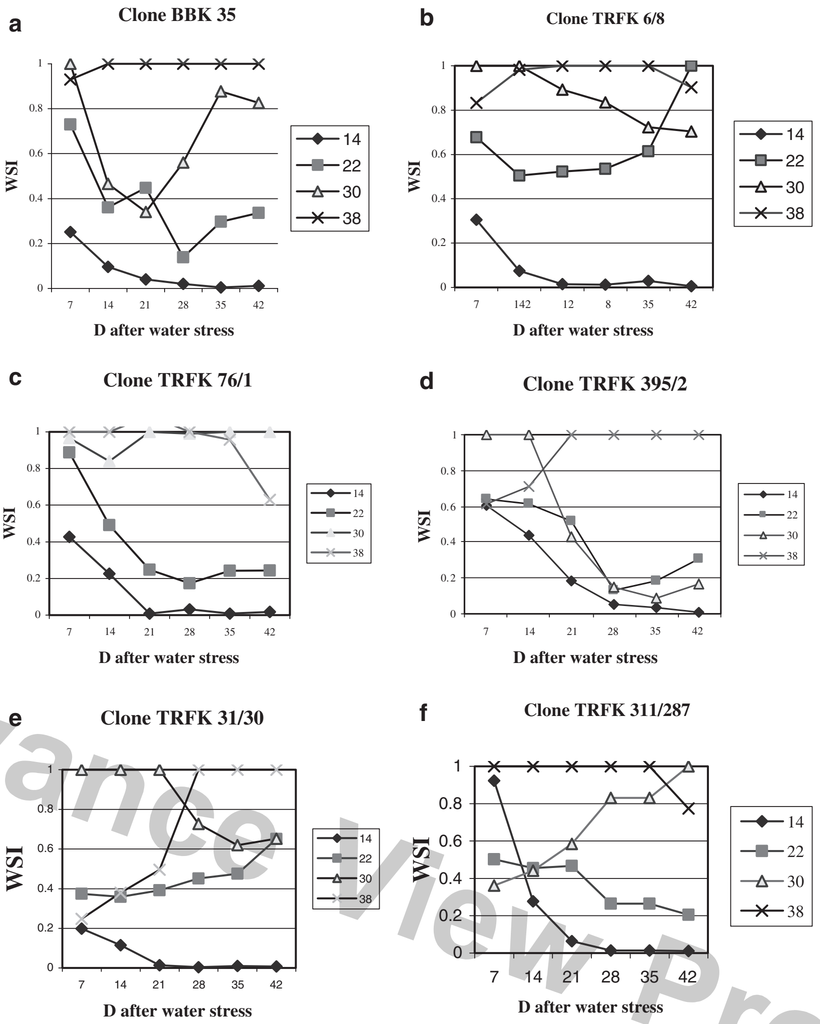

Fig. 1. Water Stress Index for Six Tea Clones (a)–(f) at 14, 22, 30, and 38% SWC.

the extraction tube placed in a centrifuge at 3500 revolutions per min for 10 min. The supernatant was carefully decanted into a 10-ml graduated tube and a cold ethanol/water mixture was added to make up to the 10 ml mark. The sample extract was further diluted by transferring 1.0 ml into a 100-ml volumetric flask, and water was added to the mark. Gallic acid standard solutions (1.0 ml) corresponding to 10, 20, 30, 40, and 50 µg of anhydrous gallic acid, was transferred in duplicate into separate graduated tubes, and a similar quantity of water for reagent blanks. Diluted sample extract (1.0 ml) was transferred into separate tubes, and 5.0 ml of Folin-Ciocalteu phenol reagent was added to each tube and mixed in. Within 5 min after the addition of Folin-Ciocalteu phenol reagent, 4.0 ml of sodium carbonate solution was added to each tube which was allowed to stand for 60 min at room temperature. Optical densities were measured in a 10-mm cell on a spectrophotometer set at 765 nm. The amounts of polyphenols in the test sample were calculated from a standard curve generated using gallic acid, and were expressed as the amounts 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, and the total polyphenol content, expressed as a per cent by mass on a sample dry matter basis, was calculated following the procedures detailed in the ISO document.[21)]

Data analysis. Regression analysis was done using GenStat (GenStat 5 release 4.2), and correlation analysis was performed with SAS (Ver.8.1 e).

