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Effects of long-term soil drought on photosynthesis and carbohydrate metabolism in mature robusta coffee (*Coffea canephora* Pierre var. *kouillou*) leaves

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Abstract

Four clones of robusta coffee representing drought-tolerant (14 and 120) and drought-sensitive (46 and 109A) genotypes were submitted to a slowly imposed water deficit. Sampling and measurements were performed when predawn leaf water potential (Ψ_{pd}) approximately −2.0 and −3.0 MPa was reached. Regardless of the clone evaluated, drought led to sharper decreases in stomatal conductance than in photosynthesis, which was accompanied by significant declines in internal to ambient CO_2 concentration ratio. Little or no effect of drought on chlorophyll *a* fluorescence parameters was observed. Regardless of the stress intensity, starch decreased remarkably. This was not accompanied by significant changes in concentration of soluble sugars, with the exception of clone 120 in which a rise in sucrose and hexose concentrations was found when Ψ_{pd} reached −3.0 MPa. At $\Psi_{pd} = -2.0$ MPa, activity of acid invertase increased only in clone 120; at $\Psi_{pd} = -3.0$ MPa, it increased in clones 14, 46 and 120, while activity of sucrose synthase declined, but only in clone 109A. Drought-induced decrease in ADP-glucose pyrophosphorylase activity was found only in clones 14 and 46, irrespective of stress intensity. At $\Psi_{pd} = -3.0$ MPa, maximal extractable and activation state of sucrose-phosphate synthase (SPS) decreased in all clones with the exception of clone 120, in which SPS activity was maintained in parallel to a rising activity of fructose-1,6-bisphosphatase. Changes in SPS activity could neither be explained by the CO_2 decrease linked to stomatal closure nor by differences in leaf water status.

Keywords: Carbon metabolism; Chlorophyll fluorescence; Coffee; Gas exchange; Sucrose-phosphate synthase; Water deficit

1. Introduction

Despite great progress in understanding the effects of drought stress on photosynthesis, there is still no unified concept of the events that reduce photosynthetic efficiency (Tezara et al., 1999; Cornic, 2000; Lawlor and Cornic, 2002; Chaves and Oliveira, 2004). Several reports have invoked the importance of stomatal closure in restricting the supply of CO_2 to metabolism, especially upon mild drought-stress conditions (Cornic, 2000), whereas other studies suggest that photosynthesis may be more directly limited by non-stomatal factors, particularly via a direct effect of drought on the ATP synthase, thus leading to a restricted ATP supply (Tezara et al., 1999; Lawlor, 2002). The accumulation of carbohydrates often observed under drought conditions could also limit photosynthesis through a diminished supply of inorganic phosphate to the Calvin cycle (Chaves, 1991). Stomatal limitation to photosynthesis is associated with a decrease in the CO_2 concentration in the cellular spaces of the leaf (C_i), which has been linked to impaired metabolism, e.g., inhibition of nitrate reductase and sucrose-phosphate synthase (SPS) (Lawlor, 2002). Stomatal, but not biochemical, limitation could be overcome by an elevated supply of external CO_2. In any case, the drought-induced decline in photosynthesis may result in an over-reduction of photosynthetic electron chain. The ensuing excitation energy should be dissipated, e.g., via non-photochemical quenching by the

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xanthophyll cycle, in order to protect the photosystem (PS) II against increased production of reactive oxygen species (Ort and Baker, 2002; Asada, 1999).

In addition to affecting photosynthesis directly or indirectly, drought stress often strongly depresses plant growth. Therefore, by restricting production and consumption of photoassimilates drought episodes will inevitably change the partitioning of carbon at both the leaf and the whole plant levels (Chaves, 1991). Modifications in the size of carbohydrate pools obviously depend on the severity and duration of water deficit, but at least under mild drought stress, a decrease in starch with a concomitant accumulation of soluble sugars is frequently observed (DaMatta et al., 1997a; Pelleschi et al., 1997; Vu et al., 1998; Basu et al., 1999). Such a shift in carbon partitioning may be adaptive since it could contribute to osmotic adjustment (Daie, 1996; Lawlor and Cornic, 2002).

The leaf activity of the two key enzymes involved in sucrose utilisation, invertase (particularly acid invertase) and sucrose synthase (SuSy), is usually increased under water restriction, which may account for a drought-induced accumulation of hexoses (Castrillo, 1992; Keller and Ludlow, 1993; Pelleschi et al., 1997). By contrast, most studies suggest that the activity of the key enzyme in sucrose synthesis, SPS, is depressed by drought conditions (Pelleschi et al., 1997; Foyer et al., 1998; Vu et al., 1998; Lawlor and Cornic, 2002; Chaitanya et al., 2003; Widodo et al., 2003). Some studies that showed increases in leaf SPS activity were performed under mild water deficit (Quick et al., 1989; Basu et al., 1999) or under very rapidly imposed water-deficit conditions (Quick et al., 1989). In any case, water shortage could ultimately lead to a sucrose accumulation in source leaves, as occurs when sink consumption is restricted (Quick et al., 1989). On the contrary, an increase for starch-hydrolysing enzymes in drought-stressed leaves has been often observed (Keller and Ludlow, 1993; Yang et al., 2001; Zeeman et al., 2004), which would in part explain the decreased starch levels with rising leaf water deficit (Chaves, 1991; DaMatta et al., 1997a; Vu et al., 1998; Basu et al., 1999).

Coffee, a tropical tree crop, is the most important commodity in international agricultural trade, generating over 90,000 million dollars each year and involving about 500 million people to manage the product, from cultivation to final consumption. Currently, robusta coffee (*Coffea canephora* Pierre) produces about 38% of coffee consumed. In south-eastern Brazil, this species is largely cultivated in drought-prone areas. Limited water supply is the major environmental stress affecting coffee production not only in Brazil but also in several other coffee growing countries (DaMatta, 2004). Previous studies have shown that there seems to be considerable clonal variation in drought tolerance within robusta coffee (DaMatta and Rena, 2001; Lima et al., 2002; DaMatta et al., 2003; Pinheiro et al., 2004). Drought-tolerant clones have been characterised by deep root systems (Pinheiro et al., 2004), and improved tissue water status (DaMatta et al., 2003; Pinheiro et al., 2004) associated with maintenance of leaf area (DaMatta et al., 2003) as soil water becomes limiting. In spite of possessing these characteristics, severe plant water deficits may occur during prolonged drought periods. A better understanding of the mechanisms that enable plants to acclimate to water shortage and maintain growth and yield during drought episodes will ultimately help in the selection of drought-tolerant clones.

