

## Physiological and biochemical response of tea [*Camellia sinensis* (L.) O. Kuntze] to water-deficit stress

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### SUMMARY

A study to determine the physiological and biochemical responses of eight tea [*Camellia sinensis* (L.) O. Kuntze] cultivars to water-deficit stress was conducted in a 'rain-out shelter' using potted plants. Three levels of soil moisture content [34, 26, or 18% (v/v) water] were applied to three plants of each cultivar in a complete randomised design, and the whole experiment was replicated three-times. The treatments were applied for 12 weeks, during which time plant water status, shoot extension rates, changes in gas exchange parameters, and leaf proline and glycinebetaine concentrations were determined. The imposition of severe water-deficit conditions [18% (v/v) soil water content] caused a significant ( $P \leq 0.05$ ) decline in the relative water content of leaves, shoot water potentials, and shoot extension rates from mean values of 84.8% to 50.6%,  $-0.80$  to  $-1.15$  MPa, and  $1.87$  to  $0.29$  mm d<sup>-1</sup>, respectively, compared to plants grown in a well-watered soil [34% (v/v) soil water content]. The three gas exchange parameters measured (stomatal conductance, evapotranspiration rate, and rate of net photosynthesis) also declined significantly ( $P \leq 0.05$ ) with decreasing soil moisture content. In contrast, water-deficit stress increased the accumulation of leaf proline and glycinebetaine from mean values of  $0.104$  to  $0.244$   $\mu\text{mol g}^{-1}$  FW, and from  $1.567$  to  $2.025$   $\mu\text{mol g}^{-1}$  DW, respectively. The eight tea cultivars differed significantly ( $P \leq 0.05$ ) in their responses to water-deficit stress. Proline accumulation was significantly ( $P \leq 0.05$ ) higher in the drought-tolerant cultivars, 'TRFK 306', 'TRFCA SFS150', and 'EPK TN14/3', suggesting that proline concentration could be used as a marker for drought-tolerance in tea.

Plants are often exposed to many abiotic and biotic stress factors. Abiotic stresses are the principal causes of crop failure and below average yields for most crops (Bray *et al.*, 2000). Abiotic stress factors include low temperatures, high salinity, or drought.

Drought, which leads to water-deficit stress, causes cellular dehydration which leads to osmotic stress and the removal of water from the cytoplasm into the extracellular spaces. To counteract these effects, plants have evolved a range of physiological and biochemical responses, including lowering the rates of cellular growth and net photosynthesis, stomatal closure, and the accumulation of organic solutes such as sugar alcohols, or osmolytes such as proline and/or quaternary ammonium compounds (Yamada *et al.*, 2005). Proline is largely responsible for changes in the osmotic potential of plant cells during drought stress. Proline also buffers cells against the effects of water-deficit stress (Yamada *et al.*, 2005). Some plant species are thought to accumulate proline under conditions of water stress in order to maintain growth under conditions of low soil water potential. Plants synthesise proline from glutamine using the enzyme, pyrroline-5-carboxylate synthase, in their

leaves (Mahajan and Tujeta, 2005). Studies on tobacco have shown that over-expression of the gene for pyrroline-5-carboxylate synthase leads to an increase in proline concentration and improved growth under drought conditions (Parvaiz and Satyawati, 2008).

Glycinebetaine (GB) is a quaternary ammonium compound that also accumulates in some plant species in response to drought stress (Ashraf and Iram, 2005). Studies have shown that the accumulation of GB in plants depends on genotype, the stage of growth, and the level of drought stress (Ashraf and Foolad, 2007). The level of accumulation of GB also varied considerably among plant species. Some, such as *Oryza sativa* and *Brassica* spp. did not accumulate the compound even when subjected to drought stress. Chen and Murata (2011) demonstrated that plants which accumulated GB normally contained lower endogenous levels of the compound and only accumulated GB when subjected to stress.

