Drought Tolerance is Associated with Rooting Depth and Stomatal Control of Water Use in Clones of Coffea canephora

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INTRODUCTION

Drought is an environmental factor that produces water deficit or water stress in plants. Internal water deficit is initiated when low water potential develops and cell turgor begins to fall below its maximum value (Kozlowski and Pallardy, 1997). There has not been a great deal of attention given to separating productivity under drought, which is important for cultivated plants, from survival mechanisms, particularly for woody species. Species or cultivars more tolerant to drought generally differ morphologically and/or physiologically, with mechanisms allowing greater production under limited water supply. These mechanisms involve maximization of water uptake by deep, dense root systems and/or minimization of water loss by stomatal closure and reduction of leaf area (Kramer and Boyer, 1995). These improve plant water status and particularly turgor maintenance, which may be achieved through osmotic adjustment and/or changes in cell wall elasticity, and is essential for maintaining physiological activity for extended periods of drought (Kramer and Boyer, 1995; Turner, 1997).

Coffee (Coffea arabica and C. canephora), a tropical tree crop, is the most important commodity in international agricultural trade, generating over US$90 billion each year and involving about 500 million people in its management, from cultivation to final product for consumption. Currently, robusta coffee (C. canephora) produces about 38% of coffee consumed (Rezende and Rosado, 2004). It is indigenous to African regions characterized by abundantly distributed rainfall and atmospheric humidity frequently approaching saturation (Willson, 1999). For this reason, robusta coffee probably evolved as a ‘water-spender’ species (DaMatta and Rena, 2001). However, in Brazil, a major area of production, it has been largely cultivated in regions where water availability constitutes the major environmental constraint affecting crop production. Even short periods of drought can substantially decrease coffee yields, and consequently irrigation is indispensable for production. Older progenies of robusta coffee differed little in response to drought, however, plant breeders have recently selected some promising clones with relatively high, and low-year-to-year variation of, bean production under rain-fed conditions. The selection has been largely empirical as relatively

Key results and conclusions

With irrigation, plant hydraulic conductance (Ks), midday ψs and stomatal conductance (gs) were measured. At the end of the experiment, carbon isotope ratio and parameters from pressure–volume curves were estimated. Morphological traits were also assessed.

Key words: Carbon isotope ratio, elastic and osmotic adjustments, robusta coffee, vapour pressure deficit, water potential, water relations, water-use efficiency.
little is known about how clones of robusta respond physiologically to drying soil. Many mechanisms have been suggested to be important. Lima et al. (2002), from a study of two clones rapidly drought stressed, proposed that drought tolerance might, at least in part, be associated with enhanced activity of antioxidant enzymes. In contrast, Pinheiro et al. (2004) did not find a general link between protection against oxidative stress and drought tolerance when four clones of robusta were subjected to long-term drought, and so did not corroborate that suggestion. DaMatta et al. (2003) found that the better crop yield of a drought-tolerant clone, compared with a drought-sensitive one, was associated with maintenance of leaf area and higher tissue water potentials, as a consequence of smaller stomatal conductance ($g_s$), which would result in less carbon isotope discrimination. Despite these efforts, the causes of the differences in clonal tolerance to drought in robusta coffee still remain largely unknown. For instance, as yet there is no consistent information about the stomatal control of water use in response to both soil and atmospheric drought stress.

In this work, clones 14, 120, 46 and 109A of robusta coffee were compared. These clones all produce a good crop when grown under irrigation; under limited soil water, however, survival and productivity (Ferrão et al., 2000a, b) as well as maintenance of tissue water status (DaMatta et al., 2000) are impaired to a greater extent in 46 and 109A, which are therefore classified as drought-sensitive, than in 14 and 120, classified as drought-tolerant. One group of plants was continuously irrigated while water was withheld from a second group to promote a drought response. Clones were grown in large containers in an attempt to develop internal water deficits slowly, thus allowing adaptation (acclimation) to occur (DaMatta, 2003). Leaf water relations were examined at a similar internal water status, permitting more reliable comparisons among clones to be made.