If drought stress progresses 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 drought (DaMatta, 2003). For example, changes in carbohydrate pools, in addition to depending on severity and duration of water deficit, should also reflect genotypic differences in the regulation of carbon metabolism and partitioning at the whole plant level. Maintenance of export capacity under drought stress, e.g., for allowing root growth, might in part explain differences in drought tolerance among genotypes. In this work, the major goal was to test the hypothesis that drought-tolerant clones differ from drought-sensitive ones at a biochemical level, through adjustments in photosynthesis and carbohydrate metabolism. For this purpose, one group of plants was continuously irrigated while water was withheld from a second group to promote a drought response. Photosynthesis, carbohydrate pools and key enzymes associated with carbohydrate metabolism were then assessed. Physiological and biochemical analyses were performed in slowly drought-stressed plants at similar internal water status, thus allowing more reliable comparisons among clones to be made.

2. Materials and methods

2.1. Plant material and experimental design

Four clones of *C. canephora* Pierre var. *kouillou* (known in Brazil as Conilon) representing drought-tolerant (14 and 120) and drought-sensitive (46 and 109A) genotypes were used. These clones all produce a good crop when grown under irrigation; under limited soil water, however, both survival and productivity as well as maintenance of tissue water status are impaired to a greater extent in the drought-sensitive than in the drought-tolerant clones. However, maintenance of water status is better, but stability of crop yield is poorer, in clone 14 than in clone 120 (Ferrão et al., 2000a,b).

Forty plants (10 per each clone) were obtained as rooted stem cuttings from the Institute for Research and Rural Assistance of the Espírito Santo State, Brazil. They were grown in Viçosa (20°45′S, 650 m a.s.l.), south-eastern Brazil, in a screen house with walls of coarse mesh screen, which permitted free exchange of air with the external environment. Plants, receiving an average midday photosynthetic photon flux (PPF) about 900 $\mu mol\ m^{-2}\ s^{-1}$, were grown individually in large pots (0.8 m high, 0.44 m internal diameter) containing 120 L of a mixture of soil, sand and manure (3:1:1, v/v/v) and a gravel layer at the bottom. When 12-months old (February 2002), plants of each clone were separated in two groups; one

continued to receive regular irrigation (control plants), and in the other, water was withheld (drought-stressed plants). For control plants, leaf predawn water potential (Ψ_{pd}), as measured periodically with a Scholander-type pressure chamber, was always above −0.1 MPa. Drought was allowed to progress until Ψ_{pd} reached about −2.0 and −3.0 MPa. On average, these target Ψ_{pd} were reached, respectively, at 15 and 19 days for clone 109A, 18 and 24 days for clone 46, 20 and 24 days for clone 120, and 23 and 29 days for clone 14. In these occasions, leaves from the third or fourth pair from the apex of plagiotropic branches were sampled for physiological and biochemical analyses. For the latter, leaf discs, collected at about 1000 h (ca. 4.5 h after the beginning of photoperiod), were rapidly frozen in liquid nitrogen and stored at −80 °C until processing.

2.2. Photosynthetic parameters

The net carbon assimilation rate (A), stomatal conductance to water vapour (g_s), and internal to ambient CO_2 concentration ratio (C_i/C_a) were measured at 0800–1000 h 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 DaMatta et al. (1997b). Chlorophyll *a* fluorescence was measured with a portable pulse amplitude modulation fluorometer (FMS2, Hansatech, King's Lynn, Norfolk, UK). Measurements of the variable to maximum fluorescence ratio (F_v/F_m) were made following dark-adaptation for 30 min. Photochemical quenching coefficient (q_p), Stern–Volmer non-photochemical quenching coefficient (NPQ) and the quantum yield of PSII electron transport (Φ_{PSII}) were estimated 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}. Further technical details have been given previously (DaMatta et al., 2002; Lima et al., 2002). All the above measurements were carried out at ambient (400 ± 10 μmol mol^{-1}) CO_2, 23 ± 2 °C air temperature and 80 ± 2% relative humidity.

2.3. Carbohydrates and aminoacids

Frozen leaf discs (ca. 20 mg FW) were homogenised using 80% (v/v) aqueous ethanol. The powdered material was shaken and incubated at 80 °C for 20 min, and then centrifuged at 16,000 × g for 10 min. The pellets were extracted further two times with aqueous ethanol. Supernatants were retained, combined, and stored at −20 °C for soluble sugar and aminoacid determinations. Glucose, fructose and sucrose were measured through the reduction of NAD^+ by glucose-6-P dehydrogenase after the sequential addition of hexokinase, phosphoglucose isomerase and invertase (Trethewey et al., 1998). Reduction of NAD^+ was continuously followed at 340 nm using an ELISA reader (Tunable Microplate Reader, VERSAmax, Sunnyvale, USA). The ethanol-insoluble pellet was used for starch extraction. The pellet was suspended in KOH, incubated at 95 °C for 1 h, neutralised with acetic acid and centrifuged at 16,000 × g for 10 min. Starch was then hydrolysed in 100 mol m^{-3} citrate buffer at pH 4.6 containing amyloglucosidase and α-amylase (Trethewey et al., 1998), and the released glucose was measured as above.

Aminoacids were assayed according to Yemm and Cocking (1955) with modifications (Yves Gibon, personal communication). The reaction mixture contained 50 μL of 1 kmol m^{-3} citrate–NaOH buffer at pH 5.2 with 0.2% (w/v) ascorbic acid, 50 μL ethanolic extract and 100 μL of 1% (v/v) ninhydrin in 70% (v/v) aqueous ethanol. The reaction was allowed to occur for 20 min at 95 °C, and the resulting colour was read at 570 nm in the ELISA reader. Aminoacid concentration was estimated using a standard curve with an equimolecular mixture of glycine, glutamic acid, phenylalanine and arginine in 70% (v/v) ethanol.