Results

Water stress tolerance

The results indicate that all clones succumbed to water stress at 14% SWC, though after varied exposure times. Water stress index (WSI) for clones TRFK 311/287 and TRFK 395/2 approached zero by the 28th day at 14% SWC, while the others approached zero earlier. Based on a SWC above 22% and given the observed threshold SWC as 20%, clones TRFK 6/8 and TRFK 31/30 had WSI ≥ 0.4, suggesting relatively high toler-

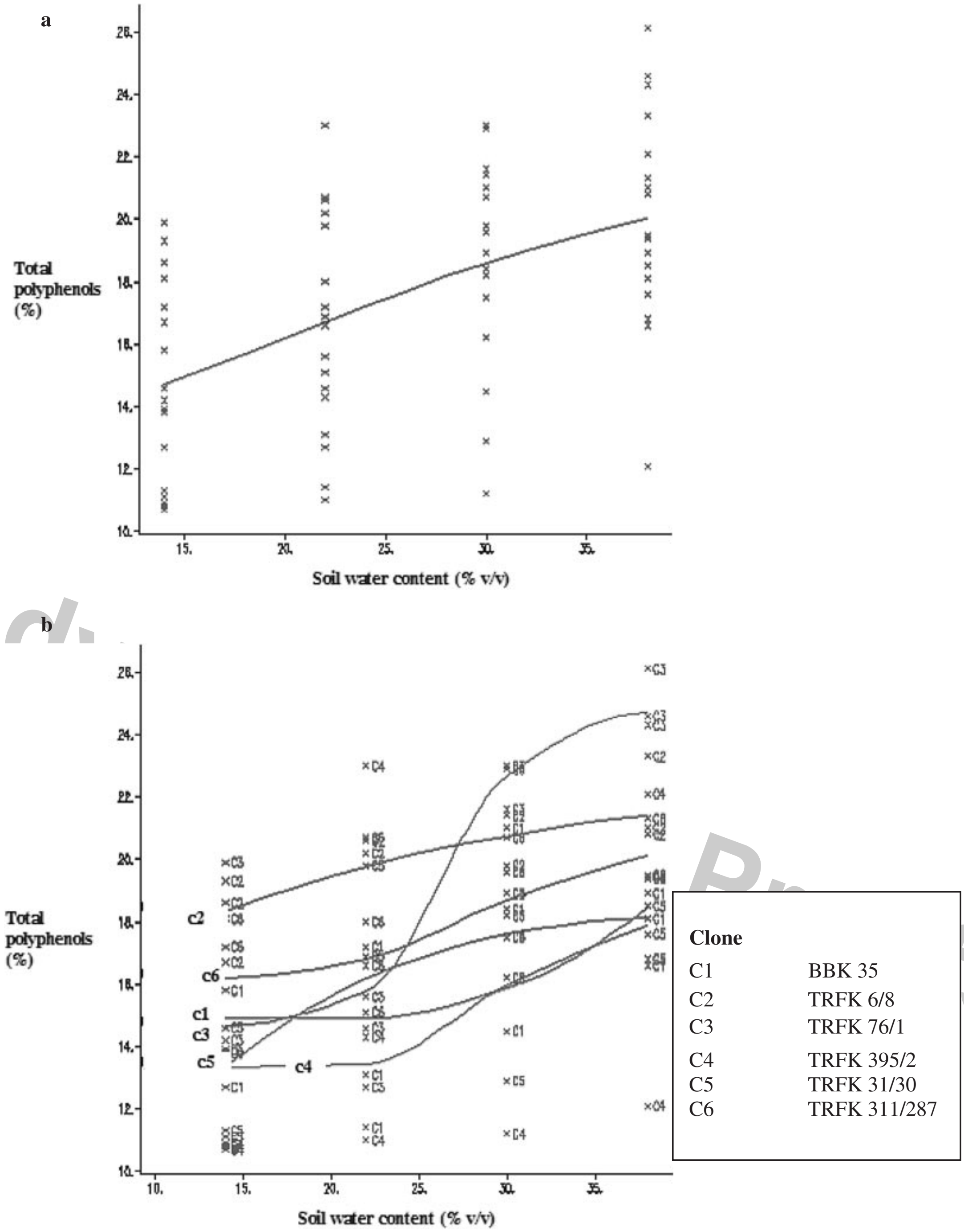

Fig. 2. Influence of Soil Water Content on Total Polyphenols.
a, in tea shoots; b, in shoots of tea clones.

ance to water stress as compared to clones TRFK 76/1, BBK 35, and TRFK 311/287 with ≥ 0.2 and TRFK 395/2 with ≥ 0.1 (Fig. 1a–f).

