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PHYSIOLOGICAL AND MORPHOLOGICAL ADAPTATIONS AS ASSOCIATED WITH DROUGHT TOLERANCE IN ROBUSTA COFFEE (*Coffea canephora* PIERRE VAR. *kouillou*)

Thesis submitted to Federal University of Viçosa, as part of the requirements for obtaining the *Doctor Scientiae* degree in Plant Physiology

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This work is dedicated to my parents Manoel and Lourdes,

To my brothers Cristina and Bruno,

To my grand-mother Yolanda,

To the memory of my grand-parents Joaquim, Duquinha and Glória,

It is also dedicated to the memory of my friend Gustavo “Calouro”.

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BIOGRAPHY

Hugo Alves Pinheiro was born in Belém-Pa, Brazil, on August 10, 1974. In 1998, he concluded his BSc course in Agricultural Sciences at the Federal Rural University of Amazônia, Belém-Pa. On February 2000, he obtained his *Master Scientiae* degree in Plant Physiology at the Federal University of Viçosa, Viçosa-MG, Brazil. On March 2000, he started his doctoral studies in Plant Physiology Program at Federal University of Viçosa.

CONTENT

ABSTRACT

PINHEIRO, Hugo Alves, D.S. Federal University of Viçosa, February, 2004. **Physiological and morphological adaptations as associated with drought tolerance in robusta coffee (*Coffea canephora* Pierre var. *kouillou*).** Major Advisor: Fábio Murilo DaMatta. Advisors: Elizabeth Pacheco Batista Fontes and Marcelo Ehlers Loureiro

Clones of *Coffea canephora* Pierre var. *kouillou* with contrasting tolerance to drought stress have been chosen on the basis of their productivities under rainfed conditions. As little is known about physiological mechanisms associated with differences in drought tolerance in those clones, this work aimed to examine morphological traits, stomatal responses to both soil and atmosphere drought, water relations, water-use efficiency (WUE) and, in addition, whether drought tolerance in *C. canephora* may be linked to protection against oxidative damage. For these purposes, four clones of *C. canephora* representing drought-tolerant (14 and 120) and drought-sensitive (46 and 109A) genotypes were grown under screen house conditions, in 120 L pots, during eight months. Drought stress was imposed by withholding irrigation until leaf water potential at predawn (Ψ_{pd}) reached about –3.0 MPa. Under full irrigation, soil-to-leaf hydraulic conductance (K_L), midday leaf water potential and total biomass were all greater in clones 109A and 120 than in the other clones. After 14 days without irrigation, Ψ_{pd} decreased significantly in clone 109A in comparison with the other clones; seven days latter, Ψ_{pd} dropped to about –2.3 MPa in clones 46 and 109A, against –0.8 MPa in clone 14 and –1.7 MPa in clone 120. Clone 109A attained –3.0 MPa at predawn earlier, followed by clone 46, clone 120, and then clone 14, in this order. Under drought stress, there was no elastic adjustment, while a slight osmotic adjustment was only noted in the clone 109A. Stomatal conductance (g_s) was strongly decreased with decreasing Ψ_{pd}; it declined modestly with increasing leaf-to-air vapour pressure deficit. Stomatal sensitivity to both soil and atmospheric drought was lower in clone 109A and similar among the other clones. Drought stress led to a significant increase in carbon isotope composition ($\delta^{13}C$) for all

clones, suggesting an increased WUE; however, absolute values of $\delta^{13}C$ were lower in clone 109A than in the other clones irrespective of the irrigation treatments. Clones 14 and 120 exhibited deeper root systems than drought-sensitive clones. This at least partially explain their better avoidance to drought as compared with the sensitive clones. On average, the larger K_L in clone 120 than in clone 14 might largely explain why the latter was better able to postpone dehydration. For all clones, water potential, g_s and K_L recovered rapidly following re-watering; these facts, associated with the remarkable stomatal sensitivity to drought, should explain greatly why *C. canephora* responds strongly to irrigation. Independently of the clone examined, little or no effect of drought on the quantum yield of electron transport, photosystem II photochemical efficiency and photochemical and non-photochemical quenching coefficients was observed. Comparatively, the clone 120 showed a more tolerant photosynthetic apparatus to both drought and paraquat-induced oxidative stress, with no clear distinction among the other clones in this regard. Drought triggered increases in superoxide dismutase (clones 109A and 120), ascorbate peroxidase (clones 14, 46 and 109A), catalase and guaiacol peroxidase (clones 46 and 109A), and glutathione reductase (clone 46). Monodehydroascorbate reductase and dehydroascorbate reductase were not induced in drought-stressed plants; their maximal activities were much lower than that of ascorbate peroxidase, irrespective of the clone investigated. Oxidative damage, however, appeared to be evident only in clone 109A. In general, the clones herein investigated were able to preserve, or even to increase, their antioxidant defences at water potentials as low as –3.5 MPa. The combination of mechanisms that effectively postpone dehydration, associated with deep root systems, should contribute to survival and/or stability of crop yield of drought-tolerant clones in regions with unpredictable precipitation. Attributes such as osmotic and elastic adjustments and protection against oxidative damage induced by drought should be of minor importance to drought tolerance in this specie.

RESUMO

PINHEIRO, Hugo Alves, D.S. Universidade Federal de Viçosa, Fevereiro de 2004. **Adaptações fisiológicas e morfológicas associadas à tolerância à seca em café robusta (*Coffea canephora* Pierre var. *kouillou*)**. Orientador: Fábio Murilo DaMatta. Conselheiros: Elizabeth Pacheco Batista Fontes e Marcelo Ehlers Loureiro

Clones de *Coffea canephora* Pierre var. *kouillou* com tolerância diferencial à seca vêm sendo selecionados com base em diferenças de produtividade, em condições de déficit hídrico. Porém, pouco se sabe sobre os mecanismos fisiológicos determinantes de tal tolerância. Objetivou-se, assim, examinar características morfológicas, as respostas estomáticas ao déficit hídrico do solo e da atmosfera, as relações hídricas, a eficiência do uso da água (E_A) e, também, examinar uma possível relação entre tolerância à seca e proteção contra o estresse oxidativo. Para isso, quatro clones (46 e 109A, sensíveis; 14 e 120, tolerantes à seca) foram cultivados em casa de vegetação, em vasos de 120 L, durante oito meses. O déficit hídrico foi imposto via suspensão da irrigação, até que o potencial hídrico na antemanhã (Ψ_{am}) atingisse –3,0 MPa. Os clones 109A e 120, sob irrigação, apresentaram maior condutância hidráulica entre a raiz e a parte aérea (K_L), maior potencial hídrico ao meio-dia e maior acúmulo de biomassa. Após 14 dias de suspensão da irrigação, Ψ_{am} foi significativamente mais negativo no clone 109A que nos outros clones; sete dias após, Ψ_{am} decresceu para –2,3 MPa nos clones sensíveis à seca, contra –0,8 MPa e –1,7 MPa nos clones 14 e 120, respectivamente. O clone 109A foi o primeiro a atingir –3,0 MPa na antemanhã, seguido pelo clone 46, clone 120 e, por fim, pelo clone 14. Não foi observado ajustamento elástico em nenhum dos clones, enquanto um ajuste osmótico de pequena magnitude foi limitado ao clone 109A, sob condições de seca. A condutância estomática (g_s) foi reduzida fortemente em resposta aos decréscimos em Ψ_{am} e, em menor extensão, aos incrementos no déficit de pressão de vapor entre folha e atmosfera. A sensibilidade estomática à seca, tanto do solo quanto da atmosfera, foi menor no clone 109A e similar entre os demais clones. A composição isotópica do carbono ($\delta^{13}C$) aumentou

significativamente, sob seca, em todos os clones, sugerindo incrementos em E_A; o clone 109A, entretanto, apresentou valores mais negativos de $\delta^{13}C$, independentemente do regime de irrigação. A profundidade do sistema radicular foi substancialmente maior nos clones tolerantes que nos sensíveis à seca. Isso pode explicar, pelo menos em parte, a manutenção de um status hídrico mais favorável nos clones tolerantes. O maior valor médio de K_L no clone 120, sob seca, poderia explicar as diferenças de status hídrico entre ele e o clone 14. Em todos os clones, g_s, K_L e o potencial hídrico recuperaram-se rapidamente após a re-irrigação das plantas sob déficit hídrico; isso, aliado à forte sensibilidade estomática à seca, pode estar associado à resposta notável dessa espécie à irrigação. Independentemente do clone estudado, a seca pouco ou nada afetou o transporte de elétrons, a eficiência fotoquímica do fotossistema II e os coeficientes de extinção fotoquímico e não-fotoquímico. Comparativamente, o clone 120 apresentou maior tolerância de seu aparelho fotossintético ao estresse oxidativo mediado pela seca ou por ação do paraquat, com poucas diferenças observadas entre os demais clones nesse contexto. Sob seca, observaram-se incrementos significativos nas atividades da dismutase do superóxido (clones 109A e 120), peroxidase do ascorbato (clones 14, 46 e 109A), catalase e peroxidase do guaiacol (clones 46 e 109A), e redutase da glutationa (clone 46). As atividades da redutase do monodesidroascorbato e da redutase do desidroascorbato não foram afetadas pelos tratamentos aplicados; as atividades dessas enzimas foram substancialmente menores que a da peroxidase do ascorbato. O déficit hídrico acarretou danos oxidativos apenas no clone 109A. De modo geral, os clones avaliados foram capazes de manter, ou mesmo de aumentar, a atividade de seus sistemas de defesa contra danos oxidativos, mesmo a potenciais hídricos da ordem de –3,5 MPa. Em suma, a combinação de mecanismos que efetivamente restringem a perda d'água, associada a sistemas radiculares profundos, deve ser decisiva para a sobrevivência e, ou, relativa estabilidade da produção dos clones de *C. canephora* tolerantes à seca, quando cultivados em ambientes sujeitos a secas prolongadas. Atributos como ajustes osmótico e elástico e proteção contra danos oxidativos mediados pela seca teriam uma importância secundária na determinação da tolerância à deficiência hídrica nessa espécie.

GENERAL INTRODUCTION

Among more than 90 species of *Coffea*, only *C. arabica* L. and *C. canephora* Pierre (robusta coffee) are economically important worldwide. The volume of business generated from robusta on the international coffee market has been steadily increasing for half a century, and presently this species accounts for about 35% of the coffee consumed worldwide. Robusta coffee is believed to have evolved in a lowland forest of the Congo river basin (Africa), with a typical equatorial climate in which average temperature is between 24 and 26 ^{o}C, and with abundant rainfall distributed over a 9-10 month period, and atmospheric humidity at a nearly constant level approaching saturation (Coste 1992, Willson 1999). Under plantation schemes, a necessary minimum annual rainfall of 1250 mm, or even 1550 mm, has been quoted, but this would require an even distribution (Coste 1992).

The most cultivated variety of *C. canephora* in the world is *robusta* and, thus, it designates the common name of this specie. However, in the main Brazilian area producing *C. canephora* (Espírito Santo State), *kouillou* is the single variety cropped. Despite a large similarity between these two varieties, *kouillou* differs from *robusta* particularly by presenting longer leaves and smaller fruits and beans. Furthermore, *kouillou* is considered to be more resistant to drought than *robusta* (Coste 1992). In Espírito Santo State, there is an increasing trend in expanding cultivation towards marginal and degraded area lands where water availability constitutes the bottleneck to crop yield. In these areas, during the dry season, which lasts from four to seven months, evaporation by far exceeds rainfall and, within the rainy season, rainfall pattern and amounts are to a great extent unpredictable. Such conditions could be expected to largely restrict the productive potential of robusta coffee. Local experience has shown that crop yield under rainfed conditions may decrease by about 40% or even as much as by 80% in dry years. Irrigation has been successfully employed, enabling the plant to be a profitable crop and preventing crop failure in dry years. However, costs involved for purchasing and installing irrigation equipment are rather expensive and immediately pose a problem for the profitability of its use, particularly in small farms where robusta coffee is

mostly cultivated. In addition, a permanent, reliable source of water for irrigation is not always available.

Robusta coffee is an obligate cross-pollination species and, therefore, a wide population variation in morphological/physiological traits to cope with drought is to be expected (DaMatta and Rena 2001). Plant breeders of INCAPER (Institute for Research and Rural Assistance of the State of Espírito Santo) have successfully explored such genetic variability, so that clones of robusta coffee with superior performance under rainfed conditions have been recently selected, what may render coffee cultivation less dependent on irrigation. Nonetheless, such selection has been conducted largely in terms of saleable crop (e.g. Ferrão et al 2000*a*, *b*), without physiologically examining how clones respond to soil drought.

The ability for a crop to produce satisfactorily in areas subjected to water deficit has been termed drought tolerance regardless of how the ability develops (Kramer and Boyer 1995). Plants may develop morphological and/or physiological mechanisms to cope with a limited water conditions. These involve maximisation in water uptake by growing deep roots and/or minimisation of water loss by way of an effective stomatal closure and reduced leaf area (Kramer and Boyer 1995), improving plant water status and turgor maintenance. In any case, in the field, water deficit is generally combined with high solar radiation, triggering an enhanced production of reactive oxygen species, which may result in oxidative damage to cell compartments, especially chloroplasts (Smirnoff 1995) and, ultimately, in decreases in crop yield. To protect the photosynthetic apparatus from oxidative stress, plants must dissipate excess light energy. This can be achieved by down-regulation of the photochemical efficiency by way of the xanthophyll cycle (Demming-Adams et al. 1996) or by maintenance of electron flux involving alternative routes such as photorespiration and the Mehler-peroxidase pathway (Asada 1999, Ort and Baker 2002). Morphological adaptations such as leaf rolling and alterations in leaf orientation can also contribute to decrease photo-inhibitory damage and excessive transpiration.

In this work, it were explored the extent and mechanisms of intra-specific variation of water use in *C. œanephora* var. *kouillou* by examining how stomatal behaviour and leaf water relations are adjusted to changes in soil water supply and evaporative demand, and how differences in drought tolerance is associated with morphological characteristics such as root depth and leaf area. In addition, it was also examined whether differences in clonal tolerance to drought stress could be associated with an enhanced protection of the photosynthetic apparatus against oxidative stress. For these purposes, drought-tolerant genotypes (clones 14 and 120) and drought-sensitive ones (clones 46 and 109A) of robusta coffee were compared.