2.4. Enzyme extraction and assays

Frozen leaf tissues (ca. 200 mg FW) were ground in a cold mortar and pestle using a suitable amount of polyvinylpolypyrrolidone with 0.5 mL extraction buffer at pH 7.4 with final concentrations 50 mol m^{-3} HEPES-KOH, 5 mol m^{-3} $MgCl_2$, 1 mol m^{-3} EDTA, 1 mol m^{-3} EGTA, 10 mol m^{-3} β-mercaptoethanol, 2 mol m^{-3} benzamidine, 2 mol m^{-3} ε-amino-*n*-caproic acid, 5 mol m^{-3} PMSF, 0.1% (w/v) BSA, 10% (v/v) glycerol, and 0.1% (v/v) Triton X-100. Following centrifugation at 15,000 × g for 15 min (4 °C), the supernatant was gel-filtered through Sephadex G-25 columns equilibrated with extraction buffer. Extracts were then stored at −80 °C until required.

Sucrose-phosphate synthase (SPS; EC 2.4.1.14) was assayed according to Doehlert and Huber (1983), under saturating (non-selective assay; V_{max}) and limiting (selective assay; V_{sel}) substrate conditions. The V_{max} assay consisted of 50 μL desalted extract, 50 mol m^{-3} MOPS-KOH at pH 7.4, 12 mol m^{-3} $MgCl_2$, 1 mol m^{-3} DTT, 6 mol m^{-3} UDP-glucose, 36 mol m^{-3} glucose-6-P, and 12 mol m^{-3} fructose-6-P, in a 70 μL final volume. The V_{sel} assay was the same as above, except that it contained 6 mol m^{-3} glucose-6-P and 2 mol m^{-3} fructose-6-P and, additionally, 5 mol m^{-3} Pi. Assays were incubated for 20 min at 25 °C, and stopped with 70 μL of 5 kmol m^{-3} KOH. Subsequently, 1 mL of 0.15% (w/v) anthrone in concentrated H_2SO_4 was added. The reaction mixture was incubated for 20 min at 40 °C. Sucrose was determined at 620 nm.

Fructose-1,6-bisphosphatase (FBPase; EC 3.1.3.11) was assayed in a reaction mixture (300 μL total volume) containing 30 μL desalted extract, 20 mol m^{-3} HEPES-KOH at pH 7.0, 5 mol m^{-3} $MgCl_2$, 500 mmol m^{-3} NAD^+, 0.25 units mL^{-1} each of glucose-6-P isomerase and glucose-6-P dehydrogenase, and 300 mol m^{-3} fructose-1,6-BP to start the reaction (Sharkey et al., 1991).

ADP-glucose pyrophosphorylase (AGPase; EC 2.2.7.27) was assayed in a reaction mixture (300 μL total volume) containing 150 μL desalted extract, 80 mol m^{-3} HEPES-KOH at pH 7.9, 2 mol m^{-3} $MgCl_2$, 10 mmol m^{-3} glucose-1,6-

BP, 10 mol m^{-3} phosphoglyceric acid, 5 mol m^{-3} DTT, 0.2 mol m^{-3} NAD^+, 1 mol m^{-3} ADP-glucose, 10 mol m^{-3} NaF, 1 unit mL^{-1} phosphoglucomutase, 2.5 unit mL^{-1} glucose-6-P dehydrogenase, and 1.5 mol m^{-3} Na-PPi to start the reaction (Müller-Röber et al., 1992).

ATP-dependent phosphofructokinase (ATP-PFK; EC 2.1.7.11) was assayed in a mixture (300 μL total volume) containing 70 μL desalted extract, 100 mol m^{-3} Tris–HCl at pH 8.0, 5 mol m^{-3} $MgCl_2$, 1 mol m^{-3} ATP, 0.1 mol m^{-3} NADH, 1 unit mL^{-1} aldolase, 10 units mL^{-1} triose-P isomerase and 1.8 unit mL^{-1} glycerol-3-P dehydrogenase, and 5 mol m^{-3} fructose-6-P to start the reaction (Burrell et al., 1994).

Acid invertase (EC 3.2.1.26) and sucrose synthase (SuSy; EC 2.4.1.13) were assayed as in Geigenberger and Stitt (1993) in a 100 μL reaction mixture. For acid invertase, the mixture contained 60 mol m^{-3} acetate buffer at pH 4.7, 100 mol m^{-3} sucrose, and 60 μL desalted extract. The reaction was allowed to occur for 3 h at 37 °C, after which it was neutralised with Na-phosphate buffer at pH 7.2, and then stopped under boiling water. Invertase was quantified through glucose released into the medium, as described above. For SuSy, the reaction mixture containing 70 μL desalted extract, 20 mol m^{-3} HEPES-KOH at pH 7.0, 100 mol m^{-3} sucrose, and 4 mol m^{-3} UDP. The reaction was allowed to proceed for 1 h at 25 °C, and was terminated in a boiling water bath. The concentration of UDP-glucose formed was assessed enzymatically by adding 80 μL of the reaction mixture into a second medium containing 200 mol m^{-3} glycine at pH 8.9, 5 mol m^{-3} $MgCl_2$, 2 mol m^{-3} NAD^+, and 0.02 unit mL^{-1} UDP-glucose dehydrogenase. Reduction of NAD^+ was measured at 340 nm.

For all enzymes, checks were made for linearity of enzyme activities over time and for proportionality between rate and amount of extracts.

2.5. Statistics

The experiment was a completely randomized design, forming a $4 \times 2 \times 2$ factorial (four clones, two watering regimes, and two sampling periods) with five plants in individual containers per treatment-combination as replication. The experimental plot was one plant per container. 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, sampling period and irrigation treatment differences using Newman–Keuls and *F*-tests.

3. Results

Four clones (14 and 120, drought-tolerant; 46 and 109A, drought-sensitive) of robusta coffee were subjected to progressive drought stress, until Ψ_{pd} attained −3.0 MPa. The decline in Ψ_{pd} upon discontinuing irrigation was faster in clone 109 (155 kPa d^{-1}) and slower in clone 14 (102 kPa d^{-1}), and intermediate in clones 46 and 120 (125 kPa d^{-1}) (data not shown).

