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MORPHOLOGICAL AND PHYSIOLOGICAL INDICATORS OF TOLERANCE TO ATMOSPHERIC STRESS IN TWO SENSITIVE AND TWO TOLERANT TEA CLONES IN SOUTH AFRICA

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SUMMARY

Tea (*Camellia sinensis*) clones (PC113 and SFS204) sensitive to very dry air and clones (PC114 and SFS150) that are tolerant, were studied at two tea estates (Tshivhase and Grenshoek) in the Northern Province of the Republic of South Africa. Among the morphological leaf traits studied, stomatal density, pore diameter and pore depth were not linked consistently to stress tolerance. Cuticle thickness was not a good indicator of stress tolerance because genetic differences between clones were confounded by the clonal response of wax production to stress. In contrast, measured leaf conductance to water vapour transport was larger and leaf water potential was lower in sensitive clones, but only with more severe atmospheric stress (Grenshoek). Also the ratio of the calculated maximum stomatal conductance in old and young leaves was higher in sensitive clones, suggesting that the loss of a larger fraction of the total stem flow by old leaves enhanced the stress experienced by the young leaves. However, this indicator was valid only under the more stressful microclimate of Grenshoek. The results indicate that even promising criteria for stress tolerance should be tested by exposure to stress during selection.

INTRODUCTION

The ultimate objective of a breeding programme for tea is the introduction of new clones superior in terms of product quality and yield. Increasingly, tea is planted

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in less favourable conditions, so susceptibility to stress becomes a selection criterion. Ideally, selection for stress tolerance should be performed at the nursery stage but estimates of stress sensitivity may be unrealistic at this stage of development. Moreover, the optimal environment of the nursery is not highly suitable for subjecting plants to stress. An alternative approach that could enhance selection efficiency is to relate morphological and physiological characteristics of clones to performance in adverse conditions (e.g. Squire, 1985; Nijs *et al.*, 2000). The latter approach is applied in the current investigation.

Tea growth is sensitive to temperature, soil drought and dry air. Shoot extension is optimal between 18 and 30 °C, and is inhibited below daily mean temperatures of 13 °C. Tea plantations require approximately 2000 mm of rainfall or irrigation per year, evenly distributed over the seasons (Carr, 1972; Herd, 1976). The current study was conducted in the Northern Province of South Africa, where hot and dry weather during September and October severely reduce yield. During this critical spring flush period, some clones suffer from dry air and exhibit wilting, desiccation, scorching and leaf fall (Nethononda, 1994). Soil drought can similarly reduce yield (Othieno, 1978; Carr *et al.*, 1987), but, contrary to atmospheric stress, this can be remedied by irrigation.

The objective of this study is to identify physiological and morphological traits that could form a basis for the selection of clones adapted to very dry air. To this end the authors have screened, in clonal material of contrasting sensitivity, a series of traits potentially related to stress tolerance: stomatal conductance, cuticle thickness, and stomatal dimensions. Leaf water potential was used as a stress measure, and the influence of local site conditions on stress sensitivity was investigated by comparing responses to hot and dry air at two tea estates.

MATERIALS AND METHODS

Experiment sites

Measurements were taken in the field from 26 September until 2 October, 1998 at two sites in the Northern Province of South Africa: the Tshivhase Tea Estate (22°56′55.3″S; 30°21′00.3″E, altitude 792–991 m asl), mean annual rainfall 1382 mm per year; and the Grenshoek Tea Estate (23°46′41.7″S; 30°05′07.7″E, 809 m asl), mean annual rainfall 1202 mm per year. Both estates are in the same climatological region. Long term weather data (minimum and maximum temperature T_{min} and T_{max}, and precipitation) derived from Nethononda (1994) are presented in Table 1. Daily maximum vapour pressure deficit (VPD) was calculated from daily T_{max} and the coinciding – typically daily minimum – RH, as $e_s(T_{max})$ (1−RH), with $e_s(T_{max})$ the saturated vapour pressure at T_{max}.

Field managers regularly irrigate the plantations to reduce soil drought. Prior to and during the study period, the Grenshoek Estate was irrigated while Tshivhase was not; owing to higher precipitation at the latter site (the two estates used similar irrigation schedules). At both sites, sensitive and tolerant clones were grown in adjacent fields on identical soils and slopes. Sampling was conducted

Table 1. Climate at the Grenshoek (Gh) and Tshivhase (Tv) tea estates (adapted from Nethononda, 1994). Monthly means recorded from 1985 to 1992 of daily minimal temperature (T_{Min}), daily maximal temperature (T_{Max}), monthly total precipitation, and daily maximum vapour pressure deficit (VPD).