The overall aim of this work was to expand our earlier studies quoted above (which explored drought tolerance mostly at a biochemical level) to improved understanding of the physiological and morphological basis of drought tolerance in robusta coffee. This would provide greater opportunities for intensifying selection of promising clones for drought-prone regions. Specific objectives were: (1) to identify the extent and mechanisms of intra-specific variation of water use by examining how stomatal behaviour and leaf water relations adjusted to changes in soil water supply and evaporative demand; and (2) to assess whether differences in drought tolerance are associated with morphological characteristics such as root depth and leaf area. For these purposes, morphological traits, leaf xylem pressure potential ($\psi_x$), $g_s$, plant hydraulic conductance ($K_h$), stable carbon isotope ratio, $\delta^{13}$C (to estimate long-term water-use efficiency; Farquhar et al., 1989), and water relations parameters derived from pressure–volume curves were evaluated.

**MATERIALS AND METHODS**

**Experimental design**

The experiment was conducted in Viçosâ (20°45’S, 650 m a.s.l.), south-eastern Brazil. Plants were grown under shade (about 45% of natural light) in a screen house with walls of coarse mesh screen, which allowed air exchange with the external environment. The experiment was a completely randomized design, with eight treatment combinations, forming a $4 \times 2$ factorial (four clones and two watering regimes) with five plants in individual pots per treatment combination as replication. The experimental plot was one plant per container. Clones (14 and 120, drought-tolerant; 46 and 109A, drought-sensitive) of *C. canephora* ‘Kouillou’ (known in Brazil as ‘Conilon’) raised as rooted stem cuttings were obtained from the Institute for Research and Rural Assistance of the Espírito Santo State (INCAPER), Brazil. Forty plants were grown in plastic, cylindrical pots (0.8 m high, 0.44 m internal diameter) containing 120 L of a mixture of soil, sand and manure (3 : 1 : 1, v/v/v) with a gravel layer at the bottom. Plants received an average midday photosynthetic photon flux of about 900 µmol m$^{-2}$ s$^{-1}$. When 12-months old, plants of each clone were separated into two groups: one continued to receive regular irrigation (control plants), and in the other water was withheld (drought-stressed plants) until $\psi_x$, at pre-dawn ($\psi_{pd}$) reached about $-3.0$ MPa. During the course of drying, $\psi_x$ and $g_s$ were measured on five occasions on the third or fourth pair of leaves from the apex of plagiotropic branches. Once the desired $\psi_{pd}$ was reached, expanded leaves (approximately half of the final size of those from control plants) were collected and their $\delta^{13}$C was determined. Expanding leaves were selected to ensure that most of the carbon analysed was incorporated into tissue during the drought treatment. Fully expanded leaves were also collected for measuring pressure–volume relationships. All the plants were then irrigated (at about 1800 h) and $\psi_x$, $g_s$ and $K_h$ were determined on the following two consecutive days ($\psi_{pd}$ measured at 12 h and 36 h after irrigation).

**Biometric measurements**

At the end of the experiment, well-watered plants were harvested and separated into above-ground parts and roots. Leaf area was measured with an area meter (Area Measurement System, Delta-T Devices, Cambridge, UK). Roots were washed thoroughly with tap water above a 0.5 mm screen sieve. Plant tissues were then oven-dried at 72°C for 72 h, after which dry matter was determined. Shoot height and root depth (after washing) were also measured.

