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Understanding the Impact of Drought in *Coffea* Genotypes: Transcriptomic Analysis Supports a Common High Resilience to Moderate Water Deficit but a Genotype Dependent Sensitivity to Severe Water Deficit

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Abstract: Water scarcity is the most significant factor limiting coffee production, although some cultivars can still have important drought tolerance. This study analyzed leaf transcriptomes of two coffee cultivars with contrasting physiological responses, *Coffea canephora* cv. CL153 and *Coffea. arabica* cv. Icatu, subjected to moderate (MWD) or severe water deficits (SWD). We found that MWD had a low impact compared with SWD, where 10% of all genes in Icatu and 17% in CL153 reacted to drought, being mainly down-regulated upon stress. Drought triggered a genotype-specific response involving the up-regulation of reticuline oxidase genes in CL153 and heat shock proteins in Icatu. Responsiveness to drought also included desiccation protectant genes, but primarily, aspartic proteases, especially in CL153. A total of 83 Transcription Factors were found engaged in response to drought, mainly up-regulated, especially under SWD. Together with the enrollment of 49 phosphatases and 272 protein kinases, results suggest the involvement of ABA-signaling processes in drought acclimation. The integration of these findings with complementing physiological and biochemical studies reveals that both genotypes are more resilient to moderate drought than previously thought and suggests the existence of post-transcriptional mechanisms modulating the response to drought.

Keywords: ABA signaling; climate changes; coffee; drought; functional analysis; leaf RNAseq; transcription factors

1. Introduction

Along with the rapid expansion in population and global warming, water scarcity has become a worldwide challenge for agriculture [1–3]. To cope with water deficits, plants trigger a wide range of responses at the molecular, biochemical, and physiological

levels [4,5]. At the molecular level, a response to drought usually includes transcriptional and post-transcriptional modulations that lead to a differential expression of genes and pathways, which ultimately promote metabolic and physiological changes associated with plant acclimation [6–8].

Integrative approaches for large-scale transcriptomic studies have shown that responses include mainly two sets of genes, one directly involved in protecting cells from stress and the other including regulatory proteins that modulate gene expression. The first group includes water channel proteins and membrane transporters [9], key enzymes for osmolyte biosynthesis (e.g., proline, sugars) [10], detoxification enzymes (e.g., catalase, superoxide dismutase, ascorbate peroxidase) [8], enzymes for fatty acid metabolism, ferritin, and lipid-transfer proteins [11], and proteins for the protection of macromolecules (e.g., late embryogenesis abundant, antifreeze proteins and chaperones) [12]. A second important group of responsive drought-induced genes that are activated by drought include transcription factors (TFs) as the widely known dehydration responsive element binding (DREB) TFs, as well as protein kinases and phosphatases implicated in the regulation of the stress signal transduction to subsequent components in the pathway towards the nucleus [13–15]. Additionally, cellular changes in shape, turgidity, or changes in concentrations of solutes and reactive oxygen species (ROS) are also assigned as early stress effects and signals triggering the plant stress responses [16,17]. However, most of these genes have been characterized in model plants such as *Arabidopsis thaliana* and are much less known in the context of tolerant crop varieties. Thus, it is crucial to identify the respective ortholog genes in each species.

Coffee (*Coffea* sp.), a valuable commodity export, is cultivated and exported by more than 80 countries, mainly developing ones, in the tropical regions of the Americas, Southeast Asia, India, and Africa [18,19]. Despite a large number of *Coffea* species have been identified, the international coffee trade is dominated by *C. canephora* (Robusta type of coffee) and especially the polyploid *C. arabica* (Arabica type of coffee). Both species are expected to be affected by climate changes [20,21], although some elite cultivars seem to display a higher resilience to different abiotic stresses than earlier assumed [18,22–25]. Additionally, the fruit development of *C. arabica* seems to be quite sensitive to rising temperatures and water shortage [26,27], and impacts in crop yields have already been noted by local farmers due, presumably, to ongoing climate changes [21]. *Coffea canephora* is also affected by drought, and countries that have heavily invested in the intensive monoculture of this species (e.g., Vietnam) are predicted to be further impaired in 90% of its total production area [28]. Moreover, the effects of drought could be potentially aggravated in coffee plantations under full sunlight exposure [18].

In this context, new resilient lines must be developed to guarantee the sustainability of the coffee crop. For that, identifying genes whose expression is associated with coffee drought tolerance is crucial. Currently, there is evidence that *C. canephora* has some tolerance to drought through enrichment of secondary compound metabolic genes, namely antioxidant genes, which play an essential role in coffee drought response [29]. Recent studies underline that at least some genotypes of *C. canephora* could be far more sensitive to thermal stress than previously thought [30,31]. Other genotypes of *C. arabica* and *C. canephora* were found to have the ability to endure harsh temperatures [23,24] and water deficit [25] to a greater extent than usually assumed. In this framework, an extended RNA-Seq analysis was performed to investigate transcriptomic leaf profile changes in two drought-responsive coffee genotypes [25,27]. Upon subjecting the plants to a gradual moderate or severe water deficit, we identified the transcriptomic mechanisms of drought tolerance in these genotypes. The identified genes should constitute potential targets for breeding drought-tolerant coffee varieties.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

In this study, we used 7-years old potted plants of two cropped genotypes (in Brazil) from the two main producing coffee species, *Coffea canephora* Pierre ex A. Froehner cv. Conilon Clone 153 (hereafter CL153) and *C. arabica* L. cv. Icatu Vermelho (hereafter Icatu), an introgressed variety resulting from a cross between *C. canephora* and *C. arabica* cv. Bourbon Vermelho that was further crossed with *C. arabica* cv. Mundo Novo. A total of 18 plants per genotype was grown in 80 L pots placed in walk-in growth chambers (EHHF 10000, ARALAB, Portugal) under controlled environmental conditions of temperature (25/20 °C, day/night), relative humidity (ca. 70%), air [CO2] (380 μL L^{-1}), photoperiod (12/12 h), and irradiance (approximately 750 μmol m^{-2} s^{-1} at the third upper part of the plants), without restrictions of water until applying the water treatments shown below, nutrients as described in [32,33] or root growth space.

2.2. Water Stress Imposition and Leaf Water Status

Water conditions were imposed as previously described [25]. Briefly, the plants were divided into three groups. In the first group, individuals were maintained well irrigated (WW) throughout the experiment. In the other two groups, water deficit was gradually imposed during two weeks by partially withholding irrigation (with a partial water replacement of the amount lost in each pot) until stability of predawn leaf water potential (Ψpd) to plant values between −1.5 and −2.5 MPa (moderate water deficit-MWD) or below −3.5 MPa (severe water deficit-SWD). WW plants were maintained under full irrigation (Ψpd > −0.35 MPa). These conditions represented approximately 80% (WW), 25% (MWD) or 10% (SWD) of maximal water availability in pots [27]. After reaching the desired Ψpd values for MWD or SWD conditions, the pot moisture was maintained for another two weeks before leaf sampling. Leaf Ψpd was determined at predawn immediately after leaf excision, using a pressure chamber (Model 1000, PMS Instrument Co., Albany, OR, USA).

2.3. RNA Extraction and Illumina Sequencing

Newly matured leaves from plagiotropic and orthotropic branches from the upper third part (well illuminated) of each plant were collected under photosynthetic steady-state conditions after 2 h of illumination, flash-frozen in liquid nitrogen and stored at −80 °C, being finely powdered in liquid N$_2$ prior to analysis. Total RNA was extracted from 18 samples (two genotypes × three water treatments × three biological replicates) using 20 mg of the frozen leaves. RNA was extracted using the Analytik-Jena InnuSPEED Plant RNA Kit (Analytik Jena Innuscreen GmbH, Berlin, Germany) following [34]. RNA quantity and quality were determined using BioDrop Cuvette (BioDrop, Manchester, UK) and Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). RNA Integrity Number (RIN) for the samples ranged from 8.89 to 9.22. The messenger RNA (mRNA) libraries were constructed with the Illumina "TruSeq Stranded mRNA Sample Preparation kit" (Illumina, San Diego, CA, USA) and sequenced on an Illumina NovaSeq6000 at Macrogen facilities (Macrogen, Geumcheon-gu, Seoul, Korea). Raw reads have been deposited in the NCBI Sequence Read Archive, BioProject accession PRJNA729673.

2.4. Quality Analysis of Sequencing Data

High-quality reads were obtained after several steps of quality checks, including trimming and removal of adaptor/primer and low-quality reads using FastQC version 0.11.8 [35] and Trimmomatic version 0.38 [36]. FastQ Screen version 0.13 [37] was used to check for contaminants against the genome of the most common model organisms and adapter databases (e.g., Mitochondria RNA, PhiX, Vector from UniVec database, FastQ Screen rRNA custom database and FastQ Screen Adapters database).

2.5. Reference-Based Mapping and Assembly

The filtered high-quality reads were mapped to the reference genome of *C. canephora* downloaded from the Coffee Genome Hub (http://coffee-genome.org/download, accessed on 4 January 2020) [38] using STAR version 2.6.1 [39]. Htseq-count v0.11.0 [40] was used to count uniquely mapped genes. Samtools version 1.9 [41] and gffread version 0.9.9 [42] were used throughout the analysis to obtain general statistics of the genome mapping.

2.6. Identification of Differentially Expressed Genes (DEGs)

Gene expression normalization of all samples was estimated with the DESeq method (median of ratios normalization) to account for sequencing depth and RNA composition, which is appropriate for the differential expression (DE) analysis across samples. A Principal Component Analysis (PCA) was performed on the expression data of genes, FKPM normalized and log10-transformed, using the ggplot2 version 3.3.2 library [43] of R software version 3.5.1 [44]. Through visual inspection of the PCA plot, the 7B replicate was considered an outlier and thus excluded from the downstream analysis (Figure S1). DESeq2 v1.28.1 [45] was used to check for differences in the relative abundance of the genes between the different water treatments. The Benjamini–Hochberg approach was used for controlling the false discovery rate, FDR [46]. Differentially expressed genes (DEGs) were defined as genes with a normalized non-zero log2 fold change (FC) expression and an FDR < 0.01. Python's matplotlib library was used to plot Venn diagrams and bar plots [47]. The functional annotation of the reference genome of *C. canephora* referred above was used to search the top responsive DEGs. To better understand the effects of drought, a specific analysis was performed among the DEGs annotated with the direct and child GO terms "response to water deprivation" (GO:0009414) and "response to desiccation" (GO:0009269). Additionally, due to its crucial importance in the acclimation response of coffee plants to changing environmental conditions [23,33,48], a specific search was performed among DEGs annotated with the following terms: "antioxidant activity" (GO:0016209), "response to oxidative stress" (GO:0006979) under Antioxidant activity; "cellular respiration" (GO:0045333), "mitochondrion" (GO:0005739), "malate dehydrogenase activity" (GO:0016615), "pyruvate kinase activity" (GO:0004743) under Cellular respiration; "fatty acid metabolic process" (GO:0006631) and LOX (GO:0004051, GO:0016702) under Lipid metabolism; and "photosynthesis" (GO:0015979), "photosystem" (GO:0009521), "photosynthetic electron transport chain" (GO:0009767), "photorespiration" (GO:0009853) and "chlorophyll biosynthetic process" (GO:0015995) under Photosynthesis.

2.7. Regulation Patterns of Transcription Factors

Blastx from the Basic Local Alignment Search Tool (BLAST) version 2.10.1 command line tools from the NCBI C++ Toolkit was used to map the DEGs against *Arabidopsis thaliana* homologs using a local Swissprot database, filtering gene hits by maximum E-Value of 1.0×10^{-3} and minimum identities of 40% [49]. Then, to study the regulation pattern of transcription factors (TFs) among the detected DEGs, a list of *A. thaliana* TFs related to drought was retrieved from DroughtDB (http://pgsb.helmholtz-muenchen.de/droughtdb/, accessed on 15 May 2021) and searched among DEGs. To complement these analyses, TFs were also searched among DEGs if annotated with "DNA-binding transcription factor activity" (GO:0003700) and "general transcription initiation factor binding" (GO:0140296) in the reference genome. To understand the enrollment of protein kinases and phosphatases in the regulation of drought, the following GO terms were also searched among DEGs: "phosphatase activity" (GO:0016791) and "protein kinase activity" (GO:0004672).

2.8. Enrichment Analysis of Gene Ontology

Gene Ontology (GO) enrichment analyses were applied to understand the functional classification of DEGs through an over-representation analysis (ORA), using gProfiler [50] under FDR < 0.05. REVIGO [51] was used to summarize results by removing redundant

GO terms with a similarity ≥0.5. Enrichment nonredundant data with these FDR and similarity cutoffs were plotted using ggplot2. This same package was used to plot heatmap with dendrograms to visualize DEGs based on the differential expression patterns between the different treatments. To prevent highly differentially expressed genes from clustering together without considering their expression pattern, log2 fold change was scaled by gene across treatments (row Z-score).

3. Results

3.1. Overall Transcriptome Profiling and Mapping Statistics

Quality analysis, data trimming, and filtering generated an average of 24.8 million (CL153) and 22.7 million (Icatu) clean reads, from an average of 25.1 and 23.0 million raw reads, respectively. Overall, an average of 90% and 84% cleaned reads from CL153 and Icatu, respectively, were mapped to the reference genome (Table S1). Statistical details for each replicate are depicted in Table S1.