At $\Psi_{pd} = -2.0$ MPa, there were large decreases in *A* (61–71%) and, to a greater extent, in g_s (78–88%) in all cones. This was accompanied by significant declines in C_i/C_a ratio (13–24%). Very similar results were observed at $\Psi_{pd} = -3.0$ MPa (Fig. 1). In spite of the sharp decreases in *A*, F_v/F_m did not respond to drought (Fig. 2A and B). Only minor changes in other photochemical parameters were observed, but solely at $\Psi_{pd} = -3.0$ MPa (Fig. 2). Relative to control plants, q_P decreased (15–30%) in all clones subjected to drought, while NPQ declined significantly in clones 14 (22%) and 46 (28%). The quantum yield of PSII electron transport decreased in clones 14 (23%) and 109A (22%) under drought; such decreases did not reach statistical significance in clones 46 (19%) and 120 (13%).

Concentrations of hexoses (Fig. 3A and B) and sucrose (Fig. 3C and D) did not change significantly in drought-stressed plants relative to control ones, with the exception of clone 120 in which an increase in hexoses (57%) and sucrose (97%) was found when Ψ_{pd} reached −3.0 MPa. By contrast, regardless of the clone investigated, starch concentration was substantially lower (31–81%) in drought-stressed plants than in control ones (Fig. 3E and F). Concentration of aminoacids remained unchanged in clones 14 and 46, but increased significantly in clones 120 (73%) at $\Psi_{pd} = -3.0$ MPa and 109A (∼80%), irrespective of the drought intensity (Fig. 3G and H).

Compared with control plants, total SPS activity (non-selective assay) was maintained at $\Psi_{pd} = -2.0$ MPa, but its activation state (selective assay) decreased in clones 46 (51%) and 109A (62%) (Fig. 4A and B). By contrast, both activity and activation state declined at $\Psi_{pd} = -3.0$ MPa, respectively 27% and 56% for clone 14, 47% and 48% for clone 46, and 65% and 94% for clone 109A (Fig. 4C and D). In clone 120, however, total and selective SPS activities were unaffected by drought (Fig. 4), but a significant build-up (∼50%) in FBPase activity occurred, regardless of the drought intensity (Fig. 5A,B). At $\Psi_{pd} = -2.0$ MPa, activity of acid invertase increased (114%) only in clone 120 (Fig. 5C); at $\Psi_{pd} = -3.0$ MPa, it increased significantly in clones 14 (236%), 46 (226%) and 120 (72%) (Fig. 5D). Activity of SuSy declined (40%) only in clone 109A at $\Psi_{pd} = -3.0$ MPa (Fig. 5F). Activity of AGPase lowered in clones 14 (74%) and 46 (∼65%) (Fig. 5G and H) regardless of stress severity, while that of PFK decreased only in clone 46 at $\Psi_{pd} = -2.0$ MPa (Fig. 5I). When not indicated, activity of a given enzyme for a particular clone remained unchanged in response to drought imposition.

4. Discussion

Upon discontinuing irrigation, plant water stress developed more slowly in clone 14 and more rapidly in clone 109A, but no clear distinction in this regard could be made between

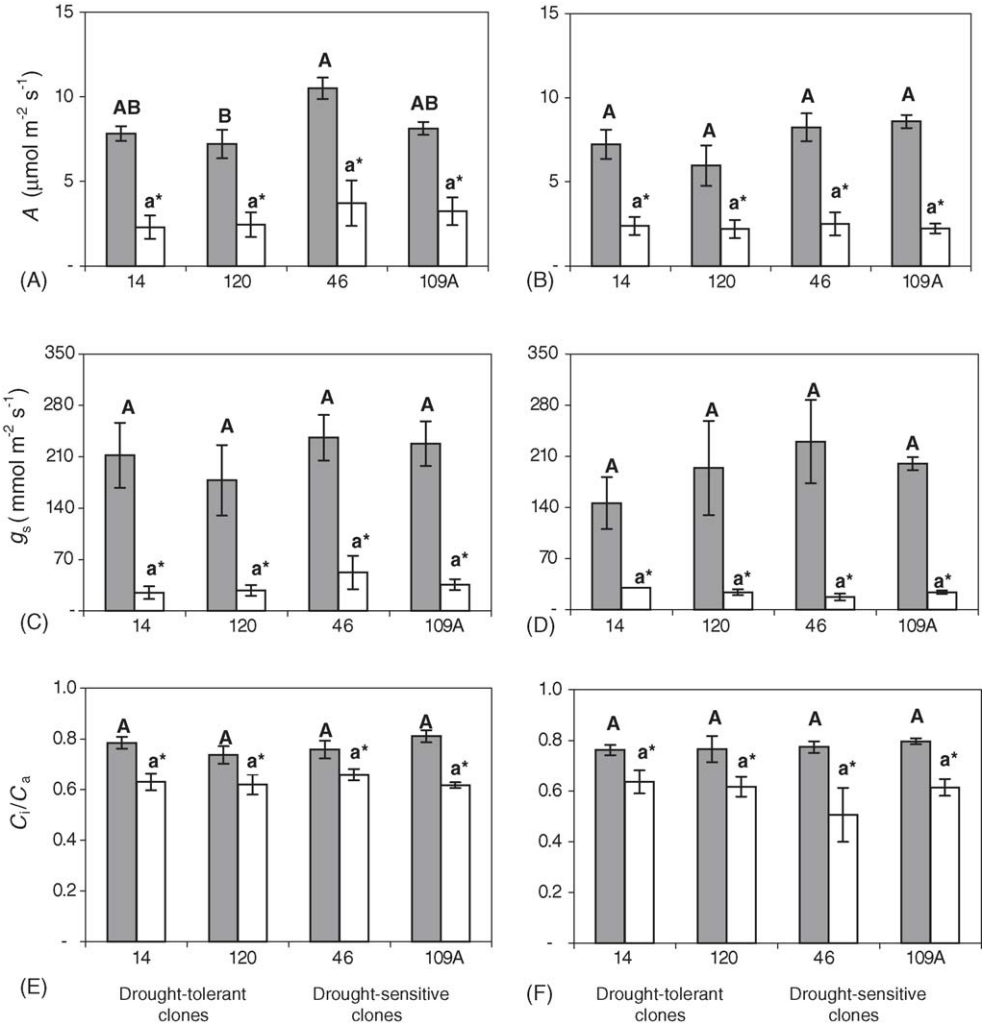

Fig. 1. Effects of drought on the net carbon assimilation rate (A; A and B), stomatal conductance (g_s; C and D), and internal to ambient CO_2 concentration ratio (C_i/C_a; E and F) of four clones of robusta coffee (grey columns, control plants; white columns, drought-stressed plants). Drought was allowed to progress until predawn leaf water potential approximately −2.0 MPa (left) and −3.0 MPa (right) was reached. 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). For a given clone, parameter means obtained either at −2.0 or at −3.0 MPa did not differ significantly to each other. Values are means ± S.E. of five replicates.