Tea [*Camellia sinensis* (L.) O. Kuntze], is a woody perennial crop that provides one of the most important beverages in the World. However, tea farming is influenced by environmental conditions and tea plants are particularly sensitive to water-deficit stress. In future, climate change may result in less predictable growing

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conditions in areas where the crop is presently cultivated. The longevity and severity of periods of drought, and the incidence of frost, have increased in most tea growing areas which has contributed to reduced levels of tea production (Wijeratne and Fordham, 1996) and an increased incidence of pests (Kamunya *et al.*, 2011; Sudo *et al.*, 2011). Drought can cause up to 33% loss in a tea crop, in addition to increasing plant mortality to approx. 19% (Cheruiyot *et al.*, 2010). To mitigate against this risk, numerous studies have been carried out to devise technologies that would ensure sustained tea production, even during periods of drought stress. The majority of these studies have attempted to identify suitable selection markers for breeding for tolerance to water stress in tea (Cheruiyot *et al.*, 2007; 2008b). However, these efforts have been constrained by a lack of adequate understanding of the physiological, biochemical, and genetic responses of tea plants to water-deficit stress, which would be useful to guide the development of drought-tolerant or drought-adapted cultivars.

This study was undertaken to determine the responses of tea plants to drought at both the physiological and biochemical levels.

## MATERIALS AND METHODS

### *Plant material and the 'rain-out shelter'*

Eight tea [*C. sinensis* (L.) O. Kuntze] cultivars, representing each of the three main types of tea (Cambod, China, and Assam), were selected for this study based on their tolerance or susceptibility to water-deficit stress (Table I).

The 18-month-old tea plants were generated through vegetative propagation in the nursery and were transplanted into 1,000-gauge black polythene tubes, 0.3 m (in diameter) × 0.3 m (deep). The soil used for planting was a 3:1 (v/v) mixture of sub-soil and top-soil and had the following physical and chemical properties: clay-loam in texture; 3.5% (w/w) N; 4 mg kg<sup>-1</sup> P; 249 mg kg<sup>-1</sup> K; 147 mg kg<sup>-1</sup> Ca; 43 mg kg<sup>-1</sup> Mg; 34 mg kg<sup>-1</sup> Mn; with a pH of 4.0. The tea seedlings (n = 3 per genotype) were allowed to establish for 4 weeks before transferring them to a 'rain-out shelter' constructed at the Tea Research Foundation of Kenya (0° 22' S, 35° 21' E; 2,180 m asl), as described by Cheruiyot *et al.* (2007). The eight cultivars were arranged in the shelter, according to treatment [34%, 26%, or 18% (v/v) soil moisture content (SMC)] in a completely randomised design, replicated three-times, giving a total of 72 experimental plots with each plot containing three plants.

All the plants were watered uniformly to field capacity [34% (v/v) SMC] before applying the experimental

water-deficit treatments [26% (v/v) or 18% (v/v) SMC] over a period of 12 weeks. The choice of SMC treatments was informed by data from previous studies using a similar soil type (Cheruiyot *et al.*, 2007; 2008a, b). Seedlings (n = 3 per genotype) in the control treatment were maintained at 34 ± 2% (v/v) SMC throughout the experimental period. Soil moisture contents were determined at 10.00 h and 15.00 h each day using a time-domain reflectometer (TDR)-soil moisture meter (TRIME-FM-2-Eijkelkamp; Agrisearch Equipment, The Hague, The Netherlands) according to the manufacturer's instructions. The desired SMC values were maintained within ± 2% (v/v) of the treatment level by daily watering. During the 12 week period of the experiment, various physiological and biochemical responses of the tea plants were determined, as described below.

### *Determination of physiological parameters*

*Relative leaf water content, shoot water potential, and shoot extension rate:* The relative water content (RWC) of leaves and the shoot extension rate (SER) were determined as described by Cheruiyot *et al.* (2007). Shoot water potentials (SWP) were measured using a portable plant water console (Model 3005; Moisture Equipment Corp., Santa Barbara, CA, USA) following the manufacturer's instructions.

*Gas exchange parameters:* Stomatal conductance ( $g_s$ ), evapotranspiration ( $E_e$ ), and the rate of net photosynthesis ( $P_n$ ) were determined on the third fully-expanded leaf using a portable photosynthesis system (TPS-2; PP Systems Inc., Amesbury, MA, USA). Measurements were taken between 11.00 – 14.00 h, when the intensity of natural light was at its maximum.