3.2. Differential Gene Changes of CL153 and Icatu in Response to Drought

In CL153, the lowest number of expressed genes was found under MWD (20052) and the highest under WW conditions (20528; Figure 1; Table S2). In Icatu, the highest (20675) and lowest (20157) number of expressed genes were observed under MWD and SWD conditions, respectively (Figure 1; Table S2). Nevertheless, MWD triggered only 5% of genes (999 DEGs) in CL153 and 4% (776 DEGs) in Icatu. Notably, the highest number of DEGs was consistently found in response to SWD: 3373 in CL153 (17% all genes); 2055 in Icatu (10% all genes) (Table S2). An average of 75% of DEGs were annotated with GO terms in both genotypes, while the remaining were uncharacterized or with unknown functions, according to the reference genome (Table S2).
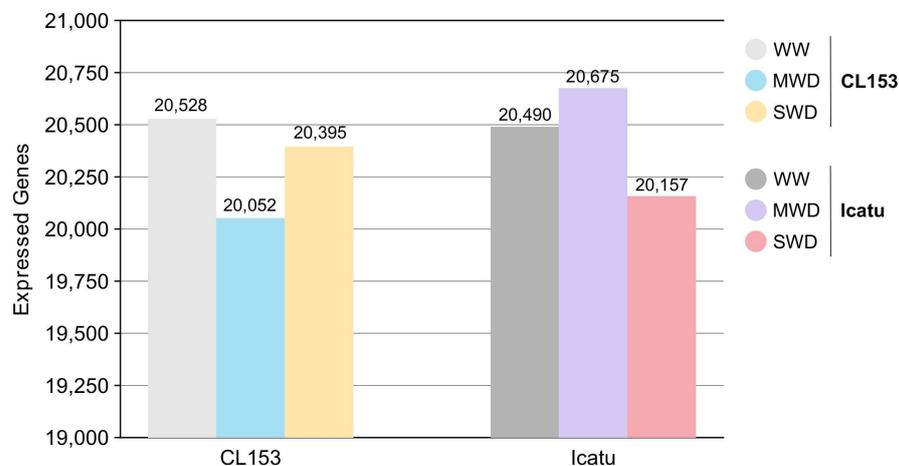

Figure 1. The total number of expressed genes *Coffea arabica* cv. Icatu (Icatu) and *C. canephora* cv. Conilon Clone CL153 (CL153) plants submitted to three different water availability conditions: well-watered (WW), moderate water deficit (MWD), and severe water deficit (SWD).

Under drought conditions, DEGs were mostly down-regulated under MWD (CL153: 58.3%, Icatu: 80.0%) and SWD (CL153: 67.0%, Icatu: 74.8%) (Figure S2). This particular response was associated with the drought severity level since only a partial number of DEGs were commonly found under both MWD and SWD, either in CL153 (down-regulated: 22.2%, 517 DEGs; up-regulated: 29.0%, 344 DEGs; Figure 2A) or in Icatu (down-regulated: 23.7%, 414 DEGs; up-regulated: 10.7%, 65 DEGs; Figure 2B). Thus, in the two genotypes, the majority of specific DEGs were found under SWD.

Besides the specificity linked with the level of water deficit, results also showed that the two genotypes reacted differently to drought as only a small number of DEGs were commonly found between genotypes and between water deficit treatments (115 down-regulated and 27 up-regulated) (Figure 2C). Both CL153 and Icatu showed a low number

of specific DEGs under MWD (57 down and 63 up in CL153; 160 down and 84 up in Icatu). In comparison, more DEGs were found under SWD, being mostly down-regulated (1046 down and 610 up in Cl153; 438 down and 183 up in Icatu; Figure 2C). This reveals a high degree of up-regulated DEGs in CL153 under SWD, whereas, in Icatu, the number of specific down-regulated DEGs was 2.4 times higher than the up-regulated ones. The same pattern was found in the heatmap of treatment-specific DEGs that showed a small genotype differentiation under MWD (Figure 3). In sharp contrast, a higher degree of variation was found under SWD, especially in CL153, where DEGs were more up-regulated in this genotype than in Icatu, in agreement with the previous analysis.
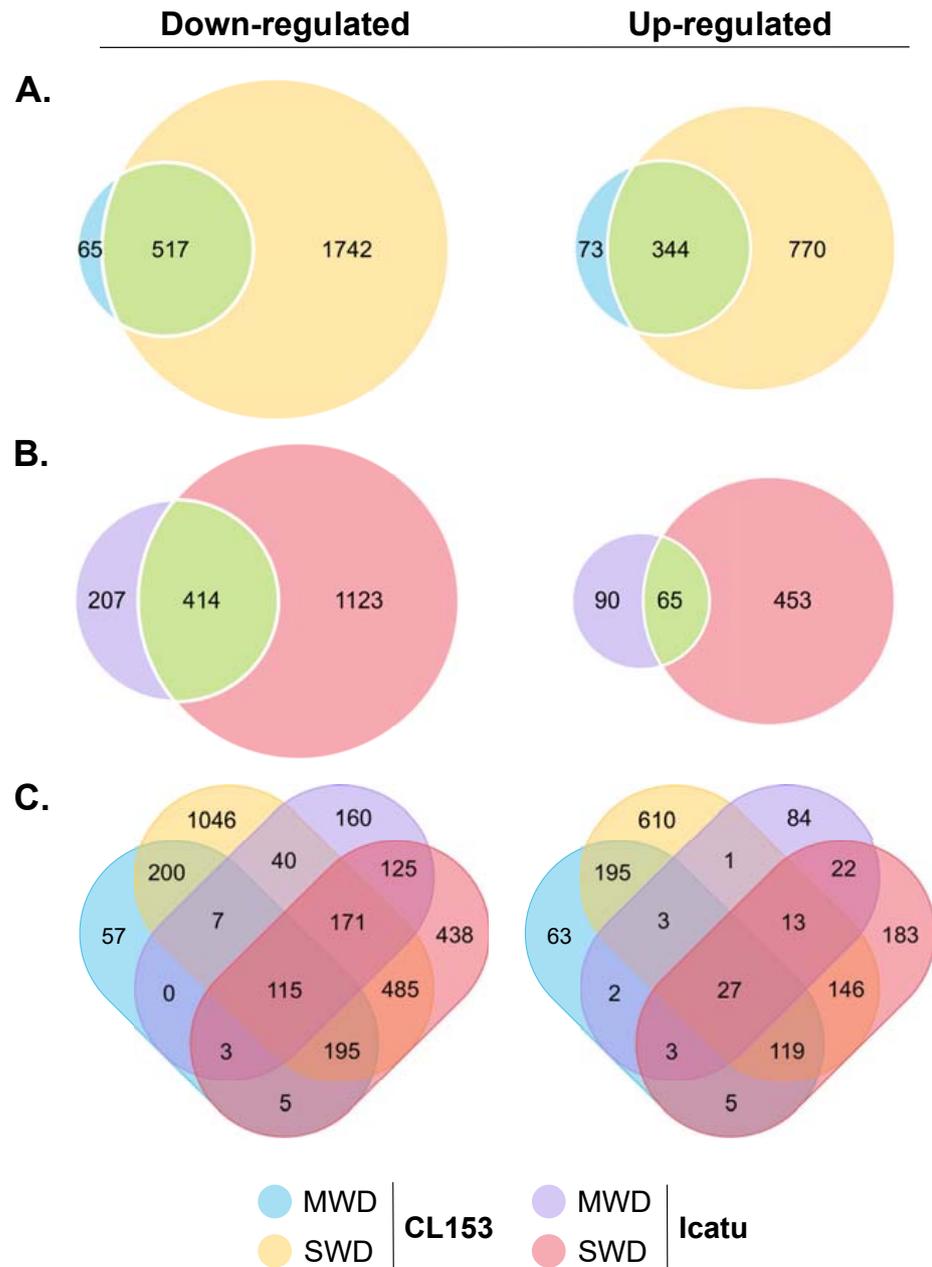
Figure 2. The total number of differentially expressed genes (DEGs) found in *Coffea arabica* cv. Icatu (Icatu) and *C. canephora* cv. Conilon Clone CL153 (CL153) plants submitted to moderate water deficit (MWD) or severe water deficit (SWD) in comparison to well-watered plants. (**A**) Number of DEGs found in CL153 that are specific to the MWD treatment (blue), specific to the SWD treatment (yellow), and commonly found in both water deficit treatments (green) being down-regulated (**left**) or right-regulated (**right**). (**B**) Number of DEGs found in Icatu that specific of the MWD treatment (purple), specific of the SWD treatment (pink) or commonly found in both water deficit treatments (green) being down-regulated (**left**) or right-regulated (**right**). (**C**) Distribution patterns of DEGs considering the two genotypes together for down-regulated (**left**) or right-regulated (**right**) DEGs.
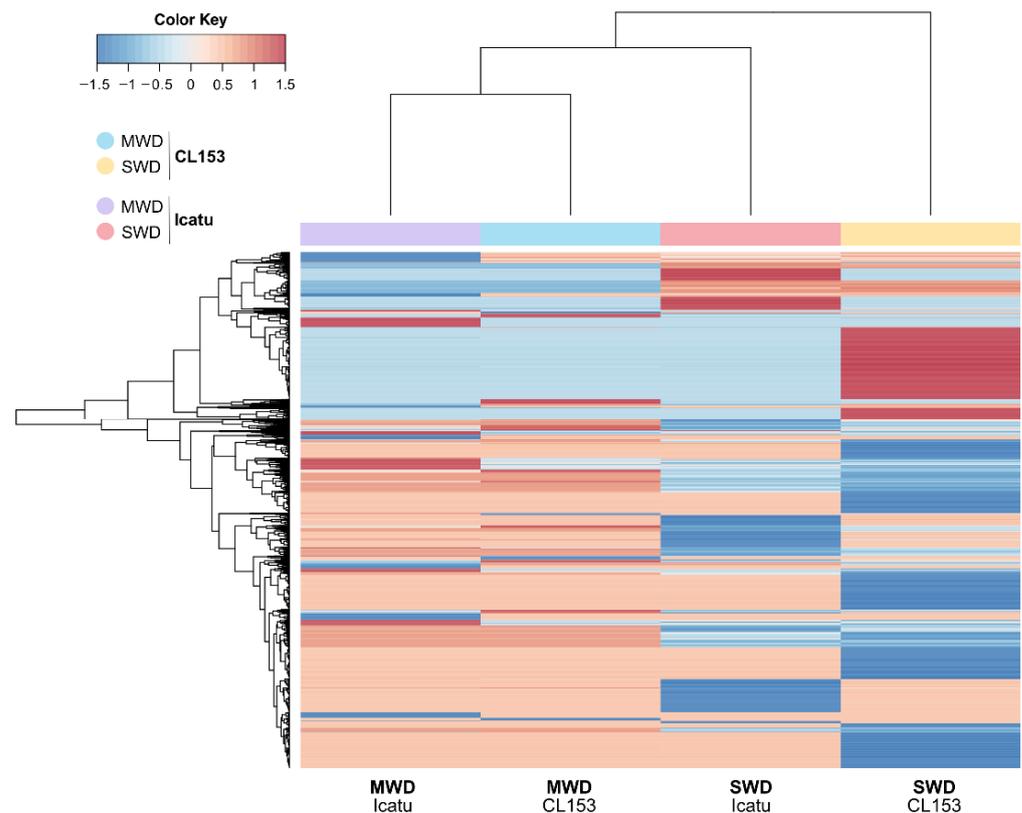
Figure 3. Clustered heat maps and dendrograms of the normalized log2 fold change (FC) visualizing the expression of significant (FDR < 0.01) treatment-specific differentially expressed genes (DEGs) considering the effect of moderate water deficit (MWD) and severe water deficit (SWD) conditions compared to well-watered plants (WW) in *Coffea arabica* cv. Icatu (Icatu) and *C. canephora* cv. Conilon Clone CL153 (CL153) plants. Values were scaled by row using Z-scores. Hot colors represent up-regulated DEGs, and cold colors represent down-regulated DEGs. Column color labels represent water treatments (colors of treatments follow Figure 1).

3.3. Identification and Classification of DEGs

Under drought, the 10 top up-regulated DEGs in CL153 were primarily involved in ox-idoreductase activity and FAD binding. For instance, under MWD, top up-regulated DEGs in CL153 included several reticuline oxidase-like genes, as well as different cytochrome *P450* genes, a carotenoid cleavage dioxygenase, and the *TF ERF027* (Tables 1 and S3).

Under SWD, the top DEGs in CL153 were involved in similar functions and at the same level of fold changes as under MWD, also showing an up-regulation of reticuline oxidase genes and mostly of an acid phosphatase gene (*PAP20*) involved in hydrolase activity and metal ion binding (Tables 2 and S4). However, while under MWD, CL153 showed a down-regulation of DEGs mostly involved in binding, auxin production, and transporter activity, under SWD, the effect was two times higher, showing a strong down-regulation of the ROP-interactive CRIB motif-containing protein 4 gene (FC = −21.55; Table 2).

Table 1. Top 10 up- and down-regulated differentially expressed genes (DEGs) under moderate water deficit (MWD) relative to well-watered (WW) *C. canephora* cv. Conilon Clone CL153 (CL153) plants. Molecular functions were retrieved from UniprotKB database (* functions exclusive to *A. thaliana* homologs). Genes are descending fold changes (FC).

Gene ID	Homolog	Protein Name	Molecular Function	FC
Up-regulated				
Cc09_g07380	AT1G30760	Putative Reticuline oxidase-like protein	oxidoreductase activity; FAD binding	11.17
Cc10_g00760		Putative Arabidopsis protein of unknown function		11.00
Cc09_g07390	AT4G20820	Putative Reticuline oxidase-like protein	oxidoreductase activity; FAD binding	10.61
Cc02_g03420	ERF027	Putative Ethylene-responsive transcription factor	DNA-binding; DNA-binding transcription factor	10.11
Cc04_g10590	CYP82C2	Putative Cytochrome P450 82A1	oxidoreductase activity; heme binding; iron ion binding	9.81
Cc06_g15980	LEA46	18 kDa seed maturation protein		9.67
Cc02_g29220	CYP75B1	Putative Cytochrome P450 750A1	oxidoreductase activity; heme binding; iron ion binding	9.24
Cc01_g19640		Putative Basic 7S globulin	aspartic-type endopeptidase activity	9.12
Cc05_g00080	EM6	Late embryogenesis abundant protein EMB564		8.96
Cc06_g06630	PUB22	E3 ubiquitin-protein ligase PUB22	ubiquitin-protein transferase activity	8.94
Down-regulated				
Cc07_g09010		Putative unknown protein		−9.32
Cc02_g16790	SAUR50	Auxin-induced protein 15A		−9.59
Cc04_g01940	HTH	Hothead	oxidoreductase activity; FAD binding; mandelonitrile lyase activity *	−9.61
Cc11_g01760		Protein of unknown function, DUF642		−9.77
Cc02_g09710	TBL36	Trichome Birefringence-like36	O-acetyltransferase activity *	−10.07
Cc00_g29810	CCR1	NAD(P)-binding Rossmann-fold superfamily protein	oxidoreductase activity *	−10.22
Cc07_g15190	DOT3	Putative BTB/POZ domain-containing protein DOT3		−10.51
Cc06_g01530	GPAT6	Glycerol-3-phosphate acyltransferase 6	transferase activity; phosphatase activity	−10.58
Cc05_g09310	PDF1	Putative uncharacterized protein		−11.24
Cc06_g08040	AZI1	Putative uncharacterized protein	protein self-association *	−12.36

Table 2. Top 10 up and down-regulated differentially expressed genes (DEGs) under severe water deficit (SWD) relative to well-watered (WW) *C. canephora* cv. Conilon Clone CL153 (CL153) plants. Molecular functions were retrieved from UniprotKB database (* functions exclusive to *A. thaliana* homologs). Genes are descending Fold Changes (FC).

