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Gene expression dynamics in *Eucalyptus* under physiological stress: Unveiling potential markers for environmental adaptability

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Abstract: We quantified the expression of five abiotic-stress-related genes (*CDPK26*, *CIPK*, *WRKY4*, *CDPK11*, and *MYB*) in *Eucalyptus grandis* × *E. urophylla* clones exhibiting Physiological Disorder in *Eucalyptus* (PDE) across two field environments. Using quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR) (three biological and two technical replicates) with melt-curve specificity and normalization to housekeeping genes, we observed in the harsher site (A1) higher expression of *CDPK26*, *CIPK*, *WRKY4* and *CDPK11* in symptomatic plants of Clone01, whereas *MYB* was comparatively higher in symptom-free plants from the milder site (A2). Across genotypes, a consistently symptomatic clone (Clone02) upregulated all five genes relative to an asymptomatic clone (Clone03), indicating genotype-dependent sensitivity. These patterns support roles for Ca^{2+} CBL–CIPK signaling and WRKY/MYB transcriptional control in field responses to water and edaphoclimatic stress. The targeted set behaves as practical markers to flag PDE-associated stress responses under operational conditions.

Keywords: *Eucalyptus* gene expression, Physiological stress in plants, Abiotic stress markers, Environmental adaptability, Edaphoclimatic variations, Stress tolerance genetics.

Introduction

Eucalyptus and *Corymbia* are cultivated in more than 95 countries, covering over 22.57 million hectares (Hill and Johnson, 1995; Parra-O et al., 2009; Zhang and Wang, 2021). Brazil, a leading global producer of pulp and an important charcoal supplier, relies heavily on these plantations for economic output and bio-based materials. However, plantation performance is constrained by biotic and abiotic factors. Light intensity, ultraviolet radiation, temperature, water availability, salinity, heavy metals and soil pH can reprogram plant physiology, reducing productivity (He et al., 2018). Interactions between environment and genome may precipitate physiological disorders, which disrupt cellular metabolism and drive differential gene expression, ultimately altering phenotype (Taiz and Zeiger, 2010). Such disorders are economically relevant and have been linked to losses under increasing climatic variability (Gechev and Petrov, 2020; Zhang et al., 2020).

In May 2007, forest stands in southern Bahia (Brazil) exhibited a syndrome subsequently termed Physiological Disorder in *Eucalyptus* (PDE). Early symptoms included intense leaf abscission, sub-canopy formation, shoot-tip deformation and leaf curling in 2.5–3-year-old trees; subsequent reports described wilting, yellowing and drying of leaves, shoot dieback, canopy curling, epicormic sprouting, bark cracking near the trunk base and branches, stem tortuosity and bifurcation, and successive tree death (Silva et al., 2010; Reis, 2011; Zauza, 2017) (Figure 1). Given the persistence of PDE and its potential links to soil–water–climate constraints, understanding how abiotic stress correlates with molecular responses in operational plantations is warranted.

Here, we test whether a targeted panel of five stress-responsive genes shows reproducible expression differences with PDE in *Eucalyptus grandis* × *E. urophylla* hybrids sampled across two contrasting environments. We test if these expression patterns distinguish symptomatic from symptom-free tissues within and across genotypes, and we frame the findings in the context of known calcium-decoding and transcriptional control pathways relevant to field stress responses.