the drought-sensitive clone 46 and the drought-tolerant clone 120. These results differ from previous observations in field (DaMatta et al., 2003) and in greenhouse (Pinheiro et al., 2004) in which clone 120 was better able to postpone tissue dehydration than clone 46. Such discrepancy might perhaps be associated with age-related differences in growth attributes among the plants of this study and those of the other ones quoted above. In any case, clone 120 has been characterised as showing a deeper root system and a more efficient antioxidant system to protection against both drought- and paraquat-induced oxidative stress than does the clone 46 (Pinheiro et al., 2004).

It was previously demonstrated that rapid imposition of water deficit (Ψ_{pd} reaching −3.0 MPa after suspending irrigation for 6 days) in clones 109A and 120 resulted in a complete suppression of A and increased C_i (Lima et al., 2002), hence characterising a severe drought treatment. In contrast, the present results indicate that, if drought progresses slowly, the coffee plants can acclimate to water shortage and, thus, they could minimise the deleterious effects of drought, e.g., by partially maintaining photosynthetic rates. In fact, the sharper decreases in g_s than in A with a concomitant decline in C_i/C_a indicate that stomatal limitations rather than stress-induced dysfunctions at the chloroplast level accounted largely for the inhibition of CO_2 assimilation under drought-stress conditions. The fast recovery of gas-exchange rates upon re-watering, as found by Pinheiro (2004) in the clones herein evaluated under similar conditions to those of this study, is also consistent with a stomatal control on photosynthesis during drought

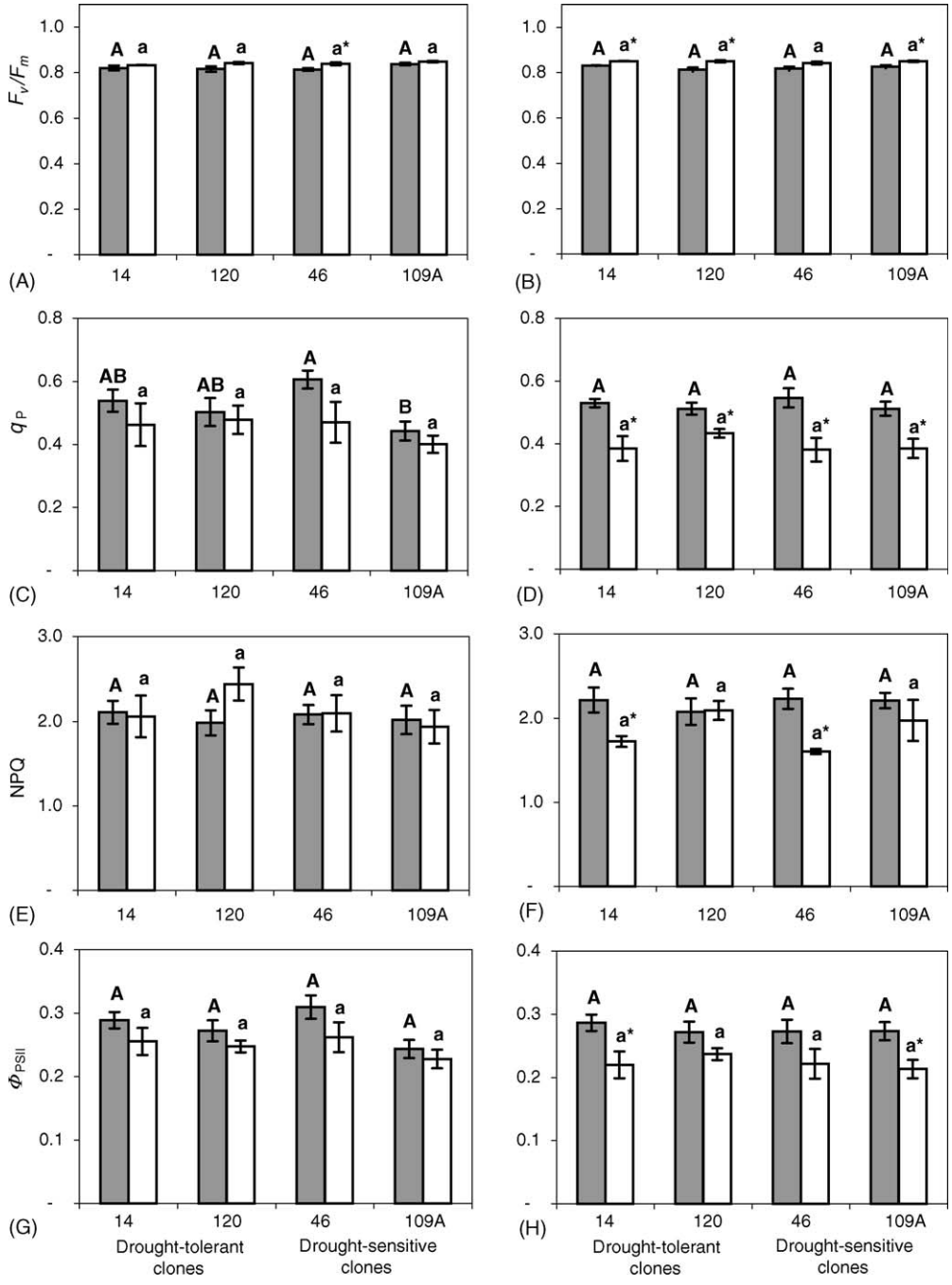

Fig. 2. Effects of drought on maximum photochemical efficiency of PSII (F_v/F_m; A and B), photochemical (q_P; C and D) and Stern–Volmer non-photochemical (NPQ; E and F) quenching coefficients, and quantum yield of PSII electron transport (Φ_{PSII}; G and H) of four clones of robusta coffee. For a given clone, NPQ was the unique parameter decreasing ($P \leq 0.05$) further at −3.0 MPa when compared with plants at −2.0 MPa, but only in clone 46. Legends and other statistical details as in Fig. 1.