Gene ID	Homolog	Protein Name	Molecular Function	FC
Up-regulated				
Cc06_g05620	PAP20	Probable purple acid phosphatase 20	hydrolase activity; metal ion binding	13.03
Cc09_g07380	AT1G30760	Putative Reticuline oxidase-like protein	oxidoreductase activity; FAD binding	12.44
Cc09_g07390	AT4G20820	Putative Reticuline oxidase-like protein	oxidoreductase activity; FAD binding	11.53
Cc04_g10590	CYP82C2	Putative Cytochrome P450 82A1	oxidoreductase activity; heme binding; iron ion binding	10.95

Table 2. *Cont.*

Gene ID	Homolog	Protein Name	Molecular Function	FC
Up-regulated				
Cc10_g00760		Putative Arabidopsis protein of unknown function (DUF241)		10.92
Cc08_g11420	EXLB1	Expansin-like B1		10.92
Cc00_g15520	CWINV2	Beta-fructofuranosidase, insoluble isoenzyme 1	hydrolase activity	10.54
Cc07_g04930	TBL19	Putative Trichome Birefringence-like 19	transferase activity *	10.20
Cc02_g24750		Carotenoid cleavage dioxygenase 7	oxidoreductase activity; metal ion binding	9.82
Cc03_g15270	AT4G20820	Putative Reticuline oxidase-like protein	oxidoreductase activity; FAD binding	9.78
Down-regulated				
Cc00_g18380	SDR2a	Momilactone A synthase	oxidoreductase activity *	−9.93
Cc10_g02800	RL1	RAD-like 1	DNA-binding transcription factor activity *	−10.15
Cc01_g12960	EXPA4	Expansin-A4		−10.31
Cc01_g05920	GASA6	Protein GAST1		−10.48
Cc04_g12550	RGI3	Probable LRR receptor-like serine/threonine-protein kinase At4g26540	ATP binding; protein kinase activity; peptide receptor activity *; peptide-binding *	−10.56
Cc01_g11300	FLA11	Fasciclin-like arabinogalactan protein 11		−10.58
Cc11_g07530	RLP55	Putative LRR receptor-like serine/threonine-protein kinase GSO1		−10.61
Cc11_g08360		Putative MLP-like protein 28		−10.72
Cc04_g15520		Putative Mitochondrial outer membrane protein porin of 36 kDa	transmembrane transporter activity	−10.93
Cc03_g10850	RIC5	Putative ROP-interactive CRIB motif-containing protein 4		−21.55

Under MWD, several heat shock proteins were among the top 10 up-regulated DEGs in Icatu, namely the glycoside hydrolase family 79 gene that showed the highest regulation (FC = 21.23), while the *TF ORG2* was the most down-regulated DEG (FC = −25.66; Tables 3 and S5).

Under SWD, Icatu top up-regulated DEGs were involved in binding and transporter activity but mainly on transferase activities involving the UDP-glycotransferase *75D1* (FC = 20.01) while down-regulating the *TF ORG2* as reported under MWD (Tables 4 and S6).

Table 3. Top 10 up and down-regulated differentially expressed genes (DEGs) under moderate water deficit (MWD) relative to well-watered (WW) *Coffea arabica* cv. Icatu (Icatu) plants. Molecular functions were retrieved from UniprotKB database (* functions exclusive to *A. thaliana* homologs). Genes are descending Fold Changes (FC).

Gene ID	Homolog	Protein Name	Molecular Function	FC
Up-regulated				
Cc05_g08230	AT5G34940	Putative Glycoside hydrolase family 79, N-terminal		21.23
Cc03_g06560	MYB59	Putative Transcription factor MYB48	transcription regulator activity; DNA binding	8.02
Cc07_g18370		Predicted protein		7.06
Cc11_g04480	HSP18.1	18.5 kDa class I heat shock protein	protein self-association *; unfolded protein binding *	6.68
Cc00_g26020		ribonuclease Ps		6.31
Cc07_g13110	ERF1B	Putative Ethylene-responsive transcription factor 15	transcription regulator activity; DNA binding	6.28
Cc11_g04470	HSP18.1	18.5 kDa class I heat shock protein	protein self-association *; unfolded protein binding *	6.13

Table 3. *Cont.*

Gene ID	Homolog	Protein Name	Molecular Function	FC
Up-regulated				
Cc02_g23670	HSP22.0	22.7 kDa class IV heat shock protein	protein self-association *; unfolded protein binding *	6.11
Cc04_g05250	HSP70-4	Heat shock 70 kDa protein	ATP binding; ATPase activity; protein binding *; protein folding chaperone *	6.05
Cc00_g04790		Hypothetical protein		5.99
Down-regulated				
Cc01_g10980	UXT1	Uncharacterized membrane protein At1g06890	transmembrane transporter activity	−9.06
Cc06_g02440		Laccase-4	copper ion binding; hydroquinone:oxygen oxidoreductase activity	−9.16
Cc06_g02050		RHO guanyl-nucleotide exchange factor 7	guanyl-nucleotide exchange factor activity	−9.17
Cc09_g03130	AT5G33370	GDSL esterase/lipase At5g33370	hydrolase activity, acting on ester bonds	−9.33
Cc07_g11210	PER64	Peroxidase 64	heme binding; metal ion binding; peroxidase activity	−9.85
Cc06_g01530	GPAT6	Glycerol-3-phosphate acyltransferase 6	acyltransferase activity; phosphatase activity *	−10.32
Cc04_g07330	ASPG1	Putative Protein Aspartic Protease in Guard Cell 1	aspartic-type peptidase activity; DNA binding	−10.55
Cc00_g31960	COBL4	Cobra-like protein 4		−10.96
Cc02_g05960	LAC5	Laccase-5	copper ion binding; hydroquinone:oxygen oxidoreductase activity	−11.3
Cc06_g19110	ORG2	Putative Transcription factor ORG2	transcription regulator activity; protein dimerization activity	−25.66

Table 4. Top 10 up and down-regulated differentially expressed genes (DEGs) under severe water deficit (SWD) relative to well-watered (WW) *Coffea arabica* cv. Icatu (Icatu) plants. Molecular functions were retrieved from UniprotKB database (* functions exclusive to *A. thaliana* homologs). Genes are sorted by FC in descending order.

Gene ID	Homolog	Protein Name	Molecular Function	FC
Up-regulated				
Cc09_g07390	AT4G20820	Putative Reticuline oxidase-like protein	oxidoreductase activity; FAD binding	20.55
Cc01_g08410		Putative Lysosomal beta glucosidase	hydrolase activity, hydrolyzing O-glycosyl compounds	20.47
Cc10_g04010		Hypothetical protein		20.47
Cc03_g09220	UGT75D1	Putative UDP-glycosyltransferase 75D1	transferase activity	20.01
Cc08_g11420	EXLB1	Expansin-like B1		10.97
Cc02_g27970		Uncharacterized protein		9.08
Cc03_g06560	MYB59	Putative Transcription factor MYB48	transcription regulator activity; DNA binding	9.04
Cc07_g18370		Predicted protein		8.24
Cc01_g04050	AT2G23950	Putative G-type lectin S-receptor-like serine/threonine-protein kinase RLK1	ATP binding; protein serine/threonine kinase activity; coreceptor activity *	8.05
Cc07_g09470		Hypothetical protein		8.04
Down-regulated				
Cc00_g31960	COBL4	Cobra-like protein 4		−11.58
Cc04_g02380	AT3G16370	GDSL esterase/lipase APG	hydrolase activity, acting on ester bonds	−11.64
Cc03_g07230	AT4G13710	Probable pectate lyase 15	metal ion binding; pectate lyase activity	−12.10
Cc02_g16500	PRP4	Hypothetical protein		−12.45

Table 4. *Cont.*

Gene ID	Homolog	Protein Name	Molecular Function	FC
Down-regulated				
Cc07_g07560	XTH6	Probable xyloglucan endotransglucosylase/hydrolase protein 6	hydrolase activity, hydrolyzing O-glycosyl compounds; xyloglucan:xyloglucosyl transferase activity	−12.86
Cc03_g09190	XTH6	Probable xyloglucan endotransglucosylase/hydrolase protein 16	hydrolase activity, hydrolyzing O-glycosyl compounds; xyloglucan:xyloglucosyl transferase activity	−12.87
Cc01_g11300	FLA11	Fasciclin-like arabinogalactan protein 11		−13.20
Cc10_g04590	SBT1.8	Putative Subtilisin-like protease	serine-type endopeptidase activity	−13.34
Cc08_g04660	SOT15	Putative Cytosolic sulfotransferase 15	sulfotransferase activity	−13.39
Cc02_g17500	RL1	Hypothetical protein	transcription regulator activity	−13.91

3.4. Regulation Patterns of DEGs Directly Linked to Water Deprivation and Desiccation

To better understand the impacts of water deficit, a specific search performed among DEGs annotated with "response to water deprivation" (GO:0009414) or "response to desiccation" (GO:0009269) found 22 additional DEGs, mostly expressed under SWD in the two genotypes (Table 5). In CL153, these drought-responsive DEGs were slightly up-regulated in MWD (5 out of 8) and more down-regulated under SWD (8 out of 14). In Icatu, these DEGs were all down-regulated under MWD (0 up and 7 down) and mostly under SWD (3 up and 9 down). Notably, the Desiccation protectant protein *Lea14* was found to be commonly up-regulated by CL153 (MWD and SWD) and Icatu (only under SWD). Additionally, a large majority of DEGs (10 out of 22) were linked to the Aspartic Protease in Guard Cell 1 gene (*ASPG1*), being all down-regulated in Icatu under the two water deficits, while some were up-regulated in CL153. Many of these drought-responsiveness DEGs were linked with the 4th chromosome of *C. canephora* (Figure S3).

Table 5. Differentially expressed genes (DEGs) under moderate water deficit (MWD) or severe water deficit (SWD) relative to well-watered (WW) *C. canephora* cv. Conilon Clone CL153 (CL153) and *Coffea arabica* cv. Icatu (Icatu) plants. Selected DEGs were annotated with the Gene Ontology (GO) terms: "response to water deprivation" (GO:0009414), "response to desiccation" (GO:0009269). Red: up-regulated DEGs; blue: down-regulated DEGs.

Gene ID	Homolog	Protein Name	CL153		Icatu	
			MWD	SWD	MWD	SWD
Response to Water Deprivation						
Cc06_g15980	AT5G06760	18 kDa seed maturation protein	9.67	9.19		
Cc04_g07360	AT3G18490	Putative Protein Aspartic Protease in Guard Cell 1	5.24			−4.43
Cc04_g07380	AT3G18490	Putative Protein Aspartic Protease in Guard Cell 1	4.35			
Cc11_g05800		Annexin D5	1.48	1.49		
Cc04_g09640	AT3G18490	Protein Aspartic Protease in Guard Cell 1	−5.00			−6.97
Cc07_g07560	AT5G65730	Probable xyloglucan endotransglucosylase/hydrolase protein 6	−6.94			−12.86
Cc04_g07330	AT3G18490	Putative Protein Aspartic Protease in Guard Cell 1	−9.13			−10.19
Cc04_g07360	AT3G18490	Putative Protein Aspartic Protease in Guard Cell 1		4.42	−3.48	
Cc04_g07380	AT3G18490	Putative Protein Aspartic Protease in Guard Cell 1		3.30		−3.23
Cc11_g05800		Annexin D5		1.49		

Table 5. *Cont.*

Gene ID	Homolog	Protein Name	CL153		Icatu	
			MWD	SWD	MWD	SWD
		Response to Water Deprivation				
Cc07_g15660	AT2G22125	C2 domain-containing protein		−1.71		−2.17
Cc04_g08280	AT5G08120	Putative movement protein-binding protein 2C		−2.83		
Cc04_g09640	AT3G18490	Protein Aspartic Protease in Guard Cell 1		−4.84	−4.24	
Cc04_g07350	AT3G18490	Putative Protein Aspartic Protease in Guard Cell 1		−5.66	−3.86	−6.28
Cc07_g07560	AT5G65730	Probable xyloglucan endotransglucosylase/hydrolase protein 6		−5.71	−4.75	
Cc02_g15480	AT4G18780	Cellulose synthase A catalytic subunit 8 (UDP-forming)		−6.4	−7.5	−8.73
Cc04_g07330	AT3G18490	Putative Protein Aspartic Protease in Guard Cell 1		−9.27	−10.55	
Cc04_g07370	AT3G18490	Putative Protein Aspartic Protease in Guard Cell 1			−1.93	−3.31
Cc01_g21050		Sucrose synthase 2				2.64
		Response to desiccation				
Cc02_g38620		Desiccation protectant protein Lea14 homolog	1.76	1.20		1.50
Cc01_g08980		Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family		−3.16		
Cc04_g03400		Putative Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family				1.60

3.5. Regulation Patterns of Photosynthetic and Other Biochemical Coffee Related DEGs

Due to the fundamental role of energy pathways (photosynthesis and respiration), antioxidative protection and membrane lipid dynamics involved in coffee acclimation to environmental stresses, a specific search was also performed among the DEGs associated with these processes. Results showed that an important set of responsive DEGs (184) associated with such crucial biochemical coffee processes were affected by drought, mostly when considering SWD, where the majority of these DEGs were detected (Table S7; Figure 4).