### *Biochemical analysis*

*Leaf proline concentrations:* Leaf proline concentrations were determined in triplicate following the method of Bates *et al.* (1973). Proline concentrations were calculated from a standard curve generated using a proline standard reagent (Sigma-Aldrich, St Louis, MO, USA) and calculated using the formula:

$$\text{Proline } (\mu\text{mol g}^{-1} \text{FW}) = \text{Proline } (\mu\text{g ml}^{-1} \text{FW}) \times \text{Volume of toluene (ml)} / 115.5 / \text{Fresh weight of leaf (g)}$$

*Leaf glycinebetaine concentrations:* The concentrations of leaf GB were determined in triplicate following the method of Grieve and Grattan (1983). The absorbance was measured at 365 nm using a UV-visible spectrophotometer (Series 6505; Jenway, London, UK).

TABLE I

*Nomenclature of the eight tea cultivars, varietal types, origins and their special attributes as determined by breeders using morphological and biochemical descriptors*

Cultivar name	Varietal type (Source)	Special attributes
'AHP S15/10'	Assam (Kenya)	High yielding, highly pubescent, susceptible to drought
'TRFCA SFS150'	Assam (Malawi)	Drought, cold and pest tolerant
'EPK C12'	China (Kenya)	Drought tolerant and susceptible to mites
'TRFK 306'	Assam (Kenya)	Anti-oxidant rich and drought tolerant
'TRFK 301/4'	Cambod (Kenya)	High yielding and moderately drought tolerant
'TRFK 301/5'	Cambod (Kenya)	High yielding but susceptible to drought.
'TRFK 303/216'	Assam (Kenya)	Susceptible to drought
'EPK TN14/3'	China (Kenya)	Drought tolerant and susceptible to mites

Leaf concentrations of GB were calculated using a standard curve and the following equation:

$$GB (\mu\text{mol g}^{-1} \text{DW}) = (A_{365} / \text{Slope} \times \text{Volume of extract}) / [\text{Volume of aliquot} \times 1/\text{DW of leaf (g)}]$$

#### Statistical analysis

Data were analysed using GENSTAT software Version 10.3 (VSN International Ltd., London, UK). Data were subjected to a factorial analysis of variance (ANOVA) to test for the effects of cultivar, SMC treatment, and their interaction on the various physiological and biochemical parameters measured. Duncan Multiple Range Test (DMRT) was used to compare means at a significance of  $P \leq 0.05$ . Correlation analysis was used to determine phenotypic relationships between the physiological and biochemical parameters. Data from all three SMC treatments were used in the correlation analysis.

## RESULTS AND DISCUSSION

### Plant water status

All eight tea cultivars used in this study responded to a reduced SMC by lowering their RWC as shown in Table II. Mean leaf RWC values differed significantly ( $P \leq 0.05$ ) between cultivars. Under control conditions [34% (v/v) SMC], the average leaf RWC for all eight tea cultivars was 84.8%, while under severe water-deficit stress conditions [18% (v/v) SMC], this declined to 50.6%. The decrease in leaf RWC can be attributed to the lower soil water content. The interaction between tea cultivar and SMC was significant ( $P \leq 0.05$ ). At 34% (v/v) SMC, leaf RWC values were not significantly ( $P > 0.05$ ) different among the eight tea cultivars. At 18% (v/v) SMC, the drought-susceptible cultivar 'TRFK 301/5' had the lowest leaf RWC value.

A decrease in SMC from 34% to 26%, or to 18% (v/v) caused significant ( $P \leq 0.05$ ) declines in SWP in all the eight tea cultivars (Table II). The three SMC treatments also differed significantly ( $P \leq 0.05$ ) in inducing plant responses to water-deficit stress. The eight tea cultivars differed significantly ( $P \leq 0.05$ ) even at the same SMC. Cultivar 'EPK C12' had the lowest SWP at both 34% and 18% SMC. The interaction between cultivar and SMC was also significant ( $P \leq 0.05$ ).

To withstand drying of the soil during drought, plants respond by lowering their water potential to values less than that of the soil. Drought-tolerant tea plants tend to maintain a high SWP in order to maintain a moderate rate of photosynthesis together with other metabolic processes and an equally high water-use efficiency. This was exhibited by the drought-tolerant tea cultivar, 'EPK TN14/3', which maintained the highest SWP under water-deficit stress conditions. Research has also shown that drought-tolerant plants maintain a high internal water status at low SMC values as a survival mechanism. As a response to water-deficit stress, most plants regulate their stomatal function in order to improve their water-use efficiency (Yordanov *et al.*, 2003).