**Water relations**

Xylem pressure potential was measured before dawn (0430–0530 h), between 0700–0900 h and at midday ($\psi_{mid}$) using a Scholander-type pressure chamber. About 12 h after irrigating both control and drought-stressed plants ($\psi_x$, typically above $-0.10$ MPa), fully expanded leaves were detached by cutting their petioles under deionized water and brought to the laboratory to produce pressure–volume curves. Fresh weight and $\psi_x$ were measured at intervals during dehydration (free transpiration technique; Hinckley et al., 1980) until a $\psi_x$ of about $-3.5$ MPa was reached. Turgid weight was estimated from the linear relationship between fresh weight and $\psi_x$ in the positive turgor range, by extrapolating to $\psi_x = 0$. The inverse of $\psi_x$ was...
plotted as a function of relative water content (RWC). From the pressure-volume curves, the osmotic potential at full (\(\psi_{m(0)}\)) and zero (\(\psi_{m0}\)) turgor, RWC at zero turgor (\(\text{RWC}_{(0)}\)) and the bulk modulus of elasticity (\(\varepsilon\); Melkonian et al., 1982) were estimated. Further details are given by DaMatta et al. (1993).

Stomatal and hydraulic conductance

Stomatal conductance to water vapour was measured with a portable, open-system infrared gas analyser (LCA-4, ADC, Hoddesdon, UK), as described in DaMatta et al. (1997). Measurements were made between 0700 and 0900 h (25 ± 2 °C air temperature, 90 ± 2% relative humidity) and between 1100 and 1300 h (30 ± 2 °C air temperature, 80 ± 2% relative humidity).

Plant hydraulic conductance \([K_L = (g_s \times \Delta_h)/(\psi_{pd} - \psi_{md})]\) was calculated using \(\psi_{pd}\) to approximate soil water potential, and \(g_s\) and \(\Delta_h\) (leaf-to-air vapour pressure deficit, estimated according to Landsberg, 1986) were measured at the same time as \(\psi_{md}\) (Hubbard et al., 1999; Donovan et al., 2000).

Carbon isotope ratio

Leaf \(\delta^{13}C\) was measured relative to the international PDB standard using a mass spectrometer (Delta-S, Finnigan MAT, Bremen, Germany), as previously described (DaMatta et al., 2002). Differences in \(\delta^{13}C\) from duplicates for each sample were below 0.2 ‰.

Statistics

Significant differences between treatment means were tested by the Newman–Keuls and F-tests, at \(P \leq 0.05\). Regression analyses were used to examine relationships between physiological and/or environmental variables. Equality of the regression models was tested using the indicator variable technique (Neter and Wasserman, 1974), at \(P \leq 0.05\). Separate regression models for clones 14, 46 and 120 did not differ statistically. Therefore, data for these clones were pooled and single regressions were fitted to the combined data.

RESULTS

Table 1. Morphological characteristics of 1-year-old clones of robusta coffee (Coffea canephora) under full irrigation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Clone 14 (mean ± standard error)</th>
<th>Clone 120 (mean ± standard error)</th>
<th>Clone 46 (mean ± standard error)</th>
<th>Clone 109A (mean ± standard error)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoot height, m</td>
<td>0.78 ± 0.065</td>
<td>0.94 ± 0.036</td>
<td>0.73 ± 0.07</td>
<td>0.92 ± 0.02</td>
</tr>
<tr>
<td>Leaf area, m²</td>
<td>1.89 ± 0.11</td>
<td>2.51 ± 0.13</td>
<td>1.91 ± 0.08</td>
<td>2.36 ± 0.10</td>
</tr>
<tr>
<td>Specific leaf area, m² kg⁻¹</td>
<td>11.18 ± 0.39</td>
<td>10.39 ± 0.96</td>
<td>12.15 ± 1.09</td>
<td>9.32 ± 0.64</td>
</tr>
<tr>
<td>Root mass to leaf area ratio, g m⁻²</td>
<td>93.5 ± 2.63</td>
<td>107.2 ± 8.91</td>
<td>113.0 ± 7.36</td>
<td>120.8 ± 22.8</td>
</tr>
<tr>
<td>Total biomass, g</td>
<td>434 ± 21</td>
<td>645 ± 31</td>
<td>455 ± 59</td>
<td>744 ± 58</td>
</tr>
<tr>
<td>Root depth, m</td>
<td>0.76 ± 0.03</td>
<td>0.75 ± 0.04</td>
<td>0.48 ± 0.04</td>
<td>0.53 ± 0.03</td>
</tr>
</tbody>
</table>

Different letters denote significant differences between clonal means \((P < 0.05);\) Newman–Keuls test). Each value represents the mean ± s.e. of five replicates.