Drought affected a similar number of DEGs related to photosynthesis in the two genotypes (50 in CL153 and 48 in Icatu), all down-regulated under both water deficits (Figure 4). While MWD barely affected this category of DEGs, they were down-regulated under SWD (from 7 to 43 in CL153 and 0 to 48 in Icatu). DEGs linked to the lipid metabolism were also mostly down-regulated in CL153 (11 out of 15 in MWD; 14 out of 21 in SWD), whereas in Icatu, they were all down-regulated (5 under MWD; 16 under SWD). Antioxidant activity related DEGs also followed this general pattern, being mostly down-regulated in both genotypes, especially under SWD (CL153: 16, Icatu: 13). The number of these DEGs significantly increased from MWD to SWD, especially in CL153 that also increased the level of up-regulated DEGs under the harshest drought condition. By contrast, DEGs associated with cellular respiration were mostly up-regulated in CL153 under SWD (20 out of 34), while in Icatu, they were all up-regulated under MWD (all 4).
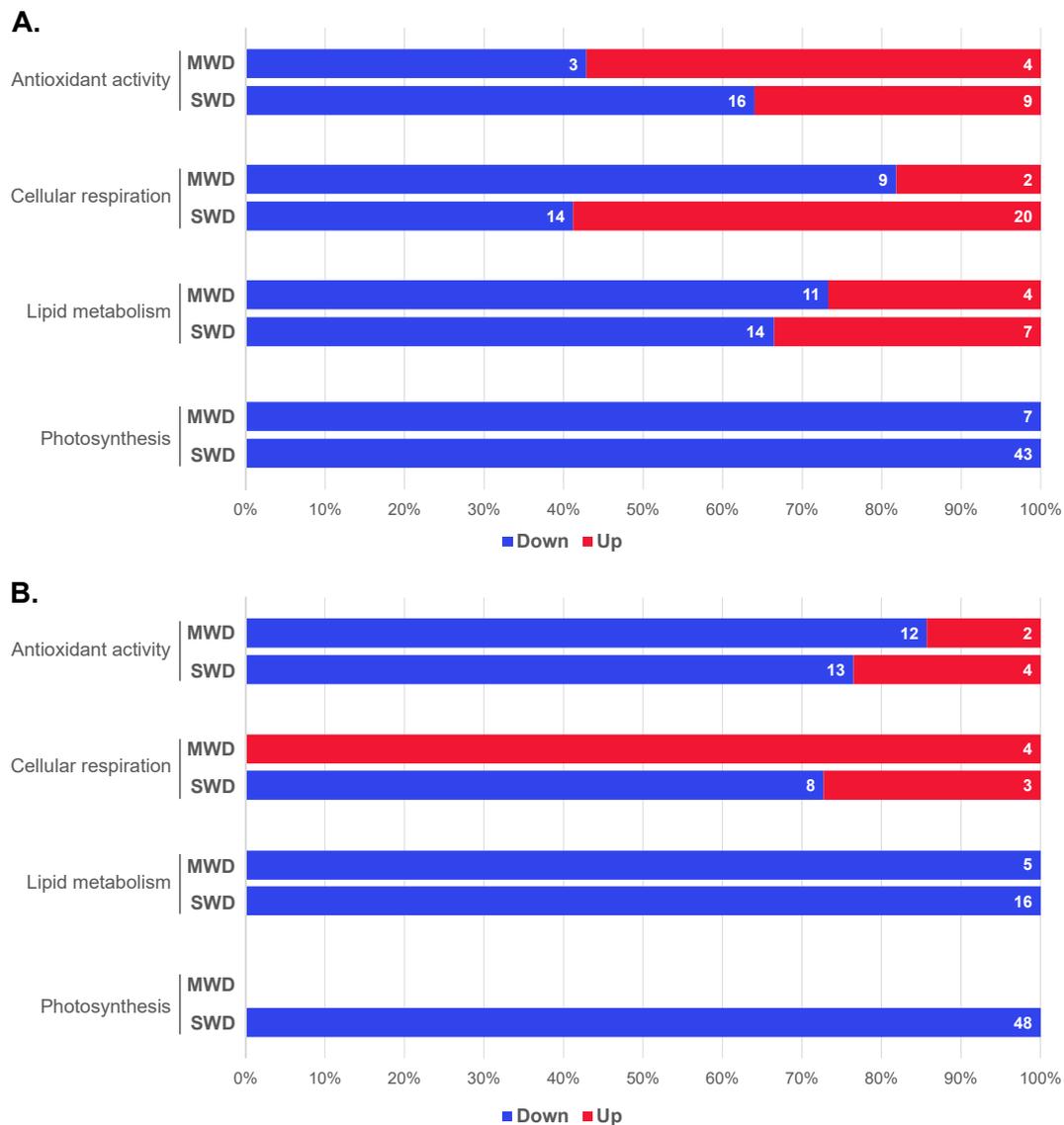
A.

B.

Figure 4. The proportion of significantly up-regulated (red) and down-regulated (blue) DEGs associated to physiological and biochemical responses in coffee that were found under moderate water deficit (MWD) and severe water deficit (SWD) relative to the well-watered (WW) control plants in (**A**) CL153 and (**B**) Icatu. DEGs represented in this plot were annotated with at least one of the following GO terms: "antioxidant activity" (GO:0016209), "response to oxidative stress" (GO:0006979) under Antioxidant activity; "cellular respiration" (GO:0045333), "mitochondrion" (GO:0005739), "malate dehydrogenase activity" (GO:0016615), "pyruvate kinase activity" (GO:0004743) under Cellular respiration; "fatty acid metabolic process" (GO:0006631), LOX (GO:0004051, GO:0016702) under Lipid metabolism; and "photosynthesis" (GO:0015979), "photosystem" (GO:0009521), "photosynthetic electron transport chain" (GO:0009767), "photorespiration" (GO:0009853), "chlorophyll biosynthetic process" (GO:0015995) under Photosynthesis.

3.6. Regulation Patterns of Transcription Factors among Responsiveness DEGs

The search of *Arabidopsis thaliana* homologs' Transcription Factors (TFs) among the DEGs found only five TFs (Table S8). The Ethylene-responsive TF (*WIN1*), the TF *MYB60*, and the ABC transporter G family member 22 (*ABCG22*) were down-regulated under SWD in the two genotypes. Among the remaining TFs (all found only in CL153), the Dehydration-responsive element-binding protein 1A (*DREB1A*) and the NAC domain-containing protein 55 (*NAC055*) were up-regulated regardless of the drought severity. In contrast, the ABC transporter G family member 22 (*ABCG22*) was down-regulated only under SWD.

Given that this analysis revealed a very low number of the TFs, we also searched DEGs annotated in the reference genome as TFs. With this search, a total of 83 TFs were found among DEGs, mostly in CL153 and predominantly under SWD: 26 and 62 TFs in CL153 plants, and 17 and 32 TFs in Icatu plants under MWD and SWD, respectively (Table S8). The majority of TFs were up-regulated in CL153, especially under SWD (34 up and 28 down) than under MWD (21 up and 5 down). The same pattern was reported for Icatu, with a high number of TFs being found under SWD (17 up and 15 down) and under MWD (6 up and 11 down). In both drought treatments, the Ethylene-responsive *TF ERF027* followed by the Dehydration-responsive element-binding protein 1D (*DREB1D*) were the most up-regulated TFs in CL153 (Table S8). In Icatu, the ethylene-responsive transcription factor 15 was up-regulated under the two water deficits levels, together with the basic leucine zipper 6 under MWD and the basic leucine zipper 5 under SWD (Table S8).

3.7. Regulation Patterns of Phosphatases and Protein Kinases DEGs

Due to its importance in stress acclimation, a specific search was performed to understand the patterns of phosphatases and protein kinases among DEGs. A total of 49 phosphatase activity-DEGs were detected with a prevalence under SWD and in higher numbers in CL153 (Table S9). Approximately one-third of them were up-regulated in CL153 (13) and one-fifth in Icatu (10) under SWD, with a notable up-regulation of the phosphatase 2C 74 in CL153 and the Major allergen (*Mal D1*) in Icatu (Table S9).

Drought had a significant impact on 272 protein kinases (Table S9). Under MWD, a similar number of protein kinases was detected in the two genotypes (38), while SWD had a higher impact on protein kinases that were mostly down-regulated by this stress (181 in CL153 vs. 96 in Icatu). Besides an uncharacterized kinase protein, SWD triggered the most up-regulation of the G-type lectin S-receptor-like serine/threonine-protein kinase (*RLK1*) in CL153, which was also the most up-regulated kinase in Icatu (Table S9).

3.8. Enriched GO Terms of Drought-Related DEGs

Overall, in both genotypes, there was an increase in enriched GO terms as drought severity increased, being predominant in down-regulated DEGs (Figure 5). Results revealed a very specific response to drought, with the catalytic activity being the only category commonly enriched in both drought treatments and both genotypes. Under MWD, GO terms categories were predominantly enriched in Icatu (16 in Icatu vs. 5 in CL153). Even so, only CL153 showed an enrichment in up-regulated DEGs linked to catalytic and DNA-binding TF activities (Figure 5). SWD had a stronger impact on a high number of categories (30 in CL153 and 31 in Icatu), with 20 of them being commonly altered in both genotypes. Under this stress, only CL153 showed enriched categories in up-regulated DEGs that were linked to the nucleus, DNA-binding TFs, and oxidoreductase activities.
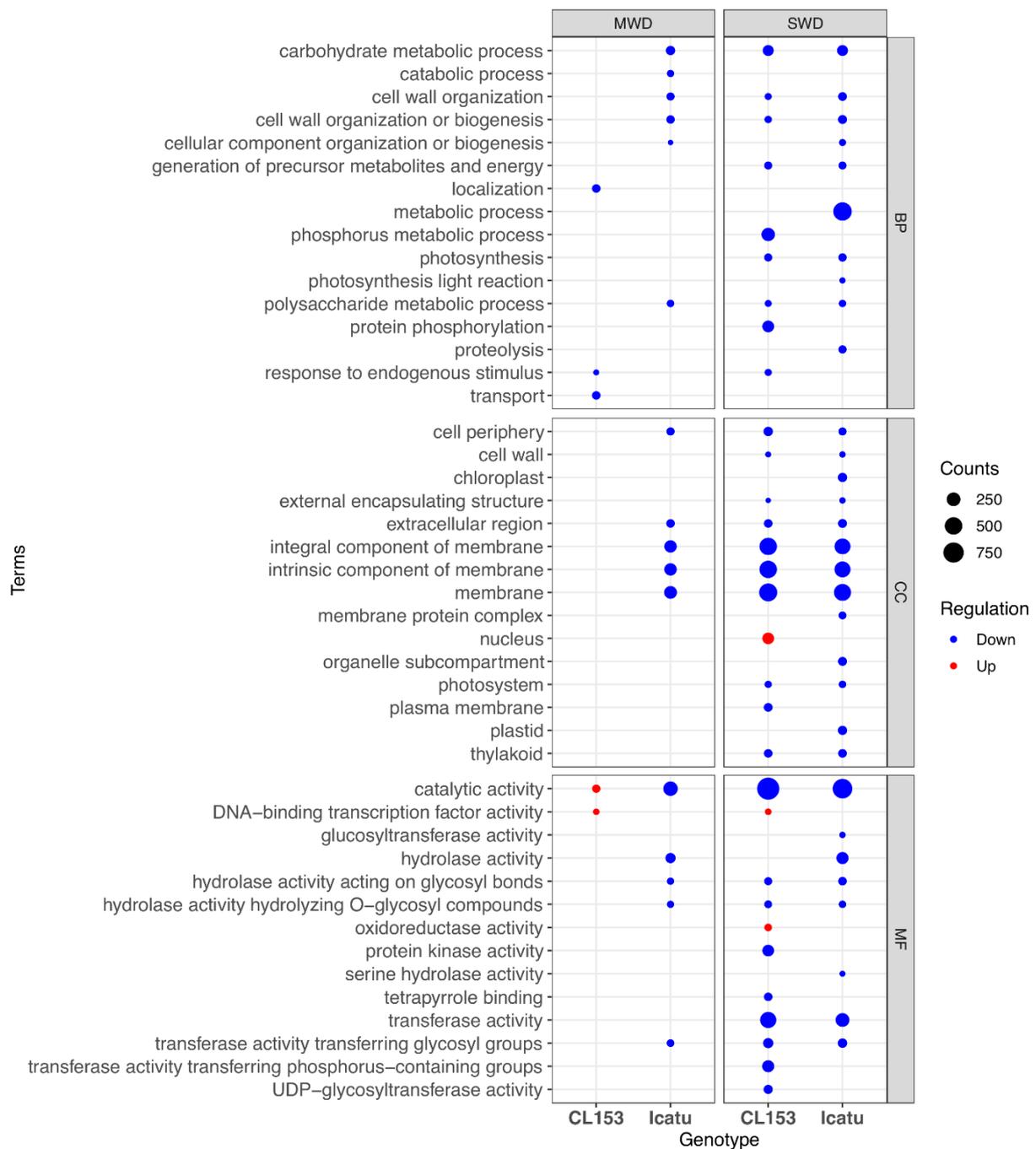
Figure 5. Over-representation analysis of Gene Ontology (GO) terms performed with gProfiler against the functional annotation of *Coffea canephora* genome. Top significantly enriched GO terms among up- and down-regulated differentially expressed genes (DEGs) were ranked by increasing log2 fold change (FC), considering the effect of moderate water deficit (MWD) and severe water deficit (SWD) conditions, compared to well-watered plants (WW) condition in *Coffea arabica* cv. Icatu (Icatu) and *C. canephora* cv. Conilon Clone CL153 (CL153) plants. Terms were filtered by REVIGO with similarity = 0.5, FDR < 0.01 and Counts > 20. GO terms are grouped by the main categories: Biological Process (BP), Molecular Function (MF), and Cellular Component (CC). Counts (size) indicate the number of DEGs annotated with each GO term, and dots are colored by type of regulation (red: up-DEGs; blue: down-DEGs).

4. Discussion

4.1. Impacts of Water Deficit on the Transcriptomic Profile of Coffea canephora (CL153) and C. arabica (Icatu)

The present study demonstrated that water deficit alters the transcriptome profile in the two coffee genotypes. Thousands of expressed genes were identified and annotated (Figure 1; Table S1) in agreement with previous drought studies in coffee [29,52,53]. However, contrary to those studies, we examined the effects of a long-term drought experiment in which stress has been imposed gradually. In addition, this study had the advantage of comparing two genotypes from different coffee species that have been found to display distinct physiological resilience to SWD conditions, particularly at the photosynthetic functioning and apparatus components [24,25]. Our findings showed that only a small number of genes was affected by MWD in the two genotypes (CL153: 5%; Icatu: 4% DEGs), whereas a substantial impact was observed under SWD (CL153: 17%; Icatu: 10% DEGs; Table S2; Figures 2 and 3). In comparison, in the drought-sensitive *C. canephora* cv. Conilon 109 only 0.59% of genes responded to drought, while 3.63% were found in the drought-tolerant *C. canephora* cv. Conilon 120, in a 14-day drought experiment [29]. Therefore, the high responsiveness values found here under MWD (in a drought level similar to [29]) suggests a high drought tolerance in the coffee cultivars of this study (CL153 and Icatu). In fact, under MWD, the potential functioning of the photosynthetic apparatus of these plants was not significantly impaired [19], although some protective mechanisms (e.g., zeaxanthin and HSP70 content, antioxidative activity) already begun to be reinforced (Semedo et al., unpublished data).