Effect of SWC on polyphenols in tea shoots

The total polyphenol content in shoots of the six tea clones varied, but it ranged from 12 to 25% on a dry-weight basis. Results in this study indicate that total polyphenol content was influenced by soil water content. Declining SWC progressively reduced shoot polyphenol

Table 1. Total Polyphenol Estimates of Fluctuation Ranges (%) Obtained from Regression Analysis ($P < 0.001$)

Clone	Fluctuation range (%)	Lowest limit attained (%)
BBK 35	4.71	13.62
TRFK 6/8	2.68	18.70
TRFK76/1	11.20	13.73
TRFK 395/2	5.49	12.36
TRFK 31/30	2.74	14.93
TRFK 311/287	4.07	15.88

Table 2. Correlation Analysis for Soil Water Content, Polyphenols, Leaf Expansion, Shoot Growth and Water-Stress Index in Tea

		Soil water content	Polyphenols	Leaf expansion	Shoot extension	Water Stress Index
Soil water content		1.000				
Polyphenols		0.63095 (0.0009)*	1.000			
Leaf expansion		0.28299 (0.1803)	0.02001 (0.9260)	1.000		
Shoot extension		0.76 107 (<0.0001)	0.73503 (<0.0001)	0.03452 (0.8728)	1.000	
Water stress index	at 5th week	0.91105 (<0.0001)	0.72431 (<0.0001)	0.04100 (0.8491)	0.83235 (<0.0001)	1.000
	at 6th week (of stress)	0.82180 (<0.0001)	0.66543 (<0.0004)	0.01710 (0.9368)	0.82192 (<0.0001)	

*P values in bracket

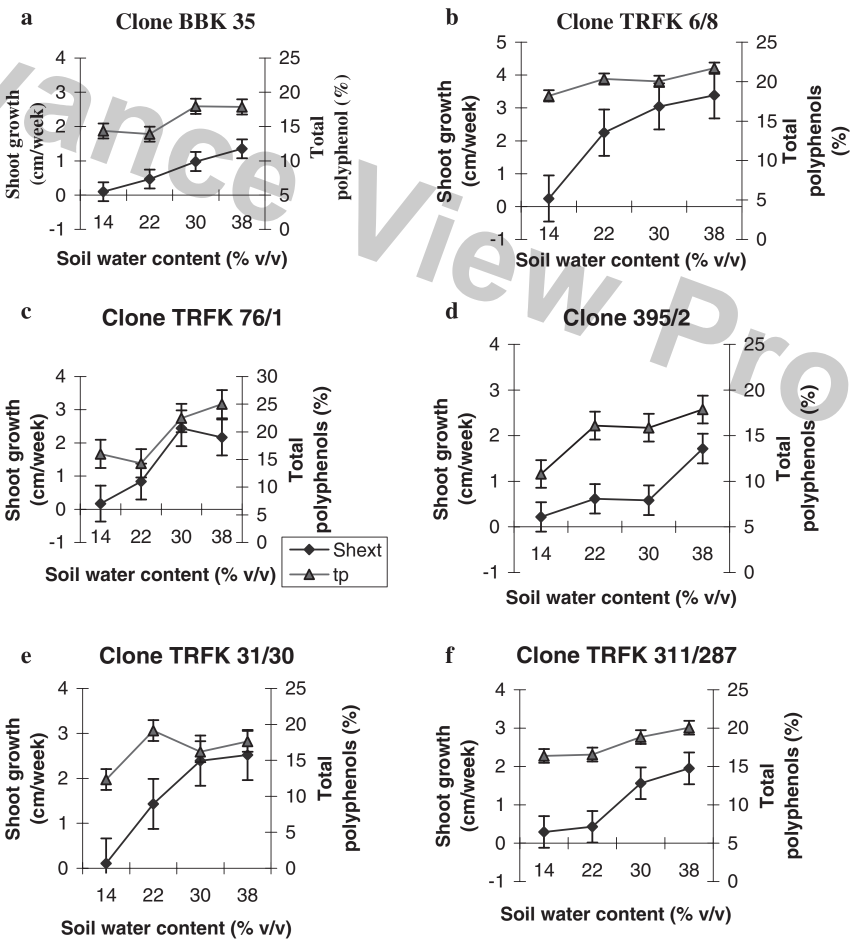

Fig. 3. Response of Shoot Growth and Shoot Polyphenols to Soil Water Content.