These clones have been shown to produce a good crop when grown under irrigation; under limited soil water, however, both survival and productivity (Ferrão et al. 2000*a, b*) as well as maintenance of tissue water status (DaMatta et al. 2000, 2003) are impaired to a greater extent in the drought-sensitive than in the drought-tolerant clones. The clones were grown in large containers in an attempt to simulate a natural development of drought down a root profile, so that the plants could acclimate to decreasing soil water availability.

REFERENCES

Asada, K., The water-water cycle in chloroplasts: scavenging of active oxygen and dissipation of excess photons, Annu. Rev. Plant Physiol. Plant Mol. Biol. 50 (1999) 601-639.

Coste, R., Coffee: The plant and the product, MacMillan, London, 1992, 328p.

DaMatta, F.M., J.S.M. Silveira, C. Ducatti, M.E. Loureiro, Eficiência do uso da água e tolerância à seca em *Coffea canephora*, *In*: I Simpósio de Pesquisa dos Cafés do Brasil, Vol. II, EMBRAPA, Brasília, 2000, pp 907-910.

DaMatta, F.M., A.B. Rena, Tolerância do café à seca, *In*: L. Zambolim, (Ed.), Tecnologias de Produção de Café com Qualidade, Universidade Federal de Viçosa, Viçosa, 2001, pp. 65-100.

DaMatta, F.M., A.R.M. Chaves, H.A. Pinheiro, C. Ducatti, M.E. Loureiro, Drought tolerance of two field-grown clones of *Coffea canephora*, Plant Sci. 164 (2003) 111-117.

Demming-Adams, B., W.W. Adams, D.H. Baker, B.A. Logan, D.R. Bowling, A.S. Verhoeven, Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation, Physiol. Plant. 98 (1996) 253-264.

Ferrão, R.G., A.F.A. Fonseca, M.A.G. Ferrão, L.P. Santos, Avaliação de clones elites de café Conilon em condição de estresse hídrico no estado do Espírito Santo, *In*: I Simpósio de Pesquisa dos Cafés do Brasil, Vol. I, EMBRAPA, Brasília, 2000*a*, pp 402-404.

Ferrão, R.G., A.F.A. Fonseca, M.A.G. Ferrão, L.P. Santos, Comportamento de clones elites de café Conilon em condições de alta tecnologia no estado do Espírito Santo, *In*: I Simpósio de Pesquisa dos Cafés do Brasil, Vol. II, EMBRAPA, Brasília, 2000*b*, pp 769-771.

Kramer, P.J., J.S. Boyer, Water Relations of Plants and Soils, Academic Press, San Diego, 1995, 495p.

Ort, D.R., N.R. Baker, A photoprotective role for O_2 as an alternative electron sink in photosynthesis? Curr. Opin. Plant Biol. 5 (2002) 193-198.

Smirnoff, N., Antioxidant systems and plant response to the environment, *In*: N. Smirnoff, (Ed.), Environment and Plant Metabolism – Flexibility and Acclimation, BIOS Scientific Publishers, Oxford, 1995, pp 217-243.

Willson, K.C., Coffee, Cocoa and Tea, CABI Publishing, Cambridge, 1999, 300 p.

CHAPTER 1

Drought tolerance is associated with root depth and stomatal control of water use in clones of *Coffea canephora* Pierre[1]

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Summary Physiological and morphological bases of drought tolerance in robusta coffee (*Coffea canephora* Pierre var. *kouillou*) were investigated. Four clones representing drought-tolerant (14 and 120) and drought-sensitive (46 and 109A) genotypes were grown in 120-l containers under screen house conditions. Drought was imposed by withholding watering until xylem pressure potential (Ψ_x) reached about –3.0 MPa at predawn. Under full irrigation, soil-to-leaf hydraulic conductance (K_L), midday Ψ_x and total biomass were all greater in clones 109A and 120 than in the other clones. For all clones, dehydration postponement was more important than dehydration tolerance. This was associated with (i) strong stomatal sensitivity to soil drying and, to a lesser extent, to leaf-to-air vapour pressure deficit; (ii) increases in leaf carbon isotope composition in stressed plants, suggesting higher water-use efficiency; and (iii) relatively high modulus of elasticity. Dehydration postponement was less developed in drought-sensitive clones than in drought-tolerant ones, which might in part to be

associated with shallower root systems in the former than in the latter. Clone 109A had the least conservative water-use characteristics and, thus, it showed a faster decline in Ψ_x than clone 46 after suspending irrigation. On average, the larger K_L in clone 120 than in clone 14 might explain why the latter was better able to postpone dehydration. There was no evidence of elastic adjustment in response to drought, whereas osmotic adjustment was small and limited to clone 109A. For all clones, Ψ_x, g_s and K_L recovered rapidly following re-watering. The combination of mechanisms that effectively postpone dehydration, associated with deep root systems, should contribute to survival and/or stability of crop yield of drought-tolerant clones in regions with unpredictable rainfall.

Keywords: carbon isotope composition, coffee, dehydration postponement, elastic and osmotic adjustments, leaf-to-air vapour pressure deficit, water potential, water relations, water-use efficiency.

Introduction

For cultivated plants tolerance to drought is generally considered as the potential for a particular species or variety to yield more in comparison to others under limited soil water conditions (Jones 1992). Plants may develop morphological and/or physiological mechanisms to cope with a limited water supply. These involve maximisation in water uptake by growing deep roots and/or minimisation of water loss by way of an effective stomatal closure and reduced leaf area (Kramer and Boyer 1995), improving plant water status and turgor maintenance. Turgor maintenance, which provides the potential for keeping physiological activity for extended periods of drought, may be achieved through an osmotic adjustment and/or changes in cell wall elasticity (Kramer and Boyer 1995, Turner 1997).

Robusta coffee (*Coffea canephora* Pierre), which accounts for about 35% of the coffee consumed worldwide, is native to typical African equatorial climate zone with abundant rainfall distributed over a 9-10 month period, and atmospheric humidity at a nearly constant level approaching saturation (Willson 1999). For this reason, robusta is thought to have evolved as a water-profligate species (DaMatta and Rena 2001). However, in Brazil it has been largely cultivated in marginal areas where water availability constitutes the major environmental constraint affecting crop production. Consequently, irrigation has been indispensable for production in those areas where even short periods of drought can largely decrease coffee yields.

Robusta coffee is an obligate cross-pollination species and, therefore, a wide population variation in morphological/physiological traits to cope with drought is to be expected (DaMatta and Rena 2001). Plant breeders have successfully explored such genetic variability, so that clones of robusta with superior performance under rainfed conditions have been recently selected, what may render coffee cultivation less dependent on irrigation. Nonetheless, such selection has been conducted largely in terms of harvestable crop (e.g., Ferrão et al 2000*a*, *b*), without examining physiologically how clones respond to soil drought.

In this work, drought-tolerant genotypes (clones 14 and 120) and drought-sensitive ones (clones 46 and 109A) of robusta coffee were compared. These clones have been shown to produce a good crop when grown under irrigation; under limited soil water, however, both survival and productivity (Ferrão et al. 2000*a, b*) as well as maintenance of tissue water status (DaMatta et al. 2003, Pinheiro et al. 2004) are impaired to a greater extent in the drought-sensitive than in the drought-tolerant clones. Comparatively, however, maintenance of water status is better, but stability of crop yield is poorer, in clone 14 than in clone 120. An initial objective of this study was to identify mechanisms of intra-specific variation of water use by examining how stomatal behaviour and leaf water relations are adjusted to changes in soil water supply and evaporative demand. A second objective was to assess whether differences in drought tolerance is associated with morphological characteristics such as root depth and leaf area. For these purposes, morphological traits, leaf xylem pressure potential (Ψ_x), stomatal conductance (g_s), hydraulic conductance (K_L), stable carbon isotope composition, $\delta^{13}C$ (to study integrated plant water use; Farquhar et al. 1989), and water relations parameters derived from pressure-volume curves were evaluated. The clones were grown in large containers in an attempt to simulate a natural development of drought down a root profile, so that the plants could acclimate to water shortage. Leaf water relations were examined at a similar internal water status, thus allowing more reliable comparisons among clones to be made.

Materials and methods

General

Clones of *C. canephora* Pierre var. *kouillou* (known in Brazil as Conilon) raised as rooted stem cuttings were obtained from the Institute for Research and Rural Assistance of Espírito Santo State (INCAPER), Brazil. Forty plants (10 per each clone) were grown under screen house conditions in Viçosa (20°45' S, 650 m a.s.l.), southeastern Brazil, with an average midday photosynthetic photon flux about 900 μmol m^{-2} s^{-1}, in large containers (0.8 m high,

0.44 m internal diameter) filled with an 120 l mixture of soil, sand and hardened manure (3:1:1, v/v/v) and a gravel layer at the bottom. The walls of the screenhouse consisted of coarse mesh screen, which permitted free exchange of air with the external environment. When plants were 12-months old, water deficit was imposed by withholding watering until Ψ_x at predawn (Ψ_{pd}) reached about –3.0 MPa. Throughout the dry-down period, five sets of measurements of Ψ_x and g_s were performed using leaves from the third or fourth pair from the apex of plagiotropic branches. Once the desired Ψ_{pd} was reached, expanding leaves (less than half of their final size) were collected and their $\delta^{13}C$ was estimated; expanded leaves were also collected for obtaining pressure-volume relationships. All the plants were then irrigated (at about 1800 h) and Ψ_x and K_L were estimated in the following two consecutive days (Ψ_{pd} measured at about 12 and 36 h after irrigation).

Biometric measurements

Three branches per tree were tagged when water was withheld, and their growth was estimated as the difference between initial and final length, as measured 21 days latter. At the end of the experiment, well-watered plants were harvested and separated into aboveground 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 were also measured.

Water relations

Xylem pressure potential was measured at predawn (0430-0530 h), between 0700-0900 h and at midday (Ψ_{md}) using a Scholander-type pressure chamber. To obtain pressure-volume curves, fully expanded leaves were collected by cutting their petioles under deionised water and brought to the laboratory. This was done at about 12 h after irrigating both control and drought-stressed plants (Ψ_x typically above –0.10 MPa). Subsequently, fresh weight and Ψ_x were taken at intervals during dehydration process (free transpiration technique) until a Ψ_x of about –3.5 MPa was reached. Turgid weight was estimated from the linear relationship between fresh weight and Ψ_x in the positive turgor range, by extrapolating to $\Psi_x = 0$. The inverse of Ψ_x was plotted as a function of relative water content (RWC). From the pressure-volume curves, the osmotic potential at full ($\Psi_{\pi(100)}$) and zero ($\Psi_{\pi(0)}$) turgor, RWC at zero

turgor ($RWC_{(0)}$) and the bulk modulus of elasticity (ε; Melkonian et al. 1982) were estimated. Further details are described in 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% air relative humidity) and between 1100 and 1300 h (30 ± 2 °C air temperature, 80 ± 2% air relative humidity).

Leaf specific hydraulic conductance from soil to leaf ($K_L = (g_s \times \Delta w)/(\Psi_{pd} - \Psi_{md})$) was calculated using Ψ_{pd} to approximate soil water potential, and g_s and Δw (leaf-to-air vapour pressure deficit, estimated according to Landsberg (1986)), measured at the same time as Ψ_{md} (Hubbard et al. 1999, Donovan et al. 2000).

Carbon isotope composition

Leaf $\delta^{13}C$ was measured relative to the international PBD 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

The experiment was set up in a completely randomised design, with eight treatment-combinations, forming a 4 x 2 factorial (four clones and two watering regimes) with five plants per treatment-combination. The experimental plot was constituted by one plant per container. Significant differences among 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. The relationship between g_s and Ψ_{pd} was non-linear and data were natural log transformed for the analysis. Based on regression analysis, ln g_s was found to be significantly linearly related to Ψ_{pd}.

A test for the equality of the regression models was performed using the indicator variable technique (Neter and Wasserman 1974), at $P \leq 0.05$. Regression models for clones 14, 46 and 120 did not differ statistically to each other. Therefore, data for these clones were pooled together and single regressions were fitted to the lumped data.

Results

The selected clones (14 and 120, drought-tolerant; 46 and 109A, drought-sensitive) could be grouped into two types of contrasting canopy morphologies, with clones 109A and 120 being taller (Table 1) with less dense crowns than clones 14 and 46. There was no significant difference in both total leaf area and specific leaf area among the clones investigated, but total dry matter and root biomass were greater in clones 109A and 120 than in clones 14 and 46, whereas root to shoot biomass ratio were larger in clone 109A than in the other clones (Table 1). In any case, the drought-tolerant clones exhibited a considerably deeper (Table 1), more regularly distributed root system down a pot profile than the drought-sensitive clones (not shown).

For control plants, Ψ_{pd} was always above –0.08 MPa. In these plants, average Ψ_{md} tended to be lower in clones 14 and 46, respectively –0.89 and –1.06 MPa, against –0.65 MPa in clones 109A and 120 (Figure 1). On average, K_L tended to be higher in these clones (about 3.0 mmol m^{-2} s^{-1} MPa^{-1}) than in clones 14 and 46 (about 1.7 mmol m^{-2} s^{-1} MPa^{-1}). Under drought stress, average K_L tended to be greater in clone 120 than in the other clones (Figure 2).

Table 1. Morphological characteristics of one-year-old clones of robusta coffee under full irrigation. Different letters denote significant differences among clonal means ($P \leq 0.05$; Newman-Keuls test). Each value represents the mean ± SE of five replicates.