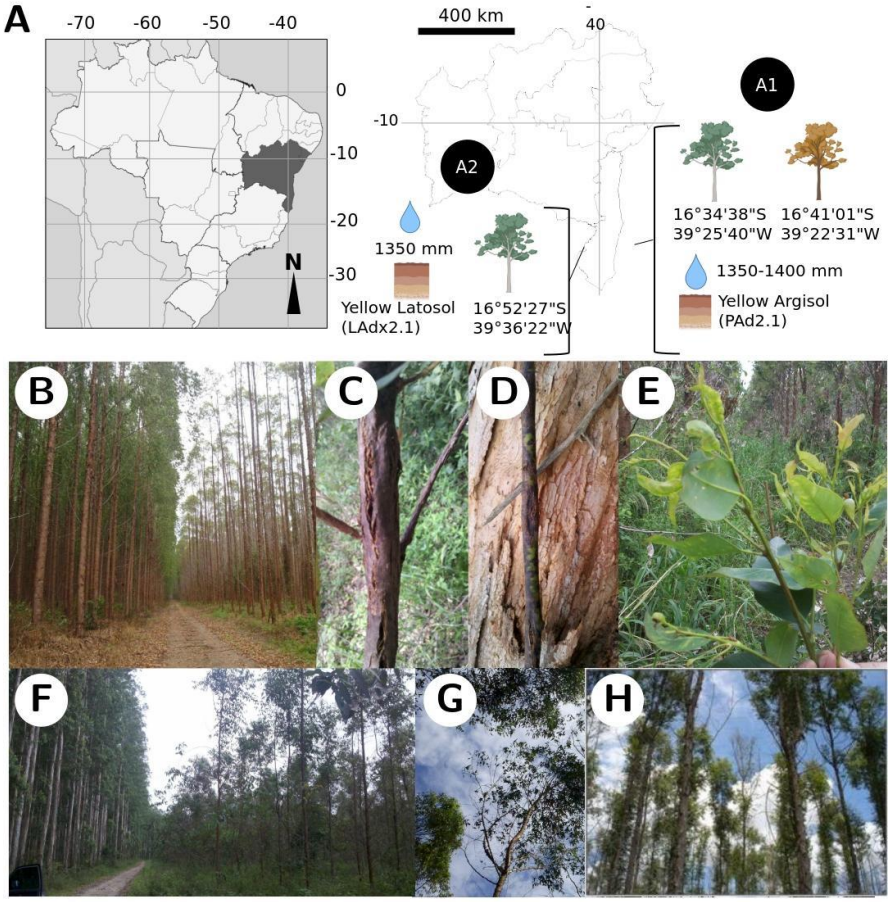

Figure 1. Location and Characteristics of Leaf Collection Site for Study and Observed Field Symptomatology. A – Overview of leaf collection site; B – Intense leaf shedding in the affected genotype; C and D – Branch cracking; E – Crinkled leaves with dry lesions and small cracks on the branches; F – Affected genotype with stunted growth; G – Forking in adult trees; H – Prolific sprouting along the trunk. Photos B and H are credited to Silva et al., 2010, while C, D, E, F, and G are credited to Laia, M.L., 2012.

Results

Gene expression analysis via qPCR revealed distinct expression patterns that were pivotal in understanding the physiological responses of *Eucalyptus* hybrids to environmental stressors. The presence of unique peaks in dissociation curve analyses confirmed the specificity of the amplified products, negating the formation of dimers. The specificity of these results lays the foundation for robust interpretations of gene expression patterns (Figure 2).

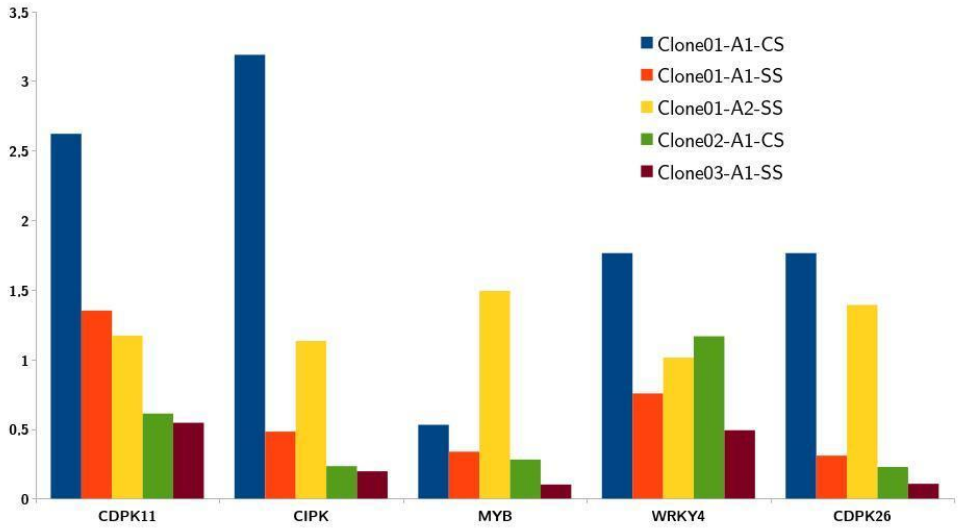

Figure 2. Relative Gene Expression of Five Genes (*CDPK11*, *CIPK*, *MYB*, *WRKY4*, and *CDPK26*) in Three *Eucalyptus* Genotypes (Clone01, Clone02, and Clone03), With and Without Symptoms (CS and SS, Respectively), Across Two Different Environments (A1 and A2).