### Shoot extension rate

Shoot extension rates (SER; in  $\text{mm d}^{-1}$ ) were adversely affected by lowering the SMC (i.e., increasing water-deficit stress). All eight tea cultivars exhibited lower SER values under 18% (v/v) SMC (Table II). SER values differed significantly ( $P \leq 0.05$ ) among cultivars, even under the same SMC conditions. At the highest SMC, 'TRFK 306' exhibited the highest SER value, while 'TRFK 303/216' had the lowest SER value. The drought-tolerant cultivar, 'EPK C12' had the highest SER value under water-stress conditions. The interaction between cultivar and SMC was significant ( $P \leq 0.05$ ). Significant differences in SER between cultivars suggested that the effects of water-deficit stress were cultivar-dependent. The overall reduction in SER under 18% (v/v) SMC conditions may be attributed to a reduction in cyclin-dependent kinase activity, which resulted in slower cell division (Mahajan and Tujeta, 2005). Growth is considered to be the most drought-sensitive physiological process in plants due to the reduction in turgor pressure. Shoot growth results from the production of daughter cells by meristematic cell division and subsequent expansion of the young cells. However, under conditions of severe drought, cell elongation is inhibited by the interruption of water flow from the xylem to the elongating cells. Drought thus impairs mitosis, cell elongation, and cell expansion, resulting in a decline in plant growth (Shakeel *et al.* 2011).

### Gas exchange parameters

The three gas exchange parameters; stomatal

TABLE II  
Effects of different soil moisture contents [34%, 26%, or 18% (v/v)] on leaf relative water contents (RWC), shoot water potentials (SWP) and shoot extension rates (SER) in eight different tea cultivars

Cultivar	RWC (%)			SWP (MPa)			SER ( $\text{mm d}^{-1}$ )		
	34%	26%	18%	34%	26%	18%	34%	26%	18%
'TRFK 306'	83.9a <sup>†</sup>	74.5ab	44.9cd	-0.69 b	-0.65a	-1.15b	3.308a	0.824cd	0.158c
'TRFK 301/4'	88.1a	74.7ab	51.4bc	-0.91cd	-0.91c	-1.16b	2.154bc	0.671de	0.343bc
'TRFCA SFS150'	84.9a	78.5a	56.3ab	-0.88c	-1.16e	-1.19b	1.690bcd	1.254ab	0.203c
'TRFK 301/5'	82.9a	73.7ab	39.2d	-0.75b	-0.77b	-1.13b	1.554bcde	0.988cd	0.504fgh
'EPK C12'	88.3a	55.8bc	51.6bc	-0.50a	-0.92cd	-0.82a	2.362b	1.156bc	0.588c
'AHP S15/10'	78.8a	73.3ab	62.1a	-0.94cd	-1.03cde	-1.21b	1.370cdef	1.380a	0.161c
'EPK TN14/3'	85.5a	70.2ab	45.8cd	-0.76b	-1.03cde	-1.44c	1.353cdef	1.258ab	0.210c
'TRFK 303/216'	86.5a	68.0b	53.6abc	-1.00e	-1.05de	-1.08b	1.139defg	0.574e	0.166c
Mean of means	84.9	71.1	50.6	-0.80	-0.94	-1.15	1.866	1.013	0.291

<sup>†</sup>Mean values (n = 9) followed by the same lower-case letters in each column are not significantly different at  $P \leq 0.05$  by Duncan's multiple range test.

SMC, soil moisture content.

RWC, SWP, and SER, relative water content, shoot water potential, and shoot extension rate, respectively.

TABLE III

Effects of different soil moisture contents [34%, 26% or 18% (v/v)] on gas exchange parameters [rate of net photosynthesis ( $P_n$ ), stomatal conductance ( $g_s$ ) and evapotranspiration rate ( $E_e$ )] in eight tea cultivars