The genes responsive to drought also differed significantly between the two coffee genotypes (Tables 1–4). In CL153, reticuline oxidase genes were predominant in the two water deficit levels, including the most up-regulation of *PAP20*. Several antioxidant genes such as peroxidase 4, thioredoxin, and FAD-related genes as reticuline oxidases have been previously identified in this genotype as being involved in stress acclimation, including high temperatures [34]. Thus, the up-regulation of *PAP20*, an acid phosphatase involved in hydrolase activity and metal ion binding, helps to alleviate the *PAP* stress signal that usually accumulates during drought and light stress, inducing the expression of stress-responsive genes [54], which could regulate the impact of water deficit in CL153. Reticuline oxidase genes were also up-regulated in CL153 even under MWD, together with cytochrome *P450*, a carotenoid cleavage dioxygenase, and the *TF ERF027* (Table 1). The up-regulation of these genes, together with the *TF ERF027*, is probably linked to a protective mechanism of CL153 to avoid oxidative damage, as previously documented in this genotype [55]. Carotenoids are essential components of the photosynthetic apparatus, being susceptible to oxidation processes that break the carotenoid backbone [56]. This cleavage reaction is catalyzed by carotenoid cleavage dioxygenases leading to apocarotenoids that usually arise through the attack of ROS [57]. Apocarotenoids have an essential role in abiotic stress response, acting as precursors of ABA synthesis that coordinates plant responses to stress, namely stomata closure to minimize water loss during drought and therefore suggests the activation of ABA-related mechanisms to minimize drought in CL153, as shown in other drought-tolerant coffee genotypes [29]. This is in line with the increase of ABA synthesis found under MWD and SWD in CL153 (although also in Icatu) plants [19]. Specifically, the strong down-regulation of the ROP-interactive CRIB motif-containing protein 4 under SWD also suggests the involvement of ABA in the response of drought in this genotype. These types of genes are usually involved in the interaction between auxin- and ABA-regulated processes, which often show an antagonistic effect, that is, positively regulating auxin signaling while negatively regulating ABA signaling [58].

In contrast, several heat shock genes were up-regulated in Icatu even under MWD, as the glycoside hydrolase family 79, in line with the higher abundance of the heat shock protein 70 kDa (HSP70) (unpublished data). Under SWD, genes involved in binding, transporter, and transferase activities were up-regulated, namely the UDP-glycotransferase 75D1. These genes are involved in a high number of developmental processes and stress

responses, including cell wall modification, plant hormone activation, and the production of antioxidants in response to stresses, including drought [59,60], which would counterbalance the down-regulation of TFs (*ORG2*) under the two water deficits (see also below). In coffee, as in other plants, ROS can be scavenged by several enzymes and non-enzyme antioxidants, such as ascorbic acid, glutathione, carotenoids, phenolic compounds, ascorbate peroxidase, or catalase [61], which are usually strongly reinforced in Icatu under drought [62] and would explain why this genotype can endure even the effects of extreme water deficit [25], beyond the transcriptomic results found here. Under drought, particularly under SWD, we found a down-regulation of DEGs associated with important physiologically and biochemically related processes, such as photosynthesis, lipid metabolism, cellular respiration, and antioxidant activity (except the cellular respiration in Icatu under MWD; Figure 4). The impact of the SWD level on the transcripts of the two genotypes was also revealed by the enrichment analysis that showed a prominent down-regulation of GO terms involved in general metabolic processes, integral components of the membrane, and especially in catalytic activities (Figure 5). In fact, up-regulated enriched GO terms were only found in CL153 and involved catalytic and DNA-binding TF activities under MWD, while under SWD, the nucleus, oxidoreductase, and DNA-binding TF activities were enriched (Figure 5). However, previous physiological, biochemical, and proteomic studies revealed an almost insensitivity of Icatu to the severe drought impact, at least in the C-assimilatory pathway functioning, as well as a stronger triggering of protective molecules [24,25,62]. This suggests the involvement of post-transcriptional regulations and indicates the need for complementary integrated studies considering several layers of plant response, from physiology and biochemistry to molecular levels. Such studies would be crucial to understand transcriptomic findings in the context of plant acclimation to environmental constraints [34].

4.2. Role of Aspartic Proteases and Protectant Proteins in Water Deprivation and Desiccation in Coffee

Among the 22 DEGs involved in response to drought, ten were related to the Aspartic Protease in Guard Cell 1 (*ASPG1*; Table 5). These genes have been identified in different plant species being the *ASPG1* usually involved in plant acclimation to drought stress [63,64]. Over the last decade, an increasing number of publications have highlighted the involvement of aspartic proteases in plant defense responses against a diversity of abiotic and biotic stresses [65,66]. For instance, plants overexpressing aspartic proteases as *APA1* have been shown to be more tolerant to water deficit due to changes in stomatal behavior induced by the regulation of the ABA signaling pathway [67]. In *Arabidopsis*, the *ASPG1* has been shown to be involved in drought stress resistance, in addition to its role in the degradation of seed storage proteins [68,69]. *Arabidopsis* mutants overexpressing *ASPG1* were shown to recover more efficiently from drought since *ASPG1* led to a significant increase in ABA sensitivity by guard cells and activation of antioxidant enzymes that prevent plants from oxidative damage [68]. A gene homologous to *ASPG1* from potato has also been shown to be down-regulated under drought and up-regulated upon re-watering, suggesting a role under drought stress [65]. Thus, the up-regulation of *ASPG1* in CL153 would help this genotype to mitigate the effects of drought. By contrast, a down-regulation of this gene was observed in Icatu, even though a previous study found an increase in ABA, and a strong stomatal conductance reduction in this genotype [25], which are related to the gene expression found here.

Drought tolerance in the coffee genotypes also involved the up-regulation of *Lea14* in CL153 (under the two water deficits) and in Icatu (under SWD). Late embryogenesis abundant proteins (*Lea*) have been found in a large number of plants being up-regulated during osmotic stress, where they bind to enzymes to prevent loss of activity functioning as cellular stabilizers during stress conditions [70]. *Lea* are also expressed under water deprivation conditions and associated with improved drought tolerance by inducing rapid stomatal closure [71,72]. Thus, the overexpression of *Lea14* could also be involved in the water scarcity response of these two coffee genotypes.

4.3. Drought-Responsiveness Transcription Factors

In addition to protective proteins, drought also triggered a high number of TFs in the two coffee genotypes (e.g., 88 TFs,) that were mainly up-regulated (Table S8). This up-regulation of TFs in response to drought was more frequent under SWD than under MWD and higher in CL153 than in Icatu, suggesting an important role in regulating drought stress tolerance, potentially through improved cellular protection, and particularly in the first genotype. TFs usually regulate genes involved in cellular protection from stress damage (e.g., osmoprotectants, antioxidants), as well as signal transduction and transcriptional regulation [73]. In coffee, several TFs have been previously observed to respond to drought exposure. For instance, a wide-throughout transcriptomic study found only 22 probable responsive TFs, namely from the *Myb* superfamily [29], being the overexpression of TFs, including the *DREB* gene family also reported [74–76]. Likewise, in our study, four TFs were found to be significantly up-regulated in CL153 under drought (*ERF027*, *DREB1D*, *DREB1A,* and *NAC055* in both water deficits) and three others in Icatu (*TF15* under the two water deficits, plus basic leucine zipper 6 under MWD, and zipper 5 under SWD) suggesting their involvement in the drought-response tolerance of these genotypes. In fact, ERF TFs usually regulate responses to abiotic stresses, including cold, drought, heat, and salt, being also involved in hormone signaling and hormone-mediated stress-responses through stress phytohormones as ABA [77,78] and ethylene [79]. TFs as *DREBs* are induced upon drought imposition, positively regulating drought-responsive genes [63,80] such as the *Lea* previously reported in this study. DREB/CBF belongs to the ERF (ethylene-responsive element binding factors) superfamily of TFs that play a pivotal role in adaptation to biotic and abiotic stresses [81]. *NAC* TFs are also relevant in ABA and ethylene pathways responding to drought stress [82]. When cells are under water deficit, ABA accumulates and binds to soluble receptors. These results in the release and activation of Open Stomata genes (members of protein kinases) that phosphorylate basic leucine zipper TFs to control gene expression in the nucleus [83]. Therefore, the overexpression of these genes could sustain an enhanced drought tolerance [84], as observed in these two coffee genotypes [25]. Expression of ERFs can be induced by ethylene and ABA under biotic and abiotic stresses that also interact with other plant hormone pathways, such as those regulated by salicylic acid or gibberellins, and suggesting coordinated interaction of hormone signaling pathways to regulate the expression of TF genes during stress responses [81]. ERFs also seem to regulate ROS-responsive genes, resulting in decreased accumulation of ROS and enhancing tolerance to multiple abiotic stresses such as drought, salt, and freezing [85].

4.4. DEGs Involved in Phosphatases and Protein Kinases Affected by Drought

Protein phosphatases and kinases are major post-translational regulators of numerous cellular processes and signaling networks [86]. Protein kinases pathways are activated by sequential phosphorylation leading to the regulation of TFs and protective enzymes in response to several stresses, including drought [87]. A high up-regulation of phosphatase genes was reported under SWD (Table S9), particularly in CL153, suggesting the involvement of these genes in the drought response. The phosphatase 2C 74 that was overexpressed in CL153 under SWD is part of a major group of protein phosphatases in plants, having important roles in various biological processes [88]. Several studies have shown that *PP2* genes are involved in the regulation of the ABA signaling pathway by modulating kinase activities in response to abiotic stresses [89]. Notably, the up-regulation of the major allergen Mal D1 phosphatase in Icatu under SWD (Table S8) could raise a significant concern since this gene was initially thought to be a major allergen in several fruits [90]. However, other studies showed that these proteins are also synthesized in response to biotic and abiotic stresses [91,92] and, thus, it would be interesting to determine if resistance to stresses could have consequences in terms of the allergenicity of the agronomic product.

G-type lectin S-receptor-like serine/threonine protein kinases are positive regulators of plant tolerance to several stresses [93,94]. For instance, transcriptomic analyses in foxtail

millet have shown their participation in the drought tolerance response [95]. Thus, the highest up-regulation of *RLK1* in both CL153 and Icatu under SWD agrees with its role in drought regulation in coffee, most likely also triggered by ABA signaling pathways induced by protein phosphatases as referred above. Ultimately, the primary response beyond ABA levels will be the stomatal closure in coffee leaves, regulating ion transport in guard cells and decreasing drought severity in coffee genotypes as reported in other species [96–98].

4.5. Coping with Drought: Lessons from Crossed Transcriptomic, Physiological, and Biochemical Studies

Climate changes are expected to include an increased frequency of water scarcity events, including the intensity of severity and duration, posing a growing threat to coffee's global supply chain. Agroforestry systems can be useful to mitigate some of these harsh effects, reducing the risk of coffee production losses and contributing to the sustainability of crops [18,31]. However, to maintain the global supply of coffee, it is also important to promote the screening and development of tolerant varieties to face the increasingly expected impacts of droughts.

At the molecular level, several studies have identified candidate genes for drought tolerance in *C. canephora* (e.g., [29,76,99–101]) and *C. arabica* (e.g., [52,76,102]). These candidate genes mainly encompass TFs as the *DREB*-like genes *DREB1D*, *ERF017*, *EDR2* [29,74,76,102], genes related to ABA synthase, ABA receptors, and protein phosphatases such as *PYL8a*, *PYR1*, *SNRK2.8* [29,103], and mainly protective genes, including those associated with ROS control and removal, such as *CuSOD1*, *CuSOD2*, *APX1*, *APX5*, *APX6*, *HSP70*, *ELIP*, *Chape20*, and *Chape60* [29,48,100,104–106]. Additionally, drought studies also found increases in protective proteins that could improve thermal dissipation processes (*PsbS*) and promote the protective cyclic electron transport around photosystems I and II when CO_2 supply to the carboxylation sites in the chloroplast is greatly diminished due to stomata closure [24,25]. These genes (and proteins) are usually overexpressed in coffee genotypes and are assumed to contribute to maintaining the photochemical efficiency under stress, at least in some genotypes [34,48,102].

The novel mRNA-Seq study reported here provided information on qualitative and quantitative differences between the two cultivars, CL153 and Icatu. Overall, comparative transcriptome analysis led to the identification of drought-responsive genes and genotype-dependent genes responsible for the different drought tolerance responses, the TFs *ERF027*, *DREB1D*, and the basic leucine zipper genes, as well as genes linked to water deprivation and desiccation, the *ASPG1* and the protectant *Lea14*. These genes will be essential for future crop improvement programs, such as the development of drought-resilient coffee varieties. However, some of the transcriptomic results found here do not fully agree with earlier physiological and biochemical studies showing a greater tolerance of Icatu than CL153 under SWD. Icatu has previously been shown to be barely affected by drought, showing minor impacts on photosynthetic functioning (e.g., Amax, Fv/Fm) and components (e.g., electron carriers, photosystems, and ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO) activity) under SWD, in contrast to CL153 which was clearly affected. This better performance of Icatu under such harsh water shortage conditions was associated with the triggering of mechanisms of (thermal) energy dissipation and ROS control over the photosynthetic machinery [24,25,62]. Such enrichment of detox pathways was also accompanied by metabolic and proteomic changes in Icatu, which included the reinforcement of thylakoid electron transport rates and some electron carriers, and the triggering of protective cyclic electron transport involving both photosystems. These processes would help maintain the photochemical use of energy while controlling the presence of reactive molecules of chlorophyll and oxygen [24,25,107]. In this context, the existence of strong post-transcriptional regulations is very likely, probably involving alternative splicing, noncoding RNAs (including siRNA, miRNA, lncRNA), RNA-binding proteins (RBPs) as well as protein modifications [108]. In the future, combining these technological

methods with bioinformatic tools and physiological experiments will allow a more holistic insight into the regulation and control of biological processes in coffee.