Discontinuing irrigation and, thus, changes in growth traits are not fully comparable between drought-stressed clones. In addition, because treatments were applied over a relatively short time, drought effects on growth were small, and so not significant (not shown). Therefore, only growth data for control plants are presented. The clones could be grouped into two types of contrasting canopy morphology, with 109A and 120 taller (Table 1) with less dense crowns than 14 and 46. There was no significant difference in total dry matter was greater in clones 109A and 120 than in 14 and 46, whereas root mass to leaf area ratio was larger in 109A than in the other clones (Table 1). Drought-tolerant clones had a considerably deeper (Table 1) and more regularly distributed root system down the profile than drought-sensitive clones (Fig. 1).

For control plants, \(\psi_{pd}\) was always above −0.08 MPa, but the average \(\psi_{pd}\) tended to be lower in clones 14 and 46 (−0.89 and −1.06 MPa, respectively) than in 109A and 120 (both −0.65 MPa; Fig. 2). On average, \(K_L\) tended to be higher in 109A and 120 (about 3.0 mmol m⁻² s⁻¹ MPa⁻¹) than in 14 and 46 (about 1.7 mmol m⁻² s⁻¹ MPa⁻¹).

Plant water stress developed faster in drought-sensitive clones. After withholding irrigation for 14 d, \(\psi_{pd}\) was significantly lower in clone 109A than in the other clones; 7 d latter, \(\psi_{pd}\) dropped to about −2.3 MPa in clones 46 and 109A, compared with −0.8 MPa in clone 14 and −1.6 MPa in clone 120. A similar trend, but clearly shifted to lower values, was found for \(\psi_{md}\) (Fig. 2). As expected from the above, clone 109A attained a \(\psi_{pd}\) of −3.0 MPa earlier than
the other clones, followed in order by 46, 120, and then 14 (Fig. 2). Under drought stress, average $K_L$ tended to be greater in clones 109A and 120 than in the other clones (Fig. 3). $\psi_x$ and $K_L$ recovered within 2 d of re-watering for all clones (Figs 2, 3).

Curvilinear decreases in $g_s$ as $\psi_{pd}$ declined were similar for clones 14, 46 and 120, but smaller in clone 109A (Fig. 4). For example, as $\psi_{pd}$ decreased from $-0.5$ to $-3.0$ MPa, $g_s$ decreased, on average, from 89 to 28 mmol m$^{-2}$ s$^{-1}$ in clones 14, 46 and 120, and from 102 to 52 mmol m$^{-2}$ s$^{-1}$ in clone 109A (estimated from the equations in Fig. 4). Similar changes were found when $g_s$ was associated with $\psi_x$, both variables being measured on the same leaf at 0700–0900 h (data not shown).

As $\Delta_w$ increased, $g_s$ decreased linearly in a similar way in clones 14, 46 and 120. By contrast, there was no relationship between $g_s$ and $\Delta_w$ in clone 109A (Fig. 5). In spite of $\Delta_w$ and leaf temperature being strongly associated to each other ($r^2 = 0.920$, $P < 0.001$), co-variance analysis revealed no direct effect of leaf temperature in the response of $g_s$ to $\Delta_w$ ($P = 0.173$).

Clonal differences in drought tolerance were not associated with osmotic or elastic adjustments, since parameters from pressure–volume curves, irrespective of treatments, were similar ($\psi_{100} = -1.83 \pm 0.08$ MPa; $\psi_{0} = -2.26 \pm 0.09$ MPa; $\epsilon = 18.4 \pm 0.6$ MPa; RWC$_{0} = 89.8 \pm 0.5 \%$) and constant for all clones (data not shown). The only exception was observed in clone 109A in which drought resulted in a slight, but significant decrease ($0.19$ MPa) in $\psi_{0}$.