Parameters	Drought-tolerant clones		Drought-sensitive clones	
	Clone 14	Clone 120	Clone 46	Clone 109A
Shoot height, m	0.78 ± 0.05 a	0.94 ± 0.03 b	0.73 ± 0.07 a	0.92 ± 0.02 b
Leaf area, m^2	1.89 ± 0.11 a	2.51 ± 0.13 a	1.91 ± 0.08 a	2.36 ± 0.10 a
Root to shoot ratio	0.69 ± 0.02 a	0.71 ± 0.04 a	0.74 ± 0.07 a	0.89 ± 0.03 b
Total biomass, g	434 ± 21 b	645 ± 31 a	455 ± 59 b	744 ± 58 a
Root dry mass, g	176 ± 9 b	268 ± 16 ab	187 ± 18 b	351 ± 34 a
Root depth, m	0.76 ± 0.03 a	0.75 ± 0.04 a	0.48 ± 0.04 b	0.53 ± 0.03 b

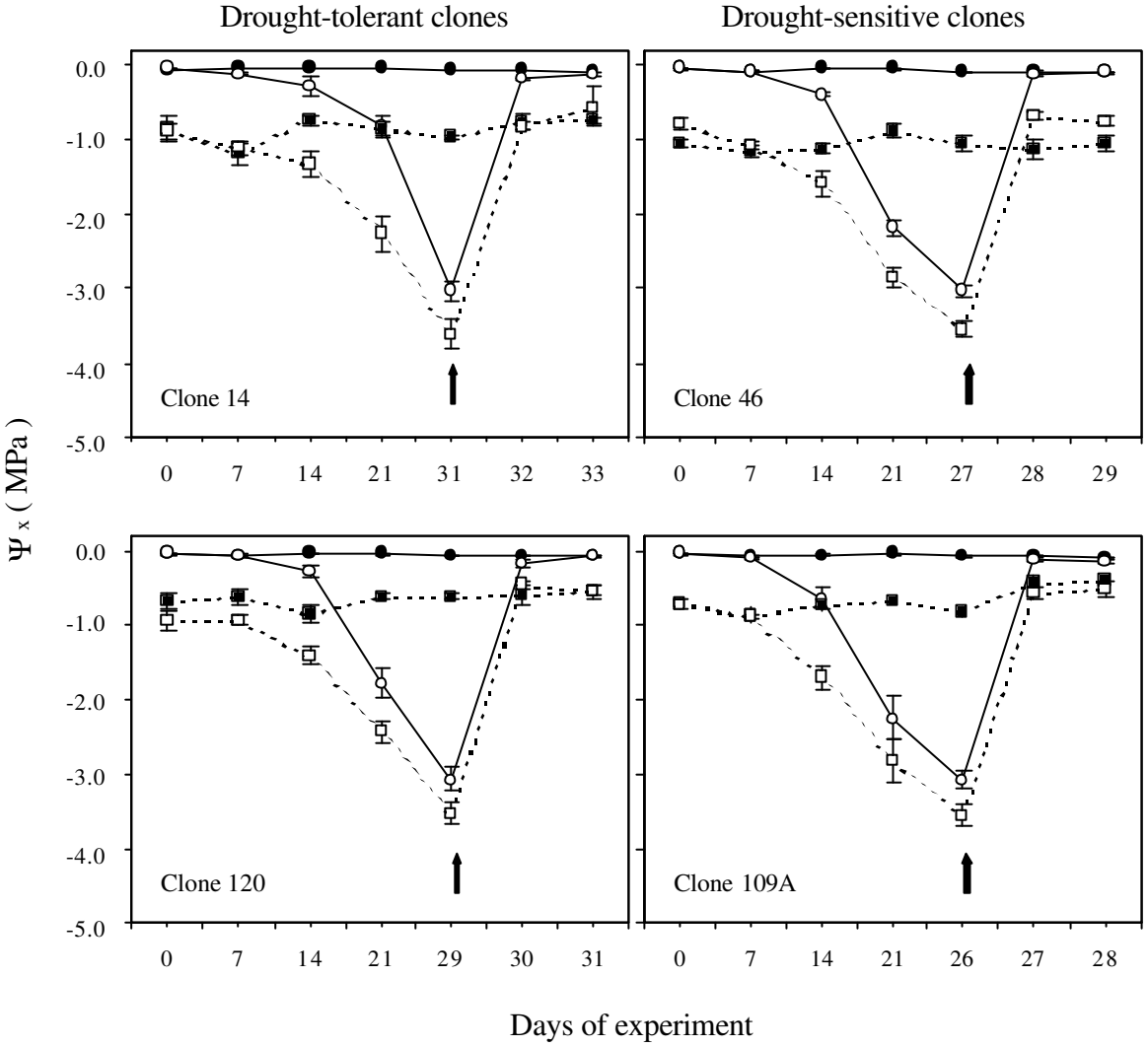

Figure 1. Time course of leaf xylem pressure potential (Ψ_x) at both predawn (circles) and midday (squares) of four clones of robusta coffee subjected to full irrigation (solid line) and drought conditions (dotted line). Arrows indicate when predawn Ψ_x reached –3.0 MPa, occasion when the drought-stressed plants were re-watered (at 1800 h); measurements were then made for more two days. Note differences in scale in abscissa. Each point represents the mean ± SE of five replicates.

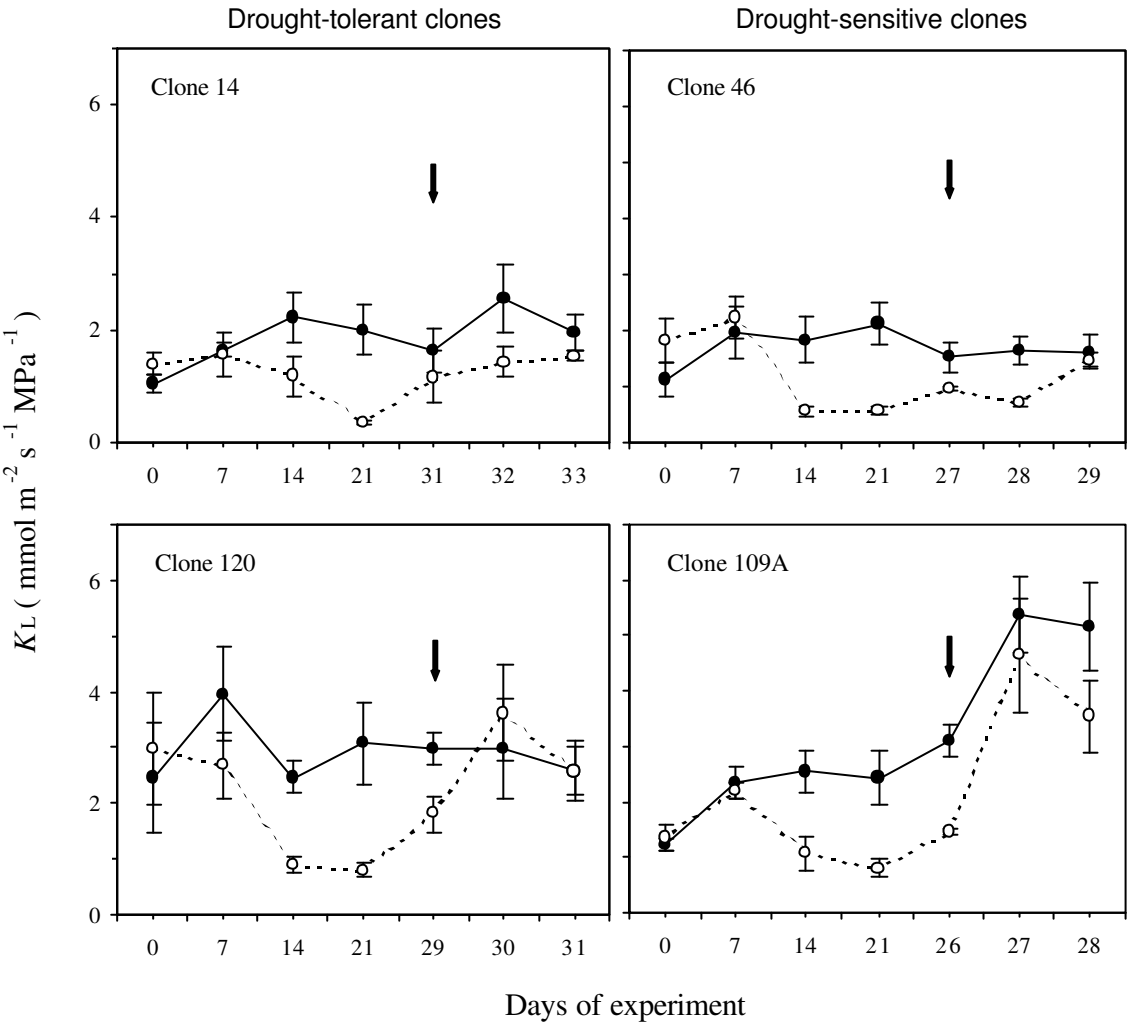

Figure 2. Time course of leaf specific hydraulic conductance from soil to leaf (K_L) of four clones of robusta coffee subjected to full irrigation (solid circles) and drought conditions (open circles). See details in Figure 1.

After withholding irrigation for 14 days, Ψ_{pd} was significantly lower in clone 109A than in the other clones; seven days latter, Ψ_{pd} dropped to about –2.3 MPa in clones 46 and 109A, against – 0.8 MPa in clone 14 and –1.7 MPa in clone 120. A similar trend, but obviously shifted to lower values, was found for Ψ_{md} (Figure 1). As could be expected from the above, clone 109A attained –3.0 MPa at predawn earlier, followed by clone 46, clone 120, and then clone 14 in this order (Figure 1). A prompt recovery of Ψ_x and K_L was found for all clones in the first two days upon re-watering (Figures 1 and 2).

Stomatal conductance decreased curvilinearly as Ψ_{pd} declined (Figure 3). Stomatal sensitivity to changes in Ψ_{pd} was similar among clones 14, 46 and 120, but lower in clone 109A than in the other clones, as indicated by the slope of regression lines. Similar regression models were found when g_s was associated with Ψ_x, both variables measured on a same leaf at 0700-0900 h (not shown).

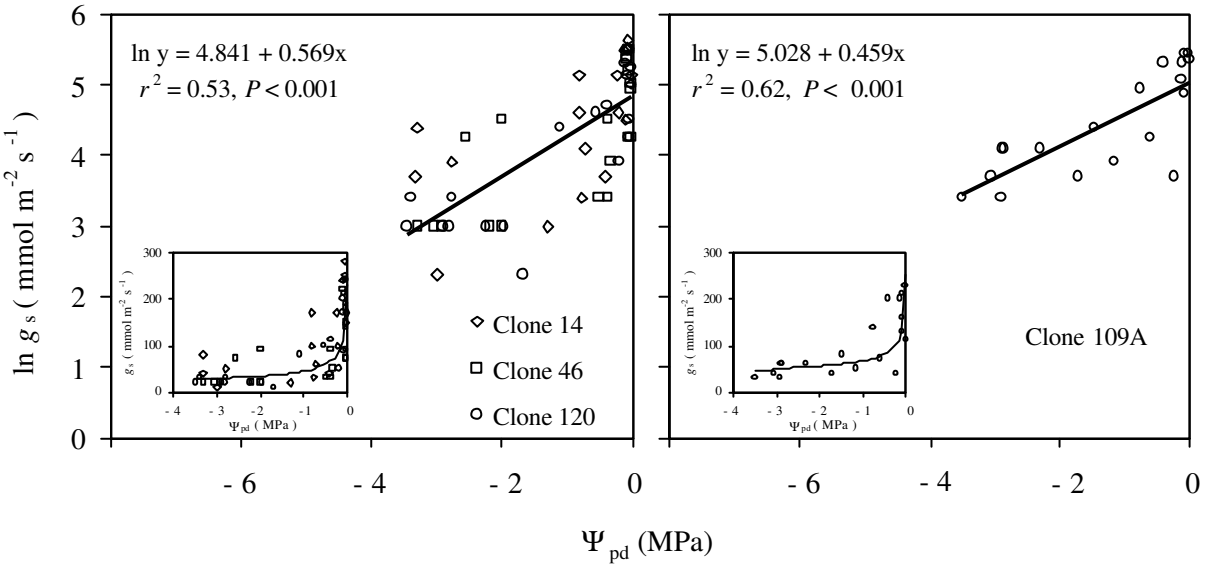

Figure 3. Stomatal conductance (g_s) in relation to leaf xylem pressure potential at predawn (Ψ_{pd}) in four clones of robusta coffee. Values of g_s were taken at 0700-0900 h and represent the entire data set from plants subjected to a dehydrating cycle after selecting a narrow range of leaf-to-air vapour pressure deficit (1.5 kPa at most). The data were fitted by a power function of the form $y = ax^b$ by nonlinear regression in the insets, and by linear regression in the main panels. The explained variance (r^2) was similar for both linear and nonlinear regressions.

As Δw increased, g_s decreased linearly in a similar way in clones 14, 46 and 120. By contrast, there was no relationship in clone 109A between g_s and Δw (Figure 4). In spite of Δw and leaf temperature being greatly associated to each other (r^2 = 0.920, P < 0.001), co-variation analysis revealed no direct effect of leaf temperature in the response of g_s to Δw (P = 0.173).

Clonal differences in drought tolerance could hardly be associated with either or both osmotic and elastic adjustments, since $\Psi_{\pi(100)}$, $\Psi_{\pi(0)}$ and ε did not differ significantly among clones under full irrigation or drought conditions, with exception of the clone 109A, in which drought resulted in a slight, significant decrease (0.19 MPa) in $\Psi_{\pi(0)}$ (Table 2). Irrespective of treatments, quite constant and similar values for $RWC_{(0)}$ (89.8 ± 0.5%) were found (Table 2).

