Plant Growth Regulation
https://doi.org/10.1007/s10725-018-0385-5

ORIGINAL PAPER

Reciprocal grafting between clones with contrasting drought tolerance suggests a key role of abscisic acid in coffee acclimation to drought stress

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Received: 28 July 2017 / Accepted: 3 March 2018

Abstract

The role of abscisic acid (ABA) in drought tolerance of *Coffea canephora* is unknown. To determine whether ABA is associated with drought tolerance and if the use of tolerant rootstocks could increase ABA and drought tolerance, we performed reciprocal grafting experiments between clones with contrasting tolerance to drought (clone 109, sensitive; and clone 120, tolerant). Plants were grown in large (120 L) pots in a greenhouse and subjected to drought stress by withholding irrigation. The non-grafted 120 plants and graft treatments with 120 as a rootstock showed a slower reduction of predawn leaf water potential (Ψ_{pd}) and a lower negative carbon isotopic composition ratio compared with the other grafting combinations in response to drought. The same 120 graft treatments also showed higher leaf ABA concentrations, lower levels of electrolyte leakage, and lower activities of ascorbate peroxidase and catalase under moderate ($\Psi_{pd} = -1.0$ or -1.5 MPa) and severe ($\Psi_{pd} = -3.0$ MPa) drought. Root ABA concentrations were higher in plants with the 120 rootstocks regardless of watering regime. The 120 shoots could also contribute to drought tolerance because treatment with 120/109 rootstock/scion combination showed postponed dehydration, higher leaf ABA concentration, and lower leaf electrolyte leakage compared with the sensitive clone. We conclude that both the shoot and root systems of the tolerant clone can increase the concentrations of ABA in leaves in response to drought. This further suggests that ABA is associated with a delayed onset of severe water deficit and decreased oxidative damage in *C. canephora*.

Keywords ABA · Coffee · Oxidative stress · Photosynthesis · Water deficit · Mass spectrometry

Abbreviations

ABA	Abscisic acid
5,8′-8′-8′-d_4 ABA	Deuterated abscisic acid
WUE	Water use efficiency
APX	Ascorbate peroxidase
CAT	Catalase
PPF	Photosynthetic photon flux
SOD	Superoxide dismutase
$\delta^{13}C$	Isotopic composition

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10725-018-0385-5) contains supplementary material, which is available to authorized users.

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Published online: 08 March 2018

g_s	Stomatal conductance to water vapor
Ψ_w	Leaf water potential
HPLC-ESI-MS/MS	High performance liquid chromatography/electrospray ionization tandem mass spectrometry
MRM	Multiple reaction monitoring

Introduction

Due to increased water deficits in arable soils, improvements are needed to develop technologies that guarantee coffee productivity in environments with restricted water. In the case of *Coffea canephora* var. *kouillou* (conilon coffee), plant breeders have empirically selected elite clones that withstand relatively severe drought spells with acceptable yields. Physiological evaluations of several of these clones have suggested that the following traits are very important: an adequate water status achieved via a combination of deep rooting, satisfactory stomatal control of transpiration and leaf area maintenance (DaMatta et al. 2003; Pinheiro et al. 2005); biochemical traits such as improved tolerance to oxidative stress (Lima et al. 2002; Pinheiro et al. 2004); and maintenance of assimilate export ability (Praxedes et al. 2006). These features ultimately contribute to the lowest relative decreases in traits related to carbon gain under drought conditions in drought-tolerant clones (Menezes-Silva et al. 2013). Transcriptomic and proteomic studies with drought-tolerant coffee genotypes have also shown a complex network of responses most likely involving abscisic acid (ABA) and nitric oxide signaling pathways. These are the major molecular determinants that explain the control of transpiration in these clones under drought conditions (Marraccini et al. 2012).

ABA is a predominant candidate for root–shoot communication during water scarcity, but the ABA-producing organ under water deficit conditions remains controversial (Vishwakarma et al. 2017). An alternative method to manipulate root-to-shoot signaling is grafting. Grafting studies have shown that in herbaceous plants, foliar ABA levels have a major influence on the levels of ABA in roots (McAdam et al. 2016). On the other hand, citrus reciprocal grafting experiments have suggested that ABA production could be stimulated in the leaves of susceptible scions under severe drought conditions when using drought-tolerant genotypes as rootstocks (Santana-Vieira et al. 2016).

In a previous study, we evaluated two clones of conilon coffee with contrasting tolerance to drought using reciprocal graftings. We demonstrated that plants having the clone 120 (tolerant) as a rootstock showed a slower decline in water potential under water withholding conditions coupled with improved stomatal closure (Silva et al. 2010). Here, we expand the work aimed at examining the role of ABA in both leaves and roots in drought tolerance in conilon coffee. Our goals were multifold: (1) to determine whether the use of tolerant clones as rootstock could increase drought tolerance in the whole plant; (2) to determine whether ABA is involved in drought tolerance as conferred by the tolerant scion or rootstock; and (3) to determine the influence of rootstock genotype on the carbon isotopic composition and activities of some antioxidant enzymes in leaves.