	T_{min} (°C)		T_{max} (°C)		Precipitation (mm)		VPD (kPa)	
	Tv	Gh	Tv	Gh	Tv	Gh	Tv	Gh
Jan	18.0	18.3	27.3	27.9	196	261	1.50	1.59
Feb	18.3	18.5	26.5	27.3	221	218	1.32	1.46
Mar	17.5	18.0	25.9	26.7	184	151	1.24	1.30
Apr	15.7	16.1	24.8	25.7	48	75	1.54	1.59
May	13.8	14.9	23.6	22.8	22	26	1.70	1.56
Jun	11.2	11.8	20.5	22.4	43	18	1.28	1.58
Jul	10.8	11.0	20.9	21.8	8	11	1.44	1.57
Aug	11.6	11.4	21.9	24.1	30	10	1.48	1.78
Sep	13.5	14.6	24.2	25.6	74	20	1.67	1.85
Oct	14.9	15.1	25.1	26.7	122	69	1.63	1.72
Nov	15.9	16.3	25.8	28.1	145	124	1.47	1.68
Dec	17.4	17.4	26.4	27.9	283	216	1.62	1.59

close to the plot borders to assure that clones of contrasting sensitivity were never further than 10 m apart. The tea bushes were pruned to a height of 0.40 to 0.50 m, at Tshivhase in July 1998, and at Grenshoek in July 1996. Pruning once every three years is standard management practice in tea plantations in South Africa.

Clones

Clones PC113, PC114, SFS204 and SFS150 were studied at Grenshoek, and PC113 and PC114 at Tshivhase. The sensitive clones PC113 and SFS204 showed stress-induced flowering, browning of leaves, abscission of old leaves, and appearance of brown clusters in the vegetation during the study period. Tolerant clones PC114 and SFS150 did not show such symptoms. Most of the measurements were on young leaves normally harvested for tea production (third expanded leaf from a shoot at the top of the canopy). Some measurements were completed on old leaves at the border of a bush, approximately 0.5 m above the ground. Readings were collected during three consecutive days at the two estates.

Environmental factors

During the experiments, air temperature (T_{air}) was measured with a solid-state temperature sensor (LM35CA), RH with a capacitive sensor (Landré Intechmij), and photosynthetically active radiation (PAR) with a GaAsp (G1116, Hamamatsu) photodiode. Data were recorded every 10 minutes with a custom-built logger placed at the centre of each study area.

Leaf water potential (ψ_{leaf}) and stomatal conductance (g_s)

Ten young leaves were selected randomly from different bushes of each clone, transported in plastic bags, and immediately sealed in a custom-built Scholander-type pressure chamber (Vanassche and Laker, 1991) with the cut ends of the

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petioles projecting from the lid. The pressure in the chamber was increased with compressed air until xylem sap appeared at the exposed end of the petiole (Scholander *et al.*, 1965). This procedure was repeated every two hours, from predawn (06.00 h) until after sunset (18.00 h). Stomatal conductance of the abaxial side of young leaves was measured with an Mk II automatic diffusion porometer (Delta-T Devices, Burwell, UK). Measurements were started after the evaporation of dew in the morning, and were repeated every two hours.

Leaf morphology

Cuticle thickness, stomatal density, and stomatal pore length and depth were determined on dried samples of both young and old leaves. Leaves were re-hydrated in hot water (95–100 °C) and cross sections were cut. The cuticle of the sections was stained with a Sudan 4 (Michrome No 413 – Edward Gurr, Ltd. London) solution (20 mg dissolved in 1 ml of 6.315% (w/v) glycerine in 95% ethanol – undissolved Sudan 4 was precipitated by centrifugation). After rinsing with the same solvent, sections were studied with a confocal microscope (BIO-RAD MRC-600 mounted on a Zeiss Axioskop microscope). Cuticles were visualized by fluorescence using green excitation light (excitation 514 nm DF 10, beam-splitter DR 540 nm LP, emission 550 nm LP) and a 40 × -water immersion objective (NA 0.9). The same staining technique was used to visualize the epidermis of the leaf for determination of stomatal density and the radius of the stomatal pore. Stomatal pore depth was measured on cross sections using a Leitz Orthoplan equipped with a 50 × -water immersion objective (NA 1.00 w) under bright field conditions. From these anatomical measurements the calculated maximum stomatal conductance (CMSC) for water vapour transport (m s^{-1}) was derived as

$$CMSC = \frac{v\pi r^2 D}{d + \left(\frac{\pi r}{4}\right)} \qquad \text{(Jones, 1992)},$$

where r = maximum pore radius (μm), d = pore depth (μm), v = stomatal density (stomata m^{-2}), and D = diffusion coefficient of water vapour in air (24×10^{-6} m s^{-1} at sea level and 20 °C). Maximum pore radius was calculated from the maximum aperture that could be achieved with the observed pore length l, i.e. l/π (the stomatal opening is then a circle, see Figure 1). Statistical analyses (ANOVA and ANCOVA) were performed with Minitab (Release 10.5 Xtra).