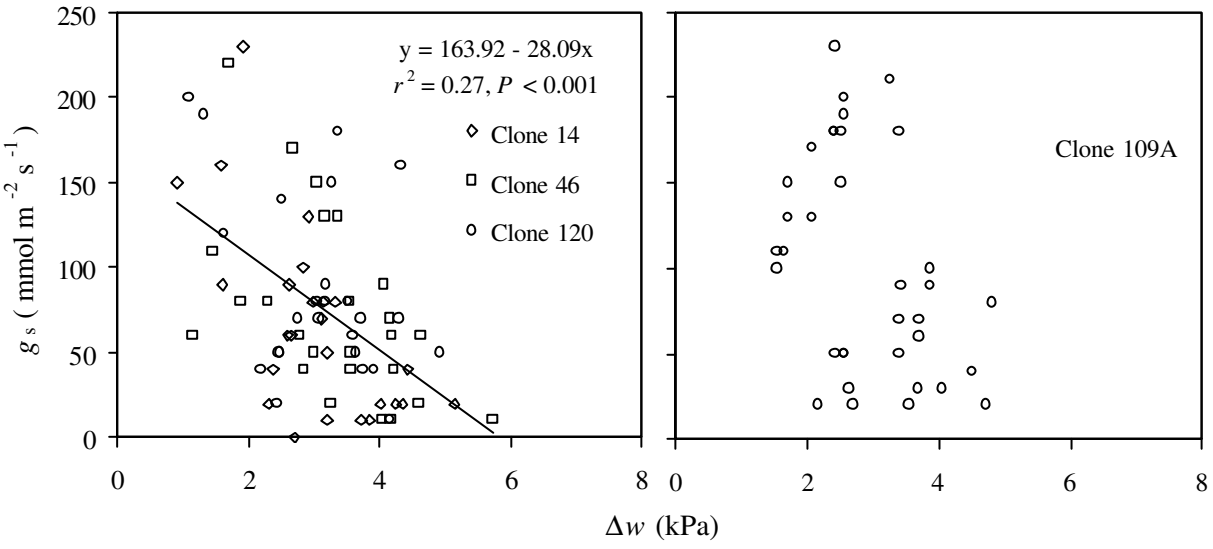

Figure 4. Stomatal conductance (g_s) in relation to leaf-to-air vapour pressure deficit (Δw) in four clones of robusta coffee under irrigated conditions. Data were obtained over several days under conditions of naturally fluctuating Δw; they were collected at 1100–1300 h in plants grown under full irrigation to insure a comparable internal water status (xylem pressure potential = -0.8 ± 0.2 MPa). For clone 109A, relationship between g_s and Δw was not significant.

After imposing water deficit, $\delta^{13}C$ increased significantly (1.48 to 2.22‰; Figure 5) in all clones, suggesting an increased long-term water-use efficiency (WUE). Nonetheless, absolute values of $\delta^{13}C$ were lower in clone 109A than in the other clones irrespective of the irrigation treatments. There was no difference in $\delta^{13}C$ among clones 14, 46 and 120 (Figure 5). Overall, g_s decreased to a greater extent than net carbon assimilation rate (data not shown) and, thus, adjustments in $\delta^{13}C$ should have been predominantly from changes in g_s.

Under continuous irrigation, clone 109A showed a larger branch growth (28%) than the averaged growth of the other clones, in which branch growth did not differ regardless of the watering treatments (Figure 6). Under drought conditions, branch growth decreased by about 45% in drought-sensitive clones against a 31% reduction in clone 14 and 37% in clone 120.

Discussion

Plant water stress developed more slowly in the drought-tolerant than in the drought-sensitive clones. Morphological traits such as leaf area, root mass or root to shoot mass ratio were not associated with that response. Instead, the much deeper root system of the tolerant clones should have enabled them to gaining a greater access to soil water and, therefore, maintaining 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. Differences in dehydration postponement were also linked to stomatal control on transpiration (see below).

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 Ψ_x (as indicated by higher Ψ_{md} values) than in clones 14 and 46, which may help to avoid limitations to photosynthesis. As a consequence, the clones 109A and 120 might have achieved a greater carbon gain, which would to some extent explain their greater biomass accumulation. 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.

Stomatal conductance decreased sharply with decreasing Ψ_x, with no apparent threshold value of Ψ_{pd} at which stomatal closure was observed. This behaviour had been already

reported for young plants of three coffee species, including robusta (Kanechi et al. 1995*b*), and also for other tree species (e.g., Menzel and Simpson 1986, Gucci et al. 1996, Arndt et al. 2001, Xu and Balocchi 2003). The positive relation between g_s and Ψ_x is expected when soil moisture changes and indirectly affects stomata through a hydraulic feedback (Jones 1998). The rapid recovery of Ψ_x after re-watering, which was accompanied by the recovery of g_s, emphasise the role of leaf water status on stomatal control, as suggested by Fuchs and Livingston (1996). When considered together, these responses should explain greatly why robusta coffee responds strongly to irrigation (DaMatta 2004).

Stomatal sensitivity to evaporative demand, as observed in clones 14, 46 and 120, might indicate the setting up of a feedforward response that would allow them to avoid large internal water deficits (Meinzer et al. 1993). Such a sensitivity appears to be weaker in robusta coffee than in *C. arabica* since in the latter g_s decreases curvilinearly with increasing Δw (Gutiérrez et al. 1994, Kanechi et al. 1995*a*). Because *C. arabica* evolved in a region relatively drier than robusta, the requirement for an efficient stomatal control on transpiration would not thus be as imperative in robusta as in *C. arabica* (DaMatta 2003). In fact, the lower sensitivity of g_s to Δw in the clone 109A might represent an adaptation to the humid climate of the equatorial region where robusta coffee is believed to have evolved. In this clone, stomatal responses to both soil and atmospheric drought should have resulted in a more prodigal use of water and, as a consequence, in more negative $\delta^{13}C$ (lower long-term WUE) regardless of the watering regime. This observation is partially in line with those of Meinzer et al. (1990*a*). They showed that genotypes of *C. arabica* with higher carbon isotope discrimination (lower $\delta^{13}C$) under full irrigation depleted soil water more rapidly and experienced symptoms of physiological stress earlier when water was withheld. One must be cautious, however, since this study was small to provide a conclusive answer about 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 reduced stomatal sensitivity and maintenance of gas exchange under drought conditions (Turner 1997). In this work, however, its amplitude was too small and limited to clone 109A. Thus osmotic adjustment could hardly explain the low stomatal sensitivity to drought in this clone. Incidentally, development of increased leaf water deficits upon discontinuing irrigation may be faster in coffee genotypes having greater amplitude of osmotic adjustment (Meinzer et al. 1990*b*, DaMatta et al. 1993). Therefore, osmotic adjustment should be of limited importance (Munns 1988) in determining drought tolerance in robusta coffee, as has also been reported for several other woody species (Fan et

Table 2. Effects of drought on osmotic potential at full ($\Psi_{\pi(100)}$) and zero ($\Psi_{\pi(0)}$) turgor, bulk volumetric modulus of elasticity (ε) and relative water content at zero turgor ($RWC_{(0)}$) of four clones of robusta coffee. Different capital letters denote significant differences among means for irrigated clones, and different small letters represent significant differences among means for drought-stressed clones by the Newman-Keuls test at $P \leq 0.05$ (clone effect). Means for drought-stressed plants marked with an asterisk differ from those for control plants by the F test at $P \leq 0.05$ (treatment effect). Each value represents the mean ± SE of five replicates.

Parameters	Drought-tolerant clones				Drought-sensitive clones			
	Clone 14		Clone 120		Clone 46		Clone 109A	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought
$\Psi_{\pi(100)}$, MPa	- 1.87 ± 0.06 A	- 1.89 ± 0.04 a	- 1.83 ± 0.06 A	- 1.89 ± 0.08 a	- 1.76 ± 0.07 A	- 1.72 ± 0.02 a	- 1.75 ± 0.07 A	- 1.94 ± 0.11 a
$\Psi_{\pi(0)}$, MPa	- 2.25 ± 0.06 A	- 2.25 ± 0.04 a	- 2.23 ± 0.07 A	- 2.37 ± 0.03 a	- 2.22 ± 0.07 A	- 2.31 ± 0.03 a	- 2.09 ± 0.06 A	- 2.38 ± 0.10 a*
ε, MPa	17.5 ± 0.8 A	18.5 ± 0.8 a	17.5 ± 0.9 A	19.0 ± 0.9 a	19.2 ± 1.0 A	18.6 ± 1.1 a	18.3 ± 1.2 A	18.5 ± 0.9 a
$RWC_{(0)}$, %	89.4 ± 0.6 A	89.3 ± 0.7 a	89.6 ± 0.5 A	89.7 ± 0.1 a	90.1 ± 1.0 A	90.8 ± 0.6 a	90.1 ± 0.6 A	89.2 ± 0.6 a

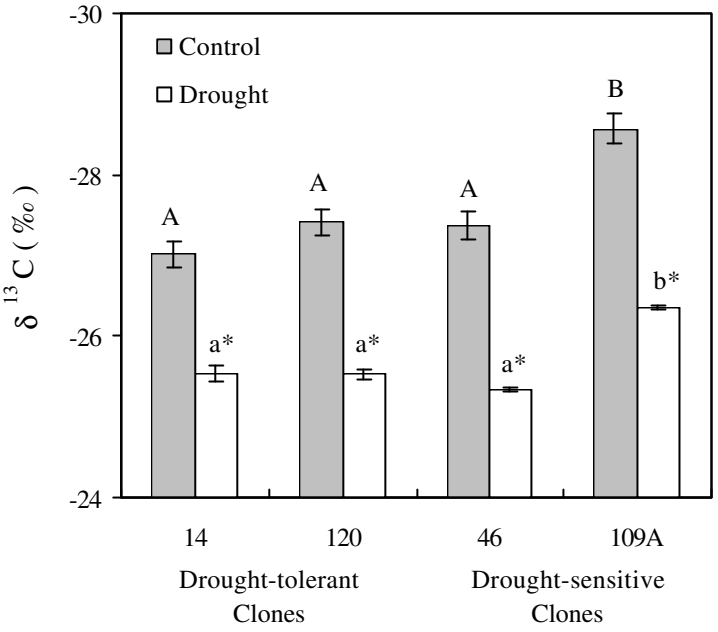

Figure 5. Effects of drought on leaf carbon isotope composition ($\delta^{13}C$) of four clones of robusta coffee. Different capital letters denote significant differences among means for irrigated clones, and different small letters represent significant differences among means for drought-stressed clones by the Newman-Keuls test at $P \leq 0.05$ (clone effect). Means for drought-stressed plants marked with an asterisk differ from those for control plants by the F test at $P \leq 0.05$ (treatment effect). Data are means ± SE of four replicates.

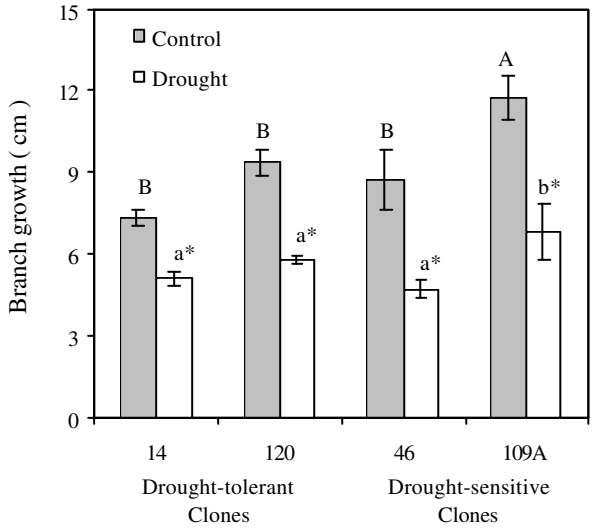

Figure 6. Effects of drought on branch growth of four clones of robusta coffee, as measured 21 days after withholding watering. Statistics as in Figure 5.

al. 1994). Where it occurs, osmotic adjustment either may not persist for long under drought, or functions over a limited range of Ψ_x-values (Blake et al. 1991).

The clones herein evaluated lost turgor at values of Ψ_x between –2.1 and –2.4 MPa. They showed relatively high values of ε (i.e., greater tissue rigidity), which resulted in high $RWC_{(0)}$, as already reported earlier (DaMatta et al. 1993, 2002, 2003). These traits were consistent with the strong stomatal sensitivity to 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 were associated with stomatal aperture, as shown by Meinzer et al. (1990*b*) working with cultivars of *C. arabica* subjected to drought. In any case, maintenance of high RWC at low Ψ_x appears to be a means of the coffee tree avoiding, instead of tolerating, dehydration (DaMatta et al. 1993).

The drought-induced reduction in branch growth could not be associated with adjustments in cell wall extensibility or in osmotic properties. Since elongation growth is sensitive to changes in turgor pressure (Kramer and Boyer 1995), the smaller decreases in growth of the drought-tolerant clones than those of the drought-sensitive ones should have been largely a consequence of the maintenance of a better water status for longer in the former. Such assumption might also explain, at least in part, the differences in branch growth between the clones 14 and 120.

In conclusion, clonal ability to postpone dehydration was more important than dehydration tolerance, and cell water relations were largely unable to adjust to drought stress in either clone. The clone 109A, although possessing greater root to shoot mass ratio, is shallow-rooted and showed a relatively poor stomatal control on transpiration; these features could explain why it experienced symptoms of drought stress earlier after suspending irrigation. The clone 46 is also shallow-rooted but exhibited a better stomatal sensitivity to both soil and atmospheric drought; hence the clone 46 was better able to delay dehydration than the clone 109A. Similarly to clone 46, stomatal sensitivity to drought was well developed in clones 14 and 120, but these clones showed a remarkably deeper root system than the drought-sensitive clones, which could explain their better avoidance to drought. In any case, the larger K_L in clone 120 than in clone 14 might be involved in the faster decrease in Ψ_{pd} in the former. Finally, the direct response of stomata to changes in Ψ_x and Δw 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 for the coffee clones, allowing for maximisation of WUE and survival as soil water availability

decreases. In this case, stomatal sensitivity to soil drying should be negatively associated with stability of crop yield under rainfed conditions. However, stomata may not respond to soil water limitations as readily and dramatically in mature field-grown trees as in young plants like those of this study with less expanded root systems (Gucci et al. 1996, DaMatta 2003). 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. Thus, combination of deep roots with relatively large K_L should contribute to dampen variations both in plant water status and productivity, as particularly observed in the clone 120 grown in seasonally-dry regions that experience satisfactory annual precipitation (Ferrão et al. 2000*a*, DaMatta et al. 2003).

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References

Arndt, S.K., S.C. Clifford, W. Waneck, H.G. Jones and M. Popp. 2001. Physiological and morphological adaptations of the fruit tree *Ziziphus rotundifolia* in response to progressive drought stress. Tree Physiol. 21:705-715.

Blake, T.J., E. Bevilacqua and J.J. Zwiazek. 1991. Effects of repeated stress on turgor pressure and cell elasticity changes in bleak spruce seedlings. Can. J. For. Res. 21:1329-1333.