Materials and methods

Plant material and growth conditions

The experiment was conducted in Viçosa (20°45′S, 42°51′W, 650 m altitude) in southeastern Brazil. Plants of two clones of *C. canephora* Pierre ex Froehner with contrasting tolerance to drought, clones 109 and 120 that were previously characterized as drought sensitive and drought tolerant, respectively (Pinheiro et al. 2004, 2005; Silva et al. 2010), were grown in a greenhouse with an average midday photosynthetic photon flux (PPF) of 900 μmol m^{-2} s^{-1} under naturally fluctuating conditions of temperature and relative humidity.

Reciprocal grafting experiments and water relations

Reciprocal grafting experiments between clone 109 and clone 120 were performed according to Silva et al. (2010). After 50 days of acclimation, grafted plants were transferred to 120-L pots containing a mixture of soil, sand, and manure (3:1:1, v/v/v) and a gravel layer at the bottom. Plants of the 109 and 120 clones and self-grafted plants were used as controls. When grafted plants were 6 months old, they were separated into two groups. One group received regular irrigation (control plants), while water was withheld from the other group (drought-stressed plants). The measured leaf predawn water potential (Ψ_{pd}) of the control plants was always above − 0.1 MPa. Ψ_{pd} was measured using a Scholander-type pressure chamber (model 1000, PMS Instruments, Albany, NY, USA), and drought was allowed to progress until the Ψ_{pd} reached about − 3.0 MPa. All samplings and measurements were made using leaves from the third or fourth pair of apex plagiotropic branches.

C isotope ratio ($\delta^{13}C$)

The carbon isotope composition ratio ($\delta^{13}C$) was measured in bulk leaf tissues relative to the international Pee Dee Belemnite standard using an isotope mass spectrometer (ANCA-GSL 20-20, Sercon, Crewe, UK) as previously described (DaMatta et al. 2002).

Electrolyte leakage

Electrolyte leakage was determined in leaves as described in Lima et al. (2002) when plants reached leaf Ψ_{pd} of − 1.5 and − 3.0 MPa.

Enzymatic assays

In all enzymatic analyses, the leaf discs were collected between 08:00 and 10:00 h when photosynthetic rates were maximal (Silva et al. 2010). Samples were flash frozen in liquid nitrogen and stored at 80 °C until analyses. Activities of the enzymes superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), and ascorbate peroxidase (APX, EC 1.11.1.11) were assayed.

Enzymes were extracted from leaf tissues using an ice-cold mortar and pestle, with polyvinylpolypyrrolidone (PVPP) and 4 mL of the following optimized extraction media: SOD (100 mM K-phosphate buffer, pH 7.8, 0.1 mM EDTA, 1 mM DTT, 10 mM β-mercaptoethanol and 0.1% Triton X-100); APX (50 mM K-phosphate buffer, pH 7.0, 2 mM EDTA, 20 mM ascorbate and 0.1% Triton X-100); and CAT (50 mM K-phosphate buffer, pH 7.8, 2 mM EDTA, 20 mM ascorbate and 0.1% Triton X-100). The resulting slurry was centrifuged at 15,000×*g* for 15 min at 4 °C. The supernatants were collected and used for the assays of protein content (Bradford 1976) and enzyme activities.

Total SOD activity was determined by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) according to Giannopolitis and Ries (1977). Each 3 mL reaction medium contained 52.5 mM K-phosphate buffer, pH 7.8; 13 mM methionine, pH 7.8; 75 µM NBT; 0.1 µM EDTA; 5 µL enzyme extract; and 2 µM riboflavin. The production of blue formazan was followed by monitoring the increase in absorbance at 560 nm. Further details are described in Martinez et al. (2001). One unit of SOD was defined as the amount of enzyme required to cause a 50% inhibition of the rate of NBT photoreduction.

Total APX activity was estimated by monitoring the decline in absorbance at 290 nm following Nakano and Asada (1981). Each 985 µL reaction medium contained 50 mM K-phosphate buffer, pH 7.0, 0.5 mM ascorbate, 0.1 mM H_2O_2 and 15 µL enzyme extract. One unit of APX was defined as the amount of enzyme required to oxidize 1 µmol ascorbate min^{-1}.

The activity of CAT was estimated by measuring the rate of decomposition of H_2O_2 at 240 nm (Havir and McHale 1989). Each 985 µL reaction medium contained 50 mM K-phosphate buffer, pH 7.0, 12.5 mM H_2O_2 and 15 µL enzyme extract. One unit of CAT was defined as the amount of enzyme required to decompose 1 µmol H_2O_2 min^{-1}.