RESULTS

Response of leaf water potential to the environment

Figure 2 summarizes the variation in ψ_{leaf} during consecutive stress days, expressed as a function of microclimatic factors. All factors had significant effects (linear or second order polynomial regression, $P < 0.05$), except PAR in Tshivhase. At Grenshoek the linear model always explained the largest part of the variance of

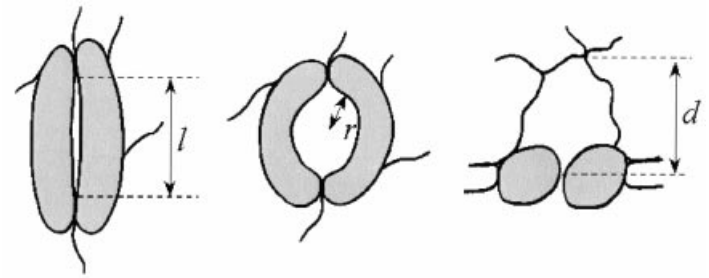

Figure 1. Schematic representation of a stomate illustrating how parameters were defined (l = pore length, r = maximum pore radius, d = pore depth).

ψ_{leaf} (highest r^2), and ψ_{leaf} declined with increasing Tair, PAR and VPD. At Tshivhase the polynomial model explained the larger part of the variance, indicating that ψ_{leaf} declined more rapidly at larger values of T_{air} and VPD. Higher sensitivity at Tshivhase is also illustrated by the more negative lowest ψ_{leaf} values recorded at that site, in spite of the less extreme values of T_{air} and VPD (*cf.* Figure 2a with 2b, and Figure 2e with 2f). Since microclimatic factors are correlated under natural conditions, not all these regressions are necessarily causal. The Grenshoek and Tshivhase sites did not only differ in maximum values of T_{air} or VPD; during the measurements, early morning air temperature was never lower than 15 °C at Grenshoek (Figure 2b), while it dropped close to 0 °C at Tshivhase (Figure 2a), inducing cold stress and highly negative ψ_{leaf} on some days.

The similarity of ψ_{leaf} in the two PC clones at Tshivhase is expressed statistically as no significant ($P > 0.05$) clonal effect in an ANCOVA of ψ_{leaf} with covariate T_{air} or VPD (Figure 2). In contrast, ψ_{leaf} at Grenshoek was consistently more negative in the stress-sensitive PC113 than in the stress-tolerant PC114, expressed statistically as a significant ($P < 0.001$) clone effect in an ANCOVA of ψ_{leaf} with covariate T_{air}, PAR or VPD. The site-dependent response of the clones was also observed in values of g_s (data not shown), which were significantly ($P < 0.05$) higher in PC113 than in PC114 at Grenshoek, but not at Tshivhase where there was no significant ($P > 0.05$) clone effect (ANOVA of g_s in young leaves with clone as single factor). In Figure 3 ψ_{leaf} is plotted as a function of g_s. At Grenshoek, ψ_{leaf} significantly declined with g_s in both clones (logarithmic regression, $P < 0.05$), while there was no such relationship at Tshivhase. Again, ψ_{leaf} at Grenshoek tended to be more negative in the stress-sensitive PC113 than in the stress-tolerant PC114, although the difference was not highly significant (ANCOVA with g_s as covariate, clone effect significant at $P = 0.056$). The latter suggests that sensitivity to stress involves other factors than g_s alone (for Grenshoek), since the above ANCOVA compares ψ_{leaf} at the same g_s.

Leaf morphology

Across the various clones and locations, cuticle thickness was not consistently correlated with stress-tolerance. At Grenshoek, the stress-sensitive PC113 pro-

