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ORIGINAL ARTICLE

Transcript and metabolic adjustments triggered by drought in *Ilex paraguariensis* leaves

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Abstract

Main conclusion **Abscisic acid is involved in the drought response of *Ilex paraguariensis*. Acclimation includes root growth stimulation, stomatal closure, osmotic adjustment, photoprotection, and regulation of nonstructural carbohydrates and amino acid metabolisms.**

Abstract *Ilex paraguariensis* (yerba mate) is cultivated in the subtropical region of South America, where the occurrence of drought episodes limit yield. To explore the mechanisms that allow *I. paraguariensis* to overcome dehydration, we investigated (1) how gene expression varied between water-stressed and non-stressed plants and (2) in what way the modulation of gene expression was linked to physiological status and metabolite composition. A total of 4920 differentially expressed transcripts were obtained through RNA-Seq after water deprivation. Drought induced the expression of several transcripts involved in the ABA-signalling pathway. Stomatal closure and leaf osmotic adjustments were promoted to minimize water loss, and these responses were accompanied by a high transcriptional remodeling of stress perception, signalling and transcriptional regulation, the photoprotective and antioxidant systems, and other stress-responsive genes. Simultaneously, significant changes in metabolite contents were detected. Glutamine, phenylalanine, isomaltose, fucose, and malate levels were shown to be positively correlated with dehydration. Principal component analysis showed differences in the metabolic profiles of control and stressed leaves. These results provide a comprehensive overview of how *I. paraguariensis* responds to dehydration at transcriptional and metabolomic levels and provide further characterization of the molecular mechanisms associated with drought response in perennial subtropical species.

Keywords Abiotic stress · De novo transcriptome · Drought stress · Cellular stress response · RNA-Seq · Transcription factors · Yerba mate

Abbreviations

AOX	Alternative oxidase
DETs	Differentially expressed transcripts
NCED	9-*cis*-epoxycarotenoid dioxygenase
RFOs	Raffinose family of oligosaccharides
TFs	Transcription factors

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Introduction

Ilex paraguariensis St. Hil. (yerba mate) is a shade-tolerant tree cultivated in understory or monoculture systems in South Brazil, Northeast Argentina, and Western Paraguay (Giberti 1995). The leaves and young shoots are processed to prepare a tea-like beverage with stimulant and pharmacological properties (Bracesco et al. 2011). The natural habitat of yerba mate is in forests under *Araucaria angustifolia* trees, where there is a low photosynthetic photon flux density and high humidity predominate. Extensive cultivation is quite recent and rapidly expanding in response to the adoption of mate-tea consumption by consumers around the world. However, scarce information is available regarding yerba mate acclimation to the new environmental conditions present in agronomic systems that are characterized by an increase in sunlight irradiance and the resulting greater water demand imposed by enhanced evapotranspiration (Acevedo et al. 2013; Caron et al. 2016). The occurrence of spring–summer drought episodes concurrently with high temperatures and radiation during the period of the greatest sprouting can cause abiotic stresses that severely reduce vegetative growth and trigger leaf abscission that constrains agronomical yields. Under these circumstances, plants can activate several morphological, physiological, and biochemical adaptation mechanisms to cope with environmental stresses (Obata and Fernie 2012; Osakabe et al. 2014). The particular strategy that a plant employs will depend on the magnitude of the strain, which can be a function of the stress duration (Tardieu et al. 2014; Blum 2016). For instance, when soil moisture reaches values slightly lower than its normal water holding capacity, plants maintain their turgor pressure for a short time to ensure steady growth. However, under mild-to-severe water deprivation, plants show decreased photosynthesis and employ several tools to minimize water loss, including stomatal closure, cell turgor maintenance, and osmotic adjustment (Shinozaki et al. 2015; Blum 2017). Even though relatively little information is available on the interaction of *I. paraguariensis* and the surrounding environment, our previous studies showed that the drought-tolerant cultivars respond by promoting stomatal closure and osmotic adjustment to reduce water loss and prevent leaf abscission (Acevedo et al. 2013). Since the harvestable products are the leaves, reduced susceptibility to abscission constitutes a significant trait to improve yerba mate. The reduction in stomatal conductance, cell membrane stability, and osmotic adjustment was associated with an increased endogenous abscisic acid (ABA) concentration, though the chemical identity of the osmoprotectant molecules remains unknown.

Although the physiological responses to water deficit are underpinned by reprogramming of the metabolism and gene expression, these protective mechanisms are still far from full characterized in yerba mate and their clarification is necessary to better understand the acclimation process to drought conditions. In this way, we observed a limited number of differentially expressed transcripts (DETs) using mRNA differential display to associate the expression profile of stressed and unstressed plants (Acevedo et al. 2016). We identified 62 DETs of which only 40.5% had strong similarities with database entries of known functions, while the others belonged to unclassified proteins or genes with no assigned functions. Currently, the availability of robust technologies such as RNA sequencing (RNA-Seq), to collect short reads that cover the full transcriptome, and analytical methods for plant metabolomics determination (Obata and Fernie 2012) enable us to achieve a more comprehensive overview and a detailed analysis of crucial components involved in plant responses to stresses. Both approaches were combined to study the stress responses in several perennials species, including *Vitis* (Agudelo-Romero et al. 2015), *Populus* (Janz et al. 2010; Hamanishi et al. 2015), *Eucalyptus*, and *Pinus* (Harfouche et al. 2014). With respect to *I. paraguariensis*, Debat et al. (2014) used next-generation sequencing data to generate a draft transcriptome from an uncharacterized pooled of leaves. In addition, several authors reported the use of metabolomic tools to strengthen the systematic classification of the genus *Ilex* and highlighted the chemotaxonomic classification of South American *Ilex* species with nuclear magnetic resonance-based metabolomics (Kim et al. 2010). However, no one has yet combined transcriptomic and metabolomic data to understand the physiological process of stress acclimation in *I. paraguariensis*.

Our study addresses dehydration tolerance through an integrative analysis of transcriptional, metabolomic, and physiological responses to drought in *I. paraguariensis*. Here, we investigated (1) how gene expression varies under water scarcity with respect to the well-watered conditions and (2) in what ways the modulation of gene expression is linked to physiological status and metabolite levels.

Availability of data and materials

The raw sequence data were generated with the Illumina HiSeq 1500 platform and was stored in the Sequence Read Archive of The National Center for Biotechnology Information (NCBI) and is identified with the accession number SRP110129. The complete set of de novo assembled transcripts was deposited in the *I. paraguariensis* repository named "Mateando" that is freely available at http://ibone.unne.edu.ar/mateando/. This repository is designed for the web-based application ATGC transcriptomics (Gonzalez et al. 2017) and allows visualization of each transcript, including sequence, functional annotation, structural features, and to compare its abundances in the different

treatments. The microsatellites sequences, the related transcript that contains it, and the SSR specific flanking primers are available in our repository.

Materials and methods

Plant material, drought assay, and sample collections

The genotype used in the experiment was previously selected for drought tolerance at the vegetative stage under field conditions. Physiological characterization was made based on the photosynthetic rate, transpiration rate, stomatal conductance, shoot water potential, and intrinsic water use efficiency (A/g). Afterward, the selected cultivar was propagated by rooting of softwood cuttings taken from field-grown plants following the protocol developed by Tarrago et al. (2004). Two-year-old *I. paraguariensis* St. Hil. cv SI-49 plants were grown in 4 L pots filled with lateritic red soils (Alfisols) and cultured under partially controlled greenhouse conditions. At the beginning of the experiment, all plants were pruned and acclimatized for seven days in a room with $27 \pm 1/22 \pm 2$ °C day/night temperatures, $50-55\%$ relative humidity, and 14 h photoperiod (420 µmol photons m^{-2} s^{-1}, provided by mercury lamps). Initially, a controlled experiment was performed to determine the potential for foliar dehydration tolerance, the maximum capacity for osmotic adjustment, and variation in the transcriptome profile using differential display analysis of plants grown at different soil moistures (Acevedo et al. 2013, 2016). Plants were watered to the drip point and subsequently subjected to a continuous soil drying episode by withholding water from pots until the water potential of the soil (Ψ_{soil}) at pre-dawn reached either -1, -2, or -3 MPa for the mild, severe, and maximum water-stress conditions, respectively. Well-watered (control) and re-watered (at $\Psi_{soil} = -3$ MPa) treatments were also included. Afterward, the severe ($\Psi_{soil\ =} -2$ MPa) water deficit regime was chosen with a non-stressed treatment (soil water content close to field capacity) to analyze the transcriptomic and metabolomic responses using more powerful tools. At this point, the plantlets consumed 65–70% of the available water and the leaf relative water content in the stressed plants diminished to $32.9 \pm 1.5\%$ with respect to the optimally hydrated state. Three biological replicates were used per treatment. This experiment was carried out under similar environmental conditions for 22 days. The soil water potential was determined using a psychrometer HR-33T with a PST-55 thermocouple (Wescor Inc., Logan, UT, USA). Leaf relative water content [RWC% = (fresh weight − dry weight)/(turgid weight − dry weight) × 100], leaf gas exchange, and fluorescence parameters were measured at midday on three fully expanded leaves with an open-flow gas exchange system LI-6400 (LI-COR Environmental, Lincoln, NB, USA), equipped with a 6400-40 leaf chamber fluorometer. Photosynthesis and fluorescence were measured with a light intensity of 1000 µmol photons m^{-2} s^{-1}, 400 µmol CO_2 mol^{-1} air, leaf temperature of 27 °C, and leaf-to-air vapor pressure deficit of 1.6 kPA. The multiphase flash protocol was used for fluorescence determination (Loriaux et al. 2013). Root growth was quantified indirectly using the electric capacitance method.