Extraction and quantification of abscisic acid by high-performance liquid chromatography/electrospray ionization tandem mass spectrometry

Extraction of ABA was performed according to Ross et al. (2004) with some modifications. Leaf discs were collected from fully expanded leaves of the third or fourth pairs (counting from the apex) of plagiotropic branches at Ψpd − 0.5, − 1.0, − 1.5 and − 3.0 MP and roots were collected when Ψ_{pd} reached − 3.0 MPa. Leaf and root lyophilized samples (50 and 200 mg, respectively) were ground in the presence of liquid nitrogen and PVPP. After that, 60 ng of 5,8′-8′-8-d_4 ABA in 1.5 mL of acetone:1% acetic acid in water (80:20) was added, mixed (700×*g*, 40 min, 4 °C), centrifuged (13,000×*g*, 10 min, 4 °C) and the pellet was extracted once with acetone:1% acetic acid in water (80:20). Pooled supernatants were lyophilized, recovered in methanol:1% acetic acid in water (10:90) and centrifuged (13,000×*g*, 2 min, 4 °C). Finally, solid phase extraction (SPE) purification (OASIS HLB C18 SPE cartridge, Waters) was done with 1% acetic acid in water:methanol (90:10, equilibration), and 1% acetic acid in methanol:water (80:20, elution). The yellow fraction was lyophilized, dissolved in acetonitrile:0.07% acetic acid in water (85:15), and analyzed in a high performance liquid chromatography/electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) system.

Chromatography separation was performed into Shimadzu HPLC (Tokyo, Japan) using a C18 Supecolsil column (150 × 4.6-mm, 5 µm, Supelco), 0.1% formic acid in water and acetonitrile (B) as mobile phase. The gradient was 15–100% B in 20 min for leaf extracts, 15–100% B in 35 min for root extracts, flow rate of 0.3 mL min^{-1}, column oven at 25 °C, and detector at 252 nm. TMS/MS analysis were carried out into Quattro II triple quadrupole mass spectrometer (Micromass, Altricham, UK) with electrospray ionization source, in the negative ion mode and collision energy of 10 eV. The Multiple Reaction Monitoring (MRM) scan was performed (Supplementary, Fig. 1) monitoring *m/z* 263 → 153 for ABA and m/z 267 → 156 for 5, 8′-8′-8-d_4 ABA. A calibration curve was done with 5,8′-8′-8′-d_4 ABA (final concentration, 25 ng/mL) and ABA (final concentration, 5–107 ng mL^{-1}).

Statistical analysis

The experiment followed a randomized block design forming a 6 × 2 factorial (six grafting treatments, two watering regimes) with five plants in containers per treatment combination as biological replicates. Data were statistically examined using analysis of variance and tested for significant ($P < 0.05$) clone and irrigation treatment differences using Newman–Keuls and F-tests.

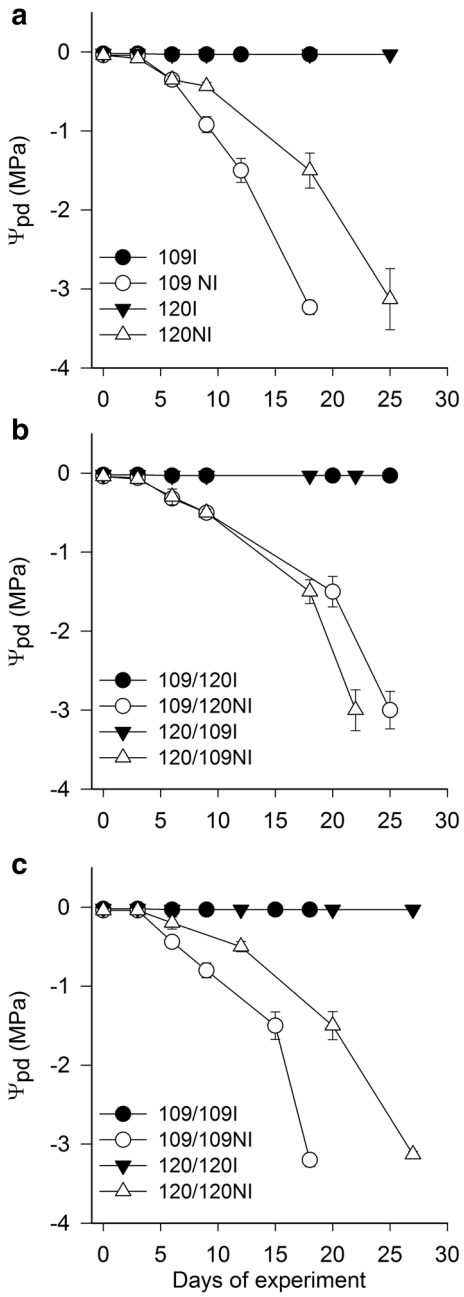

Fig. 1 Time-course of pre-dawn leaf xylem pressure potential (Ψ_{pd}) of non-grafted (**a**), self-grafted (**b**), and reciprocal graftings (**c**) of different *Coffea canephora* clones under either fully irrigated (solid figures) or drought conditions (open figures). Reciprocal grafting notation is always denoted as rootstock/scion. Each point represents the mean ± SE of five biological replicates