5. Conclusions

- Even though drought had an impact on the leaf transcriptome of both coffee genotypes, our results revealed that both genotypes are more drought-resistant than other coffee cultivars.
- Drought triggered a specific response associated with the magnitude of water deficit, which was also genotype-dependent since few DEGs and pathways were common to treatments and both genotypes. By comparison, MWD only had a minor effect on the transcripts of both genotypes.
- There was a predominance of protective genes (more in CL153) associated with antioxidant activities, including genes involved in water deprivation and desiccation, such as *Lea* and aspartic proteases.
- A significant number of TFs, including *ERF*, *DREB*, and the leucine zipper, were found to be significantly up-regulated under drought. Together with the large number of phosphatases and protein kinases we found, these results suggest the involvement of ABA signaling in the drought tolerance of these genotypes.
- Coupled with the previous physiological and metabolic results, our study provides novel and timely information showing several layers of response and suggesting the existence of post-transcriptional regulations in the two coffee genotypes, which should be further investigated.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11112255/s1, Figure S1: Principal component (PC) analysis of all log transformed gene expression data from *Coffea canephora* cv. Conilon Clone CL153 (CL153) and *C. arabica* cv. Icatu (Icatu) plants submitted to three different water availability conditions: well-watered (WW), moderate water deficit (MWD), and severe water deficit (SWD). A, B, C, D, and E correspond to biological replications. Each treatment is represented by the colors depicted in Figure 1 and contains three biological replicates, depicted in Table S1. The percentage of variance is indicated in each axis, Figure S2: The effect of moderate (MWD) and severe (SWD) water deficit on the number of up- and down-regulated DEGs in plants of *Coffea canephora* cv. Conilon Clone CL153 (CL153) and *C. arabica* cv. Icatu (Icatu), Figure S3: Clustered heat map and dendrogram of the differentially expressed genes (DEGs) associated with drought, in plants of *Coffea arabica* cv. Icatu (Icatu) and *C. canephora* cv. Conilon Clone CL153 (CL153) submitted to either moderate water deficit (MWD) or severe water deficit (SWD) in relation to well-watered (WW) plants. DEGs were selected if annotated with the Gene Ontology (GO) terms "response to water deprivation" (GO:0009414) and "response to desiccation" (GO:0009269). Hot colors represent up-regulated DEGs, and cold colors represent down-regulated DEGs. Numbers in the right column indicate the chromosome of the retrieved DEGs, Table S1: Summary of sequencing and mapping of reads from *Coffea canephora* cv. CL153 (CL153) and *C. arabica* cv. Icatu (Icatu) samples. A, B, C, D, and E correspond to biological replications. Plants were grown in two different stress water conditions, mild water deficit (MWD) and severe water deficit (SWD) and the control well-watered plants (WW). RAW READS: number of reads obtained after sequencing. CLEAN READS: number of reads passing the Illumina quality filters and downstream filters. CLEAN %: percentage of reads passing filters compared to the number of raw reads. UNIQUE: number of reads aligned to a unique position. UNIQUE %: proportion of reads aligned to a unique position compared to the number of clean reads. MULTIPLE MAP: number of reads aligned to exons of several overlapping genes. MULTIPLE MAP %: proportion of reads aligned to exons of several overlapping genes compared to the number of clean reads. UNMAPPED %: proportion of non-aligning reads compared to the number of clean reads, Table S2: Number of total expressed genes, total differentially expressed genes (DEGs), and GO annotated DEGs in *Coffea canephora* cv. CL153 (CL153) and *C. arabica* cv. Icatu (Icatu) plants submitted to three different water availability conditions: well-watered (WW), moderate water deficit (MWD), and severe water deficit (SWD). DEGs represent the number of significant genes found to be differently expressed between each water treatment and the control (MWD vs. WW and SWD vs. WW, respectively), selected by DESeq2 and filtered by log2 fold change (FC) $\neq 0$ and a false discovery rate (FDR) < 0.01,

Table S3: Full list of differentially expressed genes (DEGs) in *C. canephora* cv. CL153 (CL153) found between moderate water deficit (MWD) vs. well-watered (WW) plants. Gene identification and protein name according to *Coffea canephora* functional annotation retrieved from the Coffee Genome Hub (http://coffee-genome.org: 4 January 2020). DEGs were selected with DESeq2 and filtered by log2 fold change (FC) $\neq$ 0 and false discovery rate (FDR) < 0.01, Table S4: Full list of differentially expressed genes (DEGs) in C. canephora cv. CL153 (CL153) found between severe water deficit (SWD) vs. well-watered (WW) plants. Gene identification and protein name according to *Coffea canephora* functional annotation retrieved from the Coffee Genome Hub (http://coffee-genome.org: 4 January 2020). DEGs were selected with DESeq2 and filtered by log2 fold change (FC) $\neq$ 0 and false discovery rate (FDR) < 0.01, Table S5: Full list of differentially expressed genes (DEGs) Coffea arabica cv. Icatu (Icatu) found between moderate water deficit (MWD) vs. well-watered (WW) plants. Gene identification and protein name according to *Coffea canephora* functional annotation retrieved from the Coffee Genome Hub (http://coffee-genome.org: 4 January 2020). DEGs were selected with DESeq2 and filtered by log2 fold change (FC) $\neq$ 0 and false discovery rate (FDR) < 0.01, Table S6: Full list of differentially expressed genes (DEGs) in Coffea arabica cv. Icatu (Icatu) found between severe water deficit (SWD) vs. well-watered (WW) plants. Gene identification and protein name according to *Coffea canephora* functional annotation retrieved from the Coffee Genome Hub (http://coffee-genome.org: 4 January 2020). DEGs were selected with DESeq2 and filtered by log2 fold change (FC) $\neq$ 0 and false discovery rate (FDR) < 0.01, Table S7: Regulation pattern of among differentially expressed genes (DEGs) related to photosynthesis and biochemical processes in plants of *Coffea canephora* cv. Conilon Clone CL153 (CL153) and *C. arabica* cv. Icatu (Icatu) submitted to moderate water deficit (MWD) and severe water deficit (SWD) relative to the control, well-watered (WW) plants. DEGs were selected if annotated with the direct or respective child Gene Ontology (GO) terms: "antioxidant activity" (GO:0016209) and "response to oxidative stress" (GO:0006979) under Antioxidant activity; "cellular respiration" (GO:0045333), "mitochondrion" (GO:0005739), "malate dehydrogenase activity" (GO:0016615), "pyruvate kinase activity" (GO:0004743) under Cellular respiration; "fatty acid metabolic process" (GO:0006631), LOX (GO:0004051, GO:0016702) under Lipid metabolism; and "photosynthesis" (GO:0015979), "photosystem" (GO:0009521), "photosynthetic electron transport chain" (GO:0009767), "photorespiration" (GO:0009853) and "chlorophyll biosynthetic process" (GO:0015995) under Photosynthesis. Red represents up-regulated DEGs, and blue represent down-regulated DEGs, Table S8: Regulation pattern of transcription factors (TFs) found among differentially expressed genes (DEGs) in plants of *Coffea canephora* cv. Conilon Clone CL153 (CL153) and *C. arabica* cv. Icatu (Icatu) submitted to either moderate water deficit (MWD) or severe water deficit (SWD) in relation to well-watered (WW) plants. DEGs were selected if annotated with the Gene Ontology (GO) term "DNA-binding transcription factor" (GO:0003700). Red represents up-regulated DEGs, and blue represents down-regulated DEGs, Table S9: Regulation pattern among differentially expressed genes (DEGs) related to catalytic activities, between plants of *Coffea canephora* cv. Conilon Clone CL153 (CL153) and *C. arabica* cv. Icatu (Icatu) submitted to moderate water deficit (MWD) and severe water deficit (SWD), relative to the control of well-watered (WW) plants. DEGs were selected if annotated with the Gene Ontology (GO) terms "phosphatase activity" (GO:0016791) and "protein kinase activity" (GO:0004672), or any of its respective child terms. Red rrepresents up-regulated DEGs and, blue represents down-regulated DEGs.

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References

1. Fahad, S.; Bajwa, A.A.; Nazir, U.; Anjum, S.A.; Farooq, A.; Zohaib, A.; Sadia, S.; Nasim, W.; Adkins, S.; Saud, S.; et al. Crop production under drought and heat stress: Plant responses and management options. *Front. Plant Sci.* **2017**, *8*, 1147. [CrossRef]

2. Lamaoui, M.; Jemo, M.; Datla, R.; Bekkaoui, F. Heat and drought stresses in crops and approaches for their mitigation. *Front. Chem.* **2018**, *6*, 26. [CrossRef]

3. Lang, Y.; Wang, M.; Xia, J.; Zhao, Q. Effects of soil drought stress on photosynthetic gas exchange traits and chlorophyll fluorescence in *Forsythia suspensa*. *J. For. Res.* **2018**, *29*, 45–53. [CrossRef]

4. Chaves, M.M.; Maroco, J.P.; Pereira, J.S. Understanding plant responses to drought—From genes to the whole plant. *Funct. Plant Biol.* **2003**, *30*, 239–264. [CrossRef]

5. Khan, A.; Pan, X.; Najeeb, U.; Tan, D.K.Y.; Fahad, S.; Zahoor, R.; Luo, H. Coping with drought: Stress and adaptive mechanisms, and management through cultural and molecular alternatives in cotton as vital constituents for plant stress resilience and fitness. *Biol. Res.* **2018**, *51*, 47. [CrossRef] [PubMed]

6. Abdel-Ghany, S.E.; Ullah, F.; Ben-Hur, A.; Reddy, A.S.N. Transcriptome analysis of drought-resistant and drought-sensitive sorghum (*Sorghum bicolor*) genotypes in response to peg-induced drought stress. *Int. J. Mol. Sci.* **2020**, *21*, 772. [CrossRef] [PubMed]

7. Iquebal, M.A.; Sharma, P.; Jasrotia, R.S.; Jaiswal, S.; Kaur, A.; Saroha, M.; Angadi, U.B.; Sheoran, S.; Singh, R.; Singh, G.P.; et al. RNAseq analysis reveals drought-responsive molecular pathways with candidate genes and putative molecular markers in root tissue of wheat. *Sci. Rep.* **2019**, *9*, 1–18. [CrossRef] [PubMed]

8. Mashaki, K.M.; Garg, V.; Nasrollahnezhad Ghomi, A.A.; Kudapa, H.; Chitikineni, A.; Nezhad, K.Z.; Yamchi, A.; Soltanloo, H.; Varshney, R.K.; Thudi, M. RNA-Seq analysis revealed genes associated with drought stress response in kabuli chickpea (*Cicer arietinum* L.). *PLoS ONE* **2018**, *13*, e0199774. [CrossRef] [PubMed]

9. Jarzyniak, K.M.; Jasiński, M. Membrane transporters and drought resistance—A complex issue. *Front. Plant Sci.* **2014**, *5*, 687. [CrossRef] [PubMed]

10. Arisha, M.H.; Ahmad, M.Q.; Tang, W.; Liu, Y.; Yan, H.; Kou, M.; Wang, X.; Zhang, Y.; Li, Q. RNA-sequencing analysis revealed genes associated drought stress responses of different durations in hexaploid sweet potato. *Sci. Rep.* **2020**, *10*, 12573. [CrossRef]

11. Cochetel, N.; Ghan, R.; Toups, H.S.; Degu, A.; Tillett, R.L.; Schlauch, K.A.; Cramer, G.R. Drought tolerance of the grapevine, Vitis champinii cv. Ramsey, is associated with higher photosynthesis and greater transcriptomic responsiveness of abscisic acid biosynthesis and signaling. *BMC Plant Biol.* **2020**, *20*, 1–25. [CrossRef] [PubMed]

12. Meng, H.L.; Zhang, W.; Zhang, G.H.; Wang, J.J.; Meng, Z.G.; Long, G.Q.; Yang, S.C. Unigene-based RNA-seq provides insights on drought stress responses in *Marsdenia tenacissima*. *PLoS ONE* **2018**, *13*, e0202848. [CrossRef]

13. Agarwal, P.K.; Gupta, K.; Lopato, S.; Agarwal, P. Dehydration responsive element binding transcription factors and their applications for the engineering of stress tolerance. *J. Exp. Bot.* **2017**, *68*, 2135–2148. [CrossRef] [PubMed]

14. Chai, M.; Cheng, H.; Yan, M.; Priyadarshani, S.V.G.N.; Zhang, M.; He, Q.; Huang, Y.; Chen, F.; Liu, L.; Huang, X.; et al. Identification and expression analysis of the DREB transcription factor family in pineapple (*Ananas comosus* (L.) Merr.). *Peer J.* **2020**, *8*, e9006. [CrossRef] [PubMed]

15. Yuan, L.L.; Zhang, M.; Yan, X.; Bian, Y.W.; Zhen, S.M.; Yan, Y.M. Dynamic phosphoproteome analysis of seedling leaves in *Brachypodium distachyon* L. Reveals central phosphorylated proteins involved in the drought stress response. *Sci. Rep.* **2016**, *6*, 1–14. [CrossRef]

16. Hoang, X.L.T.; Nhi, D.N.H.; Thu, N.B.A.; Thao, N.P.; Tran, L.-S.P. Transcription factors and their roles in signal transduction in plants under abiotic stresses. *Curr. Genom.* **2017**, *18*, 483. [CrossRef] [PubMed]

17. Hrmova, M.; Hussain, S.S. Plant transcription factors involved in drought and associated stresses. *Int. J. Mol. Sci.* **2021**, *22*, 5662. [CrossRef] [PubMed]

18. DaMatta, F.M.; Rahn, E.; Läderach, P.; Ghini, R.; Ramalho, J.C. Why could the coffee crop endure climate change and global warming to a greater extent than previously estimated? *Clim. Chang.* **2019**, *152*, 167–178. [CrossRef]

19. Semedo, J.N.; Rodrigues, W.P.; Dubberstein, D.; Martins, M.Q.; Martins, L.D.; Pais, I.P.; Rodrigues, A.P.; Leitão, A.E.; Partelli, F.L.; Campostrini, E.; et al. Coffee responses to drought, warming and high [CO_2] in a context of future climate change scenarios. In *Climate Change Management*; Springer: Berlin/Heidelberg, Germany, 2018; pp. 465–477. [CrossRef]

20. Davis, A.P.; Chadburn, H.; Moat, J.; O'Sullivan, R.; Hargreaves, S.; Nic Lughadha, E. High extinction risk for wild coffee species and implications for coffee sector sustainability. *Sci. Adv.* **2019**, *5*, eaav3473. [CrossRef]