DaMatta, F.M. 2003. Drought as a multidimensional stress affecting photosynthesis in tropical tree crops. *In* Advances in Plant Physiology, Vol. 5. Ed. A. Hemantaranjan. Scientific Publishers, Jodhpur, pp 227-265.

DaMatta, F.M. 2004. Ecophysiological constraints on the production of shaded and unshaded coffee: a review. Field Crop Res. 86:99-114.

DaMatta, F.M. and A.B. Rena. 2001. Tolerância do café à seca. *In* Tecnologias de Produção de Café com Qualidade. Ed. L. Zambolim. Universidade Federal de Viçosa, Viçosa, pp 65-100.

DaMatta, F.M., R.A. Loos, E.A. Silva, M.E. Loureiro and C. Ducatti. 2002. Effects of soil water deficit and nitrogen nutrition on water relations and photosynthesis of pot-grown *Coffea canephora* Pierre. Trees 16:555-558.

DaMatta, F.M., M. Maestri, R.S. Barros and A.J. Regazzi. 1993. Water relations of coffee leaves (*Coffea arabica* and *C. canephora*) in response to drought. J. Hort. Sci. 68:741-746.

DaMatta, F.M., M. Maestri, P.R. Mosquim and R.S. Barros. 1997. Photosynthesis in coffee (*Coffea arabica* and *C. canephora*) as affected by winter and summer conditions. Plant Sci. 128:43-50.

DaMatta, F.M., J.S.M. Silveira, C. Ducatti and M.E. Loureiro. 2000. Eficiência do uso da água e tolerância à seca em *Coffea canephora*. *In* I Simpósio de Pesquisa dos Cafés do Brasil, Vol. 2. EMBRAPA, Brasília, pp 907-910.

DaMatta, F.M., A.R.M. Chaves, H.A. Pinheiro, C. Ducatti and M.E. Loureiro. 2003. Drought tolerance of two field-grown clones of *Coffea canephora*. Plant Sci. 164:111-117.

Donovan, L.A., J.B. West and K.W. McLeod. 2000. *Quercus* species differ in water and nutrient characteristics in a resource-limited fall-line sandhill habitat. Tree Physiol. 20:929-936.

Fan, S., T.J. Blake and E. Blumwald. 1994. The relative contribution of elastic and osmotic adjustment to turgor maintenance of woody plants. Physiol. Plant. 90:414-419.

Farquhar, G.D., J.R. Ehleringer and K.T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 40:503-537.

Ferrão, R.G., A.F.A. Fonseca, M.A.G. Ferrão and L.P. Santos. 2000*a*. Avaliação de clones elites de café Conilon em condição de estresse hídrico no estado do Espírito Santo. *In* I Simpósio de Pesquisa dos Cafés do Brasil, Vol. 1. EMBRAPA, Brasília, pp 402-404.

Ferrão, R.G., A.F.A. Fonseca, M.A.G. Ferrão and L.P. Santos. 2000*b*. Comportamento de clones elites de café Conilon em condições de alta tecnologia no estado do Espírito Santo. *In* I Simpósio de Pesquisa dos Cafés do Brasil, Vol. 2. EMBRAPA, Brasília, pp 769-771.

Fuchs, E.E. and N.J. Livingston. 1996. Hydraulic control of stomatal conductance in Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] and alder [*Alnus rubra* (Bong)] seedlings. Plant Cell Environ. 19:1091-1098.

Gucci, R., R. Massai, C. Xiloyannis and J.A. Flore. 1996. The effect of drought and vapour pressure deficit on gas exchange of young kiwifruit (*Actinidia deliciosa* var. *deliciosa*) vines. Ann. Bot. 77:605-613.

Gutiérrez, M.V., F.C. Meinzer and D.A. Grantz. 1994. Regulation of transpiration in coffee hedgerows: Covariation of environmental variables and apparent responses of stomata to wind and humidity. Plant Cell Environ. 17:1305-1313.

Hubbard, R.M., B.J. Bond and M.G. Ryan. 1999. Evidence that hydraulic conductance limits photosynthesis in old *Pinus ponderosa* trees. Tree Physiol. 19:165-172.

Jones, H.G. 1992. Plants and Microclimate. A Quantitative Approach to Environmental Plant Physiology. Cambridge University Press, New York, 428 p.

Jones, H.G. 1998. Stomatal control of photosynthesis and transpiration. J. Exp. Bot. 49:387-398.

Kanechi, M., N.U. Uchida, T. Yasuda and T. Yamaguchi. 1995*a*. Water stress effects on leaf transpiration and photosynthesis of *Coffea arabica* L. under different irradiance conditions. *In* Proceedings of the 16th International Scientific Colloquium on Coffee. Association Scientifique Internationale du Café, Paris, pp 520-527.

Kanechi, M., N.U. Uchida, T. Yasuda and T. Yamaguchi. 1995*b*. Water stress effects on leaf photosynthesis and diffusive conductances of three *Coffea* species (*C. arabica*, *C. canephora*, *C. liberica*). *In* Proceedings of the 16th International Scientific Colloquium on Coffee. Association Scientifique Internationale du Café, Paris, pp 804-811.

Kramer, P.J. and J.S. Boyer. 1995. Water Relations of Plants and Soils. Academic Press, San Diego, 495 p.

Landsberg, J.J. Physiological Ecology of Forest Production. 1986. Academic Press, Orlando, 198 p.

Meinzer, F.C., G. Goldstein and D.A. Grantz. 1990*a*. Carbon isotope discrimination in coffee genotypes grown under limited water supply. Plant Physiol. 92:130-135.

Meinzer, F.C., D.A. Grantz, G. Goldstein and N.Z. Saliendra. 1990*b*. Leaf water relations and maintenance of gas exchange in coffee cultivars grown in drying soil. Plant Physiol. 94:1781-1787.

Meinzer, F.C., G. Goldstein and D.A. Grantz. 1993. Carbon isotope discrimination and gas exchange in coffee during adjustment to different soil moisture regimes. *In* Stable Isotopes and Plant Carbon-Water Relations. Eds. J.R. Ehleringer, A.E. Hall and G.D. Farquhar. Academic Press, San Diego, pp 327-345.

Melkonian, J.J., J. Wolfe and P.L. Steponkus. 1982. Determination of the volumetric modulus of elasticity of wheat leaves by pressure-volume relations and the effects of drought conditioning. Crop Sci. 22:116-123.

Menzel, C.M. and D.R. Simpson. 1986. Plant water relations in lychee: diurnal variations in leaf conductance and leaf water potential. Agric. For. Meteorol. 37:267-277.

Munns, R. 1988. Why measure osmotic adjustment? Aust. J. Plant Physiol. 5:207-218.

Neter, J. and W. Wasserman. 1974. Applied Linear Statisticals Models. Regression, Analysis of Variance and Experimental Designs. Richard D. Irwin Inc., Illinois, 842 p.

Pinheiro, H.A., F.M. DaMatta, A.R.M. Chaves, E.P.B. Fontes and M.E. Loureiro. 2004. Drought tolerance as related to protection against oxidative stress in clones of *Coffea canephora* subjected to long-term drought. Plant Sci. (in press).

Tausend, P.C., G. Goldstein and F.C. Meinzer. 2000*a*. Water utilization, plant hydraulic properties and xylem vulnerability in three contrasting coffee (*Coffea arabica*) cultivars. Tree Physiol. 20:159-168.

Turner, N.C. 1997. Further progress in crop water relations. Adv. Agron. 58:293-338.

Xu, L. and D.D. Baldocchi. 2003. Seasonal trends in photosynthetic parameters and stomatal conductance of blue oak (*Quercus douglasii*) under prolonged summer drought and high temperature. Tree Physiol. 23:865-877.

White, D.A., N.C. Turner and J.H. Galbraith. 2000. Leaf water relations and stomatal behavior of four allopatric *Eucalyptus* species planted in Mediterranean southwestern Australia. Tree Physiol. 20:1157-1165.

Willson, K.C. 1999. Coffee, Cocoa and Tea. CABI Publishing, Cambridge, 300 p.

CHAPTER 2

Drought tolerance as related to protection against oxidative stress in clones of *Coffea canephora* subjected to long-term drought[1]

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Abbreviations: A, net carbon assimilation rate; APX, ascorbate peroxidase; Asc, ascorbate; CAT, catalase; C_i/C_a, internal to ambient CO_2 concentration ratio; DHA, dehydroascorbate; DHAR, DHA reductase; GPX, guaiacol peroxidase; F_v/F_m, variable to maximum fluorescence ratio; g_s, stomatal conductance; GR, glutathione reductase; GSH, glutathione; MAld, malondealdehyde; MDA, monodehydroascorbate; MDAR, MDA reductase; NPQ, Stern-Volmer non-photochemical quenching coefficient; P_E, fraction of photosynthetic photon flux absorbed in PS II antennae that was neither utilised in photochemistry nor dissipated thermally; *PPF*, photosynthetic photon flux; q_P, photochemical quenching coefficient; ROS, reactive oxygen species; SOD, superoxide dismutase; Φ_{PSII}, actual quantum yield of PSII electron transport; Ψ_w, leaf water potential.

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Abstract

Four clones of *Coffea canephora* (Robusta coffee) representing drought-tolerant (14 and 120) and drought-sensitive (46 and 109A) genotypes were submitted to slowly imposed water deficit, until predawn leaf water potential approximately –3.0 MPa was reached. Drought-tolerant clones were better able to maintain their leaf water status than drought-sensitive clones after withholding irrigation. Regardless of the clones investigated, the net carbon assimilation rate decreased under drought stress, but little or no effect of drought on the quantum yield of electron transport was observed. The photosynthetic apparatus of clone 120 was more tolerant to both drought and paraquat-mediated oxidative stress, with no clear distinction amongst the other clones in this regard. Drought triggered increases in superoxide dismutase (clones 109A and 120), ascorbate peroxidase (clones 14, 46 and 109A), catalase and guaiacol peroxidase (clones 46 and 109A), and also in glutathione reductase (clone 46) and dehydroascorbate reductase (clone 109A). Activity of monodehydroascorbate reductase was not induced in drought-stressed plants. Maximal catalytic activities of the two last enzymes were much lower than that of ascorbate peroxidase, irrespective of the clone investigated. No drought-induced decrease in enzyme activity was found, except for glutathione reductase in clone 120. In any case, oxidative damage appeared to be evident only in clone 109A. Overall, there was no apparent link between protection against oxidative stress with differences in clonal tolerance to drought.

Keywords: Acclimation; Antioxidant enzymes; Coffee; Gas exchange; Paraquat; Water deficit

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1. Introduction

Upon moderate drought conditions, photosynthesis decreases mainly due to stomatal closure. As the stress progresses, biochemical constraints may limit the photosynthetic CO_2 fixation more directly [1]. Excess energy may result, which is potentially harmful to photosystem (PS) II because of over-reduction of photosynthetic electron chain and increased production of reactive oxygen species (ROS) in chloroplasts [2]. Additionally, mitochondria and peroxisomes are also potential producers of ROS [2,3].

To protect the photosynthetic apparatus from oxidative stress, plants must dissipate excess light energy. This can be achieved by down-regulation of the photochemical efficiency by way of the xanthophyll cycle [4] or by maintenance of electron flux involving alternative

pathways such as photorespiration and the Mehler-peroxidase reaction [2,5]. However, both pathways lead to an increased production of ROS such as superoxide ($O_2^{\cdot -}$) and H_2O_2. To cope with ROS, plants are endowed with a complex enzymatic antioxidant system including superoxide dismutases (SOD), which catalyse the reaction from $O_2^{\cdot -}$ to H_2O_2, and catalase (CAT) and enzymes of the ascorbate (Asc)/glutathione (GSH) cycle, which function to detoxify the H_2O_2 produced [2,6]. A guaiacol-type peroxidase (GPX) can also participate in H_2O_2 scavenging. In addition, detoxification of ROS can be conducted by means of antioxidant molecules, both lipophilic (α-tocopherol, β-carotene) and hydrophilic (Asc, GHS). The latter can act as direct scavengers for ROS, and both compounds are also reductants in the Asc/GSH cycle [6]. Failure in antioxidant defence system may result in oxidative damage to several cell constituents such as proteins, DNA and membrane lipids [2].

If drought stress proceeds slowly, an array of time-dependent morphological and physiological acclimation responses may occur, thus largely expanding the range and kind of plant responses that can take place with soil water shortage [7]. Despite this, many studies concerning drought effects on tree crop physiology have been conducted using plants grown in small containers that limit root growth. Such an approach does not allow the phenomenon of acclimation to occur, with observations largely dependent on the rate of stress progression [7]. Another feature of many experiments resides in comparisons among species or genotypes after they suffer from a given period without irrigation. Comparisons made under such circumstances may be misleading since plants ordinarily show quite variable internal water status.

Robusta coffee (*Coffea canephora* Pierre) is largely cultivated in drought-prone areas, thus leading to crop failure in dry years. There seems to be considerable clonal variation in drought tolerance within this species [8]. Working with two clones submitted to rapidly imposed drought, Lima *et al*. [9] proposed that drought tolerance in Robusta coffee might be at least partially associated with enhanced activity of antioxidant enzymes. In fact, increased expression of the antioxidant system has been associated with decreased oxidative damage in different drought-tolerant species or genotypes [10]. In this study, the major goal was to test the hypothesis that drought-tolerant clones differ from drought-sensitive ones at a biochemical level, through enhanced protection of the photosynthetic apparatus against oxidative stress. For this purpose, leaf gas exchange and chlorophyll *a* fluorescence parameters, the enzymatic antioxidant system, Asc redox state, lipid peroxidation, as well as tolerance to oxidative stress mediated by paraquat were investigated. The clones were grown in large containers in an attempt to simulate a natural progression of drought down a root profile, so that the plants

could acclimate to water shortage. Plant responses were also measured at a similar internal water status allowing more reliable comparisons among clones to be made.

2. Material and methods

2.1. Plant material, growth conditions and sampling procedures

Four coffee clones representing drought-tolerant (14 and 120) and drought-sensitive (46 and 109A) genotypes were used. The selected clones have been shown to produce a good crop when grown under irrigation; under limited soil water, however, both survival and productivity are severely impaired in the drought-sensitive clones, with minor effects in the drought-tolerant ones, as has been observed in field experiments of the Institute for Research and Rural Assistance of the State of Espírito Santo, Brazil [Romário Ferrão, personal communication]. In addition, tolerant clones are better able to maintain their tissue water status upon discontinuing irrigation than drought-sensitive clones [11].