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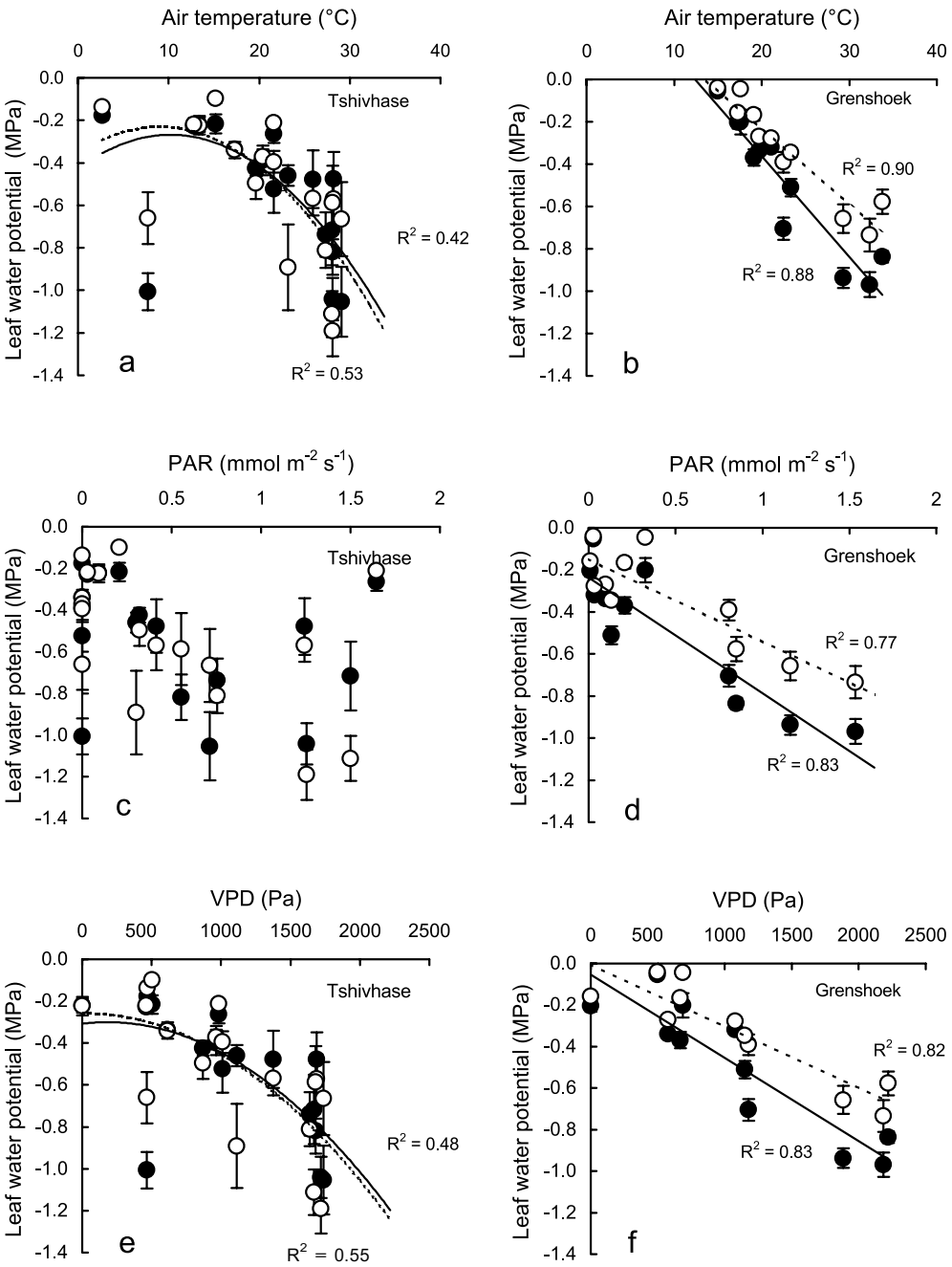

Figure 2. Relation between microclimatic factors and water potential of young leaves at Tshivhase and Grenshoek. Means ± 1 *s.e.* of replicate readings taken at the same time, and fitted curve (linear or 2nd order polynomial) for all readings combined. Clones: ● = PC113 (stress-sensitive), ○ = PC114 (stress-tolerant). a, b: air temperature; c, d: photosynthetically active radiation (PAR); e, f: vapour pressure deficit (VPD).

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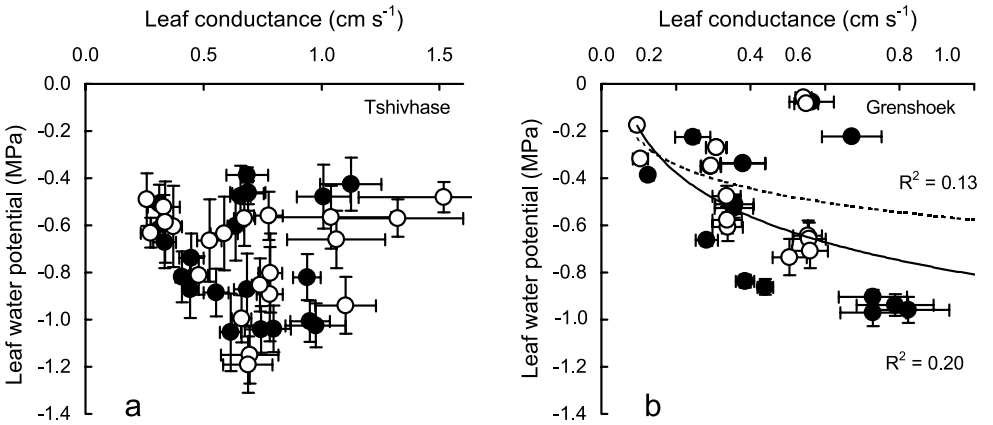

Figure 3. Relationship between stomatal conductance and water potential of young leaves at Tshivhase (a) and Grenshoek (b). Means ± 1 *s.e.* of replicate readings taken at the same time, and fitted curve (logarithmic) for all readings combined. Clones: ● = PC113 (stress-sensitive, solid line), ○ = PC114 (stress-tolerant, broken line).

duced a thicker cuticle compared with the stress-tolerant PC114 regardless of leaf age and leaf side, except on the abaxial side of young leaves where the increase in cuticle thickness was not significant (Table 2, ANOVA with factors leaf age and clone, $P > 0.05$). In the SFS clones at Grenshoek the stress-sensitive SFS204 had a thicker cuticle only on the adaxial side of its old leaves compared with the stress-tolerant SFS150, while in all other leaf types and ages the cuticle of SFS204 was thinner. At Tshivhase the old leaves of the stress-sensitive PC113 had a thinner cuticle compared with the stress-tolerant PC114, while the young leaves of PC113 had either a thicker cuticle (adaxial side) or the same cuticle thickness (abaxial side).