For transcriptomic and metabolomic analyses, mature leaf samples harvested at midday were snap frozen in liquid nitrogen upon harvest and kept at − 80 °C until processing.

RNA isolation, RNA-seq library preparation, and gene expression analysis

Total RNA samples were obtained using the SV Total RNA Isolation System (Promega Corp., Madison, WI, USA) following the manufacturer's instruction. RNA integrity was checked on a 2.5% (w/v) agarose gel stained with ethidium bromide. Purity was assessed by the A_{260}/A_{280} and A_{260}/A_{230} absorption ratios with a Multiskan™ GO Microplate Spectrophotometer (Thermo Scientific), and concentrations were determined at 260 nm. cDNA libraries for RNA sequencing were prepared as described in the TruSeq® RNA Sample Preparation Kit (Illumina Inc., San Diego, CA, USA). Insert sizes were examined with a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) using the High-Sensitivity DNA Kit (Agilent Technologies, Inc., Santa Clara, CA, USA). Finally, six cDNA libraries from both treatments were sequenced on an Illumina HiSeq 1500 platform, to produce paired-end reads (2×101 bp). For de novo transcriptome assembly, the RNA-Seq reads qualities were checked using FastQC. We used Trimmomatic to trim adapter sequences and remove low-quality reads with unknown nucleotides. The remaining reads were merged into one longer read if both paired reads could align with each other using the software "flash" (Magoc and Salzberg 2011). The final high-quality reads (both paired-end and long single-end) were used to construct a de novo transcriptome assembly, using the Trinity package, version 2.0.6 with parameters –min_kmer_cov 3 and min_glue 5.

Sixty-two transcript sequences previously obtained with the Sanger method and deposited in the GenBank were sought in the assembled transcriptome and compared to validate the assembled sequences by similarity using BLASTN (Online Resource Table S1). Afterward, the trimmed reads were mapped on the assembled transcriptome using Bowtie2. HTSeq count was employed to determine the number of aligned reads overlapping each transcript. The differential expression analysis between treatments was conducted using the normalization by TMM (trimmed mean of M values) method and negative binomial distribution with default

parameters in the Bioconductor package edgeR. Sequences with a fold change (FC) ≥ 2 in their expression between treatments and false discovery rate (FDR) < 0.05 were assigned as a differentially expressed transcripts. Finally, the assembled transcripts were annotated by sequence similarity comparisons using BLASTX (https://blast.ncbi.nlm.nih.gov/Blast.cgi); the comparison was made against NCBI non-redundant (nr) and SwissProt protein databases, with an *E* value cutoff of 10^{-5}. We compared the transcripts with the curated KEGG GENES database (http://www.genome.jp/tools/kaas/) using the Kyoto Encyclopedia of Genes and Genome (KEGG) automatic annotation server. Gene ontology (GO) terms were assigned to transcripts utilizing the BLASTX hits and Blast2Go software with default parameters. Moreover, we used the TransDecoder (https://transdecoder.github.io/) software to predict coding regions in assembled transcripts, such as untranslated regions (UTRs), exons, coding sequences (CDs), and mRNA, and used Trinotate v2.0.2 annotation suite (https://trinotate.github.io/) to identify protein domains (Pfam database) and predict signal peptide sequences (Signal P) and transmembrane domains (TMHMM). Using Trinotate, we also obtained annotations from the EggNOG database. Furthermore, up- and down-regulated transcription factors (TFs) were recognized by their GO term or by BLASTX comparison of DETs with all protein sequences of TFs in PlantTFDB 4.0 (Jin et al. 2016). Finally, to assist in the biological interpretation of changes in the transcriptomic profile, we employed the Mercator pipeline (Lohse et al. 2014) to annotate DETs sequences and MapMan software (Thimm et al. 2004) to represent these changes through colored diagrams of metabolic pathways.

All transcripts mentioned in "Discussion" are described in Online Resource Table S2 and represented in the text as T1, T2, and so forth.

Quantitative PCR analysis

To validate the reliability of expression profiles observed in the RNA-Seq data, 12 genes were randomly selected for qRT-PCR analyses. Each reaction mixture of 15 µL contained 10 ng cDNA, 7.5 µL 2X SYBR Select Master Mix (Applied Biosystems, Foster City, CA, USA), and 300 nM of the corresponding primer pair. The qPCR reactions were conducted in a 7500 Real-Time PCR System (Applied Biosystems) using a program of an initial denaturation at 95 °C for 10 min, and 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. The RNA polymerase-associated protein rtf1 (RTF) gene (KU886201, *I. paraguariensis*) was employed as an internal control (Acevedo et al. 2018). The relative expression value was calculated by the delta–delta CT method and expressed as the fold change relative to expression in the null controls. Primers used in the qRT-PCR analyses were designed with Primer3 (Online Resource Table S3). Three biological replicates and three technical replicates were considered for statistical analysis.

Metabolite profiling analysis

Metabolite extraction was performed using ground lyophilized leaf samples followed by the addition of the appropriate extraction buffer as described by Gibon et al. (2004). The levels of sucrose, fructose, and glucose in the leaf tissue were determined according to Fernie et al. (2001). Total amino acids from the soluble fraction, total protein, and starch contents were quantified following the methodology described by Cross et al. (2006). Malate and fumarate levels were determined with the method described by Nunes-Nesi et al. (2007). The contents of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl *a* + *b*), and carotenoids were determined as described by Wellburn (1994) with minor modifications. The total soluble phenols were quantified spectrophotometrically using the Folin–Ciocalteu method with tannic acid as standard. Sugars, organic acids, and amino acids were determined as described by Lisec et al. (2006). Metabolite extraction, derivatization, standard addition, and sample injection in a TruTOF gas chromatography–mass spectrometry (GC–MS) system were performed according to Osorio et al. (2012). The mass spectra were cross-referenced with those in the Golm Metabolome Database (Kopka et al. 2005).

Results

Physiological and morphological changes in response to drought

The effect of water stress on leaf relative water content and photosynthesis-related parameters was analyzed. Growth parameters including the dry weight of root, leaf, and stem were also examined. A sharp decrease in the leaf relative water content from 95.7 ± 0.6 to 62.7 ± 0.9% (Table 1) was correlated with a significant reduction in stomatal conductance ($P < 0.001$) between non-stressed and stressed plants. Due to stomatal closure, CO_2 assimilation dropped sixfold. At the same time, the maximum quantum yield of PSII remained unchanged, while the quantum efficiency of PSII and the photosynthetic electron transport rate decreased significantly. In contrast, non-photochemical quenching was elevated in stressed leaves. Neither the endogenous content of *Chl a* or *Chl b,* nor *Chl a* + *b* contents varied under dehydration, whereas the total carotenoid concentration increased from 0.16 ± 0.01 to 0.21 ± 0.01 mg g^{-1}. The stressed leaves displayed osmotic adjustment during soil drying reaching − 0.56 ± 0.04 MPa. Leaf abscission was not detected under severe dehydration. Nevertheless, the

Table 1 Photosynthetic performance of *Ilex paraguariensis* leaves under soil water deprivation

	Non-stressed	Stressed
Leaf RWC (%)	95.7 ± 0.6	**62.7 ± 0.9**
A ($molCO_2$ m^{-2} s^{-1})	8.94 ± 0.84	**1.48 ± 0.3**
F_v/F_m	0.82 ± 0.002	0.81 ± 0.002
Φ_{PSII} $(F'_m–F_t)/F'_m$	0.210 ± 0.02	**0.098 ± 0.01**
ETR	92.3 ± 10.9	**43.0 ± 6.0**
NPQ $(F^o_m–F'_m/F'_m)$	2.71 ± 0.01	**2.98 ± 0.08**
Chl a (mg gr^{-1})	0.82 ± 0.12	0.81 ± 0.04
Chl b (mg gr^{-1})	0.28 ± 0.05	0.27 ± 0.04
Chl $a+b$ (mg gr^{-1})	1.10 ± 0.17	1.08 ± 0.08
Total carotenoid content (mg gr^{-1})	0.16 ± 0.01	**0.21 ± 0.01**

Mean ± SE from three biological replicates. Values in bold font are different by t test ($P < 0.05$)

Φ_{PSII} photochemistry efficiency of PSII, *ETR* electron transport rate, *Fv/Fm* maximum quantum yield of photosystem II, *NPQ* non-photochemical quenching

previous results (unpublished data) indicated that symptoms of oxidative stress in leaves are visible when the soil water potential drops to less than − 3 MPa triggering foliar abscission (49 ± 7%) after 48 h from re-watering. The lethal leaf water potential showed that the last surviving leaves did not die until Ψ_{leaf} dropped to an average of − 3.05 MPa and Ψ_{soil} reached − 3.4 MPa.