Plants obtained as rooted stem cuttings were four months old at planting. They were grown under greenhouse conditions, with an average midday photosynthetic photon flux (*PPF*) about 900 μmol m^{-2} s^{-1}, in large containers (0.8 m high, 0.44 m internal diameter) filled with an 120 l mixture of soil, sand and hardened manure (3:1:1, v/v/v) and a gravel layer at the bottom. When the plants were 12 months old, water deficit was imposed by withholding watering until leaf water potential (Ψ_w), as measured periodically with a Scholander-type pressure chamber, reached around –3.0 MPa at predawn (about –3.5 MPa at midday). All sampling and measurements were made using leaves from the third or fourth pair from the apex of plagiotropic branches. For biochemical analyses, leaf tissue (0.3 g FW per tree for each parameter), collected at about midday, was rapidly frozen in liquid nitrogen and stored at –80°C until processing. For paraquat-mediated oxidative stress, four leaf discs (1 cm^2 each) per tree were collected and immediately assayed.

2.2. Biometric data

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 dry mass was determined after drying plant material at 72°C for 72 h. Shoot height and root depth were also measured.

2.3. Responses to paraquat-mediated oxidative stress

Leaf discs, collected from well-watered plants, were floated with adaxial side up on 0, 5 and 15 μM paraquat. In a preliminary experiment, discs vacuum-infiltrated with paraquat solutions were compared with discs to which paraquat was allowed to be absorbed freely, giving similar results. Discs were then exposed for 16 h at 25°C in the dark to allow paraquat diffusion into them, following by incubation for 3 h at *PPF* of 200 μmol m^{-2} s^{-1}. Subsequently, leaf discs were dark-incubated for 3 h at 25°C. The paraquat-dependent ROS damage was estimated according to [12], from the reduction of the variable to maximum fluorescence ratio (F_v/F_m), measured as described below.

2.4. Photosynthetic parameters

The net carbon assimilation rate (A), internal to ambient CO_2 concentration ratio (C_i/C_a) and stomatal conductance to water vapour (g_s) were measured at 0800-0900 hours under artificial, saturating *PPF* (850-900 μmol m^{-2} s^{-1}) with a portable open-system infrared gas analyser (LCA-4, ADC, Hoddesdon, UK), as described in [13]. Chlorophyll *a* fluorescence was measured with a portable pulse amplitude modulation fluorometer (FMS2, Hansatech, King's Lynn, Norfolk, UK) using actinic *PPF* of 900 μmol m^{-2} s^{-1} for 480 s and an 1-s pulse of saturating light of 6000 μmol m^{-2} s^{-1}. Measurements of the F_v/F_m ratio were made following dark-adaptation for 30 min. Further technical details have been given previously [9,14]. Photochemical quenching coefficient (q_p), Stern-Volmer non-photochemical quenching coefficient (NPQ) and the quantum yield of PSII electron transport (Φ_{PSII}) were calculated as described in [15]. The fraction of *PPF* absorbed in PSII antennae that was neither used in photochemistry nor dissipated thermally (P_E) was calculated according to [4]. All the above measurements were carried out at ambient CO_2, 25 ± 2 °C air temperature and 80 ± 2% relative humidity.

2.5. Biochemical assays

Leaf tissue was ground in a cold mortar and pestle using polyvinylpolypyrrolidone and the following extraction buffers: SOD, EC 1.15.1.1 [100 mM K-phosphate buffer, pH 7.8, 0.1 mM EDTA, 5 mM DTT, 15 mM 2-mercaptoethanol and 0.1% (v/v) Triton X-100]; CAT, EC 1.11.1.6 and Asc peroxidase (APX, EC 1.11.1.11) [50 mM K-phosphate buffer, pH 7.0, 2 mM EDTA, 20 mM Asc and 0.1% (v/v) Triton X-100]; glutathione reductase (GR, EC 1.6.4.2) [100 mM Tris-HCl buffer, pH 7.5, 50 μM EDTA, 10 mM isoascorbate, 9 mM 2-mercaptoethanol, 3 mM DTT and 0.1% (v/v) Triton X-100]; monodehydroascorbate (MDA)

reductase (MDAR, EC 1.6.5.4) and dehydroascorbate (DHA) reductase (DHAR, EC 1.8.5.1) [100 mM K-phosphate buffer, pH 7.8, 2 mM EDTA, 20 mM Asc and 0.1% (v/v) Triton X-100]; and GPX, EC1.11.1.7 [50 mM K-phosphate buffer, pH 7.4, 1 mM EDTA, 20 mM Asc and 0.1% (v/v) Triton X-100]. The resulting slurry was centrifuged at 20000 *g* for 20 min. Supernatants were collected and used for enzymatic assays and determination of protein content [16]. For MDAR and DHAR, enzyme extract was previously gel-filtered over Sephadex G-25 columns. All the above operations were conducted at 0-4 °C.

Activities of total SOD, APX and CAT were determined as described earlier [9]. Activities of GR and DHAR and activity of MDAR were assayed by measuring the rate of NADPH and NADH oxidation, respectively, both at 340 nm. A suitable amount of enzyme extract and the following reaction media were used: GR (50 mM Tris-HCl, pH 7.5, 10 mM oxidised GSH, 3 mM $MgCl_2$, and 0.15 mM NADPH) [17], MDAR (50 mM K-phosphate buffer under N_2 atmosphere, pH 7.5, 10 mM NaCl, 2 mM $MgCl_2$, 0.1 mM NADH, 400 mM sucrose, 2.5 mM Asc and 0.14 units Asc oxidase for generating MDA) [18] and DHAR (50 mM K-phosphate buffer, pH 6.1, 0.2 mM NADPH, 2.5 mM GSH, 2.5 mM DHA and 0.6 units GR) [19]. GPX activity was determined following the increase in absorbance at 470 nm using a reaction mixture containing 50 mM K-phosphate buffer, pH 7.0, 0.1 mM EDTA, 10 mM guaiacol and 10 mM H_2O_2 [20]. Where appropriate, controls were run to correct for interferences. Enzyme activities were expressed on a total protein basis since the overall leaf protein concentration, either on a dry mass or on an area basis, remained largely unaffected under drought conditions. Indeed, enzyme activities were quite similar regardless of expressed on an area or on a protein basis (data not shown).

Asc and DHA were determined as described in [21], with some modifications. Leaf tissue was ground in 6 ml 5% (w/v) trichloroacetic acid and the resulting slurry was centrifuged at 10000 *g* for 15 min at 4 °C. A 20 µl aliquot of the supernatant was diluted to 500 µl using 5% (w/v) trichloroacetic acid. Total Asc (Asc + DHA) was determined after reduction of DHA by DTT. To 500 µl diluted sample extract were added 250 µl 0.06% (w/v) DTT/ethanol and 250 µl 0.2 M Na_2HPO_4-1.2 M NaOH. The mixture was then incubated for 10 min at 25 °C. Subsequently, the following reagents, dissolved in absolute ethanol, were added: 250 µl 0.24% (w/v) *N*-ethylmaleimide, 250 µl 4% (v/v) H_3PO_4, 500 µl 0.5% (w/v) bathophenanthroline and 500 µl 0.03% (w/v) $FeCl_3$, in a 2.5 ml final volume. The mixture was vigorously mixed and incubated at 30°C for 90 min. The resulting absorbance was read at

534 nm. Asc was determined as described above, with omission of DTT. DHA was deduced from the difference of total Asc and Asc.

Malondealdehyde (MAld), assayed as described in [9], was used as an estimation of lipid peroxidation.

2.6. Statistics

The plants were distributed over a completely randomised single-tree plot design, with eight treatment-combinations, forming a 4 x 2 factorial (four clones and two watering regimes) with four or five replicates. For biochemical data, each replicate represented the mean of three determinations on the same sample. Data were statistically examined using analysis of variance and tested for significant ($P \leq 0.05$) clone and irrigation treatment differences using Newman-Keuls and F tests.

3. Results and discussion

3.1. Biometric traits and water status

The selected clones (14 and 120, drought-tolerant; 46 and 109A, drought-sensitive) could be grouped into two types of contrasting canopy morphologies, with the clones 109A and 120 being taller (Table 1) with less dense crowns than the clones 14 and 46. There was no significant difference in total leaf area among the clones evaluated, but root biomass and root to shoot biomass ratio were greater in clone 109A than in the other clones (Table 1). However, the root system was about 50% deeper in the drought-tolerant than in the drought-sensitive clones (Table 1); additionally, the tolerant clones exhibited a more regularly distributed root system down a pot profile than the drought-sensitive ones (not shown). This might be partially linked to the slower rate of decline in predawn Ψ_w in the former than in the latter clones after withholding irrigation (Table 2). In fact, in the field, where the development of the root system is much less restricted, tolerant clones appear to postpone tissue dehydration to a remarkable greater extent than sensitive clones [11]. Thus, in addition to possessing a deeper root system, the tolerant clones might also deplete accessible soil water more sparingly than the drought-sensitive clones. These features may contribute to buffer crop yield of the tolerant clones when compared with the sensitive clones in drought-prone areas [Romário Ferrão, personal communication].

3.2. Responses to paraquat-mediated oxidative stress

Paraquat was used in order to test the hypothesis that clones of Robusta coffee considered to be drought-tolerant would have higher inherent ability to cope with oxidative stress than clones considered to be drought-sensitive. As deduced from the decreases in F_v/F_m ratio (Fig. 1), clones 14 and 46 suffered from a similar oxidative stress, regardless of the paraquat concentration employed. At 5 µM paraquat, clones 109A and 120 were better protected against paraquat than clones 14 and 46. However, defence mechanisms were not as effective in clone 109A as in clone 120 since F_v/F_m decreased further in the former, but not in clone 120, as paraquat concentration was increased from 5 to 15 µM. As a whole, no clear distinction concerning differential tolerance to paraquat between drought-sensitive and drought-tolerant clones could be established. However, defence mechanisms to scavenge ROS were comparatively more effective in clone 120, affording better protection to its photosynthetic apparatus against oxidative stress.

3.3. Photosynthetic parameters

With the exception of clone 120, drought led to approximately parallel decreases in both g_s and A, 81 and 75% for clone 14, 93 and 83% for clone 46, and 58 and 51% for clone 109A, respectively. However, C_i/C_a did not decrease accordingly (Table 2). This could mean a higher inhibition of photosynthesis by non-stomatal factors than by stomatal ones. It is

Table 1. Morphological characteristics of one-year-old clones of Robusta coffee under irrigation conditions.

Parameters	Drought-tolerant clones		Drought-sensitive clones	
	Clone 14	Clone 120	Clone 46	Clone 109A
Shoot height, m	0.78 ± 0.05 a	0.94 ± 0.03 b	0.73 ± 0.07 a	0.92 ± 0.02 b
Leaf area, m^2	1.89 ± 0.11 a	2.51 ± 0.13 a	1.91 ± 0.08 a	2.36 ± 0.10 a
Root to shoot ratio	0.69 ± 0.02 a	0.71 ± 0.04 a	0.74 ± 0.07 a	0.89 ± 0.03 b
Root dry mass, g	176 ± 9 b	268 ± 16 ab	187 ± 18 b	351 ± 34 a
Root depth, m	0.76 ± 0.03 a	0.75 ± 0.04 a	0.48 ± 003 b	0.53 ± 0.03 b

Different letters denote significant differences among clonal means ($P \leq 0.05$; Newman-Keuls test). Each value represents the mean ± SE of five replicates.

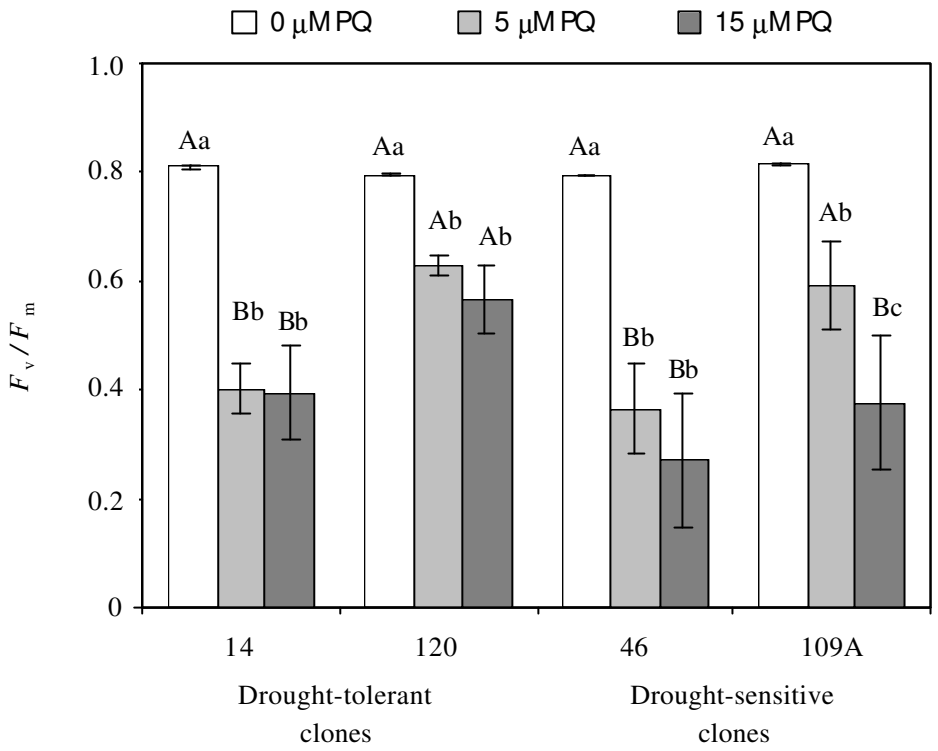

Fig. 1. Effect of paraquat-mediated oxidative stress on the variable to maximum fluorescence ratio (F_v/F_m) of leaf discs of four clones of Robusta coffee grown under continuous irrigation. Different capital letters represent significant differences among clonal means within the same paraquat concentration (clone effect). Different small letters represent significant differences among means from different paraquat concentrations within the same clone (treatment effect). Mean comparisons were performed using the Newman-Keuls test at $P \leq 0.05$. Values are means ± SE of 16 leaf discs from four plants.

unlikely that C_i has been overestimated as a result of patchy stomatal closure since (i) water deficit was imposed slowly and (ii) coffee behaves as a homobaric species and, therefore, heterogeneous stomatal closure is not to be expected [7]. In contrast, in clone 120 g_s decreased to a greater extent than A (respectively 73 and 40%), which was accompanied by a significant decrease (14%) in C_i/C_a (Table 2). Thus it may be proposed that stomatal limitations rather than stress-induced dysfunctions at the chloroplast level accounted largely for the reductions in A in clone 120.