In environment A1, the symptomatic Clone01 demonstrated upregulated expression for *CDPK11*, *CIPK*, *WRKY4*, and *CDPK26* genes. This upregulation suggests an active stress response, potentially linking these genes to key physiological mechanisms triggered by stress conditions. Intriguingly, the *MYB* gene expressed higher levels in the symptom-free Clone01 within the less stressful environment A2, suggesting that *MYB* expression may serve as an indicator of plant health under varying environmental pressures.
Further examination revealed that symptomatic Clone01 in environment A1 consistently showed increased gene expression across all evaluated genes. This pattern underscores the relevance of these genes in the manifestation of symptoms and possibly reflects the genotype's struggle to cope with the environmental challenges presented by A1.
A comparison between Clone02, which exhibits symptoms irrespective of the environment, and Clone03, which remains asymptomatic, showed that all evaluated genes were upregulated in the symptomatic genotype. Such differential expression patterns could be indicative of the intrinsic genetic resilience of Clone03 or the susceptibility of Clone02 to environmental stress.
An analysis of Clone01's behavior in environment A2 illuminated the environment's role in gene induction and repression. Notably, genes *CDPK26*, *WRKY4*, *CIPK*, and *MYB* showed higher expression in Clone01 when comparing symptomatic to symptom-free plants, except for *CDPK11*. This trend hints at an environmental influence on gene expression, with environment A2 possibly exerting a stress-inducing effect, leading to heightened gene activity as a coping mechanism.
Across the predefined comparisons, *CDPK11*, *CIPK*, *WRKY4* and *CDPK26* showed higher expression in symptomatic Clone01 at A1 versus its symptom-free counterpart at A1, and in symptomatic Clone02 versus asymptomatic Clone03 at A1. By contrast, *MYB* was higher in symptom-free Clone01 at A2 than in symptomatic Clone01 at A1. Patterns were consistent across biological replicates (n = 3 trees per condition; mean ± SE in Figure 2). No formal hypothesis tests were applied; fold changes are descriptive (2^-ΔΔCq).

Discussion

Results indicate genotype × environment (G×E) effects: the same clone responds differently across environments, and clones diverge within a given environment. To parse environment, genotype, and G×E, we contrasted Clone01-A1-CS with Clone01-A1-SS, Clone01-A1-CS with Clone01-A2-SS, and Clone02-A1-CS with Clone03-A1-SS (CS, symptomatic; SS, symptom-free).

Gene CDPK26
Calcium (Ca^{2+}) is a central second messenger in plant stress signaling. Calcium-dependent protein kinases (CDPKs) decode Ca^{2+} transients in guard cells, regulating ion fluxes that control stomatal movements and downstream transcriptional responses (Boudsocq and Sheen, 2013; Kudla et al., 2010). In *Eucalyptus*, *EgrCDPK26* has been associated with stomatal aperture control under water limitation (Martins et al., 2018), and the Arabidopsis homolog *AtCPK26* has been functionally described (Xu et al., 2010). Stress-inducible expression of CDPK26-like genes has also been reported in other species (e.g., *Vitis amurensis*) (Dubrovina et al., 2013).
Across our sites (A1, A2), periods of alternating rainfall and water deficit likely modulated stomatal regulation. Consistent with its role, higher *CDPK26* expression is interpreted as activation of drought-related signalling to limit water loss via stomatal closure; when acclimation is insufficient, stress symptoms emerge.
In our contrasts, symptomatic plants at A1 showed higher *CDPK26* than symptom-free counterparts of the same genotype. In A2, symptom-free Clone01 exhibited higher *CDPK26* than Clone01 at A1 (both CS and SS), suggesting greater demand for this pathway in A2. The tolerant genotype (Clone03-A1-SS) displayed lower *CDPK26*, whereas the consistently susceptible genotype (Clone02-A1-CS) showed higher *CDPK26*, indicating genotype-dependent activation.
Overall, the patterns support genotype × environment modulation of *CDPK26*. We treat *CDPK26* as a context-dependent indicator of stress signalling rather than a sole determinant of PDE.