Beside physiological adjustments, *I. paraguariensis* plants also undergo morphological adaptation to drought. We observed that stressed plants modified their growth pattern to recover available water by prioritizing root growth. The variation in root dry weight, during the 22-day experiment ranged from 1.84 ± 0.12 to 5.07 ± 0.7 g per plant (non-stressed and stressed plants, respectively), while the dry mass of leaves and stems from the newly sprouted buds declined substantially in stressed plants.

Finally, signs and symptoms of disease were not visible during the stress treatment.

Changes in transcripts due to water deficit

Leaf RNA samples isolated from three well-watered (controls) and three stressed plants were used to construct six cDNA libraries associated with drought response. A total of 210,734,128 paired-end reads (2 × 101 bp) were produced that had an average GC content of 43.6%. Among them, 94.9% had Q scores > Q30. After discarding the low-quality reads, 195,454,899 clean paired-end reads (2 × 76 bp) constituting 29.71 gigabase pairs were obtained. Results from FASTQC, such as *k-mer* frequencies and sequence duplication levels, were all as expected, so we were confident assembling a de novo transcriptome with adequate coverage and representation. A transcriptome with 161,411,996 bases (39.82% GC content), was constructed with the Trinity package. The transcriptome contained 143,795 transcript contigs with a 1962 bp N50 length and a 1122 bp average transcript length. Sixty-two transcripts previously sequenced with the Sanger method and stored in GenBank were used to validate the nucleotide accuracy of the sequences assembled using BLASTN (Online Resource Table S1). All sequences were present and reached nucleotide identities between 99.5 and 100% along the aligned fragment of both sequences. Remarkably, these sequences were previously obtained from the same genotype and from a similar drought stress treatment using mRNA differential display (Acevedo et al. 2013, 2016), highlighting their accuracy.

For each library, more than 98.3% of the trimmed reads were mapped on the assembled transcriptome. A principal component analysis (PCA) was conducted with the number of reads assigned to each transcript in each library/sample. Principal component 1 (PC1) explained 46.6% of the total variation in the data, while PC2 described another 20.2%. The PCA plot shows that within treatments, different samples were similar to each other in the same treatment and highlights the differences between unstressed and stressed plants (Online Resource Fig. S1).

In total, 4920 DETs were modulated after water deprivation. Among them, 2156 DETs were up-regulated and 2764 DETs were down-regulated. Aside, 1210 sequences varied more than fourfold in their expression ratio, and of these, 522 DETs were induced, while 688 DETs were repressed, in response to drought.

Functional classification

The assembled transcripts were searched against the SignalP, SwissProt, Pfam, TMHHM, EggNOG, and KEGG databases. More than 52% of the transcriptome was annotated by sequence similarity comparisons and protein domains searches. Among them, 5.4% of DETs had a BLAST hit in the SwissProt database. The proportion of DETs with significant similarity to sequences in SignalP, Pfam, TMHHM, and EggNOG databases was 1.4, 5.5, 5.6, and 11.1%, respectively.

As per the GO classification, the matched DETs (23.1%) were classified into the three functional categories: biological process, molecular function, and cellular component (Fig. 1). Genes involved in "metabolic process" and "cellular process" were abundant in the biological process category. Under the molecular function category, "binding" and "catalytic activity" were the highly represented groups; while for the cellular component category, DETs mostly belonged to the "cell part" and "organelles" groups.

Similarly, all DETs were further classified by KEGG into environmental information processing, genetic information

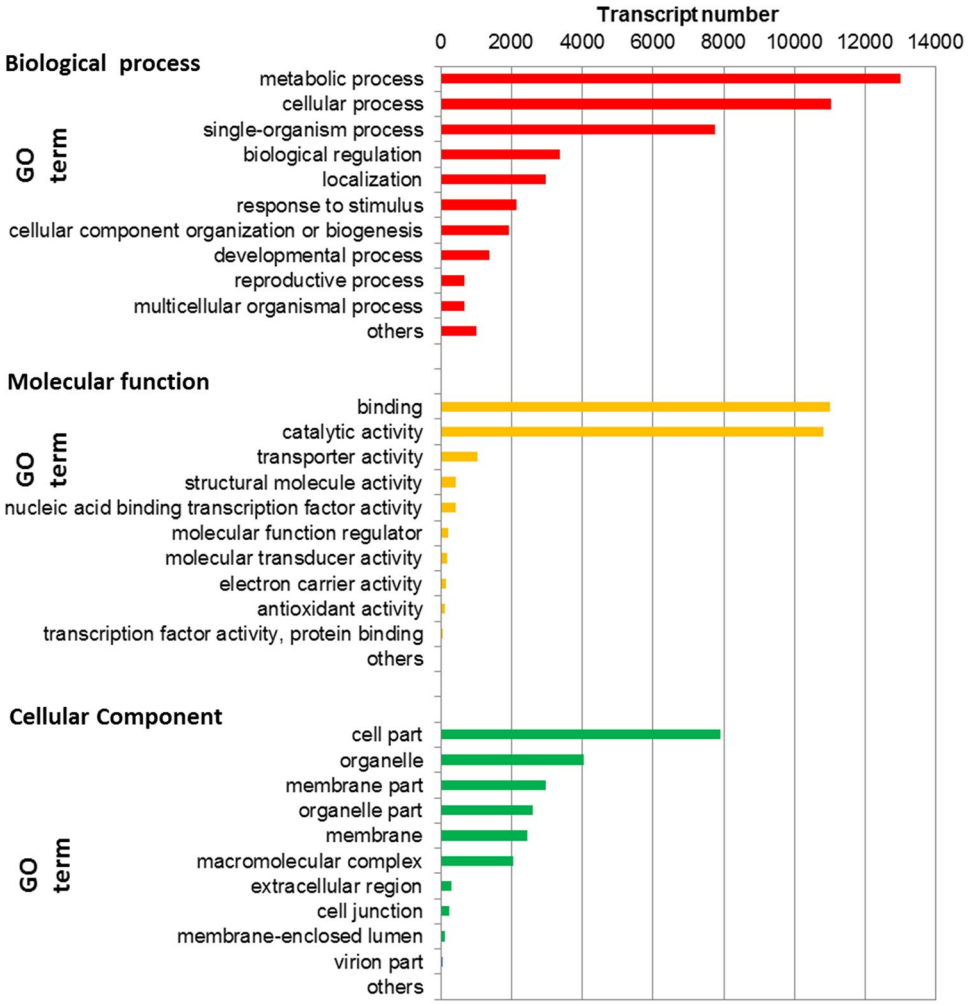

Fig. 1 GO-functional enrichment analyses of overrepresented GO terms in drought-regulated DETs. The cut-off threshold was FDR < 0.05

processing, cellular processes, organism system, and metabolism (Online Resource Fig. S2). The last functional category (e.g., metabolism) constituted the most abundant group of up-regulated (62%) and down-regulated (67%) DETs. In general, the transcriptomic profile was similar in both cases. The proportion of up-regulated DETs belonged to the organism system, and cellular process increased in response to dehydration, while those included in metabolism and genetic information were mostly repressed.