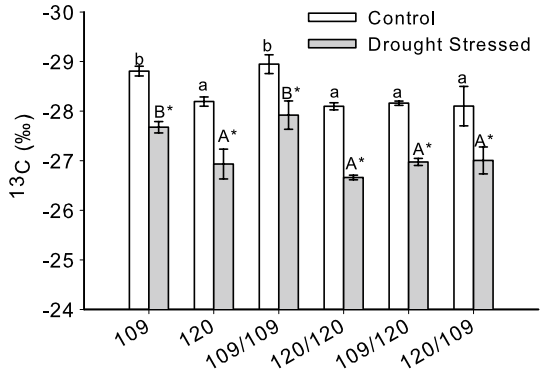

Fig. 2 Effect of water deficit on the leaf isotopic carbon dioxide ($\delta^{13}C$) composition of grafted and non-grafted *Coffea canephora* clones. Reciprocal grafting notation is always denoted as rootstock/scion. Different small letters denote significant differences among means for irrigated clones, and different capital 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 five replicates

Results

Water relations

Upon drought imposition, the predawn leaf water potential (Ψ_{pd}) declined faster in the non-grafted 109A plants relative to the non-grafted 120 plants (Fig. 1a), and self-grafting did not alter this pattern (Fig. 1c). After water withholding, Ψ_{pd} reached − 3.0 MPa, which is considered a severe deficit in coffee, at different times depending on the graft combination. It took approximately 18 days in the 109 and self-grafted 109 plants (Fig. 1c), 21 days in the 109/120 rootstock/scion plants (Fig. 1b), and 25 days in the 120 (Fig. 1a), self-grafted 120 (Fig. 1c) and the 120/109 rootstock/scion plants (Fig. 1b).

Carbon isotopic composition

We analyzed the carbon isotopic composition of leaves from the non-grafted and reciprocal grafted plants sampled at control and severe drought ($\Psi_{pd} = -3.0$ MPa) conditions. Under control conditions, the $\delta^{13}C$ ranged from − 29 to − 28.3‰ (Fig. 2). Under water deficit conditions, the $\delta^{13}C$ increased (became less negative) regardless of the plant treatment combinations. Overall, plants that had the 120 clone as the scion and/or rootstock displayed higher $\delta^{13}C$ in both control and water deficit conditions.

ABA quantification

ABA in both leaves and roots was detected by HPLC-ESI-MS/MS (Supplementary Fig. 2). MS/MS fragmentation of ABA at *m/z* 263 (Supplementary Fig. 2a) and 5, 8′-8′-8′-d_4 ABA at *m/z* 267 (Supplementary Fig. 2b) generated the intense product ion at *m/z* 153 and 156, respectively. Detection of ABA and internal standard 5,8′-8′-8′-d_4 ABA in samples under control and water deficit conditions was

proceeded by MRM mode monitors mass transition *m/z* 263 → 153 for ABA (Supplementary Fig. 2c) and *m/z* 267 → 156 for 5,8′-8′-8′-d_4 ABA (Supplementary Fig. 2d). Under mild drought stress conditions ($\Psi_{pd} = -0.5$ MPa; Fig. 3a), there were no differences in the ABA content in leaves. Under moderate water deficit ($\Psi_{pd} = -1.0, -1.5$) (Fig. 3b, c), up to three times higher levels of ABA were detected in all grafting treatments compared with the control. Plants with the sharpest delay in water deficit progression (Fig. 1c) showed higher ABA content under moderate water stress compared with their respective controls in contrast to the more sensitive reference treatment (109A/109A). Under severe water stress ($\Psi_{pd} = -3.0$ MPa, Fig. 3d), the ABA content was similar in plants from all grafting treatments except for those from the grafting treatment 120/109. This showed the highest ABA levels compared with the others.