In addition to cuticle thickness, stomatal dimensions were measured as a second possible morphological indicator of stress tolerance (Table 3). Stomata were found only on the abaxial side of the leaves. Young leaves always had higher stomatal densities, but lower pore diameters and depths except in Tshivhase where pore depth of young and old leaves were not significantly different (ANOVA with factors leaf age and clone, $P > 0.05$). Similar to cuticle thickness, there was no consistent pattern in stomatal density that allowed discrimination between sensitive and tolerant clones. Contrary to expectations, at Grenshoek the stress-sensitive PC113 even had a much lower stomatal density than the stress-tolerant PC114, whereas stomatal density in the SFS clones was as anticipated, i.e. higher in the sensitive SFS204. The pattern also reversed between Grenshoek and Tshivhase, where the sensitive PC113 had more stomata per unit area than the tolerant PC114, opposite to Grenshoek. The fact that clones and locations where stomatal density was higher, were not systematically characterized by more negative ψ_{leaf} (cf. Figures. 2b, d and f, Figure 3a), suggests that leaf water balance under atmospheric stress is not highly determined by stomatal density. The same

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Table 2. Thickness of leaf adaxial and abaxial cuticle in sensitive and non-sensitive clones at Tshivhase and Grenshoek. Means ± 1 *s.e.* for young and old leaves (n = 30).

	Cuticle thickness (μm) at Grenshoek			
	Sensitive		Tolerant	
Clone	PC113		PC114	
	Young	Old	Young	Old
Adaxial	2.91 ± 0.07	3.32 ± 0.11	2.62 ± 0.06	2.82 ± 0.06
Abaxial	2.81 ± 0.09	3.13 ± 0.08	2.71 ± 0.07	2.42 ± 0.06
Clone	SFS204		SFS150	
	Young	Old	Young	Old
Adaxial	3.23 ± 0.07	4.92 ± 0.12	3.38 ± 0.08	4.62 ± 0.13
Abaxial	2.37 ± 0.05	2.92 ± 0.08	2.94 ± 0.08	3.55 ± 0.09
	Cuticle thickness (μm) at Tshivhase			
	Sensitive		Tolerant	
Clone	PC113		PC114	
	Young	Old	Young	Old
Adaxial	2.90 ± 0.08	3.07 ± 0.06	2.56 ± 0.05	3.81 ± 0.07
Abaxial	2.10 ± 0.05	2.47 ± 0.05	2.14 ± 0.04	3.23 ± 0.06

Table 3. Stomatal characteristics of sensitive and tolerant clones at Tshivhase and Grenshoek. Means ± 1 *s.e.* for young and old leaves (n = 30).

Clone		Tshivhase		Grenshoek	
		Stomatal density (10^6 m^{-2})		Stomatal density (10^6 m^{-2})	
		Young	Old	Young	Old
PC113	(Sensitive)	271 ± 10	153 ± 5	233 ± 7	170 ± 5
PC114	(Tolerant)	244 ± 7	164 ± 4	332 ± 18	201 ± 7
SFS204	(Sensitive)			334 ± 10	200 ± 5
SFS150	(Tolerant)			218 ± 13	149 ± 4
		Stomatal pore diameter (μm)		Stomatal pore diameter (μm)	
		Young	Old	Young	Old
PC113	(Sensitive)	15.0 ± 0.3	18.5 ± 0.2	16.2 ± 0.7	23.2 ± 0.5
PC114	(Tolerant)	15.2 ± 0.3	18.6 ± 0.2	15.9 ± 1.2	20.6 ± 0.6
SFS204	(Sensitive)			11.2 ± 0.6	20.3 ± 0.5
SFS150	(Tolerant)			13.4 ± 0.4	18.4 ± 0.5
		Stomatal depth (μm)		Stomatal depth (μm)	
		Young	Old	Young	Old
PC113	(Sensitive)	29.6 ± 1.1	27.9 ± 1.0	19.8 ± 0.6	26.9 ± 0.8
PC114	(Tolerant)	29.5 ± 0.9	31.0 ± 1.7	18.1 ± 0.7	25.0 ± 0.8
SFS204	(Sensitive)			19.1 ± 0.7	25.3 ± 0.8
SFS150	(Tolerant)			19.7 ± 0.6	26.5 ± 1.0