After imposing water deficit, $\delta^{13}$C increased significantly (1.48 to 2.22 %; Fig. 6) in all clones, suggesting increased long-term water-use efficiency (WUE). However, absolute values of $\delta^{13}$C were lower in 109A than in the other clones irrespective of the irrigation treatments. There was no difference in $\delta^{13}$C between clones 14, 46 and 120 (Fig. 6). Overall, $g_s$ decreased more than net carbon assimilation rate, which did not differ among clones (data not shown).
and, thus, changes in $\delta^{13}$C would have been predominantly from changes in $g_s$.

**DISCUSSION**

Plant water stress developed more slowly in the drought-tolerant than in the drought-sensitive clones. Morphological traits such as leaf area and root mass to leaf area ratio were not associated with that response. Instead, the much deeper root system of the tolerant clones enabled them to gain greater access to water towards the bottom of the pots and, therefore, to maintain a more favourable internal water status longer than in drought-sensitive clones. Differences between drought-tolerant and drought-sensitive clones in postponing tissue dehydration are even more evident in the field (DaMatta et al., 2000, 2003), where the development of the root system is much less restricted.

Hydraulic conductance is positively associated with rates of water use, as has been found in genotypes of *C. arabica* (Tausend et al., 2000). Thus the larger $K_L$, as observed in clones 109A and 120 under full irrigation might at least partially explain their smaller variations in $\psi_a$ (as indicated by higher $\psi_{md}$ values) than in clones 14 and 46, which may help to avoid limitations to photosynthesis. As a consequence, clones 109A and 120 might have achieved a greater carbon gain, which would to some extent explain their greater biomass accumulation under well-watered conditions. This would be advantageous with non-limiting soil water or with brief periods of water deficit, but disadvantageous with long-term drought since a high $K_L$ may hasten the development of severe internal water deficit. This could be partially offset by a deeper root system, as is the case of clone 120. However, because $K_L$ was estimated using data from instantaneous gas-exchange measurements, rather than transpiration integrated over the morning, the above considerations should be interpreted cautiously.

Stomatal conductance decreased sharply with decreasing $\psi_a$, with no apparent threshold value of $\psi_{md}$ at which

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**Fig. 3.** Time-course of hydraulic conductance from soil to leaf ($K_L$) of four clones of robusta coffee subjected to full irrigation (solid circles) and drought conditions (open circles). See Fig. 1 for details.
stomatal closure was observed. The positive relationship between gs and y is expected when soil moisture changes and indirectly affects stomata through a hydraulic feedback (Jones, 1998). The rapid increase of y after re-watering, which was accompanied by increased gs, emphasizes the role of leaf water status on stomatal control, as suggested by Fuchs and Livingston (1996). In addition, stomatal sensitivity to evaporative demand, as observed in clones 14, 46 and 120, might indicate a feedforward response that would avoid large internal water deficits. Such sensitivity appears to be weaker in C. canephora than in C. arabica since, in the latter, gs decreases curvilinearly with increasing Δw (Gutiérrez et al., 1994; Kanechi et al., 1995). When considered together, these responses largely explain why C. canephora responds strongly, and better than C. arabica, to irrigation (DaMatta, 2004a).

Less negative δ13C can arise because of low gs or high carbon assimilation, both leading to a high WUE (Farquhar et al., 1989). The observed increases in δ13C in all drought-stressed clones should therefore reflect an increase in
long-term WUE. However, in clone 109A stomata closed less in response to both soil and atmospheric drought, probably resulting in a more prodigal use of water and also in a relatively more negative $\delta^{13}$C (and thus lower long-term WUE) than in the other clones regardless of the watering regime. These observations are partially in line with those of Meinzer et al. (1990a), who showed that genotypes of *C. arabica* with higher carbon isotope discrimination (more negative $\delta^{13}$C) under full irrigation resulted from higher $g_s$ rather than lower carbon assimilation, depleted soil water more rapidly, and experienced symptoms of physiological stress earlier when water was withheld. One must be cautious, however, since this present study was too small to demonstrate conclusively the usefulness of $\delta^{13}$C as an index for ranking clones of robusta coffee in terms of drought tolerance.