The ABA content was also quantified in the roots of the grafted plants under control and severe drought conditions

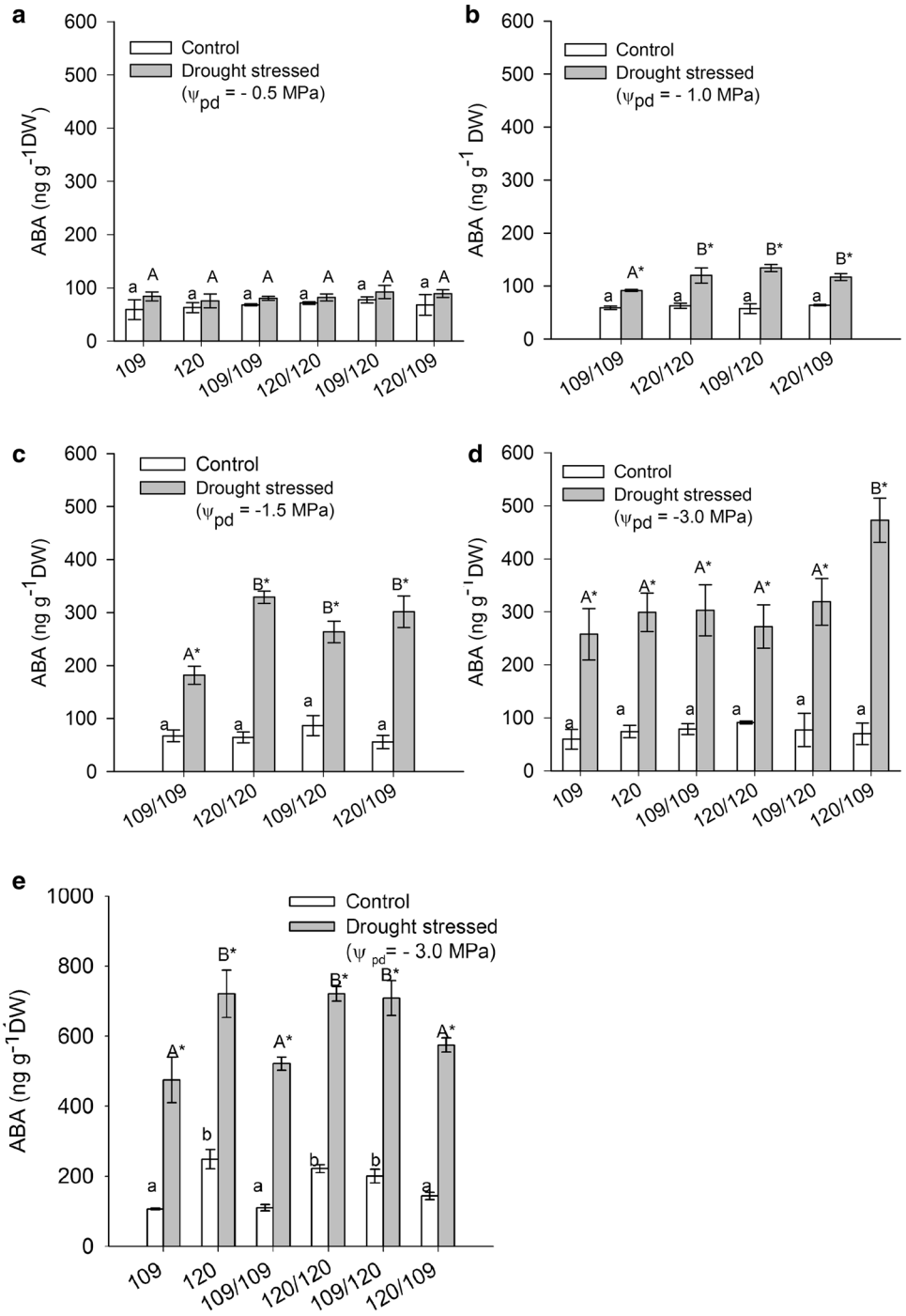

Fig. 3 Response of clones and grafts to water deficit. ABA concentrations from leaf tissue collected in plants under irrigated condition and submitted to a water deficit of (**a**) $\Psi_{pd} = -0.5$ MPa, (**b**) $\Psi_{pd} = -1.0$ MPa, (**c**) $\Psi_{pd} = -1.5$ MPa and (**d**) $\Psi_{pd} = -3.0$ MPa. (**e**) Root tissue was collected in plants under irrigated condition and submitted a water deficit ($\Psi_{pd} = -3.0$ MPa). Reciprocal grafting notation is always denoted as rootstock/scion. Different small letters denote significant differences among means for irrigated clones, and different capital 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 five replicates

(Fig. 3e). The drought-tolerant 120 clone displayed higher root ABA content in both the self-grafted and non-grafted treatments. In reciprocal grafting experiments, the tolerant scion did not affect the root ABA content in sensible rootstock. Notably, the combination grafts with more pronounced postponement of internal water deficit were those showing the highest content of ABA in their roots regardless of watering treatments except for the 120/109A rootstock/scion combination. Under severe drought stress, plants from all treatments exhibited an average increase (~ 70% relative to control plants) in ABA root content.