Finally, an overview of the different genes modulated by drought in relation to their correspondent metabolic pathways was generated using MapMan. The obtained data indicate that *I. paraguariensis* plants undergo an extensive reprogramming of the transcriptome in response to dehydration. Many genes related to photosynthesis coding for the structural components of the photosystems and the biosynthesis of pigments were repressed, indicating a general diminution of the photosynthetic process. Similarly, central carbon metabolism was affected by drought conditions, as indicated by an overall decrease in the expression of genes involved in glycolysis and tricarboxylic acids cycle. Many genes related to lipid metabolism were down-regulated as a result of the water shortage. Drought also modulated several pathways involved in biosynthesis and degradation of amino acids. Concurrently, several genes related to plant defences against pathogens were induced.

Expression of hormone biosynthesis, signal transduction, and transcription factor-related genes

There were 374 DETs involved in signal perception and hormones biosynthesis identified (Fig. 2). Among them, 64 DETs were found that encoded receptor-like kinases (RLKs) superfamily members including serine/threonine kinase proteins, MIC-related transcriptional activators, and the UDP-N-acetylglucosamine (UAA) transporter family. Several DETs involved calcium regulation (47), the G-protein family (26), light receptors (26), MAP kinases (11), and phosphoinositides (8). Furthermore, DETs encoding redox proteins, such as thioredoxin (21), ascorbate/glutathione (35), glutaredoxin (5), peroxiredoxin (4), and dismutase/catalase (6) were modulated in stressed leaves. Finally, 157 DETs

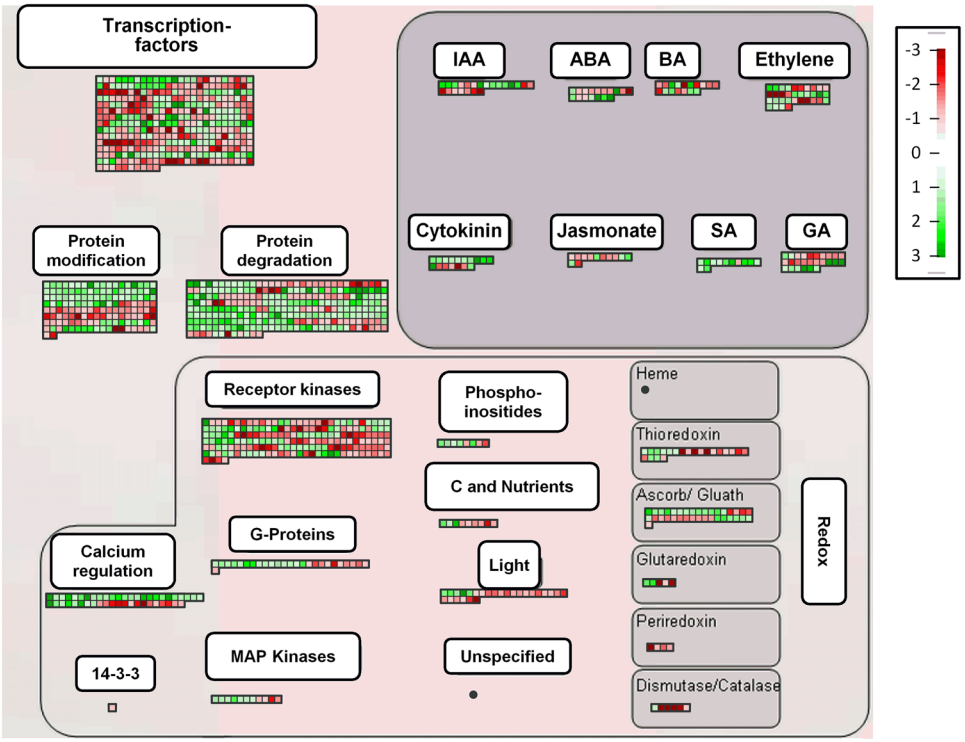

Fig. 2 MapMan regulation overview of genes modulated by drought in *I. paraguariensis* plants. Each square corresponds to a gene. Red and green indicate lower and higher expression than the control, respectively. The scale bar is shown in log2. Sequences with a fold change (FC) ≥ 2 in its expression between treatments and FDR < 0.05 were assigned as a differentially expressed transcript

involved in hormone biosynthesis pathways, such as ABA, ethylene, jasmonate, salicylic acid, IAA, brassinosteroids, cytokinins, and gibberellins.

In addition, we identified 2588 putative *I. paraguariensis* transcription factor genes (TFs) comprising 52.6% of all isolated DETs. The TFs were classified into 53 families. Among them, 1464 were repressed by drought, whereas 1124 were induced in response to the stress (Online Resource Table S4). Most of the TFs were related to abiotic stress responses (64%) and plant defences against pathogens (20%), while the others were associated with signalling, the circadian clock, metabolism, growth, and development.

Confirmation of gene expression with qRT-PCR analysis

To confirm RNA-Seq results, quantitative reverse transcription (qRT) PCR was conducted on 12 randomly selected DETs (six induced and six repressed transcripts) based on transcriptional profile analysis.

qRT-PCR analyses validated differences in gene expression between stressed and control samples identified by RNA-Seq, including the increased expression of LEA (late embryogenesis abundant protein Dc3), HB-7 (homeobox–leucine zipper protein ATHB-7), XERO1 (dehydrin xero 1), EXLB1 (expansin-like B1), GOLS2 (galactinol synthase 2), and TCM_029457 (uncharacterized protein TCM_029457) DETs, and the reduced expression of ACO (1-aminocyclopropane-1-carboxylate oxidase), UGT76E11 (UDP-glucosyltransferase 76E11), PPT2 (phosphoenolpyruvate/phosphate translocator 2), GSTU25 (glutathione S-transferase U25), FRO7 (ferric reduction oxidase 7), and SWEET3 (bidirectional sugar transporter SWEET3) DETs (Online Resource Table S3 and Online Resource Fig. S3). We further validated the changes in gene expression identified by RNA-Seq by comparing fold changes in sequence reads with fold changes determined by qRT-PCR for each condition. In both cases, the fold changes determined by either method fell on a single-fitted straight line with $R^2 = 0.95$. This concordance between the RNA-Seq and qRT-PCR data confirmed the authenticity of the DETs and validated the findings from our transcriptome study.

Metabolic changes induced by drought

We next extended the analysis to major primary pathways of plant metabolism by combining single metabolite analyses with gas chromatography–mass spectrometry (GC–MS)-based metabolic profiling (Osorio et al. 2012). The biochemical analyses of *I. paraguariensis* leaf extracts enabled detection of 60 compounds (Figs. 3, 4). The greatest number of drought-responsive compounds represented amino acids (21), followed by organic acids or their anions (13), carbohydrates (13), amines (2), nucleobases (2), polyols (1), and other metabolites (8).

A principal component analysis indicated differences in the metabolic profiles of control and stressed leaves. PC1 explained 52.5% of the variation and allowed separation of