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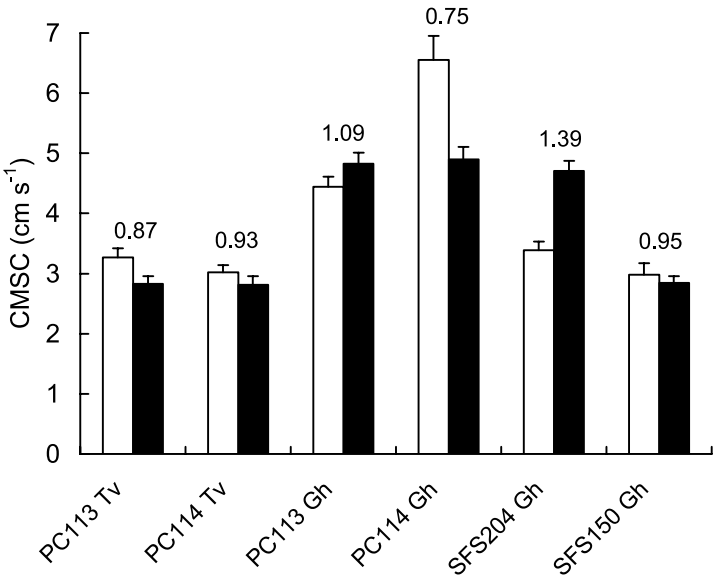

Figure 4. Calculated maximum leaf stomatal conductance (CMSC) for different clones at Tshivhase (Tv) and Grenshoek (Gh). Means ± 1 *s.e.* (n = 30). ■ = old leaves; □ = young leaves. Values above the bars represent the ratio $CMSC_{Old}/CMSC_{Young}$.

was true for stomatal dimensions. Pore diameter was either identical in sensitive and tolerant clones (i.e. in young leaves of PC clones at Grenshoek, and in young and old leaves of PC clones at Tshivhase), or the differences between sensitive and tolerant clones were inconsistent (higher pore diameter in old leaves of sensitive clones of both PC and SFS, but lower pore diameter in young leaves of sensitive SFS clones, in all cases at Grenshoek). Differences in stomatal depth between sensitive and tolerant clones were small (< 10% in most cases), and either not significant (SFS clones) or unrelated to sensitivity (PC clones).

The stomatal dimensions in Table 3 were used to derive the calculated maximal stomatal conductance (CMSC). In contrast to g_s, which varies with instantaneous microclimate, CMSC is an indicator of the potential maximal water loss at full stomatal aperture. No general relationship was found between clone sensitivity and CMSC (Figure 4). Nevertheless, at Grenshoek a pattern emerged of stress-sensitive clones (PC113 and SFS204) having larger CMSC in the old relative to the young leaves, and vice versa for the stress-tolerant clones (PC114 and SFS150). This suggests that the water loss of old leaves (which represent most of the foliage) may affect the stress on young leaves, by consuming either a smaller or a larger fraction of the stem flow. The fact that, also for ψ_{leaf}, the stress-sensitive and stress-tolerant clones were differentiated only at Grenshoek (Figures. 2b, 2d and 2f), similar to CMSC, supports such a mechanism. No clear pattern of stress-sensitivity and $CMSC_{old}/CMSC_{young}$ ratio was found at Tshivhase (Figure 4), the location where differences in stress-sensitivity were not expressed in ψ_{leaf} (see Figures. 2a and 2e).

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DISCUSSION

Drought stress arises when the interplay between meteorological, biological, soil, and management factors dislocates the water balance of the plant. Leaf morphological traits such as cuticle thickness, stomatal density or dimensions, and maximum or effective stomatal aperture, directly affect the water balance by determining the 'loss' side of the equation, so these traits could become critical under high atmospheric demand. In their evaluation of sensitive and tolerant clones, the authors had so far considered ψ_{leaf} as an instantaneous physiological measure of stress intensity at the leaf level, and the traits mentioned above as factors influencing this intensity. However, cause and effect cannot always be separated: for example, the morphological leaf traits which determine water loss in a given environment are themselves shaped by the prevailing stress regime. Also the 'instantaneous' stress measure ψ_{leaf} partly reflects stress history. Analysis of stress tolerance and its possible predictors, therefore, has to consider that both the response (ψ_{leaf}) and the underlying factors (leaf morphological traits) integrate information from the physical environment over time, probably with different time constants.