stress. It is 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 (DaMatta, 2003). Nonetheless, these observations do not dismiss that biochemical limitations, which could be assessed under saturating CO_2, might be also in part constraining photosynthesis in drought-stressed plants, despite the observed decrease in C_i/C_a ratio. In any case, as shown by others in robusta coffee (DaMatta et al., 2002; Lima et al., 2002; Pinheiro et al., 2004), the maximum quantum yield of PSII photochemistry, as analysed by F_v/F_m, did not decrease under drought conditions, suggesting that photoinhibition did not occur. Also, the observed decrease in A was not accompanied by increases in NPQ, and hence no drought-induced alteration in thermal dissipation

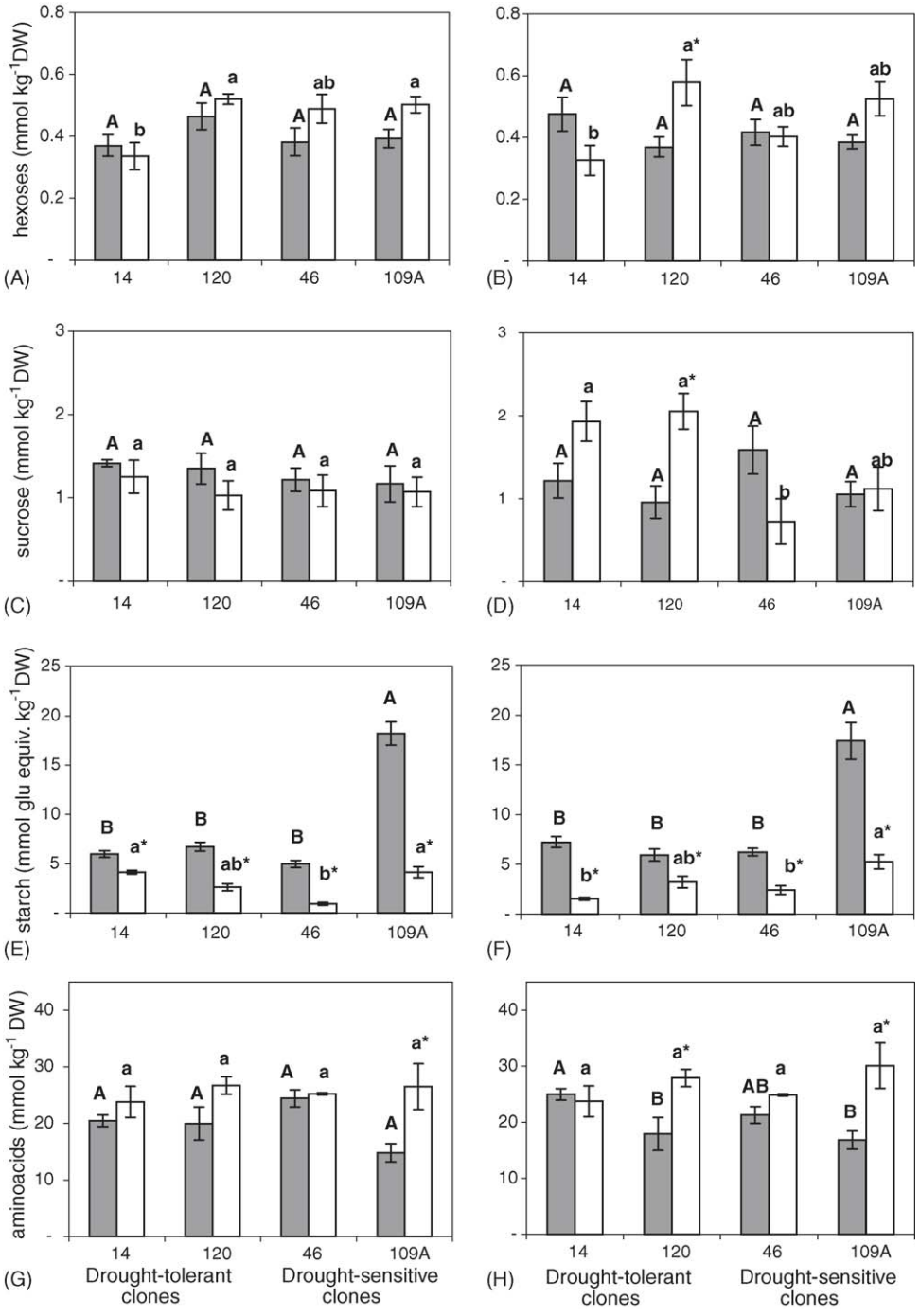

Fig. 3. Effects of drought on the concentrations of hexoses (A and B), sucrose (C and D), starch (E and F) and aminoacids (G and H) of four clones of robusta coffee. For a given clone, starch concentration was the unique parameter decreasing ($P \leq 0.05$) further at −3.0 MPa when compared with plants at −2.0 MPa, but only in clone 14. Legends and other statistical details as in Fig. 1.

should have occurred. Drought led to a slight increase in the excitation pressure on PSII (as indicated by the decrease in q_P), implying that a fraction of the PSII traps was closed during actinic irradiance, thus bringing about a decrease in Φ_{PSII}. Reductions in Φ_{PSII} paralleling an unchanged F_v/F_m were likely associated with a down-regulation of PSII during steady-state photosynthesis. Such a reduction should represent a photoprotective mechanism by adjusting the rate of photochemistry to match that of ATP and NADPH consumption (Havaux et al., 1991; Cruz et al., 2003). Therefore, changes in Φ_{PSII} should have been a consequence, rather than a cause, of the partial loss of the photosynthetic capacity.