Osmotic adjustment has been associated with maintenance of gas exchange under drought conditions (Turner, 1997). In our work, however, its amplitude was small and limited to clone 109A and could hardly explain the low stomatal sensitivity to drought in this clone. It should be noted that leaf water deficits may develop faster upon discontinuing irrigation in coffee genotypes having higher amplitude of osmotic adjustment (DaMatta, 2004b). Therefore, osmotic adjustment seems of limited importance (Munns, 1988) in determining drought tolerance in robusta coffee; this has also been reported for several other woody species (Fan et al., 1994). Where it occurs, osmotic adjustment either may not persist for long under drought, or functions over a limited range of $\psi_s$ values (Blake et al., 1991).

The clones we evaluated lost turgor at values of $\psi_s$ between $-2.1$ and $-2.4$ MPa. They showed relatively high values of $\epsilon$ (i.e. greater tissue rigidity), which resulted in high RWC$_{(0)}$, as reported for robusta coffee in both pot (DaMatta et al., 1993, 2002) and field (DaMatta et al., 2003) studies. These traits were consistent with the strong stomatal sensitivity to soil water deficit mediated by rapid loss of turgor as a consequence of inelastic leaf tissues (White et al., 2000). One must be cautious, however, because changes in relative symplast volume rather than changes in leaf turgor *per se* have been associated with stomatal aperture, as shown by Meinzer et al. (1990b) working with cultivars of *C. arabica* subjected to drought. In any case, maintenance of high RWC at low $\psi_s$ appears to be a means of the coffee tree avoiding, instead of tolerating, dehydration.

In summary, clonal ability to postpone dehydration was more important than dehydration tolerance, and cell water relations were largely unable to adjust to drought stress in any clone. Clone 109A, although possessing a greater root mass to leaf area ratio, is shallow-rooted and showed relatively poor stomatal control of transpiration; these features could explain why it experienced symptoms of drought stress earlier than other clones after irrigation was suspended. Clone 46 is also shallow-rooted, but its stomata closed more with both soil and atmospheric drought than in clone 109A; hence clone 46 dehydrated more slowly than 109A. Similarly to clone 46, stomatal sensitivity to drought was well developed in clones 14 and 120, but these clones showed substantially deeper root systems than the drought-sensitive clones, which could explain their better avoidance of drought. In any case, the larger $K_L$ in clone 120 than in clone 14 might be involved in the faster decrease in $\psi_{st}$ in the former. The direct response of stomata to changes in $\psi_s$ and $\Delta_w$ should have important consequences for clonal ability to support relatively long periods of soil drought associated with high atmospheric evaporative demand. Such behaviour would be advantageous, allowing for maximization of WUE and survival as soil water availability decreases. In this case, stomatal sensitivity to soil drying should be negatively associated with the stability of crop yield under rainfed conditions. However, stomata of mature field-grown trees may not respond to soil water limitations as readily and dramatically as those of the young plants in this study with less expanded root systems (Gucci et al., 1996). If so, a deeper root system compensating for water loss during the day would be of paramount importance. Of course, this should be possible if the plant maintains a sufficient $K_L$, as appears to be the case with clone 120. In this clone, the combination of deep roots with relatively large $K_L$ (this study), better protection against oxidative stress (Pinheiro et al., 2004), and maintenance of capacity for sucrose synthesis—and possibly export, allowing extra root growth (Praxedes et al., 2005)—under drought, should contribute to dampen variations in its productivity, as has been observed in test-trials under rainfed conditions (Ferrão et al., 2000a). In any case, clones with better yield stability under drought (120), or better able to survive drought episodes through a more conservative use of water (14), may be of greater value than clones selected for improved environments (46 and 109A), particularly under low-input conditions typical of many farming systems of drought-prone regions (DaMatta, 2004b).

![Figure 6](image-url)
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LITERATURE CITED