content and the degree of that response varied with the clone (Fig. 2a and b). The total polyphenol content in clones TRFK 6/8 and TRFK 31/30 showed almost linear responses to SWC changes, and were relatively higher at low SWC as compared to other clones (Fig. 2b). Regression analysis also indicated the range in which shoot polyphenols fluctuated in each clone when subjected to varying SWC. Clones TRFK 6/8 and

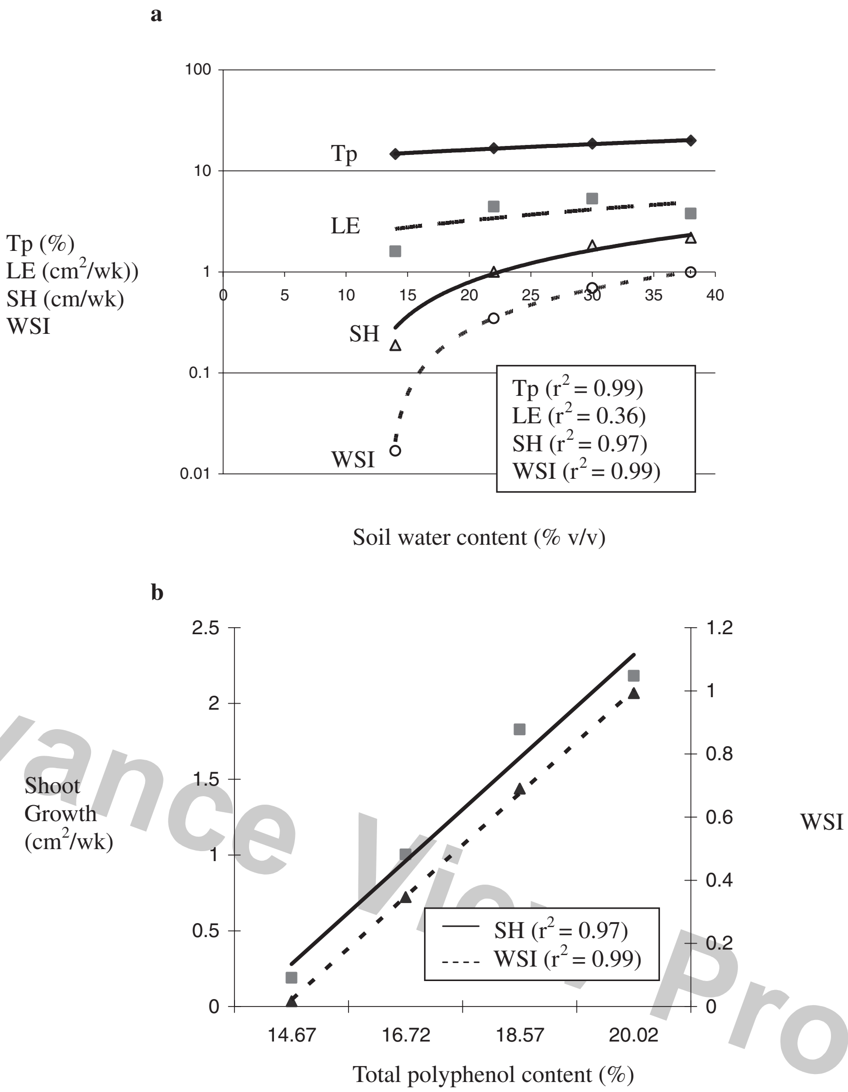

Fig. 4. Soil Water Content and Total Polyphenol Content.
a, Influence of soil water content on total polyphenol (Tp), leaf expansion (LE), shoot growth (SH), and WSI in tea; b, linear relation between shoot polyphenol, shoot growth, and WSI in tea.

TRFK 31/30 had 2.68 and 2.74% ranges respectively, which were low compared to clone TRFK 76/1, with a high range of 11.20% (Table 1). This response was similar to the calculated water stress tolerance index (Fig. 1), where clones TRFK 6/8, TRFK 31/30, and TRFK 311/287 had higher indexes. Total polyphenols positively correlated with shoot growth ($P < 0.001$) and the calculated WSI, while there was no significant correlation with leaf expansion (Table 2). There was also a linear relationship between shoot growth, WSI, and polyphenols content (Figs. 3 and 4).