Table 2. Effects of drought on leaf water potential (Ψ_w) at predawn, rate of decrease of predawn Ψ_w (RDWP), net carbon assimilation rate (A), stomatal conductance (g_s), internal to ambient CO_2 concentration ratio (C_i/C_a), maximum photochemical efficiency of PSII (F_v/F_m), quantum yield of PSII electron transport (Φ_{PSII}), photochemical (q_P) and Stern-Volmer non-photochemical (NPQ) quenching coefficients, and fraction of absorbed light that was neither used in photochemistry nor dissipated thermally (P_E) of four clones of Robusta coffee.

Parameters	Drought-tolerant clones				Drought-sensitive clones			
	Clone 14		Clone 120		Clone 46		Clone 109A	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought
Ψ_w, MPa	-0.05 ± 0.01 A	-2.93 ± 0.07 a*	-0.05 ± 0.01 A	-2.95 ± 0.08 a*	-0.08 ± 0.00 A	-2.95 ± 0.02 a*	-0.06 ± 0.01 A	-2.97 ± 0.06 a*
RDWP, kPa d^{-1}	----	93 ± 4 a	----	100 ± 2 a	----	112 ± 3 b	----	120 ± 8 b
A, µmol m^{-2} s^{-1}	10.7 ± 0.5 A	2.7 ± 0.7 ab*	6.1 ± 1.1 B	3.7 ± 0.7 a*	9.8 ± 0.6 A	1.7 ± 0.3 b*	8.6 ± 0.3 A	4.2 ± 0.4 a*
g_s, mmol m^{-2} s^{-1}	158 ± 13 A	28 ± 8 a*	147 ± 23 A	36 ± 8 a*	145 ± 23 A	12 ± 4 a*	118 ± 8 A	52 ± 7 a*
C_i/C_a	0.62 ± 0.04 A	0.60 ± 0.02 a	0.63 ± 0.04 A	0.54 ± 0.01 a*	0.64 ± 0.03 A	0.58 ± 0.01 a	0.58 ± 0.04 A	0.62 ± 0.01 a
F_v/F_m	0.841 ± 0.003 A	0.837 ± 0.012 a	0.842 ± 0.002 A	0.854 ± 0.002 a	0.827 ± 0.011 A	0.836 ± 0.005 a	0.846 ± 0.003 A	0.855 ± 0.002 a
Φ_{PSII}	0.344 ± 0.025 A	0.225 ± 0.022 a*	0.279 ± 0.022 A	0.261 ± 0.023 a	0.276 ± 0.052 A	0.290 ± 0.009 a	0.276 ± 0.031 A	0.223 ± 0.010 a
q_P	0.590 ± 0.035 A	0.417 ± 0.044 a*	0.513 ± 0.033 A	0.454 ± 0.037 a	0.502 ± 0.079 A	0.495 ± 0.021 a	0.494 ± 0.020 A	0.405 ± 0.033 a
NPQ	1.846 ± 0.147 A	1.792 ± 0.108 a	2.235 ± 0.091 A	1.617 ± 0.059 a*	1.870 ± 0.192 A	1.549 ± 0.107 a	2.021 ± 0.131 A	1.857 ± 0.126 a
P_E	0.237 ± 0.015 A	0.317 ± 0.028 a*	0.264 ± 0.016 A	0.314 ± 0.022 a	0.262 ± 0.033 A	0.298 ± 0.016 a	0.279 ± 0.023 A	0.328 ± 0.015 a

Different capital letters denote significant differences among means for irrigated clones, and different small letters represent significant differences among means for drought-stressed clones by the Newman-Keuls test at $P \leq 0.05$ (clone effect). Means for drought-stressed plants marked with an asterisk differ from those for control plants by the F test at $P \leq 0.05$ (treatment effect). Each value represents the mean ± SE of five replicates.

In spite of the strong decreases in A, only minor changes in photochemical parameters were observed in drought-stressed plants (Table 2), as already shown for Robusta coffee [9,14]. In clone 14, a slight decline (35%) in Φ_{PSII} was greatly associated with decreased availability of electron acceptors downstream of PSII, as deduced from the 29% reduction in q_P. In clone 120, only a discrete depression (28%) in thermal energy dissipation, analysed as NPQ, was found. Overall, the present results indicate that electron transport under drought stress was maintained, similarly as in control plants. Since photosynthetic CO_2 fixation declined sharply, a surplus of excitation light energy should have occurred, which could potentially lead to photoinhibition and photodamage. However, P_E increased significantly (34%) only in clone 14. Because treatment effect on F_v/F_m was not found (suggesting that photoinhibition did not occur) and leaf gas exchange and photochemical parameters returned to control levels rapidly upon rewatering (measured 12 h latter; data not shown), it may be suggested that drought stress in this experiment did not cause a long-term effect on the photosynthetic apparatus. In any case, since electron transport continued at high rates in drought-stressed plants, an increase in the capacity of alternative electron acceptor routes such as photorespiration and Mehler-peroxidase pathway is to be expected [2,5,10].

3.4. Drought-induced defence mechanisms against oxidative stress

Under drought conditions, SOD activity was not induced in clones 14 and 46, but increased about 45% in clones 109A and 120, as compared with control plants (Fig. 2). This increase leads to enhanced production of H_2O_2 [6,10]. Thus in clone 109A, the possible H_2O_2 build-up could be attended by increases in activities of APX (45%), CAT (74%) and GPX (43%) (Fig. 3). By contrast, protection against oxidative stress mediated by SOD might be at first glance limited in clone 120 since no co-ordinate increase in H_2O_2 scavenging system was observed (Fig. 3). However, there was no apparent oxidative damage in clone 120, conversely to what happened in clone 109A (see below).

Non-SOD scavenging of $O_2^{\cdot -}$ may also occur through direct reactions with Asc or GSH [6,10]. This suggests that non-enzymatic $O_2^{\cdot -}$ removal might be possible, particularly in clones 14 and 46 in which SOD activity was not induced by drought. Since this alternative pathway also leads to H_2O_2 production, corresponding increases in antioxidants and/or enzymes involved in H_2O_2 removal are to be expected [22]. In clone 14 of the six enzymes herein evaluated involved in post-SOD reactions only APX, which has a much higher affinity for H_2O_2 than either CAT or GPX, increased (38%) as a result of drought (Fig. 3). This

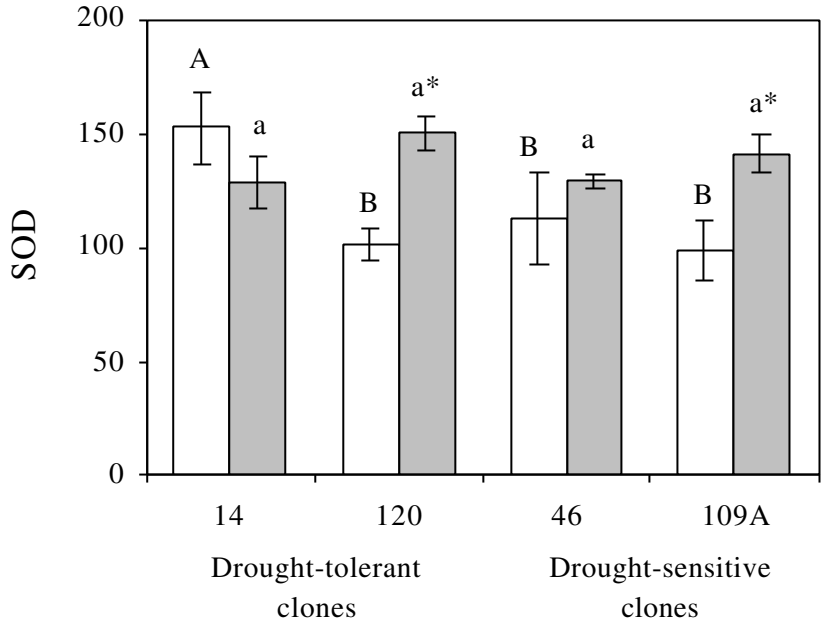

Fig. 2. Effects of drought on the activity of superoxide dismutase (SOD), expressed as enzyme unit mg^{-1} protein, of four clones of Robusta coffee (white columns, control plants; grey columns, drought-stressed plants). One unit of SOD was defined as the amount of enzyme that inhibited the reduction of nitro-blue tetrazolium by 50%. Different capital letters denote significant differences among means for irrigated clones, and different small letters represent significant differences among means for drought-stressed clones by the Newman-Keuls test at $P \leq 0.05$ (clone effect). Means for drought-stressed plants marked with an asterisk differ from those for control plants by the F test at $P \leq 0.05$ (treatment effect). Values are means ± SE of four replicates.

evidences a key role of APX in allowing the clone 14 to cope with potential increases in H_2O_2 under drought conditions. With regard to clone 46, besides APX, other enzymes (CAT, GPX and possibly GR) were affected by drought (Fig. 3) in order to control H_2O_2 levels. However, it should be pointed out that only measurements of metabolic fluxes across biochemical pathways could provide evidence for an imbalance between production and assimilation of H_2O_2. In any case, although analyses *in vitro* of the enzyme activities do not allow assessment

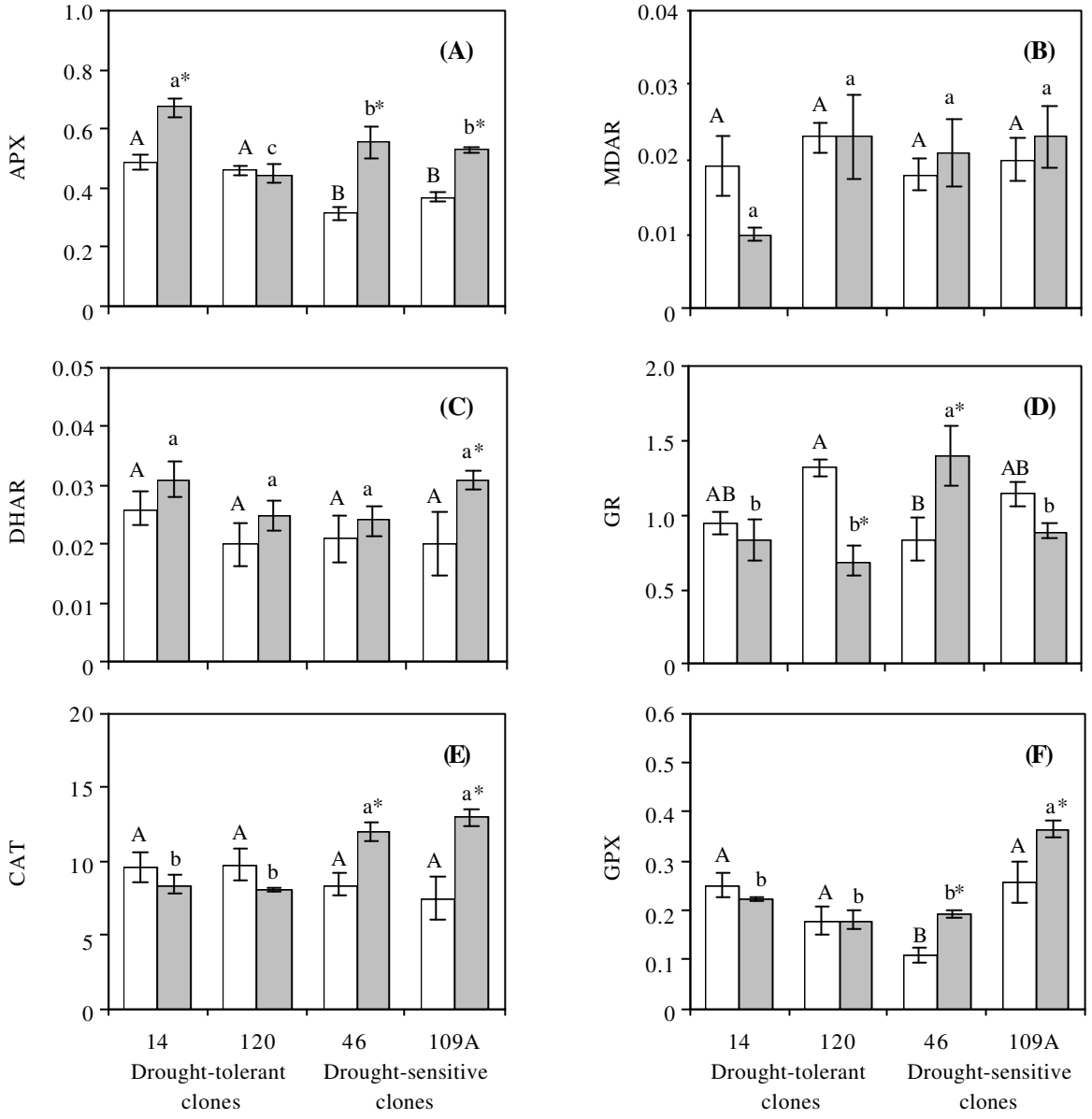

Fig. 3. Effects of drought on antioxidant enzymes, ascorbate peroxidase – APX (A), monodehydroascorbate reductase – MDAR (B), dehydroascorbate reductase – DHAR (C), glutathione reductase – GR (D), catalase – CAT (E), and guaiacol peroxidase – GPX (F), of four clones of Robusta coffee (white columns, control plants; grey columns, drought-stressed plants). Enzyme activities are expressed as: APX, µmol Asc min^{-1} mg^{-1} protein; MDAR, µmol NADH min^{-1} mg^{-1} protein; DHAR and GR, µmol NADPH min^{-1} mg^{-1} protein; CAT, µmol H_2O_2 min^{-1} mg^{-1} protein; GPX, µmol guaiacol min^{-1} mg^{-1} protein. Statistics as in Fig. 2.