Oxidative damage and antioxidant defenses

Oxidative stress was evaluated through electrolyte leakage and the activity of antioxidant enzymes in leaves from grafted and ungrafted plants. Under moderate water stress, there was increased electrolyte leakage when the 109A clone was used as the scion compared with the respective control conditions (Fig. 4a). When the 120 clone was used as the scion, the electrolyte leakage in grafted plants with moderate water stress was similar to their respective controls (Fig. 4a). Under severe drought stress, the electrolyte leakage increased in all plants, particularly in the 109A and 109A/109A rootstock/scion combination (Fig. 4b). Every time the 120 plants were used, whether as the scion or rootstock, the electrolyte leakage in scion leaves lessened.

Under control or moderate drought stress conditions, the activities of APX and SOD were similar among the treatments (Fig. 5a, c). CAT activity increased significantly in plants from all grafting treatments at $\Psi_{pd} = -1.5$ MPa (Fig. 5e). Under severe stress, there was a general increase in APX (Fig. 5b), SOD (Fig. 5d) and CAT (Fig. 5f) activities in plants from all grafting treatments.

Discussion

Reciprocal grafting between two *C. canephora* genotypes with differential drought tolerance allowed us to demonstrate a clear contribution of the tolerant rootstock (120) to increased drought tolerance in the sensible scion (109A). This delay in dehydration could not be explained based on differences in leaf area or shoot/root ratio. There were no differences in these parameters between the non-grafted clones and reciprocal grafting treatments (Silva et al. 2010). Overall, the results suggest that ABA is involved in this increased drought tolerance because when the 120 clone was used as the rootstock, there was increased ABA content in the 109A scion, especially in the early phases of drought ($\Psi_{pd} = -1.0$ and -1.5 MPa). In addition, the root ABA levels were higher in plants having the 120 clone as their rootstock regardless of the watering treatments.

The highest concentrations of ABA in the leaves of plants having the 120 clone as rootstock may have been derived from the ABA transport from roots to leaves through the xylem. This paper reports the level of ABA detected in leaves and roots for the first time for *C. canephora* under abiotic stress. The concentrations of ABA in sample and calibration curves ranged from 50 to 800 ng DW^{-1} and 5–107 ng mL^{-1}, respectively. These values are below (de Sa et al. 2014) or the same as the concentration ranges of other plants species (Floková et al. 2014; Escandón et al.

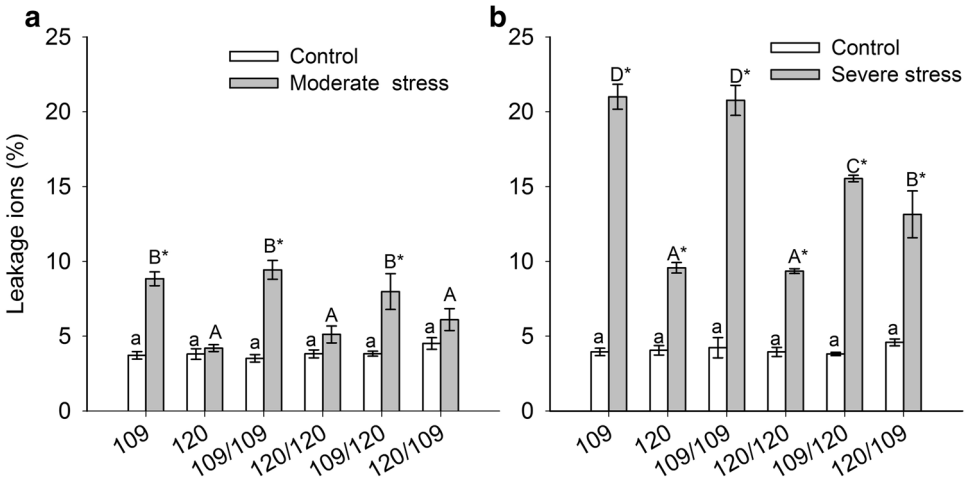

Fig. 4 Electrolyte leakage (%) of leaves from reciprocal grafted plants submitted to moderate ($\Psi_{pd} = -1.5$ MPa) (**a**) and severe water deficit ($\Psi_{pd} = -3.0$ MPa) (**b**). Reciprocal grafting notation is always denoted as rootstock/scion. Different small letters denote significant differences among means for irrigated clones, and different capital 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 five replicates