Gene CDPK11
The *CDPK11* gene functions as a vital positive regulator within the ABA-mediated CDPK/calcium signaling pathways, orchestrating stomatal regulation and water balance during abiotic stress (Zhu et al., 2007). The expression of *CDPK11* under diverse abiotic stressors, such as ABA fluctuations, salinity, drought, and temperature changes, underscores its integral role in plant adaptation and survival strategies (Singh et al., 2017). This gene's activity, confirmed by Zhu et al. (2007), highlights its pivotal role in various physiological processes, including seed germination, seedling growth, guard cell function, and plant stress tolerance to salinity, providing a comprehensive in-plant genetic demonstration of the CDPK/calcium modulation in ABA signal transduction.
Water deficit triggers a cascade of cellular alterations that can act as signals to induce adaptive responses. While the mechanisms that detect these changes are not fully understood, the role of ABA as a critical physiological signal inducing drought responses is well-established. The heightened transcription level of *CDPK11* in diseased plants may relate to the lower soil water availability, suggesting that it acts as a positive regulator in ABA signaling pathways, striving to maintain the plant's water balance under abiotic stress.

Gene WRKY4

The *WRKY4* gene is part of a vast family of transcription factors pivotal for a myriad of developmental processes and defense mechanisms in plants. WRKY factors have regulatory roles in defending against both biotic and abiotic stresses, including pathogen attacks, temperature stress, nutrient deficiency, saline stress, cold, UV-B radiation, and water scarcity. While their involvement in biotic stress responses is well-documented, the precise mechanisms of how WRKY factors mediate abiotic stress tolerance are less defined and warrant further investigation (Yang et al., 2018).

Fan et al. (2018) have explored the classification of WRKY genes in *Eucalyptus*, reporting their expression in response to various plant hormones and cold and salinity stress. However, the need for more comprehensive data derived from bioinformatics analyses and diverse stress conditions remains. Zheng et al. (2013) have examined Th*WRKY4* in *Tamarix hispida*, a gene analogous to *WRKY4* in this study, revealing its operational model in abiotic stress response. Overexpression of Th*WRKY4* conferred increased tolerance to salt stress, oxidative stress, and ABA treatment in transgenic plants. This gene functions as a transcription factor that positively modulates abiotic stress tolerance and is implicated in the modulation of reactive oxygen species, highlighting its potential role in stress signal transduction pathways.

In our observations, *WRKY4* was upregulated in symptomatic plants across both environments for all compared genotypes (Clone01-A1-CS vs. Clone01-A1-SS; Clone01-A1-CS vs. Clone01-A2-SS; Clone02-A1-CS vs. Clone03-A1-SS). This trend is consistent with involvement of *WRKY4* in stress responses under field conditions. The consistent upregulation in symptomatic plants across environments suggests that *WRKY4* is associated with the manifestation of PDE across both environments examined, although we refrain from environment-specific attribution.

WRKY factors are implicated in the defense against thermal stress, nutrient deficiency, saline stress, cold, UV-B, and water deficit. The elevated expression in diseased plants points to a defense mechanism aimed at enhancing tolerance to these stresses through gene expression modulation. Plants, unable to relocate to more favorable environments, have developed a suite of regulatory mechanisms to perceive, transduce, and respond to stress signals at molecular, cellular, and physiological levels.

The discussion around *WRKY4* provides insights into the gene's relevance in the context of PDE, suggesting that its expression correlates with symptomatic conditions and could serve as an informative marker for environmental stress in *Eucalyptus*.

Gene CIPK

CBL-interacting protein kinases (CIPKs) are Ser/Thr kinases that, together with CBL sensors, decode Ca^{2+} signals to regulate ion homeostasis, hormone responses and stress adaptation (Hashimoto et al., 2012). They act as key links between calcium transients and downstream transcriptional programs.