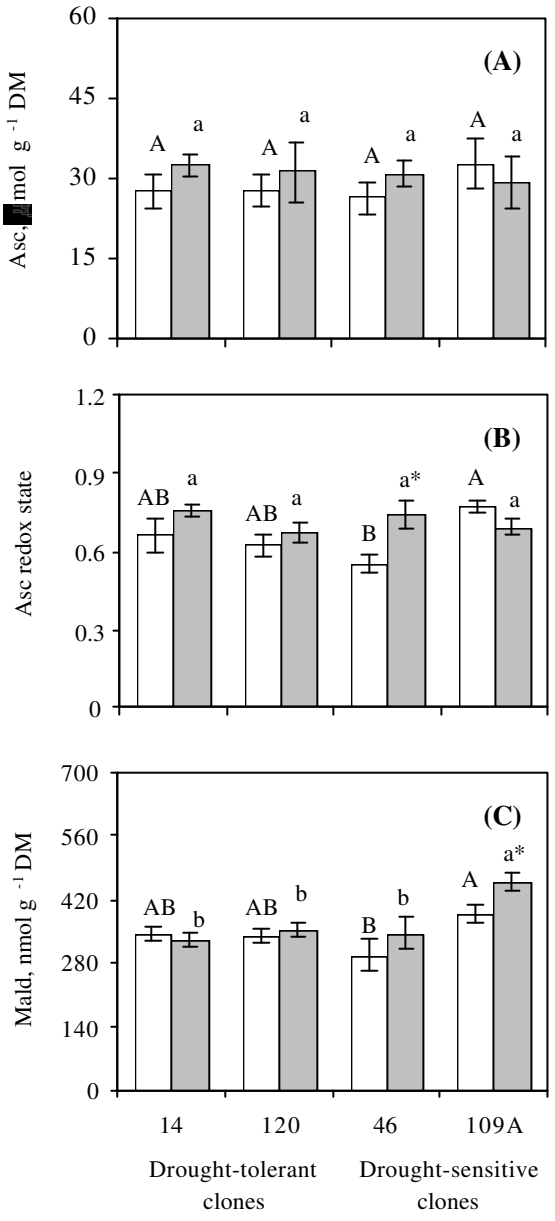

Fig. 4. Effects of drought on concentration of ascorbate – Asc (A), Asc redox state (B), and malondealdehyde – MAld (C), of four clones of Robusta coffee (white columns, control plants; grey columns, drought-stressed plants). Statistics as in Fig. 2.

of the actual activities *in vivo*, they do reflect the potential and, thus, the capacity for the use of different substrates.

Changes in APX were not accompanied by alterations in MDAR (Fig. 3B), while DHAR tended to increase in response to drought, but only in clone 109A it increased (55%) significantly (Fig. 3C). GR activity increased (67%, clone 46), decreased (48%, clone 120) or remained unaffected (clones 14 and 109A) in drought-stressed plants when compared with irrigated ones (Fig. 3D). In addition, maximal extractable activities of MDAR, DHAR and GR were considerably lower than that of APX (Fig. 3). When taken together, these data support the hypothesis that direct photoreduction of MDA (probably by ferredoxin), which is formed upon peroxidation of Asc, has predominated over enzymatic routes. Similar assumptions have been reported for other species [23,24,25].

Differences in Asc levels were not significant among clones regardless of the watering regime (Fig. 4A). Drought caused a decrease in DHA in clone 46 (data not shown) and, as a result, an increase in Asc redox state (Asc/Asc + DHA) was only observed in this clone (Fig. 4B). In clone 109A, drought induced an 18% increase in lipid peroxidation (estimated as MAld), but not in the other clones. Also, absolute values of MAld in clone 109A were about 34% larger than those averaged over the drought-stressed plants of the other clones (Fig. 4C). This could be an indication that leaf tissues from clone 109A suffered from oxidative stress. By contrast, acclimatory changes in antioxidant system appeared to be sufficient to prevent oxidative damages in the other clones investigated.

It was previously demonstrated that rapid imposition of water deficit (predawn Ψ_w = –3.0 MPa) in clones 109A and 120 resulted in considerable increases in some antioxidant enzyme activities, especially in clone 120 (e.g. 2- and 5.6-fold for SOD in clones 109A and 120, respectively), but changes in enzyme activities did not prevent cellular damages, particularly in clone 109A [9]. The present results indicate that, if water deficit proceeds slowly, the coffee plants can acclimate to drought and, as a consequence, they could largely avoid oxidative stress. This is in agreement with the fact that plants are more tolerant of a water shortage when the stress is imposed gradually [26,27].

4. Conclusions

Under the experimental conditions, drought-tolerant clones were better able to maintain their leaf water status than drought-sensitive clones after withholding irrigation. In part, this might be associated with a deep root system in the former. Induction of defence mechanisms

against oxidative stress triggered by drought was greater in drought-sensitive than in drought-tolerant clones. This might be an indication of increased oxidative processes suffered by the sensitive clones. However, there was no apparent link between oxidative damage with differences in clonal tolerance to drought. For example, no clear distinction between the drought-tolerant clone 14 and the drought-sensitive clone 46, in terms of defence mechanisms to cope with oxidative stress, could be established. Clone 120 showed a comparatively more tolerant photosynthetic apparatus to both drought and paraquat-mediated oxidative stress. The data presented allow other physiological insight into high degree of drought tolerance in Robusta coffee [8,28] taking into consideration that no long-term effect of drought on the photosynthetic apparatus had occurred; additionally, the clones herein investigated were able to preserve, or even to increase, their antioxidant defences at Ψ_w as low as –3.5 MPa.

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References

[1] D.H. Lawlor, The effects of water deficit on photosynthesis, In: N. Smirnoff, (Ed.), Environment and Plant Metabolism – Flexibility and Acclimation, BIOS Scientific Publishers, Oxford, 1995, pp. 129-156.

[2] K. Asada, The water-water cycle in chloroplasts: scavenging of active oxygen and dissipation of excess photons, Annu. Rev. Plant Physiol. Plant Mol. Biol. 50 (1999) 601-639.

[3] C.H. Foyer, M Lelandais, K.J. Kunert, Photooxidative stress in plants, Physiol. Plant. 92 (1994) 698-717.

[4] B. Demming-Adams, W.W. Adams, D.H. Baker, B.A. Logan, D.R. Bowling, A.S. Verhoeven, Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation, Physiol. Plant. 98 (1996) 253-264.

[5] D.R. Ort, N.R. Baker, A photoprotective role for O_2 as an alternative electron sink in photosynthesis? Curr. Opin. Plant Biol. 5 (2002) 193-198.

[6] R. Mittler, Oxidative stress, antioxidants and stress tolerance, Trends Plant Sci. 9 (2002) 405-410.

[7] F.M. DaMatta, Drought as a multidimensional stress affecting photosynthesis in tropical tree crops, In: A. Hemantaranjan, (Ed.), Advances in Plant Physiology, Vol. 5, Scientific Publishers, Jodhpur, 2003, pp.227-265.

[8] F.M. DaMatta, A.B. Rena, Tolerância do café à seca, In: L. Zambolim, (Ed.), Tecnologias de Produção de Café com Qualidade, Universidade Federal de Viçosa, Viçosa, 2001, pp. 65-100.

[9] A.L.S. Lima, F.M. DaMatta, H.A. Pinheiro, M.R. Totola, M.E. Loureiro, Photochemical responses and oxidative stress in two clones of *Coffea canephora* under water deficit conditions, Environ. Exp. Bot. 47 (2002) 239–247.

[10] N. Smirnoff, Antioxidant systems and plant response to the environment, In: N. Smirnoff, (Ed.), Environment and Plant Metabolism – Flexibility and Acclimation, BIOS Scientific Publishers, Oxford, 1995, pp. 217-243.

[11] F.M. DaMatta, A.R.M. Chaves, H.A. Pinheiro, C. Ducatti, M.E. Loureiro, Drought tolerance of two field-grown clones of *Coffea canephora*, Plant Sci. 164 (2003) 111-117.

[12] L. Slooten, K. Capiau, W. Van Camp, M. Van Montagu, C. Sybesma, D. Inzé, Factors affecting the enhancement of oxidative stress in transgenic tobacco overexpressing manganese superoxide dismutase in the chloroplasts, Plant Physiol. 107 (1995) 737-750.

[13] F.M. DaMatta, M. Maestri, P.R. Mosquim, R.S. Barros, Photosynthesis in coffee (*Coffea arabica* and *C. canephora*) as affected by winter and summer conditions, Plant Sci. 128 (1997) 43-50.

[14] F.M. DaMatta, R.A. Loos, E.A. Silva, M.E. Loureiro, Limitations to photosynthesis in *Coffea canephora* as a result of nitrogen and water availability, J. Plant Physiol. 159 (2002) 975-981.

[15] U. Schreiber, W. Bilger, C. Neubauer, Chlorophyll fluorescence as a nonintrusive indicator of rapid assessment of in vivo photosynthesis, In: E.D.M. Schulze, M.M. Caldwell, (Eds.), Ecophysiology of Photosynthesis, Springer-Verlag, Berlin, 1995, pp. 49-70.

[16] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248-254.

[17] C.H. Foyer, B. Halliwell, The presence of glutathione and glutathione reductase in chloroplasts: A proposed role in ascorbate acid metabolism, Planta 133 (1976) 21-25.

[18] I. Yamazaki, I.H. Piette, Mechanism of free radical formation and disappearance during the ascorbic acid oxidase and peroxidase reactions, Biochim. Biophys. Acta 50 (1961) 62-69.

[19] S. Shigeoka, Y. Nakano, S. Kitaoka, Metabolism of hydrogen peroxide in *Euglena gracilis* by L-ascorbic acid peroxidase, Biochem. J. 186 (1980) 377-380.

[20] H. Costa, S.M. Gallego, M.L. Tomaro, Effect of UV-B radiation on antioxidant defense system in sunflower cotyledons, Plant Sci. 162 (2002) 939-945.

[21] N. Arakawa, K. Tsutsumi, N.G. Sanceda, T. Kurata, C. Inagaki, A rapid and sensitive method for the determination of ascorbic acid using 4,7-diphenyl-1,10-phenanthroline, Agric. Biol. Chem. 45 (1981) 1289–1290.

[22] A. Polle, Dissecting the superoxide dismutase-ascorbate-glutathione-pathway in chloroplasts by metabolic modeling. Computer simulations as a step towards flux analysis, Plant Physiol. 126 (2001) 445-462.

[23] S.C. Grace, B.A. Logan, Acclimation of foliar antioxidant systems to growth irradiance in three broad-leaved evergreen species, Plant Physiol. 112 (1996) 1631-1640.

[24] J.M. Cheeseman, L.B. Herendeen, A.T. Cheeseman, B.F. Clough, Photosynthesis and photoprotection in mangroves under field conditions, Plant Cell Environ. 20 (1997) 579-588.

[25] C.L.M. Sgherri, M. Maffei, F. Navari-Izzo, Antioxidative enzymes in wheat subjected to increasing water deficit and rewatering, J. Plant Physiol. 157 (2000) 273-279.

[26] C.L.M. Sgherri, F. Navari-Izzo, Sunflower seedlings subjected to increasing water deficit stress: oxidative stress and defense mechanisms, Physiol. Plant. 93 (1995) 25-30.

[27] B. Loggini, A. Scartazza, E. Brugnoli, F. Navari-Izzo, Antioxidative defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought, Plant Physiol. 119 (1999) 1091-1099.

[28] F.M. DaMatta, M. Maestri, R.S. Barros, Photosynthetic performance of two coffee species under drought, Photosynthetica 34 (1997) 257-264.

GENERAL CONCLUSIONS

Clonal ability to postpone dehydration was more important than dehydration tolerance. Overall, this was associated with (i) strong stomatal sensitivity to soil drying and, to a lesser extent, with leaf-to-air vapour pressure deficit; (ii) increases in leaf carbon isotope composition in stressed plants, suggesting higher water-use efficiency; and (iii) relatively high modulus of elasticity. The clone 109A, although possessing greater root to shoot mass ratio, is shallow-rooted and showed a relatively poor stomatal control on transpiration; these features could explain why it experienced symptoms of drought stress earlier after suspending irrigation. The clone 46 is also shallow-rooted but exhibited a better stomatal sensitivity to both soil and atmospheric drought; hence the clone 46 was better able to delay dehydration than the clone 109A. Similarly to clone 46, stomatal sensitivity to drought was well developed in clones 14 and 120, but these clones showed a remarkably deeper root system than the drought-sensitive clones, which could explain their better avoidance to drought. In any case, the larger soil-to-leaf hydraulic conductance in clone 14 might be involved in the slower decrease in its predawn xylem pressure potential than in the clone 120. The prompt recovery of water potential, stomatal conductance and soil-to-leaf hydraulic conductance following re-watering, altogether with the remarkable stomatal sensibility to soil drought, should explain greatly why robusta coffee responses strongly to irrigation.

Induction of defence mechanisms against oxidative stress triggered by drought was greater in drought-sensitive than in drought-tolerant clones. This might be an indication of increased oxidative processes suffered by the sensitive clones. However, there was no apparent link between oxidative damage with differences in clonal tolerance to drought. For example, no clear distinction between the drought-tolerant clone 14 and the drought-sensitive clone 46, in terms of defence mechanisms to cope with oxidative stress, could be established. Overall, the present data lend support to explain the high degree of drought tolerance in robusta coffee taking into consideration that no long-term effect of drought on the photosynthetic apparatus had occurred. Additionally, the clones herein investigated were able to preserve, or even to

increase, their antioxidant defences at water potentials as low as −3.5 MPa. In any case, clonal differences in drought tolerance in robusta coffee should be greatly determined by the combination of mechanisms that effectively postpone dehydration and, associated with deep root systems, they should contribute to survival and/or stability of crop yield of drought-tolerant clones in regions with unpredictable rainfall. Attributes such as osmotic and elastic adjustments and protection against oxidative damage induced by drought are unlikely to be of major importance to drought tolerance in this specie.