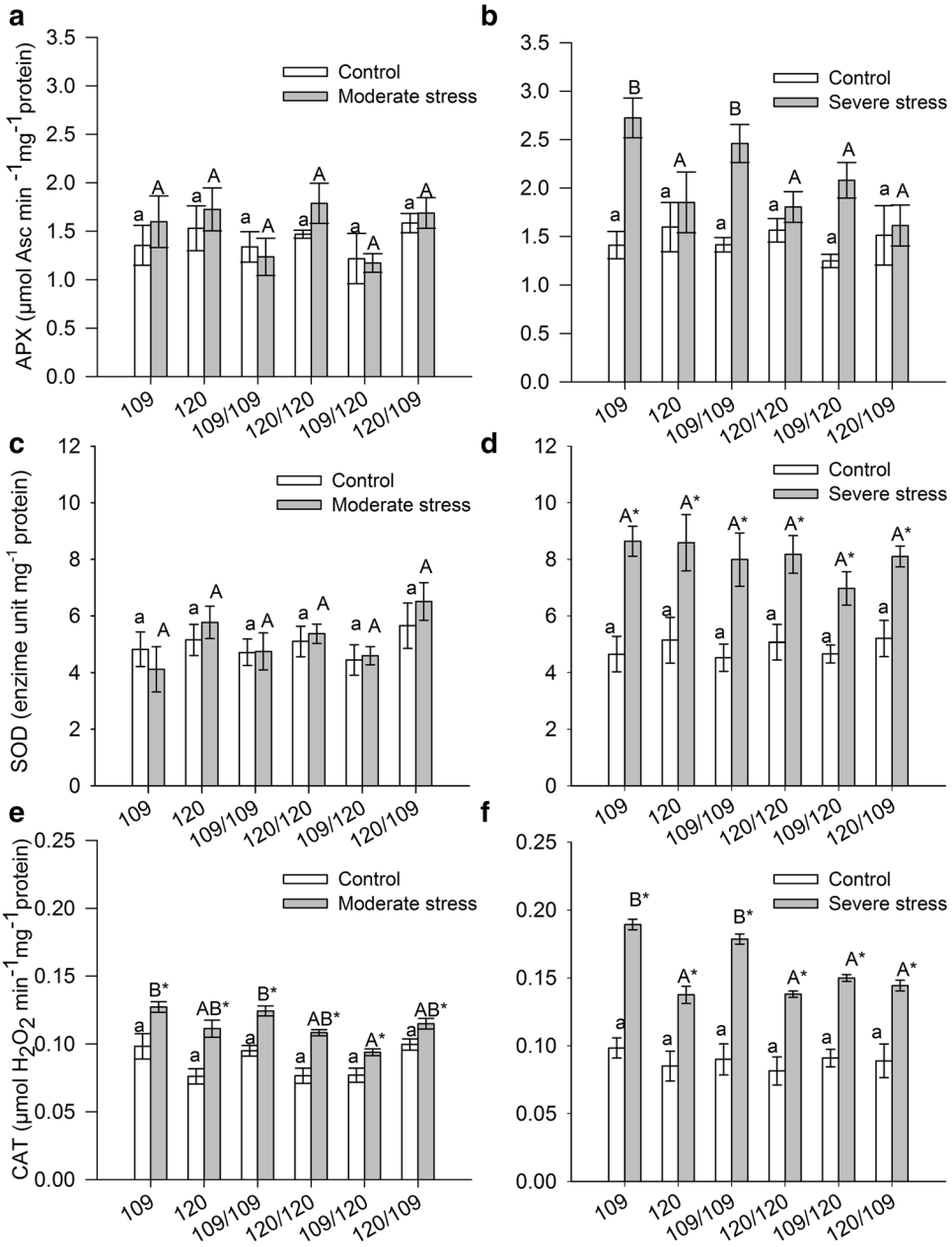

Fig. 5 Effects of moderate ($\Psi_{pd} = -1.5$ MPa) and severe ($\Psi_{pd} = -3.0$ MPa) drought on the activity of superoxide dismutase (SOD) (**a, b**), ascorbate peroxidase (APX) (**c, d**), and catalase (CAT) (**e, f**) of leaves. Reciprocal grafting notation is always denoted as rootstock/scion. Different small letters denote significant differences among means for irrigated clones, and different capital 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 five replicates

2016), demonstrating the efficiency of the method for ABA analysis of the leaves and roots of coffee trees.

The role of ABA in controlling stomatal conductance (g_s) is strongly supported by many experiments and experimental approaches in *Arabidopsis thaliana* (Osakabe et al. 2014). In a previous study we demonstrated a significant reduction in g_s in plants with the 120/120, 109/120 and 120/109 rootstock/scion grafting combinations at relatively high values of Ψ_{pd} (−0.5 MPa) (Silva et al. 2010). Nevertheless, our work showed no increase in ABA content under these conditions, suggesting that the changes in ABA content were not associated with earlier changes in g_s. Rather, the redistribution of ABA in the mesophyll cells likely induced stomata closure without additional ABA synthesis (Merilo et al. 2015) because there was a smaller postponement for the 120/109A rootstock/scion combination plants to reach moderate and severe water deficit. Alternatively, other signals such as a change in pH or changes in hydraulic conductivity of the xylem might influence g_s or trigger the redistribution of ABA (Tombesi et al. 2015; Korovetska et al. 2016). Previous work (Marraccini et al. 2012) showed that genes related to ABA perception and signaling are induced in response to drought stress in coffee genotypes, thus suggesting that there is also an active ABA signaling pathway in coffee leaves in response to drought in addition to the increased ABA content per se.