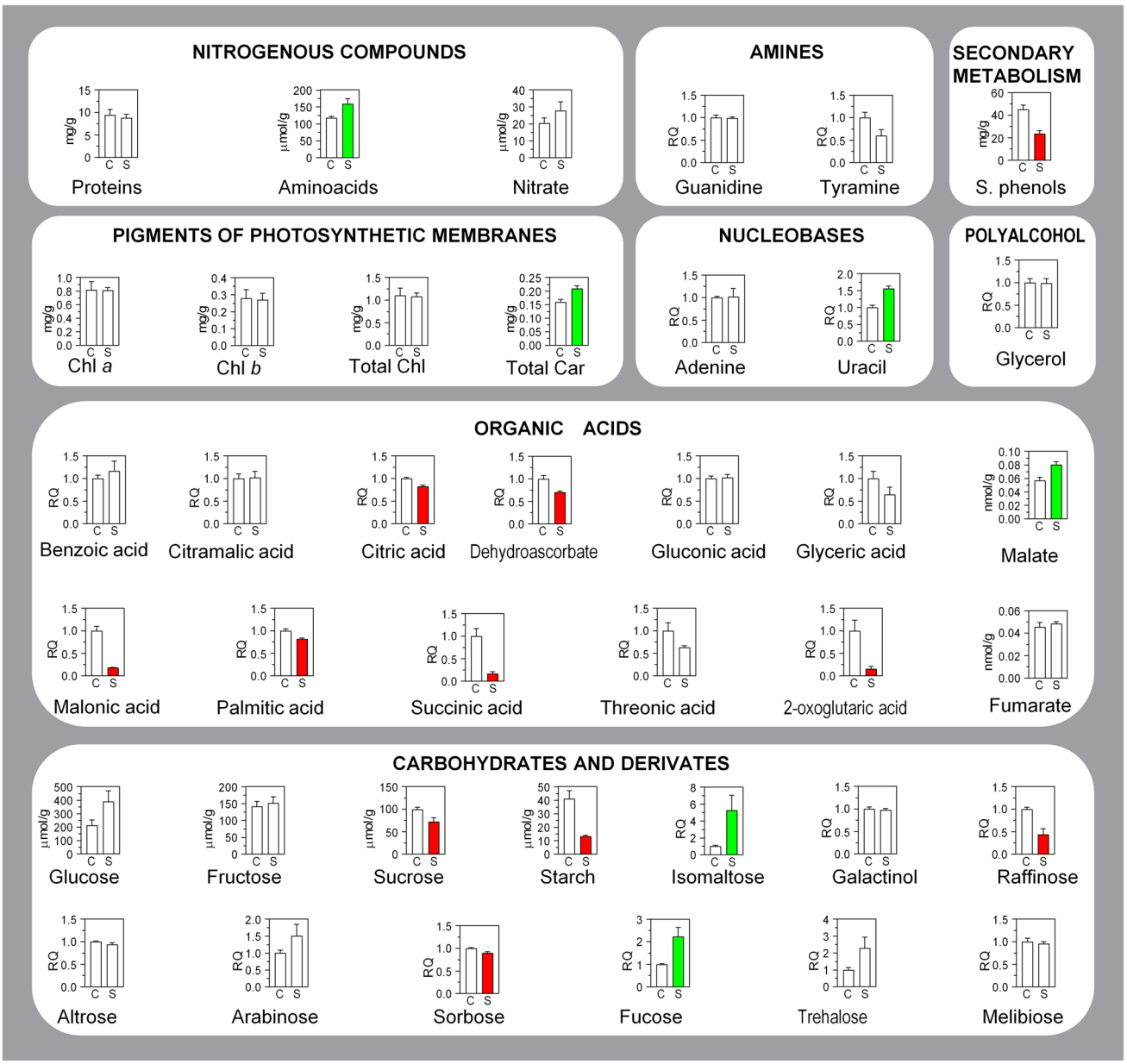

Fig. 3 Drought-induced changes in the accumulation levels of metabolites identified in leaves of non-stressed and stressed plants. Bars represent the average of three replicates ± SD. Values are expressed either in absolute or relative terms. Different colors between treatments indicate significant differences (*t* test, $P < 0.05$). Red and green colors indicate lower and higher levels than the well-watered control, respectively. *C* non-stressed (control), *S* stressed plants

control and stressed treatments, while the PC2 explained 21.4% of the total variation (Fig. 5). In this context, the non-stressed (control) profile was characterized by concentrations of starch, soluble phenols, organic acids, and aspartic acid. In contrast, the stressed profile was predominantly characterized by isomaltose, fucose, uracil, total carotenoids, and the majority of amino acids except for aspartic acid.

Significant changes in the accumulation of 23 isolated metabolites, total amino acids, total carotenoids, and soluble phenols were observed in response to the drought treatment. Concentrations of 12 compounds increased, and 11 decreased, following the stress treatment. We also found significant increases in the total level of amino acid (Fig. 3). In this sense, the accumulation of total amino acids in leaves from stressed plants was increased 26%, probably due to the accumulation of asparagine, glutamine, ornithine, phenylalanine, pyroglutamic acid, serine, threonine, and valine (Fig. 4). In contrast, the concentration of aspartic acid diminished twofold, while the endogenous contents of glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine,

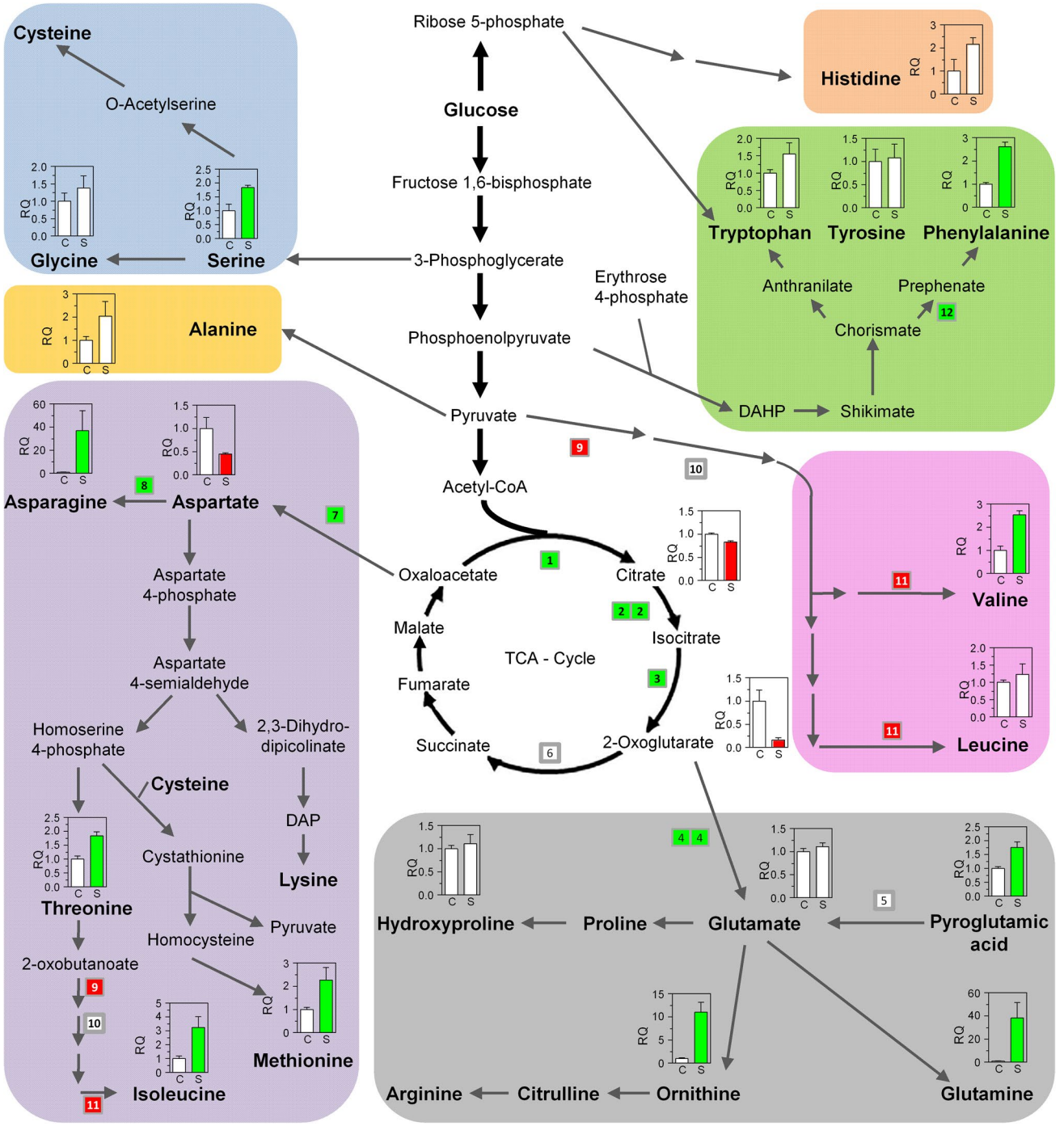

Fig. 4 Schematic diagram of drought-induced changes in amino acids metabolism. The changes in the abundance of the metabolites in response to dehydration are shown in relative scale. Transcripts encoding different enzymes are represented in boxes. Red and green color indicate lower and higher levels than the well-watered control, respectively. White color shows non-significant difference with respect to the well-watered conditions. 1: citrate synthase; 2: aconitase; 3: isocitrate dehydrogenase; 4: glutamate dehydrogenase; 6: 2-oxoglutarate dehydrogenase; 7: aspartate transaminase; 8: asparagine synthetase; 9: acetohydroxyacid synthase; 10: ketol-acid reductoisomerase; 11: branched-chain aminotransferase; 12: chorismate mutase

methionine, tryptophan, and tyrosine, remained unchanged. Notably, the level of glutamine, a vital amino acid in the nitrogen assimilation pathway, increased 38-fold, whereas, proline and glycine–betaine were not detected. Total proteins and nitrate contents remained unchanged (Fig. 3).