Previous studies have reported decreased tea production under high VPD and dry air, e.g. Carr (1972), Herd (1976), and Tanton (1982a; b). Notably $T_{air} > 30\,^{\circ}C$ (Carr, 1972; Herd, 1976) and VPD exceeding 2.3 kPa (Tanton, 1982b) can depress shoot extension. When the present measurements were made, such conditions were approximated only at Grenshoek, but the stress symptoms visually observed in the sensitive clones indicated that both estates experienced severe atmospheric stress prior to the experimental period. In addition, at both Tshivhase and Grenshoek night air temperature fell below 12.5 °C, which, according to Tanton (1982a), may reduce shoot extension. In spite of the broadly similar range of conditions during the experiments at Grenshoek and Tshivhase, clonal differences in ψ_{leaf}, g_s, and the ψ_{leaf}–g_s relationship (Figures 2 and 3), were observed only at Grenshoek, while at Tshivhase the sensitive and tolerant clones behaved similarly. The key to this site-dependent expression of stress tolerance may be in the climate at these locations, because both leaf physiology and leaf morphogenesis are strongly determined by environmental conditions (Jones, 1985). Grenshoek has slightly higher T_{air} and higher VPD than Tshivhase (Table 1), and is subject to cold, dry winter winds which do not occur in Tshivhase. Also, extreme VPDs in spring are more frequent at Grenshoek (Nethononda, 1994). Harsher growth conditions at Grenshoek are reflected in more strongly developed cuticular wax layers, providing more efficient defence mechanisms (but only in the sensitive PC113, Table 2, see also discussion below). They are also reflected in the lower sensitivity of the clones to atmospheric stress at that site (linear curves in Figures 2b, 2d and 2f, *vs.* polynomial curves in 2a and 2c), since plants typically acclimatize to higher stress incidence by reducing sensitivity (Larcher, 1995). Given these different stress regimes at Grenshoek and Tshivhase, the recurring contrast between the clones with respect to ψ_{leaf}, g_s, and the ψ_{leaf}-g_s relationship,

could indicate that frequent exposure to stress is required for clonal differences in stress tolerance to become expressed. By contrast, the more favourable conditions at Tshivhase are less likely to induce efficient defence mechanisms, such that both intrinsically sensitive and intrinsically tolerant clones are affected by stress to a similar, but large, extent (*cf.* the same, highly negative, ψ_{leaf} in PC113 and PC114 in Figures 2a and 2e, compared with Figures 2b and 2f). Although ψ_{leaf} is treated as a measure of stress in this study and not as a predictor of stress sensitivity, the fact that it discriminates between sensitive and tolerant clones at Grenshoek suggests it could also serve as an indicator of sensitivity (again, only in an environment with high stress incidence such as Grenshoek). In explaining the differences between Tshivhase and Grenshoek, the effects of pruning could be significant. Since the plants had been more recently pruned in Thsivhase (see Methodology), lower transpiration rates associated with lower leaf area index may have reduced atmospheric stress to a level where intrinsic differences in stress tolerance are no longer expressed (hence the similar curves for PC113 and PC114 at Tshivhase). Such a mechanism is unlikely, however, since the most extreme values of ψ_{leaf} recorded at Tshivhase were more negative and occurred at lower PAR, T_{air} and VPD compared with Grenshoek (Figures 2a, 2c, and 2e, *vs.* Figures 2b, 2d, and 2f). On the other hand, accumulation of stress by repetitive wounding of the plant during consecutive pruning events would explain the more negative ψ_{leaf} stress level in the more recently pruned plants at Tshivhase. However, because pruning is carried out only once every three years, and the recovery of the productivity of tea bushes after pruning is rapid, there do not appear to be lasting adverse effects. Clearly, more studies are needed to determine possible influences of pruning on stress sensitivity, but the current data from Grenshoek and Tshivhase suggest that the degree of local stress exposure is more important for expression of sensitivity. In principle, more negative ψ_{leaf} at Tshivhase could also be due to the lack of irrigation at that estate, at the time of the experiment. Since Tshivhase had higher precipitation, however, absence of irrigation does not imply that soil drought stress levels were higher. Both estates had similar irrigation schedules.

To what extent can the stress level of the clones be accounted for by stomatal regulation? In other words, do plants that limit transpiration by having lower stomatal conductance exhibit less negative ψ_{leaf}? Sensitivity of ψ_{leaf} to changes in g_s was clearly stronger at Grenshoek (where strongly negative ψ_{leaf} was associated with high g_s, Figure 3b), than at Tshivhase, where no such association was observed (Figure 3a). Similarly, Squire (1978) found no dependence of shoot water potential on stomatal conductance in tea. Low sensitivity of ψ_{leaf} to g_s might arise because reduction of g_s, which should alleviate stress by lowering transpiration, simultaneously increases leaf temperature, to which clones can differ in sensitivity (e.g. Smith *et al.* 1994). However, this does not explain differences in sensitivity between sites. Low sensitivity of ψ_{leaf} to stomatal conductance is not an index of low efficiency *per se*: closer inspection of Figure 3a reveals that the high g_s values of around 10 mm s^{-1} coincided in a number of cases with highly negative values of