Across species, CIPK genes are stress responsive. In cassava, 26 *CIPK* loci respond to NaCl, PEG, heat and cold (Mo et al., 2018). In pineapple, eight *CBL* and 21 *CIPK* genes show tissue-specific and stress-induced expression, underscoring roles in ABA signalling and abiotic tolerance (Aslam et al., 2019). These reports support a conserved CBL–CIPK module in environmental responses.

In our field contrasts, *CIPK* expression was higher in symptomatic plants than in symptom-free counterparts within A1, and the consistently susceptible genotype showed higher *CIPK* than the tolerant one at A1. Healthy Clone01 at A2 displayed higher *CIPK* than Clone01 at A1 (both CS and SS), except for Clone01-A1-CS, suggesting greater pathway demand in A2. We interpret these patterns as genotype-dependent activation of a calcium-decoding module under less favourable site conditions.

Because stresses seldom occur in isolation, we treat *CIPK* as a context-dependent indicator of stress signalling rather than a PDE-specific determinant.

Gene MYB

The *MYB* superfamily of transcription factors is expansive, with roles at the heart of plant developmental processes and defense responses. These genes regulate cellular differentiation, organ formation, leaf morphogenesis, secondary metabolism, and responses to abiotic stress. The *MYB* family is distinguished by its conserved DNA-binding *MYB* domain, which typically comprises one to four imperfect repeats (Li et al., 2015).

Research has illuminated the roles of MYB proteins such as AtMYC2 and AtMYB2 as significant transcription factors in the ABA-dependent gene expression under drought and saline stress (Abe et al., 2002). AtMYB102 has been identified as a key component in integrating signaling pathways in *Arabidopsis* responses to injury, osmotic stress, and ABA (Denekamp and Smeekens, 2003). Further studies have revealed that *Arabidopsis* MYB proteins (AtMYB44, AtMYB60, and AtMYB61) are implicated in regulating stomatal aperture in response to water stress (Cominelli et al., 2005; Jung et al., 2007; Liang et al., 2005).

Ta*MYB*73 in wheat has been shown to induce stress signaling genes and enhance salt tolerance in transgenic *Arabidopsis* (He et al., 2011). Overexpression of Os*MYB*48-1 in rice improved stress response gene expression, increasing salinity tolerance as well as drought stress tolerance (Xiong et al., 2014). Fang et al. (2018) reported that the MYB transcription factor AtDIV2 plays negative roles in saline stress and is necessary for ABA signaling in *Arabidopsis*. Yang et al., (2012) isolated an R2R3-type MYB gene, OsMYB2, from rice and functionally characterized its role in abiotic stress tolerance, finding that OsMYB2 encodes a stress-responsive MYB transcription factor that plays a regulatory role in rice tolerance to salt, cold, and dehydration stress.

In this study, when plants of the same genotype were compared across different environments (A1 and A2), asymptomatic individuals in environment A2 exhibited nearly twice the expression of those symptomatic in A1. Similar patterns were

observed in comparisons between asymptomatic individuals. These findings underscore the influence of environmental factors on the differential gene expression of *MYB*. The harsher conditions in environment A2 likely necessitate a more robust expression of this gene for plant survival.
Conversely, when comparing plants within the same environment, symptomatic plants showed higher gene expression levels. The elevated expression of *MYB* in environment A2 relative to A1 may indicate that A2 presents more challenging conditions for plant growth, and thus, *MYB* is expressed at lower levels in A1.
Overall, the *MYB* gene serves as an intriguing indicator of environmental responsiveness, potentially acting as a marker for unfavorable conditions leading to PDE. The genes discussed in this study could represent a significant advancement in understanding PDE. They may also be utilized in genetic improvement efforts, such as progeny or clonal testing, for early selection of more adapted genotypes. These targets could inform early selection in progeny or clonal testing, pending broader validation.