21. Van der Vossen, H.; Bertrand, B.; Charrier, A. Next generation variety development for sustainable production of arabica coffee (*Coffea arabica* L.): A review. *Euphytica* **2015**, *204*, 243–256. [CrossRef]
22. Ramalho, J.C.; DaMatta, F.M.; Rodrigues, A.P.; Scotti-Campos, P.; Pais, I.; Batista-Santos, P.; Partelli, F.L.; Ribeiro, A.; Lidon, F.C.; Leitão, A.E. Cold impact and acclimation response of Coffea spp. plants. *Theor. Exp. Plant Physiol.* **2014**, *26*, 5–18. [CrossRef]
23. Rodrigues, W.P.; Martins, M.Q.; Fortunato, A.S.; Rodrigues, A.P.; Semedo, J.N.; Simoes-Costa, M.C.; Pais, I.P.; Leitão, A.E.; Colwell, F.; Goulão, L.; et al. Long-term elevated air [CO_2] strengthens photosynthetic functioning and mitigates the impact of supra-optimal temperatures in tropical *Coffea arabica* and *Coffea canephora* species. *Glob. Chang. Biol.* **2016**, *22*, 415–431. [CrossRef]
24. Dubberstein, D.; Lidon, F.C.; Rodrigues, A.P.; Semedo, J.N.; Marques, I.; Rodrigues, W.P.; Gouveia, D.; Armengaud, J.; Semedo, M.C.; Martins, S.; et al. Resilient and sensitive key points of the photosynthetic machinery of Coffea spp. to the single and superimposed exposure to severe drought and heat stresses. *Front. Plant Sci.* **2020**, *11*, 1049. [CrossRef] [PubMed]
25. Semedo, J.N.; Rodrigues, A.P.; Lidon, F.C.; Pais, I.P.; Marques, I.; Gouveia, D.; Armengaud, J.; Silva, M.J.; Martins, S.; Semedo, M.C.; et al. Intrinsic non-stomatal resilience to drought of the photosynthetic apparatus in *Coffea* spp. is strengthened by elevated air [CO_2]. *Tree Physiol.* **2021**, *41*, 708–727. [CrossRef] [PubMed]
26. DaMatta, F.M.; Ramalho, J.D.C. Impacts of drought and temperature stress on coffee physiology and production: A review. *Braz. J. Plant Physiol.* **2006**, *18*, 55–81. [CrossRef]
27. Ramalho, J.C.; Pais, I.P.; Leitão, A.E.; Guerra, M.; Reboredo, F.H.; Máguas, C.M.; Carvalho, M.L.; Scotti-Campos, P.; Ribeiro-Barros, A.I.; Lidon, F.J.C.; et al. Can elevated air [CO_2] conditions mitigate the predicted warming impact on the quality of coffee bean? *Front. Plant Sci.* **2018**, *9*, 287. [CrossRef] [PubMed]
28. Haggar, J.; Schepp, K. NRI Working Paper Series: Climate Change, Agriculture and Natural Resources Natural Resources Institute Coffee and Climate Change Impacts and options for adaption in Brazil, Guatemala, Tanzania and Vietnam Coffee and Climate Change Impacts and options for adaption in Brazil, 2012, Guatemala, Tanzania and Vietnam. Available online: www.nri.org (accessed on 20 September 2021).
29. De Freitas Guedes, F.A.; Nobres, P.; Rodrigues Ferreira, D.C.; Menezes-Silva, P.E.; Ribeiro-Alves, M.; Correa, R.L.; DaMatta, F.M.; Alves-Ferreira, M. Transcriptional memory contributes to drought tolerance in coffee (*Coffea canephora*) plants. *Environ. Exp. Bot.* **2018**, *147*, 220–233. [CrossRef]
30. Kath, J.; Byrareddy, V.M.; Craparo, A.; Nguyen-Huy, T.; Mushtaq, S.; Cao, L.; Bossolasco, L. Not so robust: Robusta coffee production is highly sensitive to temperature. *Glob. Chang. Biol.* **2020**, *26*, 3677–3688. [CrossRef]
31. Venancio, L.P.; Filgueiras, R.; Mantovani, E.C.; do Amaral, C.H.; da Cunha, F.F.; dos Santos Silva, F.C.; Althoff, D.; dos Santos, R.A.; Cavatte, P.C. Impact of drought associated with high temperatures on *Coffea canephora* plantations: A case study in Espírito Santo State, Brazil. *Sci. Rep.* **2020**, *10*, 19719. [CrossRef]
32. Ramalho, J.C.; Fortunato, A.S.; Goulao, L.F.; Lidon, F.C. Cold-induced changes in mineral content in leaves of *Coffea* spp. Identification of descriptors for tolerance assessment. *Biol. Plant.* **2013**, *57*, 495–506. [CrossRef]
33. Ramalho, J.C.; Rodrigues, A.P.; Semedo, J.N.; Pais, I.P.; Martins, L.D.; Simões-Costa, M.C.; Leitão, A.E.; Fortunato, A.S.; Batista-Santos, P.; Palos, I.M.; et al. Sustained photosynthetic performance of *Coffea* spp. under long-term enhanced [CO_2]. *PLoS ONE* **2013**, *8*, 82712. [CrossRef]
34. Marques, I.; Fernandes, I.; Paulo, O.S.; Lidon, F.C.; DaMatta, F.M.; Ramalho, J.C.; Ribeiro-Barros, A.I. A transcriptomic approach to understanding the combined impacts of supra-optimal temperatures and CO_2 revealed different responses in the polyploid *Coffea arabica* and its diploid progenitor *C. canephora*. *Int. J. Mol. Sci.* **2021**, *22*, 3125. [CrossRef]
35. Andrews, S. FastQC: A Quality Control Tool for High Throughput Sequence Data. 2010. Available online: http://www.bioinformatics.babraham.ac.uk/projects/fastqc (accessed on 20 May 2020).
36. Bolger, A.M.; Lohse, M.; Usadel, B. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* **2014**, *30*, 2114–2120. [CrossRef]
37. Wingett, S.W.; Andrews, S. FastQ Screen: A tool for multi-genome mapping and quality control. *F1000Research* **2018**, *7*, 1338. [CrossRef]
38. Denoeud, F.; Carretero-Paulet, L.; Dereeper, A.; Droc, G.; Guyot, R.; Pietrella, M.; Zheng, C.; Alberti, A.; Anthony, F.; Aprea, G.; et al. The coffee genome provides insight into the convergent evolution of caffeine biosynthesis. *Science* **2014**, *345*, 1181–1184. [CrossRef]
39. Dobin, A.; Davis, C.A.; Schlesinger, F.; Drenkow, J.; Zaleski, C.; Jha, S.; Batut, P.; Chaisson, M.; Gingeras, T.R. STAR: Ultrafast universal RNA-seq aligner. *Bioinformatics* **2013**, *29*, 15–21. [CrossRef] [PubMed]
40. Anders, S.; Pyl, P.T.; Huber, W. HTSeq—A Python framework to work with high-throughput sequencing data. *Bioinformatics* **2015**, *31*, 166–169. [CrossRef] [PubMed]
41. Li, H.; Handsaker, B.; Wysoker, A.; Fennell, T.; Ruan, J.; Homer, N.; Marth, G.; Abecasis, G.; Durbin, R. The sequence alignment/map format and SAMtools. *Bioinformatics* **2009**, *25*, 2078–2079. [CrossRef] [PubMed]
42. Pertea, G.G. gffread: GFF/GTF Utility Providing Format Conversions, Region Filtering, FASTA Sequence Extraction and More. 2015. Available online: https://github.com/gpertea/gffread (accessed on 15 January 2021).
43. Wickham, H. *ggplot2: Elegant Graphics for Data Analysis*; Springer: New York, NY, USA, 2016; ISBN 978-3-319-24277-4.
44. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2018.

45. Love, M.I.; Huber, W.; Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **2014**, *15*, 550. [CrossRef] [PubMed]

46. Benjamini, Y.; Hochberg, Y. On the Adaptive Control of the False Discovery Rate in Multiple Testing with Independent Statistics. *J. Edu. Behav. Stat.* **2016**, *25*, 60–83. [CrossRef]

47. Hunter, J.D. Matplotlib: A 2D graphics environment. *Comput. Sci. Eng.* **2007**, *9*, 90–95. [CrossRef]

48. Martins, M.Q.; Rodrigues, W.P.; Fortunato, A.S.; Leitão, A.E.; Rodrigues, A.P.; Pais, I.P.; Martins, L.D.; Silva, M.J.; Reboredo, F.H.; Partelli, F.L.; et al. Protective response mechanisms to heat stress in interaction with high [CO_2] conditions in *Coffea* spp. *Front. Plant Sci.* **2016**, *7*, 947. [CrossRef]

49. Chen, C.; Huang, H.; Wu, C.H. Protein bioinformatics databases and resources. *Meth. Mol. Biol.* **2017**, *1558*, 3–39. [CrossRef]

50. Raudvere, U.; Kolberg, L.; Kuzmin, I.; Arak, T.; Adler, P.; Peterson, H.; Vilo, J. G:Profiler: A web server for functional enrichment analysis and conversions of gene lists (2019 update). *Nucleic Acids Res.* **2019**, *47*, W191–W198. [CrossRef]

51. Supek, F.; Bošnjak, M.; Škunca, N.; Šmuc, T. Revigo summarizes and visualizes long lists of gene ontology terms. *PLoS ONE* **2011**, *6*, 21800. [CrossRef] [PubMed]

52. Mofatto, L.S.; de Araújo Carneiro, F.; Vieira, N.G.; Duarte, K.E.; Vidal, R.O.; Alekcevetch, J.C.; Cotta, M.G.; Verdeil, J.-L.; Lapeyre-Montes, F.; Lartaud, M.; et al. Identification of candidate genes for drought tolerance in coffee by high-throughput sequencing in the shoot apex of different *Coffea arabica* cultivars. *BMC Plant Biol.* **2016**, *16*, 1–18. [CrossRef] [PubMed]

53. Thioune, E.-H.; Strickler, S.; Gallagher, T.; Charpagne, A.; Decombes, P.; Osborne, B.; McCarthy, J. Temperature Impacts the response of *Coffea canephora* to decreasing soil water availability. *Trop. Plant Biol.* **2020**, *13*, 236–250. [CrossRef]

54. Estavillo, G.M.; Crisp, P.A.; Pornsiriwong, W.; Wirtz, M.; Collinge, D.; Carrie, C.; Giraud, E.; Whelan, J.; David, P.; Javot, H.; et al. Evidence for a SAL1-PAP chloroplast retrograde pathway that functions in drought and high light signaling in *Arabidopsis*. *Plant Cell* **2011**, *23*, 3992–4012. [CrossRef] [PubMed]

55. Marques, I.; Fernandes, I.; David, P.H.C.; Paulo, O.S.; Goulao, L.F.; Fortunato, A.S.; Lidon, F.C.; DaMatta, F.M.; Ramalho, J.C.; Ribeiro-Barros, A.I. Transcriptomic leaf profiling reveals differential responses of the two most traded coffee species to elevated [CO_2]. *Int. J. Mol. Sci.* **2020**, *21*, 9211. [CrossRef]

56. Giuliano, G.; Al-Babili, S.; Von Lintig, J. Carotenoid oxygenases: Cleave it or leave it. *Trends Plant Sci.* **2003**, *8*, 145–149. [CrossRef]

57. Hou, X.; Rivers, J.; León, P.; McQuinn, R.P.; Pogson, B.J. Synthesis and function of apocarotenoid signals in plants. *Trends Plant Sci.* **2016**, *21*, 792–803. [CrossRef]

58. Choi, Y.; Lee, Y.; Kim, S.Y.; Lee, Y.; Hwang, J.-U. Arabidopsis ROP-interactive CRIB motif-containing protein 1 (RIC1) positively regulates auxin signalling and negatively regulates abscisic acid (ABA) signalling during root development. *Plant Cell Environ.* **2013**, *36*, 945–955. [CrossRef]

59. Cao, Y.; Luo, Q.; Tian, Y.; Meng, F. Physiological and proteomic analyses of the drought stress response in Amygdalus mira (Koehne) Yü et Lu roots. *BMC Plant Biol.* **2017**, *17*, 1–16. [CrossRef] [PubMed]

60. Dong, N.-Q.; Sun, Y.; Guo, T.; Shi, C.-L.; Zhang, Y.-M.; Kan, Y.; Xiang, Y.-H.; Zhang, H.; Yang, Y.-B.; Li, Y.-C.; et al. UDP-glucosyltransferase regulates grain size and abiotic stress tolerance associated with metabolic flux redirection in rice. *Nat. Commun.* **2020**, *11*, 1–16. [CrossRef]

61. Fortunato, A.; Lidon, F.C.; Batista-Santos, P.; Leitão, A.E.; Pais, I.P.; Ribeiro, A.I.; Ramalho, J.C. Biochemical and molecular characterization of the antioxidative system of Coffea spp. under cold conditions in genotypes with contrasting tolerance. *J. Plant Physiol.* **2010**, *167*, 333–342. [CrossRef] [PubMed]

62. Ramalho, J.C.; Rodrigues, A.P.; Lidon, F.C.; Marques, L.M.C.; Leitão, A.E.; Fortunato, A.S.; Pais, I.P.; Silva, M.J.; Scotti-Campos, P.; Lopes, A.; et al. Stress cross-response of the antioxidative system promoted by superimposed drought and cold conditions in *Coffea* spp. *PLoS ONE* **2018**, *13*, e0198694. [CrossRef] [PubMed]

63. Maruyama, K.; Takeda, M.; Kidokoro, S.; Yamada, K.; Sakuma, Y.; Urano, K.; Fujita, M.; Yoshiwara, K.; Matsukura, S.; Morishita, Y.; et al. Metabolic Pathways involved in cold acclimation identified by integrated analysis of metabolites and transcripts regulated by *DREB1A* and *DREB2A*. *Plant Physiol.* **2009**, *150*, 1972. [CrossRef] [PubMed]

64. Xia, Y.; Suzuki, H.; Borevitz, J.; Blount, J.; Guo, Z.; Patel, K.; Dixon, R.A.; Lamb, C. An extracellular aspartic protease functions in *Arabidopsis* disease resistance signaling. *EMBO J.* **2004**, *23*, 980. [CrossRef]

65. Gong, L.; Zhang, H.; Gan, X.; Zhang, L.; Chen, Y.; Nie, F.; Shi, L.; Li, M.; Guo, Z.; Zhang, G.; et al. Transcriptome profiling of the potato (*Solanum tuberosum* L.) plant under drought stress and water-stimulus conditions. *PLoS ONE* **2015**, *10*, e0128041. [CrossRef] [PubMed]

66. Figueiredo, L.; Santos, R.B.; Figueiredo, A. Defense and Offense Strategies: The Role of Aspartic Proteases in Plant–Pathogen Interactions. *Biology* **2021**, *10*, 75. [CrossRef] [PubMed]

67. Sebastián, D.I.; Fernando, F.D.; Raúl, D.G.; Gabriela, G.M. Overexpression of Arabidopsis aspartic protease APA1 gene confers drought tolerance. *Plant Sci.* **2020**, *292*, 110406. [CrossRef] [PubMed]