In addition, a depletion of the nonstructural carbohydrates pool was observed. In effect, drought treatment induced

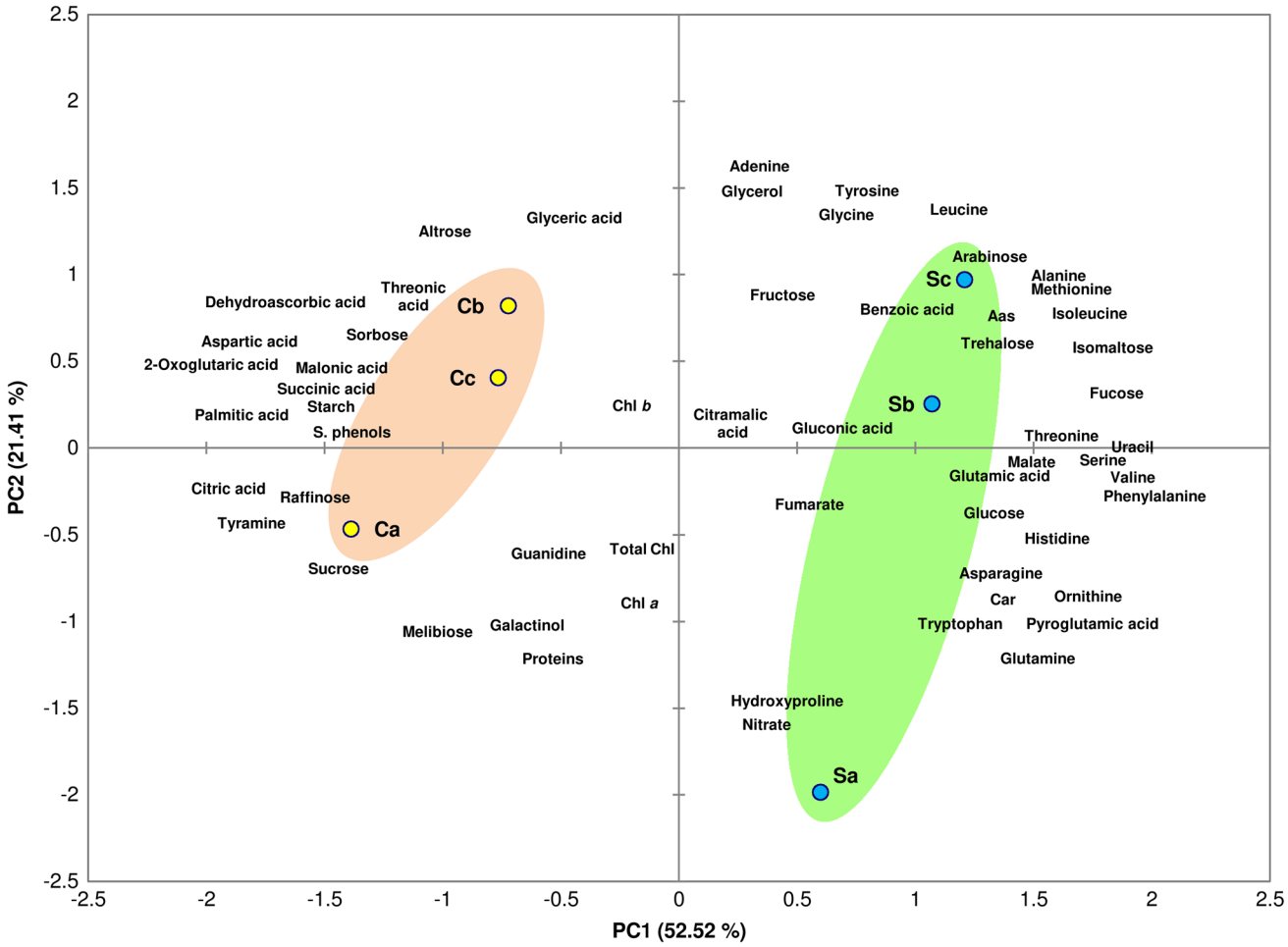

Fig. 5 Principal component analysis biplot of the correlation between the control and stressed treatments and their metabolic profiles. *PC1* principal component 1, *PC2* principal component 2, *Ca—Cc and Sa—Sc* control and drought stressed plants, respectively

a decrease in the levels of starch by 68% and increased the availability of isomaltose (Fig. 3). Likewise, the endogenous sucrose content diminished 27%, without considerable changes to glucose or fructose concentrations. The fucose content increased more than twofold, while the altrose, arabinose, alpha-trehalose, and melibiose concentrations remained unchanged. Significant decrease in sorbose and raffinose concentrations were detected. Concerning organic acid concentrations, we observed that malate was up-regulated in responses to dehydration, while citric acid, dehydroascorbate, malonic acid, palmitic acid, succinic acid, and 2-oxoglutaric acid were diminished. Finally, a significant reduction in the soluble phenols concentration was observed in stressed leaves.

Discussion

Plant acclimation to water deficits

Considering the previous (Acevedo et al. 2013, 2016) and current results, we observed that *I. paraguariensis* displays a typical isohydric plant phenotype (Tardieu and Simonneau 1998) and tends to close stomata at a relatively high plant water status (Ψ_{soil} decrease from −0.04 to −0.7 MPa) to reduce water loss. The onset of osmotic adjustment requires a reduction of RWC to at least 78% and proceeds to the maximum when the dehydration strain

becomes more severe and leaf RWC drops to 62.7%. Consequently, the lethal leaf water potential of this cultivar is lower than would be expected for a subtropical rainforest plant, and was equivalent to the temperate *Quercus rubra* (Augé et al. 1998), a dominant species native to a variety of mesic and dry-mesic sites.

Under severe water shortage conditions ($\Psi_{soil} = -2$ MPa), the conservation of the photosynthetic apparatus is particularly notable and an exciting feature of this genotype that recovers CO_2 assimilation upon re-watering (Acevedo et al. 2013). Simultaneously, we observed an active modification of the transcriptomic and metabolomic profiles that allow the plant to maintain functionality in a severely dehydrated state. Dehydration survival is not only ecologically important in natural forests, but is critical for yields in large-scale plantations of perennials, such as yerba mate and tea, where the harvestable products are the leaves. Besides physiological functions, *I. paraguariensis* also displayed morphological modification in response to drought by stimulating root growth.

Hormones and signalling

The response of plants to drought conditions is a complex process that begins with the stress recognition and activation of signal transduction pathways that modify the pattern of gene expression to bring about molecular and physiological changes to overcome the water shortage (Osakabe et al. 2014).

The sensing of stresses initiates several complex signalling pathways in plants, including alteration of intracellular Ca^{2+} concentration, production of secondary signalling molecules, and activation of kinase cascades (Verma et al. 2016). In *I. paraguariensis*, five DETs encoded Ca^{2+}-dependent protein kinases (SnRK2, CDPK1, and CDPK2) related to ABA signalling that were induced by drought. Likewise, numerous DETs encoding for phosphoinositide phospholipase C activity [T1] (Online Resource Table S2), G-proteins such as XLG1 [T2] and ARA3 [T3] involved in ethylene mediated signal transduction, and MAP kinases related to osmotic stress [T4] and plant defences to pathogens [T5, T6, T7, and others] were up-regulated indicating active signalling mechanisms.

Our previous results (Acevedo et al. 2013) revealed that ABA accumulates rapidly in *I. paraguariensis* leaves exposed to drought stress and this decreases transpiration through stomatal closure. The current study indicates that several genes related to biosynthesis, signalling, and the ABA-induced response were up-regulated in stressed leaves. Collectively, these results suggest that the ABA pathway provides central regulation of the water-stress response in *I. paraguariensis* plants. The leaf transcriptome contained two transcripts encoding 9-cis-epoxycarotenoid dioxygenase (NCED), a key enzyme in the ABA biosynthesis that is regulated by drought (Iuchi et al. 2001). Using qRT-PCR, we determined that the total expression of NCED genes in leaves from well-watered plants remained significantly lower than in stressed ones; *IpNCED1* was the most abundant variant (Online Resource Fig. S4). In contrast, the *IpNCED2* gene was induced by drought and is likely related to the rise of ABA under water deprivation.

Another differentially expressed transcripts encoded a member of the SNF1-related protein kinases [SnRK2: T8, T9, and T10] and protein phosphatase 2C [T11, T12, and T13] revealing active regulation of ABA signalling. In turn, ABA-induced inhibition of protein phosphatase 2C leads to SnRK2 activation by autophosphorylation, which allows the SnRK2 s to relay the ABA signal to downstream target proteins, including AREB/ABF transcription factors, anion channels, and NADPH oxidases (Cutler et al. 2010). Moreover, the expression of this ABA-signalling components is related to various abiotic stresses in *Zea mays* (Li et al. 2017) and *Arabidopsis thaliana* (Chan 2012). Finally, the induction of transcripts that encode an ABA-responsive element binding protein [AREB, T14] and basic leucine zipper transcription factor [T15] that binds to ABRE is responsible for the regulation of the ABA-inducible transcription in the stressed leaves. In addition to its central role in modulating the response to osmotic stress, ABA is considered a central regulator of plant defences (Pandey et al. 2015). In effect, ABA, salicylic acid, jasmonic acid, and ethylene are known to play significant roles in mediating plant defences (Rejeb et al. 2014). We observed the induction of a DET [T16] encode ENHANCED DISEASE SUSCEPTIBILITY1 (EDS1), which along with PHYTOALEXIN-DEFICIENT4 (PAD4), is an essential regulator of basal resistance to invasive obligate biotrophic and hemibiotrophic pathogens (Feys et al. 2005). Considering that under field conditions, *I. paraguariensis*, like many perennial plants, is associated with endophytic microorganisms (Pérez et al. 2016), the activation of plant defence signalling could be related to stress-induced restructuring of the leaf-associated microbiome (Timm et al. 2018). Moreover, the expression of several DETs [T19–T29] involved in hormone homeostasis suggested probable crosstalk linked to plant defence responses.