Soil-climate interactions and PDE expression
Soil type and climate likely modulate PDE symptom expression via their effects on plant water and oxygen status. In the study region, alternating dry spells and intense rainfall have been reported operationally; although not quantified here, such variability plausibly interacts with Yellow Latosol (LAdx2.1) and Yellow Argisol (PAd2.1) properties (water-holding, aeration, compaction) to shape stress exposure. Within this context, the observed expression patterns—higher *CDPK26* and related calcium-decoding targets in symptomatic contrasts, with comparatively higher *MYB* in symptom-free plants at the milder site—are consistent with activation of stomatal and ion-homeostasis programmes under fluctuating water availability. We therefore interpret PDE as a genotype × environment outcome modulated by soil–water dynamics rather than a single-cause response. Future work should pair continuous soil-moisture and microclimate monitoring with transcriptome-wide assays to resolve mechanisms and strengthen predictive value for management.

Materials and Methods

In this study, "physiological stress" means abiotic, environment-driven disturbances (e.g., water deficit or excess, heat, salinity, pH and related edaphic constraints) that disrupt cellular metabolism and trigger stress-response signaling and gene-expression changes, without a primary biotic cause.

Characterization of environments
We sampled in southern Bahia where PDE is operationally reported. A1 (Porto Seguro; Yellow Argisol, PAd2.1; ~1350–1400 mm yr^{-1}) historically shows higher PDE incidence; A2 (Itabela; Yellow Latosol, LAdx2.1; ~1300 mm yr^{-1}) shows lower incidence. Sites were chosen to contrast soil physical properties (water holding/aeration) and field symptom prevalence, enabling genotype × environment contrasts under commercial management. Sampling coordinates are shown in Figure 3. A1 (with symptoms): 16°34′38″S, 39°25′40″W; A1 (without symptoms): 16°41′01″S, 39°22′31″W; A2 (without symptoms): 16°52′27″S, 39°36′22″W. (Figure 1A, Figure 3).