68. Yao, X.; Xiong, W.; Ye, T.; Wu, Y. Overexpression of the aspartic protease ASPG1 gene confers drought avoidance in Arabidopsis. *J. Exp. Bot.* **2012**, *63*, 2579. [CrossRef] [PubMed]

69. Cao, S.; Guo, M.; Wang, C.; Xu, W.; Shi, T.; Tong, G.; Zhen, C.; Cheng, H.; Yang, C.; Elsheery, N.I.; et al. Genome-wide characterization of aspartic protease (AP) gene family in *Populus trichocarpa* and identification of the potential PtAPs involved in wood formation. *BMC Plant Biol.* **2019**, *19*, 1–17. [CrossRef] [PubMed]

70. Devnarain, N.; Crampton, B.G.; Olivier, N.; van der Westhuyzen, C.; Becker, J.V.W.; O'Kennedy, M.M. Transcriptomic analysis of a Sorghum bicolor landrace identifies a role for beta-alanine betaine biosynthesis in drought tolerance. *S. Afr. J. Bot.* **2019**, *127*, 244–255. [CrossRef]

71. Magwanga, R.O.; Lu, P.; Kirungu, J.N.; Lu, H.; Wang, X.; Cai, X.; Zhou, Z.; Zhang, Z.; Salih, H.; Wang, K.; et al. Characterization of the late embryogenesis abundant (LEA) proteins family and their role in drought stress tolerance in upland cotton. *BMC Genet.* **2018**, *19*, 1–31. [CrossRef]

72. Yu, Z.; Wang, X.; Tian, Y.; Zhang, D.; Zhang, L. The functional analysis of a wheat group 3 late embryogenesis abundant protein in *Escherichia coli* and *Arabidopsis* under abiotic stresses. *Plant Signal. Behav.* **2019**, *14*, 1667207. [CrossRef] [PubMed]

73. Wei, S.; Xia, R.; Chen, C.; Shang, X.; Ge, F.; Wei, H.; Chen, H.; Wu, Y.; Xie, Q. ZmbHLH124 identified in maize recombinant inbred lines contributes to drought tolerance in crops. *Plant Biotechnol. J.* **2021**. [CrossRef]

74. Alves, G.S.C.; Torres, L.F.; Déchamp, E.; Breitler, J.C.; Joët, T.; Gatineau, F.; Andrade, A.C.; Bertrand, B.; Marraccini, P.; Etienne, H. Differential fine-tuning of gene expression regulation in coffee leaves by CcDREB1D promoter haplotypes under water deficit. *J. Exp. Bot.* **2017**, *68*, 3017–3031. [CrossRef] [PubMed]

75. Alves, G.S.C.; Torres, L.F.; de Aquino, S.O.; Reichel, T.; Freire, L.P.; Vieira, N.G.; Vinecky, F.; This, D.; Pot, D.; Etienne, H.; et al. Nucleotide diversity of the coding and promoter regions of DREB1D, a candidate gene for drought tolerance in Coffea species. *Trop. Plant Biol.* **2018**, *11*, 31–48. [CrossRef]

76. Torres, L.F.; Reichel, T.; Déchamp, E.; de Aquino, S.O.; Duarte, K.E.; Alves, G.S.C.; Silva, A.T.; Cotta, M.G.; Costa, T.S.; Diniz, L.E.C.; et al. Expression of DREB-like genes in *Coffea canephora* and *C. arabica* subjected to various types of abiotic stress. *Trop. Plant Biol.* **2019**, *12*, 98–116. [CrossRef]

77. Lata, C.; Prasad, M. Role of DREBs in regulation of abiotic stress responses in plants. *J. Exp. Bot.* **2011**, *62*, 4731–4748. [CrossRef] [PubMed]

78. Phukan, U.J.; Jeena, G.S.; Tripathi, V.; Shukla, R.K. Regulation of Apetala2/Ethylene response factors in plants. *Front. Plant Sci.* **2017**, *8*, 150. [CrossRef]

79. Xie, Z.; Nolan, T.M.; Jiang, H.; Yin, Y. AP2/ERF Transcription Factor regulatory networks in hormone and abiotic stress responses in *Arabidopsis*. *Front Plant Sci.* **2019**, *10*, 228. [CrossRef] [PubMed]

80. Singh, D.; Laxmi, A. Transcriptional regulation of drought response: A tortuous network of transcriptional factors. *Front. Plant Sci.* **2015**, *6*, 895. [CrossRef] [PubMed]

81. Müller, M.; Munné-Bosch, S. Ethylene Response Factors: A Key Regulatory Hub in Hormone and Stress Signaling. *Plant Physiol.* **2015**, *169*, 32–41. [CrossRef] [PubMed]

82. Bian, Z.; Gao, H.; Wang, C. NAC Transcription factors as positive or negative regulators during ongoing battle between pathogens and our food crops. *Int. J. Mol. Sci.* **2020**, *22*, 81. [CrossRef]

83. Furihata, T.; Maruyama, K.; Fujita, Y.; Umezawa, T.; Yoshida, R.; Shinozaki, K.; Yamaguchi-Shinozaki, K. Abscisic acid-dependent multisite phosphorylation regulates the activity of a transcription activator AREB1. *Pro. Natl. Acad. Sci. USA* **2006**, *103*, 1988. [CrossRef]

84. Hussain, R.M.; Ali, M.; Feng, X.; Li, X. The essence of NAC gene family to the cultivation of drought-resistant soybean (*Glycine max* L. Merr.) cultivars. *BMC Plant Biol.* **2017**, *17*, 55. [CrossRef]

85. Wu, L.; Zhang, Z.; Zhang, H.; Wang, X.C.; Huang, R. Transcriptional modulation of ethylene response factor protein JERF3 in the oxidative stress response enhances tolerance of tobacco seedlings to salt, drought, and freezing. *Plant Physiol.* **2008**, *148*, 1953–1963. [CrossRef]

86. Mitula, F.; Tajdel, M.; Cieśla, A.; Kasprowicz-Maluśki, A.; Kulik, A.; Babula-Skowrońska, D.; Michalak, M.; Dobrowolska, G.; Sadowski, J.; Ludwików, A. Arabidopsis ABA-activated kinase MAPKKK18 is regulated by protein Phosphatase 2C ABI1 and the Ubiquitin–Proteasome pathway. *Plant Cell Physiol.* **2015**, *56*, 2351–2367. [CrossRef]

87. Jeong, S.; Lim, C.W.; Lee, S.C. The pepper MAP kinase CaAIMK1 positively regulates ABA and drought stress responses. *Front. Plant Sci.* **2020**, *11*, 720. [CrossRef]

88. Yang, Q.; Liu, K.; Niu, X.; Wang, Q.; Wan, Y.; Yang, F.; Li, G.; Wang, Y.; Wang, R. Genome-wide identification of PP2C genes and their expression profiling in response to drought and cold stresses in *Medicago truncatula*. *Sci. Rep.* **2018**, *8*, 1–14. [CrossRef]

89. Li, L.; Zhu, T.; Song, Y.; Feng, L.; Farag, E.A.H.; Ren, M. ABSCISIC ACID INSENSITIVE5 interacts with RIBOSOMAL S6 KINASE2 to mediate ABA responses during seedling growth in *Arabidopsis*. *Front. Plant Sci.* **2021**, *11*, 598654. [CrossRef]

90. Fernández-Rivas, M.; Bolhaar, S.; González-Mancebo, E.; Asero, R.; van Leeuwen, A.; Bohle, B.; Ma, Y.; Ebner, C.; Rigby, N.; Sancho, A.I.; et al. Apple allergy across Europe: How allergen sensitization profiles determine the clinical expression of allergies to plant foods. *J. Allergy Clinic. Immunol.* **2006**, *118*, 481–488. [CrossRef] [PubMed]

91. Van Loon, L.C.; Rep, M.; Pieterse, C.M.J. Significance of Inducible defense-related proteins in infected plants. *Annu. Rev. Phytopathol.* **2006**, *44*, 135–162. [CrossRef]

92. Pagliarani, G.; Paris, R.; Arens, P.; Tartarini, S.; Ricci, G.; Smulders, M.J.; van de Weg, W.E. A qRT-PCR assay for the expression of all Mal d 1 isoallergen genes. *BMC Plant Biol.* **2013**, *13*, 1–13. [CrossRef] [PubMed]

93. Teixeira, M.A.; Rajewski, A.; He, J.; Castaneda, O.G.; Litt, A.; Kaloshian, I. Classification and phylogenetic analyses of the Arabidopsis and tomato G-type lectin receptor kinases. *BMC Genom.* **2018**, *19*, 1–20. [CrossRef] [PubMed]

94. Sun, X.L.; Yu, Q.Y.; Tang, L.L.; Ji, W.; Bai, X.; Cai, H.; Liu, X.F.; Ding, X.D.; Zhu, Y.M. GsSRK, a G-type lectin S-receptor-like serine/threonine protein kinase, is a positive regulator of plant tolerance to salt stress. *J. Plant Physiol.* **2013**, *170*, 505–515. [CrossRef] [PubMed]

95. Yu, T.-F.; Zhao, W.-Y.; Fu, J.-D.; Liu, Y.-W.; Chen, M.; Zhou, Y.-B.; Ma, Y.-Z.; Xu, Z.-S.; Xi, Y.-J. Genome-wide analysis of CDPK family in foxtail millet and determination of SiCDPK24 functions in drought stress. *Front. Plant Sci.* **2018**, *9*, 651. [CrossRef]

96. Geiger, D.; Scherzer, S.; Mumm, P.; Stange, A.; Marten, I.; Bauer, H.; Ache, P.; Matschi, S.; Liese, A.; Al-Rasheid, K.A.S.; et al. Activity of guard cell anion channel SLAC1 is controlled by drought-stress signaling kinase-phosphatase pair. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 21425–21430. [CrossRef]

97. Lee, S.C.; Lan, W.; Buchanan, B.B.; Luan, S. A protein kinase-phosphatase pair interacts with an ion channel to regulate ABA signaling in plant guard cells. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 21419–21424. [CrossRef]

98. Lim, J.; Lim, C.W.; Lee, S.C. Pepper novel serine-threonine kinase CaDIK1 Regulates drought tolerance via modulating ABA sensitivity. *Front. Plant Sci.* **2020**, *11*, 1133. [CrossRef]

99. Marraccini, P.; Freire, L.P.; Alves, G.S.; Vieira, N.G.; Vinecky, F.; Elbelt, S.; Ramos, H.J.; Montagnon, C.; Vieira, L.G.; Leroy, T.; et al. RBCS1 expression in coffee: *Coffea* orthologs, *Coffea arabica* homeologs, and expression variability between genotypes and under drought stress. *BMC Plant Biol.* **2011**, *11*, 1–24. [CrossRef] [PubMed]

100. Marraccini, P.; Vinecky, F.; Alves, G.S.C.; Ramos, H.J.O.; Elbelt, S.; Vieira, N.G.; Carneiro, F.A.; Sujii, P.S.; Alekcevetch, J.C.; Silva, V.A.; et al. Differentially expressed genes and proteins upon drought acclimation in tolerant and sensitive genotypes of *Coffea canephora*. *J. Exp. Bot.* **2012**, *63*, 4191. [CrossRef]

101. Vieira, N.G.; Carneiro, F.A.; Sujii, P.S.; Alekcevetch, J.C.; Freire, L.P.; Vinecky, F.; Elbelt, S.; Silva, V.A.; DaMatta, F.M.; Ferrão, M.A.G.; et al. Different molecular mechanisms account for drought tolerance in Coffea canephora var. Conilon. *Trop. Plant Biol.* **2013**, *6*, 181–190. [CrossRef]

102. De Oliveira Santos, M.; Coelho, L.S.; Carvalho, G.R.; Botelho, C.E.; Torres, L.F.; Vilela, D.J.M.; Andrade, A.C.; Silva, V.A. Photochemical efficiency correlated with candidate gene expression promote coffee drought tolerance. *Sci. Rep.* **2021**, *11*, 1–14. [CrossRef]

103. Bertrand, B.; Bardil, A.; Baraille, H.; Dussert, S.; Doulbeau, S.; Dubois, E.; Severac, D.; Dereeper, A.; Etienne, H. The greater phenotypic homeostasis of the allopolyploid *Coffea arabica* improved the transcriptional homeostasis over that of both diploid parents. *Plant Cell Physiol.* **2015**, *56*, 2035. [CrossRef] [PubMed]

104. Dussert, S.; Serret, J.; Bastos-Siqueira, A.; Morcillo, F.; Déchamp, E.; Rofidal, V.; Lashermes, P.; Etienne, H.; JOët, T. Integrative analysis of the late maturation programme and desiccation tolerance mechanisms in intermediate coffee seeds. *J. Exp. Bot.* **2018**, *69*, 1583–1597. [CrossRef] [PubMed]

105. Martins, M.Q.; Fortunato, A.S.; Rodrigues, W.P.; Partelli, F.L.; Campostrini, E.; Lidon, F.C.; DaMatta, F.M.; Ramalho, J.C.; Ribeiro-Barros, A.I. Selection and validation of reference genes for accurate RT-qPCR Data normalization in Coffea spp. under a climate changes context of interacting elevated [CO_2] and temperature. *Front. Plant Sci.* **2017**, *8*, 307. [CrossRef]

106. Toniutti, L.; Breitler, J.-C.; Guittin, C.; Doulbeau, S.; Etienne, H.; Campa, C.; Lambot, C.; Pinilla, J.-C.H.; Bertrand, B. An altered circadian clock coupled with a higher photosynthesis efficiency could explain the better agronomic performance of a new coffee clone when compared with a standard variety. *Int. J. Mol. Sci.* **2019**, *20*, 736. [CrossRef]

107. Rodrigues, A.M.; Jorge, T.; Osorio, S.; Pott, D.M.; Lidon, F.C.; DaMatta, F.M.; Marques, I.; Ribeiro-Barros, A.I.; Ramalho, J.C.; António, C. Primary metabolite profile changes in *Coffea* spp. promoted by single and combined exposure to drought and elevated CO_2 concentration. *Metabolites* **2021**, *11*, 427. [CrossRef] [PubMed]

108. Zapalska-Sozoniuk, M.; Chrobak, L.; Kowalczyk, K.; Kankofer, M. Is it useful to use several "omics" for obtaining valuable results? *Mol. Biol. Rep.* **2019**, *46*, 3597–3606. [CrossRef] [PubMed]