In spite of the remarkable inhibition of A, hexose and sucrose concentration was maintained in drought-stressed

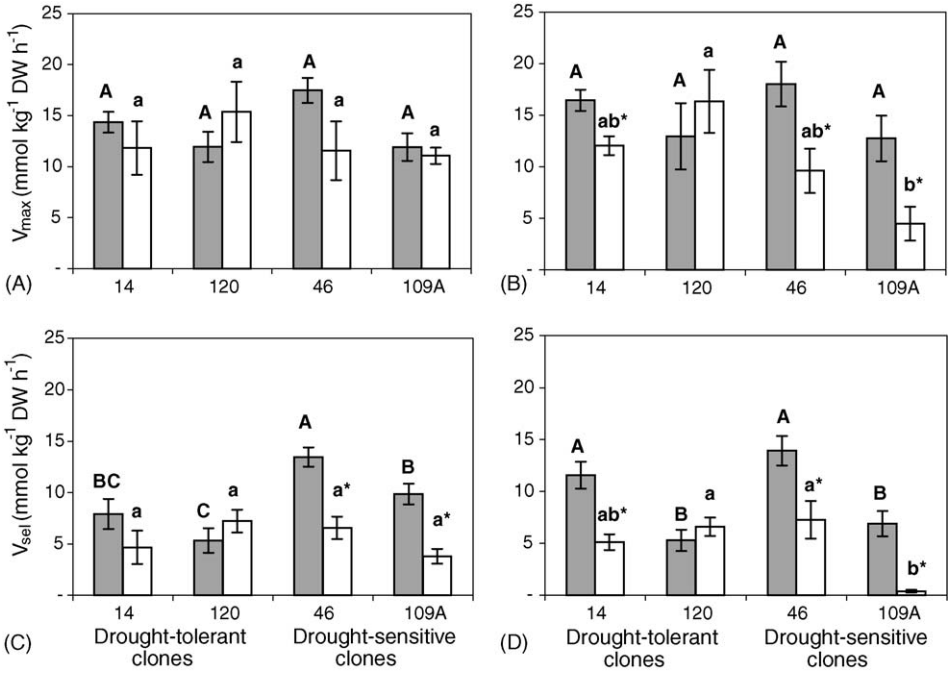

Fig. 4. Effects of drought on the sucrose-phosphate synthase (SPS) under saturating (V_{max}; A and B) and selective (V_{sel}; C and D) substrate conditions of four clones of robusta coffee. For a given clone, both V_{max} and V_{sel} decreased ($P \leq 0.05$) further at −3.0 MPa when compared with plants at −2.0 MPa, but only in clone 109A. Legends and other statistical details as in Fig. 1.

plants relative to control ones in clones 14, 46 and 109A. In those plants, a decrease of sucrose synthesis and a rise of degradation were to be expected due to partial inhibition of SPS and the increase in acid-invertase activity, particularly at $\Psi_{pd} = -3.0$ MPa. However, as the stress progressed, capacity for sucrose synthesis should have been maintained for longer in clone 14 than in clones 46 and 109A, taking into consideration that SPS activation state (as indicated by the V_{sel} activity) was kept in that clone at $\Psi_{pd} = -2.0$ MPa as compared with control plants. In any case, it is unlikely that SuSy has played a major role in sucrose degradation since its activity declined marginally, but only in clone 109A, as also reported for rice (Yang et al., 2001). On the other hand, different results were found by Castrillo (1992) in common bean and by Keller and Ludlow (1993) in pigeonpea, in which SuSy activity increased more than invertase activity under water-deficit conditions. Since sucrose did not decline in response to drought treatment, it might be proposed that export was reduced owing to a decreased sink demand. In fact, in the clones herein evaluated, shoot growth was fully inhibited at some value of Ψ_{pd} above −2.0 MPa (Pinheiro, 2004). Additionally, starch breakdown should have increased in drought-stressed plants, as reported by others (Chaves, 1991; Yang et al., 2001), regardless of whether AGPase activity was maintained or not under drought; thus, starch hydrolysis could partially buffer fluctuations in sugar concentrations when photosynthesis is limiting. In clone 120, by contrast, it is unlikely that drought stress has impaired sucrose synthesis since SPS activity was maintained, and FBPase increased, relative to control plants. At $\Psi_{pd} = -3.0$ MPa, but not at −2.0 MPa, there were an increase in sucrose concentration in clone 120 that was also accompanied by an augmentation in hexoses, consistent with a rise in acid-invertase activity. Sucrose is a compatible solute that could act as an osmoprotectant during desiccation (Schwall et al., 1995) and, therefore, its accumulation might be associated with drought tolerance, as has been proposed for wheat in which drought-tolerant varieties accumulate sucrose to a significantly greater extent than do the drought-sensitive ones (Kerepesi and Galiba, 2000). Furthermore, the likely maintenance of capacity for translocation from leaves under water-deficit conditions (Daie, 1996; Lawlor and Cornic, 2002), and the association between SPS activity and assimilate export (Galtier et al., 1993; Daie, 1996; Balibrea et al., 2000; Li et al., 2002) might support some additional root growth in drought-stressed clone 120. Indeed, this clone exhibits a relatively deeper, more extended root system under field, rain-fed conditions than the other clones here investigated (José S. Silveira, personal communication).

At $\Psi_{pd} = -2.0$ MPa, maximal extractable SPS activity was maintained relative to control plants, but its activation state decreased in clones 46 and 109A. This means that changes in the kinetic properties (changes in V_{max}) rather than changes in protein amount (changes in V_{sel}) should probably have occurred in the drought-sensitive clones. Different results were reported for spinach leaves under short-term water-deficit conditions in which V_{sel} increased paralleling maintenance of V_{max} (Quick et al., 1989). By contrast, at $\Psi_{pd} = -3.0$ MPa, both V_{sel} and V_{max} decreased in parallel as observed in clone 46, or V_{sel} decreased relatively more than V_{max} as found in clones 14 and 109A. Thus, in these clones