Cultivar	$P_n$ ( $\mu\text{mol O}_2 \text{ m}^{-2} \text{ s}^{-1}$ )			$g_s$ ( $\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )			$E_e$ ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ )		
	34%	26%	18%	34%	26%	18%	34%	26%	18%
'TRFK 306'	21.10 ab <sup>†</sup>	12.11bc	12.47a	260 cd	243b	235c	2.07bc	1.94c	1.97cd
'TRFK 301/4'	17.72c	12.04bc	11.95a	245d	263b	253abc	1.86c	1.73c	1.80de
'TRFCA SFS150'	27.10ab	19.60ab	5.42b	285bcd	280ab	250abc	2.13b	1.87c	2.08bc
'TRFK 301/5'	29.45a	21.79a	5.50b	315abcd	243b	280ab	1.84c	1.94c	2.27ab
'EPK C12'	20.03bcd	15.85abc	5.28b	370a	295ab	290a	2.71a	2.57ab	2.53a
'AHP S15/10'	21.88bc	21.25a	14.95a	320abc	276ab	245bc	2.65a	2.78a	2.02ec
'EPK TN14/3'	17.40c	11.15bc	5.28b	277cdef	257b	240bc	2.15b	1.8ghi	1.72e
'TRFK 303/216'	20.20c	11.00c	6.33b	340ab	327a	260abc	2.54a	2.46b	2.01cd
Mean of means	21.86	15.59	7.74	301.5	273	256.6	2.24	2.14	2.05

$P_n$ ,  $g_s$ , and  $E_e$  are the rate of net photosynthesis, stomatal conductance, and rate of evapotranspiration, respectively.

<sup>†</sup>Mean values ( $n = 9$ ) followed by the same lower-case letters in each column are not significantly different at  $P \leq 0.05$  by Duncan's multiple range test.

SMC, soil moisture content.

conductance ( $g_s$ ), evapotranspiration ( $E_e$ ) and rate of net photosynthesis ( $P_n$ ) were also affected by water-deficit stress. Stomatal conductance, which is a measure of the maximum rate of passage of water vapour or  $\text{CO}_2$  dioxide from the leaf through the stomata to the atmosphere, declined with a decrease in SMC (Table III). Stomatal conductance differed significantly ( $P \leq 0.05$ ) both between cultivars and the three SMC treatments. However, the interaction between tea cultivar and SMC showed no significant difference. The drought-tolerant cultivar, 'EPK C12', had the highest  $g_s$  values under 34% (v/v) and 18% (v/v) SMC, respectively. In contrast, cultivar 'TRFK 306' had the lowest  $g_s$  value under 18% (v/v) SMC.

Stomatal closure has been identified as the first response to water-deficit stress in plants (Mansfield and Atkinson, 1990). This is because stomatal guard cells receive the first chemical signal, abscisic acid (ABA), synthesised in the roots when plants detect a reduced SMC. In this study, the significant decline in  $g_s$  with a decrease in SMC in all eight tea cultivars may be attributed to anatomical changes in stomatal density, a strategy employed by plants to enhance their ability to minimise water loss (Waheed *et al.*, 2012). Stomatal closure can also result from direct evaporation of water from the guard cells through a metabolically-active process involving reversal of the ion fluxes responsible for stomatal opening. This process is regulated by ABA which mediates the root-shoot signalling process. Studies have shown that the transport of ABA into the root xylem is regulated by environmental conditions such as xylem pH. When plants experience water-deficit stress, the pH of the xylem sap is raised. This leads to an accumulation and loading of ABA into the root xylem and its transport to the shoots where it causes stomatal closure, thus reducing stomatal conductance (Wilkinson and Davies, 2002).

All eight tea cultivars recorded significant declines in the rate of evapotranspiration ( $E_e$ ) with a decrease in SMC (Table III). This response has been reported in several plant species including tea (Damayanthi *et al.*, 2010), *Amaranthus* spp. (Liu and Stutzel, 2002), and *Vigna subterranea* (Jorgensen *et al.*, 2011).  $E_e$  values differed significantly ( $P \leq 0.05$ ) between the eight tea cultivars and SMC. The drought-tolerant cultivar, 'EPK TN14-3', had the lowest  $E_e$  value under 18% (v/v) SMC. However, the interaction between cultivar and SMC was not significant. The results obtained in this study showed a significant positive correlation (Table IV) between  $E_e$  values and leaf RWC, suggesting that SMC affected the rate of evapotranspiration. The decline in  $g_s$  also caused a reduction in  $E_e$ . A reduction in  $E_e$  allows a plant to conserve water.