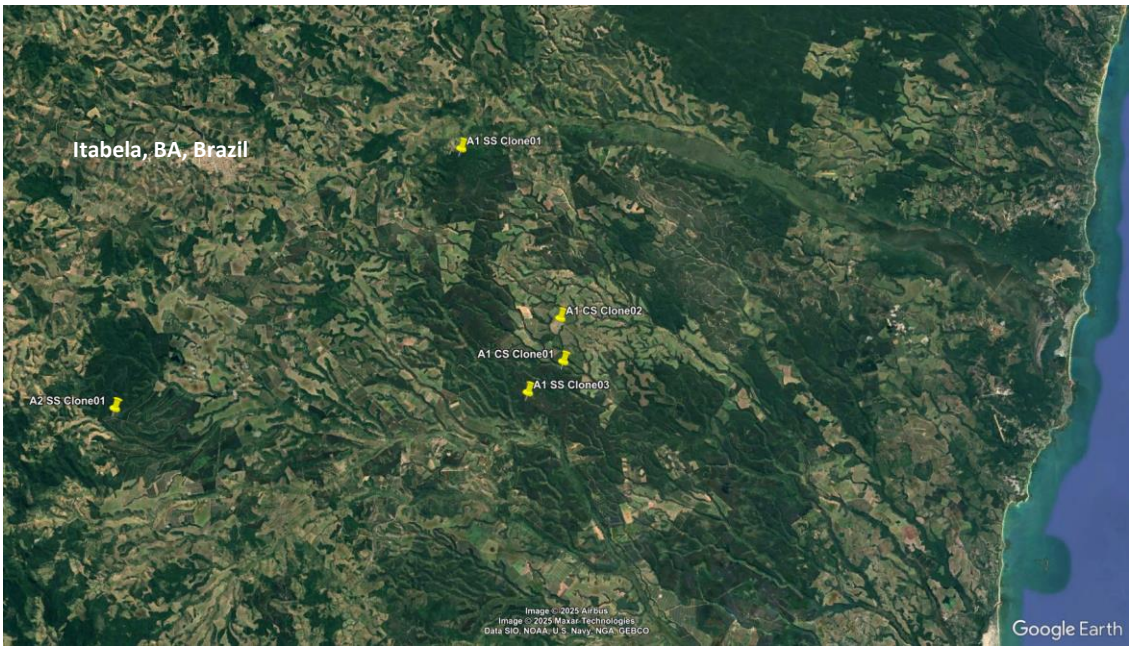

Figure 3. Sampling locations and environment classification in southern Bahia, Brazil. Google Earth satellite image with pins marking stands in A1 (Porto Seguro; Yellow Argisol, PAd2.1) and A2 (Itabela; Yellow Latosol, LAdx2.1). Coordinates: A1 (with symptoms) 16°34′38″S, 39°25′40″W; A1 (without symptoms) 16°41′01″S, 39°22′31″W; A2 (without symptoms) 16°52′27″S, 39°36′22″W. Pins indicate PDE symptom prevalence at the time of sampling.

Plant material

Leaf tissue with and without symptoms was collected from commercial plantations of *Eucalyptus grandis* x *Eucalyptus urophylla* hybrids in the southern state of Bahia affected by physiological disorder (Figure 1B-H). Three trees with symptoms and three without symptoms were randomly selected for sampling. Three leaf samples were collected from each selected tree. Tissue samples were immediately placed in liquid nitrogen and subsequently stored at -80 °C until total RNA extraction. The collected samples were labeled as follows: Clone01-A1-CS (Clone01 genotype, A1 environment, with symptoms); Clone01-A1-SS (Clone01 genotype, A1 environment, without symptoms); Clone01-A2-SS (Clone01 genotype, A2 environment, without symptoms); Clone02-A1-CS (Clone02 genotype, A1 environment, with symptoms); Clone03-A1-SS (Clone03 genotype, A1 environment, without symptoms).

For each condition (genotype × environment × symptom class), we randomly selected three non-adjacent trees (≥30 m apart) of similar age and canopy position to avoid pseudo-replication and micro-site bias. From each tree we collected three fully expanded leaves from the mid-canopy on the sun-exposed side, pooled per tree to constitute one biological replicate (n = 3 per condition).

Total RNA extraction from samples

Leaf samples (approximately 100 mg) were mechanically macerated using a Mini Beadbeater (Biospec Products) in 2.0 mL polyethylene tubes, precooled with liquid nitrogen, with three 3.2 mm diameter stainless steel spheres. Total RNA was purified using the RNeasy Plant Mini Kit (QIAGEN) according to the manufacturer's instructions. To remove genomic DNA contaminants, samples were treated with DNase I (Invitrogen). All samples were analyzed on a Lambda Bio spectrophotometer (PerkinElmer) at 260, 280, and 230 nm wavelengths and on a Qubit 3 fluorometer (Invitrogen), following the manufacturer's instructions.

Gene selection

We selected five targets that occupy complementary nodes of abiotic-stress signaling: *CDPK11/26* (Ca^{2+} ABA-mediated guard-cell/osmotic signaling), *CIPK* (CBL–CIPK calcium decoding and ion homeostasis), and *WRKY4/MYB* (transcriptional regulators integrating ABA/ROS/water-deficit cues). Prior work links these orthologs to drought/salinity or osmotic responses in trees and crops; here we test whether their field expression discriminates symptomatic from symptom-free tissues across genotypes and environments (e.g., Zhu et al., 2007; Martins et al., 2018; Hashimoto et al., 2012; Fan et al., 2018; Abe et al., 2002). Gene sequences and primer targets were retrieved from NCBI GenBank; accession numbers are listed in Table 1 (GI column).

Table 1. Direct (F) and reverse (R) oligonucleotides, size of each oligonucleotide in bases (b), amplicon size in base pairs (pb), melting temperature (Tm), and accession number of the mRNA of the respective gene deposited in GenBank (GI)