Discussion

The results of this study indicate that declining soil water content limits growth of tea, and that there is an association between shoot growth and total polypnenol content. The results also show that drought-tolerant clones have high phenolic content with restricted fluctuation to changes in SWC.

Water stress lowered total polyphenol content in tea shoots (Figs. 2, 3, and 4), and there were significant correlations between SWC and phenolic content and WSI and shoot growth in tea (Table 2). This was expected, because water is one of the raw materials in photosynthesis, and it directly impacts on organic synthesis for both growth and secondary metabolites. Although a significant correlation between polyphenol content and shoot growth was observed, there was none with leaf expansion. Leaf is the source of photo assimilates, which yield the precursors of secondary metabolites such as malonyl-CoA and *p*-coumaroyl-CoA[3,22,23] which are dependent on light and SWC.[17] In resource-limited environments, the theory of functional balance explain why shoot growth and not leaf growth correlates with phenolic content.[24] An organ that has close proximity to a limiting resource is less affected by, unavailability of that resource than a far-off sink within the plant.

Of significance was the potential amount and extent of variation of total polyphenol content in each clone, which might provide a basis for clonal selection, improvement and/or management of tea for better yield and quality. Clones TRFK 6/8, TRFK 31/30, and TRFK 311/287 had higher polyphenol content than the other clones in the study. Similarly, the same clones had higher WSI, indicating that they were more tolerant to water stress. Given the close correlation of shoot growth (as influenced by SWC) and WSI to total polyphenols, these results suggest an association of plant polyphenols with water stress in tea. This observation agrees with results of Yaginum *et al.*[25] who noted increased polyphenols in light and water-stress resistant safflower and cucumber seedlings as compared to those which responded weakly to the stresses. Further investigation of flavonoid contents in safflower revealed that a strong antioxidant was responsive to both light and water stress while a weak antioxidant remained unchanged.[26] This provides pointers, that although total tea polyphenol content may be related to water-stress tolerance in tea, flavonoid derivatives, particularly the rich tea catechins might be more useful and should be the subject of further investigation. Besides absolute amounts of polyphenols, results of this study show varied fluctuation of polyphenol content with changes in SWC, and suggest that clones with more stable polyphenols are more tolerant to water stress (Table 1).

This implies that tea cultivars which have less fluctuation in phenolic content are less affected by changes in SWC and may reflect tolerance to drought. There is a potential that some polyphenol derivatives will prove useful indicators of drought tolerance in tea and will hasten the development of better-adapted cultivars to water-stress environments.

Acknowledgment

The authors wish to thank Egerton University and the Tea Research Foundation of Kenya for funding and laboratory facilities for this work. This was part of post-graduate research work submitted to the Graduate School at Egerton University by the first author.