We identified a significant number of transcription factor (TF) families associated with abiotic (16), biotic (7), or combined abiotic and biotic (6) stress responses (Online Resource Table S4). Among them, NAC, AP2/ERF, C2H2, bZIP, and WRKY were the major TF families associated with drought tolerance. The NAC TFs are considered one of the largest families of TFs in plants and contribute to regulating plant growth and developmental processes, including abiotic stress responses (Joshi et al. 2016). Likewise, the AP2/ERF TFs that comprise the AP2, DREB, and ERF subfamilies are induced by pathogens attack, abiotic stresses,

and stress-related hormones (Licausi et al. 2013). C2H2 participates in various aspects of healthy plant growth and in osmotic stress responses. A recent study indicates that *C2H2* genes are expressed in leaves and roots of the model forest species *Populus trichocarpa* when subjected to abiotic stresses and are considered as candidate genes for future genetic engineering in *Populus* (Liu et al. 2015). Llorca et al. (2014) confirmed that bZIP TFs play a significant role in abiotic stress responses through ABA signalling. Among the TFs associated with modulation of plant growth, we highlight the differential expression of NF-Y TFs, since our results clearly showed that one of the main traits linked to *I. paraguariensis* drought tolerance is its capacity to stimulate root growth at the expense of the shoot proliferation. In this way, the differential expression of several NF-Y TFs under dehydration may contribute to this response by promoting lateral root development through an ABA-dependent mechanism (Zanetti et al. 2017).

Photosynthesis and related processes

Drought perturbs photosynthesis through CO_2 limitations resulting from stomatal closure and biochemical restrictions associated with a buildup of reducing power (Pinheiro and Chaves 2011). To cope with energy imbalances in photosynthetic metabolism and prevent reactive oxygen species (ROS) formation, chloroplasts employ several strategies that were recently reviewed (Vanlerberghe et al. 2016). The transcriptomic profile of drought stressed *I. paraguariensis* leaves revealed that several genes encoding the principal components of the light reactions, Calvin–Benson cycle, and photorespiratory pathway were down-regulated in response to water deprivation (Online Resource Table S5 and Online Resource Fig. S5), suggesting that drought impairs these processes. In effect, our results showed that a severe reduction in the water available in the soil induced stomatal closure concurrently with ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) activase transcription repression that together reduced CO_2 assimilation by 85% and reduced starch concentration threefold. Moreover, we detected an almost complete depletion in the expression of light reaction-related genes, indicating great coordination between the light-dependent reactions and Calvin–Benson cycle under water stress. Likewise, the relative contents of glycine and serine increased, which also suggest that photorespiration was affected. This variation can alter the rate of rubisco oxygenation (Novitskaya et al. 2002), and it should be supported by the repression of several genes encoding photorespiratory pathway enzymes. This "slowdown" response would compromise the primary role of photorespiration to dissipate excess reducing equivalents preventing the over-reduction of the electron transport chain (Voss et al. 2013). Accessory photosynthetic pigments provide another mechanism that decreases the production of ROS. In this way, the chlorophyll fluorescence and P700 measurements revealed a significant reduction in electron transport rate and efficient heat dissipation (high non-photochemical quenching) in the stressed leaves that was supported by the rise in the total carotenoid content (Table 1). A positive linear correlation was previously established between the amount of zeaxanthin present in the leaf and the overall level of thermal energy dissipation activity (Adams and Demmig-Adams 2004). Although the endogenous content of this xanthophyll was not quantified, this mechanism was supported by the observed up-regulation of transcripts [T30 and T31] encoding β-carotene 3-hydroxylase, which catalyzes the conversion of β-carotene in zeaxanthin through β-cryptoxanthin. In addition, leaves synthesize other secondary metabolites, such as chlorogenic acid, caffeic acid, and rutin, which should contribute directly to the antioxidant activity by preventing lipid peroxidation (Blum-Silva et al. 2015).

Our results also indicate that *I. paraguariensis* employs the malate valve to shuttle redox equivalents from the chloroplast to the cytosol and mitochondria. The oxaloacetate/malate shuttle is composed of an oxaloacetate/malate exchanger located in the chloroplast inner envelope membrane and isozymes of malate dehydrogenase (MDH) in the stroma and cytosol (Taniguchi and Miyake 2012). To shuttle reductant from the chloroplast, NADP–MDH reduces stromal oxaloacetate to malate using NADPH. Malate is then exported to the cytosol by an oxaloacetate/malate transporter. Cytosolic NAD–MDH produces NADH by oxidizing the exported malate to oxaloacetate, which is then returned to the stroma by the oxaloacetate/malate transporter (Fridlyand et al. 1998). Finally, NADH is oxidized in the mitochondrial electron transport chain activating the alternative oxidase (AOX) pathway to dissipate the excess energy under drought (Vanlerberghe et al. 2015). In this context, we observed significantly higher expression of transcript T32, an ortholog of the *A. thaliana* gene At5g12860, which encodes the 2-oxoglutarate/malate transporter (OMT). OMT is considered a crucial component of the oxaloacetate/malate transporter (Taniguchi and Miyake 2012). Simultaneously, other transcripts related to dicarboxylate transporter had their expression increased threefold in response to dehydration. Consequently, there was a significative rise in malate concentration (Fig. 3). Besides, AOX-related transcript [T33] expression increased 64-fold during drought. AOX is considered essential to maintaining respiration in the light during drought and it aids maintenance of photosynthesis by promoting energy balance in the chloroplast (Vishwakarma et al. 2015; Vanlerberghe et al. 2016). Besides, there is some evidence indicating that, unless in *Nicotiana tabacum* (Wang and Vanlerberghe 2013) and *Nothophagus* (Sanhueza et al. 2013), AOX plays an essential role during the recovery phase from drought stress. The combination of these

mechanisms to prevent photodamage probably allows the yerba mate plants to recover rapidly after a severe drought stress episode. Our previous results showed that carbon fixation in recovered plants did not vary significantly from continuously well-watered control plants 48 h after re-watering (Acevedo et al. 2013).

Carbohydrate metabolism

The endogenous nonstructural carbohydrate contents in leaves declined during drought stress (Fig. 3) due to reduced photosynthesis in conjunction with greater C demand for root growth, which took place in response to water shortage. The reduction of C fixation induced several DETs encoding protein kinases involved in sugar-mediated signalling pathways (Ramon et al. 2008), which included transcripts for hexokinase 1 [T34 and T35], and hexokinase 2 [T36] that function as glucose sensors. The hexokinases connect nutrient, light, and hormone signalling systems to regulate growth in response to environmental changes (Moore et al. 2003; Granot et al. 2013). Furthermore, drought modified the expression of several genes related to the nonstructural carbohydrates metabolism, including synthesis, transport, and hydrolysis of starch and sucrose, synthesis of sugars from the raffinose family, and glucose phosphorylation. Transcripts that encode the chloroplastic glucose-1-phosphate adenyl transferase small subunit (APS1) [T37 and T38], a key enzyme in the starch synthesis, were up-regulated. Simultaneously, a threefold reduction was observed in the expression of the transcript [T39] that codifies the large subunit of ADP-glucose pyrophosphorylase (APL1), which catalyzes the first rate-limiting step in starch synthesis (Crevillén et al. 2003). We also observed the induction of DETs involved in starch hydrolysis that encode α-amylase [T40] and chloroplastic ß-amylases, BAM1 [T41, T42, and T43] and BAM3 [T44 and T45]. The isolated α-amylase transcript had high homology with the *A. thaliana AMY1* (At4g25000) gene which is induced by ABA, heat stress, and biotic stress (Doyle et al. 2007).