The mean rate of net photosynthesis ( $P_n$ ) also declined with decreasing SMC (Table III).  $P_n$  differed significantly ( $P \leq 0.05$ ) between treatments, with the highest mean  $P_n$  value recorded in all eight tea cultivars subjected to 34% (v/v) SMC. There were also significant differences ( $P \leq 0.05$ ) between cultivars. The interaction between SMC and tea cultivar also differed significantly ( $P \leq 0.05$ ). The sensitivity of  $P_n$  to a decrease in SMC was confirmed by a highly significant positive correlation ( $r_p = 0.801^{***}$ ) between leaf RWC and  $P_n$  (Table IV). The decline in  $P_n$  can therefore be attributed to changes in cellular water status, resulting in a reduction in stomatal conductance, which restricted the flow of  $\text{CO}_2$  from mesophyll cells to the chloroplast stroma and hence reduced photosynthesis (Damayanthi *et al.*, 2010). The variation in  $P_n$  values among tea cultivars may be attributed to genotypic variation. Studies have shown that  $P_n$  values in higher plants are influenced by stomatal limitations in addition to metabolic impairment. Water-deficit stress leads to a rapid decline in the abundance of transcripts

TABLE IV

Coefficients of phenotypic correlation ( $r_p$ ) between various physiological and biochemical parameters in eight tea cultivars

Parameter <sup>‡</sup>	SWP	SER	$g_s$	$E_e$	$P_n$	Proline	GB
RWC	0.680***	0.724***	0.431*	0.393*	0.801***	-0.525**	-0.501*
SWP		0.647***	0.420*	0.338ns	0.582**	-0.350*	-0.603**
SER			0.308ns	0.352*	0.703***	-0.437*	-0.516**
$g_s$				0.714***	0.462*	-0.599**	-0.446*
$E_e$					0.488*	-0.487*	-0.310ns
$P_n$						-0.434*	-0.586**
Proline concentration							0.218ns

<sup>‡</sup>RWC is relative water content, SWP is shoot water potential, SER is shoot extension rate,  $g_s$  is stomatal conductance,  $E_e$  is evapotranspiration rate,  $P_n$  is net photosynthetic rate, and GB is leaf glycinebetaine concentration. Asterisks \*, \*\*, and \*\*\* denote statistical significance at  $P \leq 0.05$ ,  $P \leq 0.01$  and  $P \leq 0.001$ , respectively. ns, not significant.



(mRNA) for the small subunit of ribulose-1,5-bisphosphate carboxylase (Rubisco), resulting in reduced photosynthetic enzyme activity and hence a decline in the rate of photosynthesis (Bota *et al.*, 2004).

#### Leaf proline and glycinebetaine concentrations

Leaf proline concentrations differed significantly ( $P \leq 0.05$ ) with changes in SMC (Table V). All eight tea cultivars accumulated more proline at 18% (v/v) SMC, while proline concentrations differed significantly ( $P \leq 0.05$ ) among tea cultivars. Variations in proline accumulation between genotypes can be attributed to variations in their response to water-deficit stress. Research has shown that drought-tolerant plants tend to accumulate more proline compared to drought-susceptible plants (Ashraf and Foolad, 2007). The drought-tolerant tea cultivar, 'TRFK 306', had significantly ( $P \leq 0.05$ ) higher proline concentrations under all SMC treatments. The other drought-tolerant tea cultivars ('TRFCA SFS150' and 'EPK TN14/3') accumulated higher levels of proline under 18% (v/v) SMC. The drought-susceptible cultivar, 'AHP S15/10', accumulated lower concentrations of proline than the tolerant cultivars, 'TRFK 306' and 'TRFCA SFS150', under all three SMC conditions. Cultivar 'TRFK 306' had a unique biochemical profile that enabled it to synthesise and accumulate anthocyanins and anthocyanidins, giving its leaves their predominantly purple colour (Kerio *et al.*, 2012). However, the relationship between proline synthesis and the accumulation of anthocyanins or anthocyanidins in this cultivar has not been studied.

The present study also showed a significant negative correlation ( $r_p = -0.525^*$ ) between proline concentration and leaf RWC, which indicated that water-deficit stress induced the accumulation of proline (Table IV). The correlation between SWP and proline concentration was also negative and significant ( $r_p = -0.350^*$ ). This corroborates earlier reports showing that the accumulation of proline lowered the SWP in order to facilitate additional uptake of water from the environment (Yamada *et al.*, 2005).