References

1) Han, D.-W., Suh, H., Park, Y. H., Cho, B. K., Hyon, S.-H., and Park, J.-C., Preservation of human saphenous vein against reactive oxygen species-induced oxidative stress by green tea polyphenol pretreatment. *Artif. Organs*, **27**, 1137–1142 (2003).
2) Mandel, S., Weinreb, O., Amit, T., and Youdim, M. B. H., Cell signaling pathway in the neuroprotective actions of the green tea polyphenol (−)-epigallocatechin-3-gallate: implications for neurodegenerative diseases. *J. Neurochem.*, **88**, 1555–1569 (2004).
3) Schijlen, E. G. W. M., Vos, C. H. R., Tunen, A. J., and Bovy, A. G., Modification of flavonoid biosynthesis in crop plants: a review. *Phytochemistry*, **65**, 2631–2648 (2004).
4) Yun, J.-H., Pang, E.-K., Kim, C.-S., Yoo, Y.-J., Cho, K.-S., Chai, J.-K., Kim, C.-K., and Choi, S.-H., Inhibitiory effects of green tea polyphenol (−)-epigallocatechin gallate on the expression of matrix metalloproteinase-9 and on the formation of osteoclasts. *J. Period. Res.*, **39**, 300–307 (2004).
5) Jung, Y. D., and Lee, M. E., Inhibition of tumour invasion and angiogenesis by epigallocatechin gallate (EGCG), a major component of green tea. *Int. J. Exp. Pathol.*, **82**, 309–316 (2001).
6) Sato, D., and Matsushima, M., Preventive effects of urinary bladder tumors induced by *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine in rat by green tea leaves. *Int. J. Urol.*, **10**, 160–166 (2003).
7) Mahaboob, S. K., Protective effect of black tea extract on the level of lipid peroxidation and antioxidant enzymes in liver of mice with pesticide-induced liver injury. *Cell Biochem. Funct.*, **24**, 327–332 (2006).
8) Chen, J. J., Ye, Z. Q., and Koo, M. W. L., Growth inhibition and cell cycle arrest effects of epigallocatechin gallate in the NBT-II bladder tumour cell line. *BJU Int.*, **93**, 1082–1086 (2004).
9) Stephens, W., and Carr, M. K. V., A water stress index for tea (*Camellia sinensis*). *Exp. Agric.*, **25**, 545–558 (1989).
10) Wijeratne, M. A., Vulnerability of Sri Lanka tea production to global climate change. *Wat. Air, Soil pollut.*, **92**, 87–94 (1996).
11) Ng'etich, W. K., Effects of different applied nitrogen rates on yield and plant survival during periods of water stress. *Tea*, **20**, 61–65 (2000).
12) Sabry, S. R., Smith, L. T., and Smith, G. M., Osmoregulation in spring wheat under drought and salinity stress. *J. Genet. Breed.*, **49**, 55–60 (1995).
13) Hare, P. D., Cress, W. A., and Van Staden, J., Dissecting the roles of osmolyte accumulation during stress. *Plant Cell Environ.*, **21**, 535–553 (1998).
14) Sarker, C. B., Hara, M., and Uemura, M., Comparison of response of two C3 species to leaf water relation, proline synthesis, gas exchange and water use under periodic water stress. *J. Plant Biol.*, **47**, 33–41 (2004).
15) Slama, I., Messedi, D., Ghnaya, T., Savoure, A., and Abdelly, C., Effects of water deficit on growth and proline metabolism in *Sesuvium portulacastrum. Environ. Exp. Bot.*, **56**, 231–238 (2006).
16) Singh, H. P., Ravindranath, S. D., and Singh, C., Analysis of tea shoot catechins: spectrophotometric quantitation and selective visualization on two-dimensional paper chromatograms using diazotized sulphanilamide. *J. Agric. Food Chem.*, **47**, 1041–1045 (1999).
17) Stewart, A. J., Chapman, W., Jenkins, G. I., Graham, I., Martin, T., and Crozier, A., The effect of nitrogen and phosphorus deficiency on flavonol accumulation in plant tissues. *Plant Cell Environ.*, **24**, 1189–1197 (2001).
18) Anon., Tea Growers Handbook, Tea Research Foundation of Kenya, Nairobi (1986).
19) Ng'etich, W. K., and Wachira, F. N., Use of a non-destructive method of leaf area estimation in triploid and

diploid tea plants (*Camellia sinensis*). *Tea*, **13**, 11–17 (1992).
20) Younis, M. E., El-Shahaby, O. A., Abo-Hamed, S. A., and Ibrahim, A. H., Effects of water stress on growth, pigments and $^{14}CO_2$ assimilation in three sorghum cultivars. *J. Agr. Crop Sci.*, **185**, 73–82 (2000).
21) ISO TC 34/SC 8/WG. Tea: methods for determination of substances characteristic of green and black tea. 1. Determination of total polyphenols in tea: colorimetric method using Folin-Ciocalteu reagent. ISO, Geneva (2003).
22) Magoma, G. N., Wachira, F. N., Obanda, M., Imbuga, M., and Agong, S. G., The use of catechins as biochemical markers in diversity studies of tea (*Camellia sinensis*). *Genet. Res. Crop Evol.*, **47**, 107–114 (2000).
23) Wheeler, D. S., and Wheeler, W. J., The medicinal chemistry of tea. *Drug Develop. Res.*, **61**, 45–65 (2004).
24) Thornley, J. H. M., A balanced quantitative model for root:shoot ratios in vegetative plants. *Ann. Bot.*, **68**, 211–264 (1972).
25) Yaginuma, S., Shiraishi, T., Ohya, H., and Igarashi, K., Polyphenol increases in safflower and cucumber seedlings exposed to strong visible light with limited water. *Biosci. Biotechnol. Biochem.*, **66**, 65–72 (2002).
26) Yaginuma, S., Shiraishi, T., and Igarashi, K., Developmental transition of the flavonoid contents in safflower leaves during stress-loaded cultivation. *Biosci. Biotechnol. Biochem.*, **67**, 1691–1698 (2003).