Water use efficiency (WUE) is closely associated with integrated internal partial pressure of CO_2 over time. This can be estimated from the relative abundance of stable carbon isotopes ^{13}C and ^{12}C ($\delta^{13}C$) in plant material. Considering that real-time gas exchange measurements may not reflect plant performance over time, analysis of the carbon isotopic composition in leaf dry mass is important because it is a more sensitive indicator of photosynthetic capacity, stomatal behavior, and WUE over time (Farquhar et al. 1989; Taylor et al. 2013). Higher $\delta^{13}C$ reflected greater ^{13}C discrimination that in turn was associated with higher drought tolerance and higher ABA concentrations. Here, higher $\delta^{13}C$ values were observed in the 120/109 rootstock/scion combination compared with the 109 autografts, suggesting that there is an important contribution of 120 rootstock in the WUE improvement of the 109 scion. These results suggest that increased ABA content in the 120 roots would promote g_s decrease in the 109 scion, ultimately delaying the reduction in Ψ_{pd}. The higher levels of ABA observed in the plants with the 120 clone root system could also contribute to decreased leaf oxidative damage under water deficit because at severe water stress levels, all treatments with the 120 shoots had lower electrolyte leakage—this was also seen for the 120/109 rootstock/scion plants. The influence of rootstock on the antioxidant system in leaves was also observed in young apple trees in response to drought stress (Liu et al. 2012). These authors also suggest that the choice of grafting rootstock can enhance drought resistance by improving the plant's antioxidant system.

Here, the major activities of antioxidant enzymes were not associated with increased tolerance to drought because greater cellular damage results in higher APX and CAT enzyme activities. The major cell damage and antioxidant enzyme activity observed in the sensitive clone suggest that changes in enzyme activities could not control cell damage in the sensitive clone. Alternatively, it may be a consequence of injury caused by drought rather than a mechanism of drought tolerance itself. In close agreement with these results, we also observed an increase in *CcCAT2* (catalase coding gene) transcript abundance in leaves of the drought-sensitive and drought-tolerant clones of *C. canephora* clones subjected to drought ($\Psi_{pd} = -3.0$ MPa) (Marraccini et al. 2012). *CcAPX1* (APX coding gene) expression increased in clones of *C. canephora* according to the enzymatic activity observed in the present work, while *CcAPX2* expression was stable upon water deficit (Vieira et al. 2013). The ABA content differences between tolerant and sensitive plants were more pronounced under moderate deficit, which may suggest that a severe water deficit could also induce ABA catabolism, resulting in no net change in steady-state ABA levels (Weng et al. 2016). Alternatively, this might also imply that ABA may act upstream in the mechanisms that confer drought tolerance in coffee plants. All observations corroborate the fact that water deficit progressed more slowly in plants showing higher levels of ABA in their roots and leaves, allowing these plants to gradually acclimate to drought. Ultimately, increased ABA levels could minimize the deleterious effects of drought, thereby resulting in less oxidative damage (this study) and improved photosynthetic performance (Silva et al. 2010). Moreover, ABA could participate long-term as a systemic signaling molecule, inducing the expression of genes that may contribute to drought tolerance. The increased expression of dehydrins, ascorbate peroxidase, prephenate-dehydrogenase, HSPs, catalases and oxidoreductases genes in tolerant *C. canephora* clones under water deficit is known (Marraccini et al. 2012; Vieira et al. 2013). ABA could also play a role in regulating root growth by interacting with other plant hormones (Zhao et al. 2015). Thus, ABA seems to be a messenger that mediates root-to-shoot signaling in *C. canephora*.

In conclusion, the high concentrations of ABA in the shoots of tolerant clones under moderate drought conditions are involved in drought tolerance, allowing the plant to manage a progressive acclimation process and lower oxidative damage. On the other hand, when the tolerant clone is used as a rootstock, it can also increase the drought tolerance of the sensitive scion by providing ABA to the leaves of sensitive plants. In turn, g_s in the sensitive scion can be modulated, leading to a decrease in the rate of stress progression and minimizing the oxidative damage caused by water deficit. We conclude that both the shoot and root systems of coffee tolerant clones contribute to the increase in ABA content in leaves in response to drought. This suggests that ABA from leaves and roots is associated with improved drought tolerance in conilon coffee genotypes.

Acknowledgements The authors thank Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) No. 2012/12663-1, CEPID Redoxoma No. 2013/07937-8, Conselho Nacional para o Desenvolvimento Científico Tecnológico (CNPq) No. 301307/2013-0, No. 159068/2014-2, No. 301404/2016-0, Pro-Reitoria de Pesquisa da Universidade de São Paulo (PRPUSP) NAP Redoxoma No. 2011.1.9352.1.8 and John Simon Guggenheim Memorial Foundation (P.D.M. Fellowship) for financial support. Vânia Aparecida Silva thanks Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for BIPDT Grant.

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