The expression of two mRNAs [T46 and T47] encoding a protein with putative sucrose-phosphate synthase activity (SPS3) was repressed in drought stressed plants, indicating a decrease in the sucrose pool. Since SPS3 play a significant role in catalyzing the rate-limiting step of sucrose biosynthesis from UDP-glucose and fructose-6P, downregulation of its related transcripts likely affected sucrose availability for growth. Notwithstanding, the activation of several genes encoding sucrose synthases and invertases [T48, T49, and T50] provides carbon for plant metabolism (Barratt et al. 2009; Ruan et al. 2010). In addition, transcripts encoding cell-wall acid invertase 2 [T51] and chloroplastic neutral/alkaline invertase [T52] were induced in drought stressed *I. paraguariensis* leaves. Similarly, a transcript related to sucrose (and maltose) transport into cells [T53] and phloem loading for long-distance transport [T54] were induced in the stressed leaves (Chandran et al. 2003; Sherson et al. 2003). As a result, the sucrose content in leaves decreases by 25% (Fig. 3).

In summary, the expression patterns suggest an impairment of starch and sucrose biosynthesis and an enhancement of starch-derived soluble sugars in leaves. Our results are consistent with those obtained through transcriptome studies of dehydration tolerance in mature non-senescent *Populus trichocarpa* leaves (Tang et al. 2015). Drought also affects the expression of genes related to the carbon metabolism in leaves. In effect, the transcriptome and the metabolome results suggest that *I. paraguariensis* down-regulates the carbon metabolism to sustain the basal activity in the leaves and redirects the assimilates (sucrose) to support root growth.

As in *P. trichocarpa* (Tang et al. 2015), drought concurrently induces differential expression of genes responsible for the synthesis of raffinose family oligosaccharides (RFOs). RFOs are synthesized from sucrose by the subsequent addition of galactinol moieties to form raffinose, stachyose, verbascose, and ajugose by the sequential action of different synthases (ElSayed et al. 2014). We observed that transcript levels of galactinol synthase, raffinose synthase, and stachyose synthase increased under drought. Among them, the cytosolic galactinol synthase, a critical regulator of the pathway, is involved in the first step of RFOs biosynthesis, where it forms galactinol through the transfer of a galactosyl moiety from UDP-galactose to myo-inositol. Increasing evidence indicates that galactinol synthase is vital in the physiology of plant stress tolerance and photosynthate translocation (Zhou et al. 2012). Nishizawa et al. (2008) found that *Coffea canephora* plants with high galactinol and raffinose contents were less susceptible to oxidative stress. The up-regulation of the biosynthetic-related genes was also reported in leaves of plants subjected to severe drought (Mofatto et al. 2016). ABA also induces the expression of galactinol synthase and raffinose synthase through the regulation of WRKY TFs that mediate oxidative stress responses (Valluru and Van den Ende 2011). Raffinose is ubiquitous in plants and may function as a compatible solute that maintains cell turgor and stabilises cell proteins (Taji et al. 2002; Janecek et al. 2011). In this regard, *I. paraguariensis* employs osmotic adjustments to overcome the deleterious effects of dehydration (Acevedo et al. 2013); however, the compatible solutes involved remain unknown, since neither proline nor glycine betaine was detected. Although additional studies are necessary to confidently identify all of the puzzle pieces, considering the up-regulation of the related enzymes and the endogenous contents of galactinol and raffinose, the release of stachyose as an osmoprotectant is hypothesized. Finally, we observed a twofold increase in fucose (a deoxyhexose) content during drought. Fucose is

considered a major constituent of plant cell-wall surfaces and plays a central role in cell-wall protection (Ebert et al. 2017).

Nitrogen and amino acid metabolism

The endogenous content of nitrate and proteins did not vary under stress, while the total amount of free amino acids increased (Fig. 3). Analysis of the transcript levels for the primary enzymes involved in amino acid biosynthesis revealed that several were elevated under drought stress, indicating that de novo synthesis was a relevant source of induced variation. Glutamate and glutamine are considered central molecules in amino acid metabolism (Hildebrandt et al. 2015). The primary source of glutamate in *I. paraguariensis* leaves appeared to be the transfer of the amide group from glutamine to the 2-oxoglutarate, a key intermediate in the tricarboxylic acids cycle. Two lines of evidence could support this possibility. First, the expression of several transcripts encoding citrate synthase [T55], aconitase [T56, T57], and isocitrate dehydrogenase [T58], which catalyze the early steps in the tricarboxylic acids cycle and lead to 2-oxoglutarate formation, were induced under water deprivation (Fig. 4). Second, the transcript T59 was also up-regulated and it encodes glutamate dehydrogenase, which catalyzes the reductive amination of 2-oxoglutarate to give glutamate. Therefore, the metabolomic results indicated that the level of 2-oxoglutarate decreased, while the endogenous content of glutamate remained constant in response to stress. Furthermore, *I. paraguariensis* accumulated large quantities of pyroglutamic acid from the degradation of glutathione, which can be converted to glutamate by the action of 5-oxoprolinase [T60] in the cytoplasm (Ohkama-Ohtsu et al. 2008). The up-regulation of several DETs encoding γ-glutamyl transpeptidase and γ-glutamyl cyclotransferase confirms the conversion of glutathione into pyroglutamic acid.

The transcriptomics study indicated that cytosolic glutamine synthetase [T61, T62, and T63], which combines ammonium and glutamate to produce glutamine, was induced during drought. Our metabolic data supports this transcriptomic finding, since glutamine was sharply elevated (38-fold) during drought stress. In this regard, Wang et al. (2016) used proteomics data during heat stress to find that glutamine synthetase was induced to aid the plant in using glutamate rapidly to generate ample quantities of glutamine. Alternatively, N assimilated into glutamate and glutamine may be incorporated into aspartate and asparagine (Kirma et al. 2012). Aspartate serves as the nitrogen donor in numerous aminotransferase reactions and is the precursor of a large family of amino acids. We observed accumulation of methionine (2.3-fold) and threonine (1.9-fold) that were synthesized from aspartate and these increases were associated with a reduction (2.2-fold) in the endogenous content of aspartate. The metabolomic results also showed a significant increase in valine (2.5-fold) and isoleucine (3.2-fold), both of which result from protein degradation, while the endogenous leucine content remained unchanged. In this context, Huang and Jander (2017) used a combination of amino acid assays and genetic approaches to demonstrate that ABA-regulated protein degradation causes branched-chain amino acid accumulation in osmotically stressed *A. thaliana* plants. Considering that drought decreases CO_2 assimilation, it is possible to speculate that the primary target of amino acid breakdown is to fuel the respiratory chain to produce available energy (Araújo et al. 2011). We observed a significant increase in the content of asparagine (37-fold) in concordance with the induction of various transcripts that encode asparagine synthetase during stress. Asparagine is relatively inert and serves primarily as a nitrogen transport and storage compound during the stress and at the early stages of leaf rehydration before the recovery of photosynthetic activity (Martinelli et al. 2007). Asparagine buildup was indeed observed in several water-stressed plants (Sieciechowicz et al. 1988).

Finally, we also detected an increase in the phenylalanine concentration, which is linked to the earlier results in the sense that the expression of the chorismate mutase gene (GenBank: KT428840) is induced in response to dehydration (Acevedo et al. 2016). Chorismate is the final product of the shikimate pathway and is the initiator metabolite for the synthesis of phenylalanine, tyrosine, and secondary metabolites derived from these amino acids that are produced in response to environmental signals as part of a general defence response (Less et al. 2010).

Conclusions

In summary, our analysis of whole plant physiology and leaf transcriptional and metabolic changes triggered by drought showed that (1) ABA is involved in the drought response mechanism in *I. paraguariensis*; (2) the acclimation response includes root growth stimulation, stomatal closure, osmotic adjustment (probably triggered by sugars and amino acids release), photoprotection of the photosynthetic apparatus (increased carotenoids content), and regulation of nonstructural carbohydrates and amino acid metabolism; (3) considering the identification of several protein kinases and transcription factors families associated with abiotic, biotic, or combined abiotic and biotic stress responses, we hypothesize that the stress generated by drought affects the symbiotic equilibrium between yerba mate and its microbiome resulting in a secondary strain that the host plant should overcome it. Hence, future research will focus on elucidating if water scarcity affects the equilibrium in the endophytic

communities or provides an advantage allowing proliferation of opportunistic microorganism that then become a potential threat to the plant.

Author contribution statement RMA, OAR, and PAS conceived and designed the experiments. RMA and EHA performed the experiments. RMA, SG, MR, NP, and PAS analyzed the transcriptomics data. ARS and ANN performed the metabolomics data. RMA, ANN, OAR, and PAS wrote the manuscript. PAS coordinate its revision. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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