An increase in GB concentration was also observed in most tea cultivars subjected to 18% (v/v) SMC, compared to plants under 34% (v/v) SMC (Table V). The concentrations of GB accumulated by all eight tea cultivars were comparable under 34% (v/v) SMC. However, there were significant variations in GB

concentrations among the eight cultivars at 18% (v/v) SMC. The drought-tolerant tea cultivars, 'EPK TN14/3', 'EPK C12', and 'TRFCA SFS150' accumulated the highest concentrations of leaf GB under 18% (v/v) SMC. However, cultivar 'AHP S15/10' accumulated the highest concentration of GB under 18% (v/v) SMC, whereas 'TRFK 301/5' accumulated the lowest GB concentration under these conditions. Both these tea cultivars are extremely susceptible to drought and this observation confirmed the genotypic specificity of GB accumulation. This observation also confirms that the accumulation of GB was not always high in drought-tolerant cultivars, as reported by Ashraf and Foolad, (2007). Though not significant, the positive correlation between leaf GB and proline concentrations may indicate that an increase in GB led to an increase in proline concentration. This may support earlier reports that the synthesis of GB enhanced drought-protection and increased the activities of enzymes, including those associated with sugar and amino acid metabolism, leading to increases in total soluble sugar and free amino acid concentrations (Quan *et al.*, 2004).

#### Phenotypic correlations between physiological and biochemical parameters

Correlation coefficients between the various physiological and biochemical parameters are presented in Table IV. Several pairs of parameters were significantly correlated ( $P \leq 0.05$ ). Leaf RWC values were significantly and positively correlated ( $r_p = 0.724^{***}$ ) with SER. This would be expected, since leaf water status determines the rate of carbon accumulation in actively-growing shoots (Bianco and Avellone, 2014). Leaf RWC values were also significantly and positively correlated ( $r_p = 0.801^{***}$ ) with  $P_n$ . This was because tea is a highly water-dependent plant, and any slight change in water status affected  $P_n$ . As the SMC declined, the RWC of leaves also declined due to stomatal closure, which in turn led to a decline in  $P_n$ .

#### CONCLUSIONS

From the results obtained here, we conclude that water-deficit stress had significant effects on the internal water status, gas exchange parameters, and rates of shoot extension of all eight tea cultivars. Water-deficit stress also increased the accumulation of osmolytes, particularly proline and glycinebetaine. The accumulation of proline

TABLE V  
Effects of different soil moisture contents (34%, 26%, or 18%) on leaf proline and glycinebetaine (GB) concentrations in eight tea cultivars

Cultivar	Proline ( $\mu\text{mol g}^{-1}$ FW)			GB ( $\mu\text{mol g}^{-1}$ DW)		
	34%	26%	18%	34%	26%	18%
'TRFK 306'	0.205a <sup>†</sup>	0.222a	0.534a	1.565 a	1.475c	1.737bc
'TRFCA SFS150'	0.086bc	0.124bc	0.331b	1.707a	1.670bc	2.034abc
'EPK TN14/3'	0.109b	0.154b	0.254c	1.619a	2.285a	2.372ab
'TRFK 301/4'	0.080bc	0.121bc	0.206d	1.496a	1.956b	1.755bc
'AHP S15/10'	0.046c	0.056d	0.201de	1.607a	1.787bc	2.511a
'TRFK 303/216'	0.100b	0.083cd	0.182def	1.619a	1.571c	2.050abc
'TRFK 301/5'	0.125b	0.213a	0.162efg	1.434a	1.611c	1.568c
'EPK C12'	0.079bc	0.082cd	0.084h	1.492a	1.762bc	2.172abc
Mean of means	0.104	0.132	0.244	1.567	1.765	2.025

<sup>†</sup>Mean values ( $n = 9$ ) followed by the same lower-case letters in each column are not significantly different at  $P \leq 0.05$  by Duncan's multiple range test.

SMC, soil moisture content; GB, leaf glycinebetaine concentration.

in leaves was shown to be significantly higher in drought-tolerant tea cultivars and may be used as a marker to select for drought tolerance. However, the potential to use leaf proline concentrations as a marker to predict drought stress tolerance in tea would require screening a larger pool of germplasm that segregated for drought-tolerance. Attempts should also be made to derive a water-stress index for tea, based on data for the

accumulation of total polyphenols, specific catechins, and the two main osmolytes (proline and glycinebetaine) in leaves, all of which respond to variations in SMC.

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