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ψ_{leaf} of around -1 MPa, but in other cases only with moderate values of ψ_{leaf} between -0.4 and -0.6 MPa. This was not the case at Grenshoek, where high g_s always coincided with highly negative ψ_{leaf}. Possibly the degree of stress control owing to stomatal regulation cannot be compared between Grenshoek and Tshivhase, since the same g_s might occur at different levels of atmospheric stress at the two estates. Comparison of stomatal regulation between clones at the same site (Grenshoek) shows that the stress-tolerant PC114 is more efficient than the stress-sensitive PC113 in two ways: average g_s is lower, and ψ_{leaf} is less negative when compared at the same g_s. Since in this comparison the microclimate was identical, differences can be ascribed solely to the plant. The authors therefore conclude that higher tolerance in PC114 arises from (i) better regulation of g_s, e.g. via stomatal aperture, and (ii) better regulation of cell water potential components contributing to ψ_{leaf}, although it cannot be excluded that PC114 also extracted water more effectively, for example, because of its root architecture or a higher amount of fine roots per unit area of leaf (*cf.* Nixon *et al.* 2001). The fact that no evidence for stomatal regulation was found in Tshivhase (Figure 3a) again suggests that the more favourable conditions at that site do not precondition the plant to respond adequately to stress.

Relating stress tolerance to leaf morphology is complicated because leaf anatomical features themselves are modified by exposure to stress (Squire and Callander, 1981; Jones, 1985). In tea, inherently thin cuticular wax layers can be expected since it is an understorey crop, originating from the humid tropics. In such a climate, high cuticular water loss would not greatly affect plant performance. In the South African spring with its frequent extremely high VPDs, low cuticular wax production could be detrimental, and clonal differences in stress-tolerance may reflect cuticular thickness. However, thicker cuticular wax layers in some clones, protecting against stress, could be genetically programmed or induced by exposure to stress (Bondada *et al.*, 1996; Prior *et al.*, 1997). Table 2 does not support a general pattern of inherently thinner cuticles in sensitive clones, when they are compared to their tolerant counterparts at the same site (i.e. exposed at the same stress level). At Grenshoek, the thicker cuticle of PC113 relative to PC114 even supports the opposite, suggesting that stress-sensitive clones could perceive a given local VPD and T_{air} regime as more stressful than stress-tolerant clones, and they consequently produce more wax. However, the SFS clones at Grenshoek do not confirm this hypothesis, nor do the PC clones at Tshivhase. The former could be due to incapacity to produce more wax, the latter due to a lower background stress incidence which does not lead to a differentiation of wax production between clones. A second question is how the cuticular thickness of a given clone responds to a change in location (i.e. to stress level), and whether this response depends on the sensitivity of that clone. For most leaf types and leaf sides (old and young, abaxial or adaxial), PC113 produces a thicker cuticle at Grenshoek than at Tshivhase, suggesting that stress-sensitive clones respond to less favourable conditions by producing more wax. PC114 does not respond in the same way, suggesting that stress-tolerant clones do not perceive the

Grenshoek conditions as being significantly more stressful than those at Tshivhase and, consequently, do not enhance wax synthesis (unless PC114 was incapable of increasing its wax production). While these responses can be explained from the viewpoint of local stress regime and its perception by sensitive versus tolerant plants, the fact remains that an overall relationship between clonal sensitivity and cuticular wax thickness is lacking, which precludes the use of the latter as a predictor of stress tolerance.

That $CMSC_{old}/CMSC_{young}$ was a potentially promising indicator of stress tolerance but, similar to ψ_{leaf} and g_s, only for the more severe stress regime at Grenshoek, opens perspectives for plant breeders. The hypothesis underlying the $CMSC_{old}/CMSC_{young}$ index is supported by Sandanam *et al.* (1981), who directly observed stress-sensitive tea plants losing more water by their old leaves. It should be noted that in some cases CMSC itself apparently can be inconsistent with g_s: for example, in the young leaves at Grenshoek, g_s is higher in PC113 than PC114, while CMSC is lower. Clearly, the potential maximum conductance expressed in CMSC is lower in PC113 as a consequence of lower stomatal density (Table 3), so the higher g_s during the measurements implies that the stomata were more open, confirming the poorer stomatal regulation in PC113. The reason why indicators based on CMSC, like $CMSC_{old}/CMSC_{young}$, could nevertheless be useful probably lies in the fact that they integrate over a longer period than the more instantaneous g_s. As a consequence, the linkage with stress damage in the field could be stronger (especially if the indicator at the same time refers to the whole plant water balance like $CMSC_{old}/CMSC_{young}$). Using an energy balance approach, Nijs *et al.* (2000) found that the canopy conductance to water vapour transport, which also integrates water loss from both old and young leaves, similarly allows discrimination between sensitve and tolerant tea clones exposed to atmospheric stress.

CONCLUSIONS

Growth in low-stress conditions, typical of the nursery, does not seem to promote the morphological or physiological expression of indicators of stress tolerance, not in $CMSC_{old}/CMSC_{young}$ nor in any of the other parameters examined by the authors. Testing the validity of promising selection criteria like g_s, ψ_{leaf} or $CMSC_{old}/CMSC_{young}$, is therefore likely to benefit from subjecting plants to stress scenarios during selection. The current work being limited to four clones, these criteria would need to be established across a wider range of clones.

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