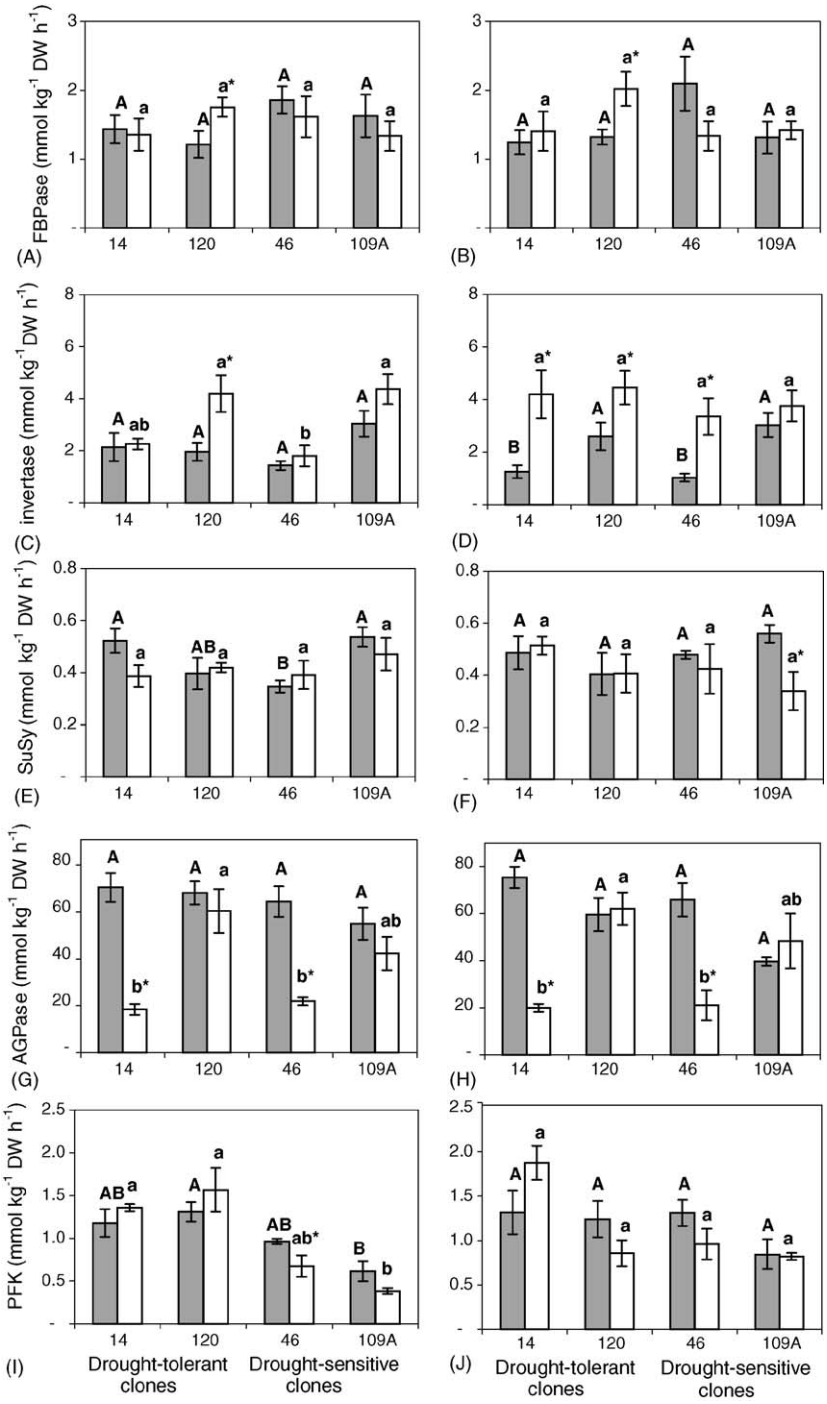

Fig. 5. Effects of drought on the activities of fructose-1,6-bisphosphatase (FBPase; A and B), acid invertase (C and D), sucrose synthase (SuSy; E and F), ADP-glucose pyrophosphorylase (AGPase; G and H), and ATP-dependent phosphofructokinase (PFK; I and J). Legends and statistics as in Fig. 1.

SPS showed no metabolic compensation for decreased maximal extractable SPS activities by changes in kinetic properties that would favour an increase in activity in substrate-limited conditions. Such decreases would serve to reduce the flux of carbon to sucrose in a situation of declining photosynthetic capacity and export (Foyer et al., 1998).

Both light and CO_2 are required for activation of SPS (Stitt and Quick, 1989; Daie, 1996). In several investigations, a decrease in C_i is often invoked as to explain the decrease in SPS activity under mild water-deficit conditions (e.g., Vassey and Sharkey, 1989; Vassey et al., 1991; Pelleschi et al., 1997; Lawlor and Cornic, 2002; Aoki et al., 2003). In this case, reduction in SPS activity could be reversed after exposing leaf tissues to elevated C_a levels over several hours (Vassey et al., 1991). However, the CO_2 effect on SPS is likely rather complex, and the present results show that. While the

clones 14, 46 and 109A exhibited decreases in SPS activity (both V_{sel} and V_{max}) accompanied by declines in C_i/C_a ratio, no decrease in enzyme activity occurred in clone 120 in spite of C_i/C_a was reduced. Moreover, at $\Psi_{pd} = -2.0$ MPa, V_{max} was maintained, whereas V_{sel} was decreased, in clones 46 and 109A even though C_i/C_a was reduced in these clones. According to Daie (1996), drought-induced decrease in SPS activity would not be compatible with observations showing SPS activation under mild drought-stress conditions in which C_i levels would be limiting. In any case, regardless of the mechanism of SPS activation/deactivation, changes in SPS activity would have important implications for photosynthetic rates because of the resulting alterations in the availability of inorganic phosphate for exchange across the chloroplast membrane (Stitt and Quick, 1989).

In summary, the most expressive difference among the clones submitted to a slowly imposed drought resided in the maintenance of both SPS activity and activation state, and increased FBPase activity paralleling a significant augmentation of sucrose and hexose concentrations in the clone 120, but not in the other clones, when Ψ_{pd} reached -3.0 MPa. It should be emphasised that a few works showing no decrease in leaf SPS activity in drought-stressed plants were performed under mild water deficit (e.g., Basu et al., 1999; Yang et al., 2001) or under rapidly imposed water-deficit conditions (Quick et al., 1989). In the present work, changes in SPS activity could neither be explained by the CO_2 decrease linked to stomatal closure, nor by differences in internal water status since enzyme activity was assessed at similar leaf water potential. Finally, while drought tolerance in clone 14 might be more directly associated with survival mechanisms (e.g., more conservative use of water via a low soil-to-leaf hydraulic conductance (Pinheiro, 2004)), in clone 120 drought tolerance might be also linked to biochemical traits such as better protection against oxidative stress (Pinheiro et al., 2004) and maintenance of SPS activity with rising leaf water deficit. This might be associated with assimilate export, which would allow an extra root growth under limited water supply. Moreover, the drought-induced accumulation of soluble sugars and aminoacids should contribute to osmotic adjustment in this clone. Taken together, these characteristics should help the clone 120 in keeping its productivity, contrarily to what is observed with the other clones, as has been empirically found in drought-prone regions.

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