Genes	Primers Sequence	*T*m	Size (b)	Amplicon (bp)	GI
CDPK26	F- GCATTCACGTGCAAGTCATC		20	72	XM_010055039
	R- CCCCTCAATTGAAGATGTGG		20		
CDPK11	F- CAGTGGCAGCATAACATT CG		20	141	XM_010063698
	R- GCGAGAAATTCGCCATAGTC		20		
WRKY4	F- TGGGCAGAAACAGGTTAAGG		20	108	XM_010046160
	R- ATATGGCCGTCAAGAGAACG		20		
CIPK	F- TTTGGAACGGAAGGAGACAG		20	125	XM_010051144
	R- TCTTGTCACGGCAAAGTCTG		20		
MYB	F-TCTTGTGCTGGAACAACCAG		20	90	XM_010045792
	R- TCCCACGAAATGGTCCTAAG		20		
IDH	F - AGTTTGAGGCTGCTGGAATC		20	100	XM_010064015
	R - CTTGCATGCCCACACATAAC		20		
UBQ	F - GAGGGACATCTATCTCTATGAC		22	131	XM_010071338
	R - CAACAGTAAGCACACGAG		18		

The nucleotide sequences of these genes were retrieved from GenBank (Sayers et al., 2020) and used in the Phytozome project (Goodstein et al., 2011) as "baits" to check if the *Eucalyptus grandis* BRASUZ1 genome (Myburg et al., 2014) has any homologs. Once the nucleotide sequence of the homolog was retrieved, it was used as a template for primer production. With the aid of the Primer3Plus program (Untergasser et al., 2007) set to produce primers for qPCR reactions, a pair of oligonucleotides for each gene of interest was produced. The main characteristics of these primers were: amplicon size between 70 and 200 bp; oligonucleotide size between 18 and 23 bases; melting temperature between 58 and 62 °C, with a minimum difference between the two primers of a pair not exceeding 3 °C; and no occurrence of dimers between them. After obtaining the sequences of each pair of primers, they were subjected to an *in silico* similarity analysis in GenBank using the BLASTN tool. This analysis confirmed the specificity of the primer pair for a single gene in the studied species.

Finally, all oligonucleotide pairs were subjected to conventional PCR to evaluate their specificity using DNA extracted from the same leaf samples as the template.

RT-qPCR Assays

RT-qPCR reactions were performed on a StepOne RealTime PCR System (Applied Biosystems) using the EXPRESS-One Step SYBR GreenER Kit, with premixed ROX (Invitrogen). The assay included three biological replicates, each with two technical replicates. Negative controls (NTC), where RNA was replaced by an equal volume of water, were included following the manufacturer's recommendations. The reaction volume was standardized to 10 µL and consisted of 2.0 µL of RNA (10 ng), 0.75 µL of nuclease-free water (Ambion), 1 µL of the forward oligonucleotide, 1 µL of the reverse oligonucleotide, 0.25 µL of SYBR Green PCR Superscript mix, and 5.00 µL of SYBR Green PCR Supermix with Rox. The cDNA production step was performed with one cycle at 45 °C for 30 min and one at 95 °C for 10 min. Then, the samples underwent 40 cycles at 95 °C for 15 s followed by 65 °C for 75 s. A dissociation curve from 60 °C to 95 °C was generated at the end of each reaction. Data were pre-analyzed using Step-One Plus software version 2.3 (Applied Biosystems) and exported to a spreadsheet. The amplification and dissociation curves generated by the system were used in qualitative analyses.

Differential gene expression analysis

Expression levels were assessed based on the number of amplification cycles needed to reach a fixed threshold (cycle threshold (Cq)) in the exponential phase of PCR. From the Cq values obtained, gene expression levels were measured using the relative quantification calculation 2^-ΔΔCq (Livak and Schmittgen, 2001). The average of the technical replicates was used in the analyses. Subsequently, the values were normalized based on the average expression of the endogenous genes, and the standard error of the mean was calculated for each “without symptoms” and “with symptoms” set.

Conclusion

Field RT-qPCR across two contrasting environments and three genotypes revealed reproducible PDE-associated shifts in five abiotic-stress genes: symptomatic tissues tended to upregulate *CDPK26*, *CIPK*, *WRKY4* and *CDPK11*, whereas *MYB* tracked healthier status in the milder site. Consistency within genotype–environment contrasts supports these targets as practical, biologically coherent markers of PDE-related stress signaling under operational conditions. We explicitly frame them as indicators rather than exclusive determinants, given that many loci respond under stress; accordingly, we outline RNA-seq and in-situ environmental monitoring as next steps to resolve network-level mechanisms and further validate predictive value.

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The datasets generated during and/or analysed during the current study are publicly available.

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