








Molecular responses to freshwater limitation in the mangrove tree *Avicennia germinans* (Acanthaceae)

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Abstract

Environmental variation along the geographical space can shape populations by natural selection. In the context of global warming and changing precipitation regimes, it is crucial to understand the role of environmental heterogeneity in tropical trees adaptation, given their disproportional contribution to water and carbon biogeochemical cycles. Here, we investigated how heterogeneity in freshwater availability along tropical wetlands has influenced molecular variations of the black mangrove (*Avicennia germinans*). A total of 57 trees were sampled at seven sites differing markedly in precipitation regime and riverine freshwater inputs. Using 2,297 genome-wide single nucleotide polymorphic markers, we found signatures of natural selection by the association between variations in allele frequencies and environmental variables, including the precipitation of the warmest quarter and the annual precipitation. Additionally, we found candidate loci for selection based on statistical deviations from neutral expectations of interpopulation differentiation. Most candidate loci within transcribed sequences were functionally associated with central aspects of drought tolerance or plant response to drought. Moreover, our results suggest the occurrence of the rapid evolution of a population, probably in response to sudden and persistent limitations in plant access to soil water, following a road construction in 1974. Observations supporting rapid evolution included the reduction in tree size and changes in allele frequencies and in transcript expression associated with increased drought tolerance through the accumulation of osmoprotectants and antioxidants, biosynthesis of cuticles, protection against protein degradation, stomatal closure, photorespiration and photosynthesis. We describe a major role of spatial heterogeneity in freshwater availability in the specialization of this typically tropical tree.

KEYWORDS

Avicennia germinans (Black Mangrove), drought-tolerance, ecological genomics, nextRAD, RNA-Seq, tropical tree

1 | INTRODUCTION

Natural ecosystems are characterized by wide environmental heterogeneity over geographical space. These spatial variations in several abiotic conditions can shape population specializations, particularly in widespread species, through changes in the frequency of genotypes and phenotypes (Kawecki & Ebert, 2004). Therefore, environmental gradients represent natural laboratories for the study of environmental selection (De Frenne et al., 2013). As sessile organisms, incapable of escaping from unfavourable conditions, plants are ideal models for investigating adaptation through phenotypic plasticity and/or adaptive genetic variation. They are often subject to a wide range of environmental factors, such as water, light, temperature and nutrient availability. Among the various environmental factors that determine adaptive phenotypic and genotypic diversity in plants, freshwater availability plays a prominent role (Choat et al., 2018; Phillips et al., 2010). Accordingly, population differentiation in drought tolerance has been widely identified in various studies, providing valuable insights into the evolutionary consequences of spatial variation in freshwater availability in plant species (Aranda et al., 2014; Donovan, Ludwig, Rosenthal, Rieseberg, & Dudley, 2009; Etterson, 2004; Heschel & Riginos, 2005; Keller et al., 2011; Ramírez-Valiente et al., 2018). Reduced freshwater availability resulting from the combination of high atmospheric temperature with low rainfall and air humidity represents a major threat to plants, especially trees and the forest ecosystems that they form (Asner et al., 2016; Bennett, McDowell, Allen, & Anderson-Teixeira, 2015; McDowell & Allen, 2015). These changes influence major components of resource use by reducing the soil water potential, which limits the water and nutrient supply to leaves, and by raising the air vapour pressure deficit (VPD), which increases water loss through transpiration (McRae, 1980; Novick et al., 2016). In response to these conditions, plants close their stomata (McAdam & Brodribb, 2015; Tyree & Sperry, 1989), thereby decreasing the chance of death from hydraulic failure (Rowland et al., 2015) despite causing negative impacts on photosynthesis and productivity (Lawlor, 2002).

Currently, there is great interest in understanding the genotypic and phenotypic bases of tree resistance to drought, as these mechanisms are key to improving predictions of the environmental consequences of extreme events and to developing plans to mitigate forest loss (Corlett, 2016; da Costa et al., 2010; Phillips et al., 2009). Substantial advances in the understanding of phenotypic characteristics that enhance drought tolerance in trees have been achieved recently (Bartlett, Scoffoni, & Sack, 2012; Bennett et al., 2015; Hacke, Sperry, Wheeler, & Castro, 2006; Powell et al., 2017); however, little is known about the molecular basis of water stress tolerance, particularly in tropical species (Holliday et al., 2017), which contribute disproportionately to the global carbon cycle (Corlett, 2016).

In this study, we investigated the role of environmental selection along a gradient of freshwater availability in shaping the molecular variation of a typically tropical and abundant tree, *Avicennia germinans* (L.) L. (Acanthaceae). During dry seasons, VPD, soil salinity and water deficits can reach extreme levels in tropical zones, hindering the maintenance of water and ion homeostasis and limiting carbon

gain and plant growth (Clough, Sim, Inlet, Bay, & Rivers, 1989; Lin & Sternberg, 1992). Thus, we hypothesized that spatial heterogeneity in freshwater availability shapes adaptive variation in the allele frequencies and the expression profiles of transcripts of tropical trees associated with the response to and tolerance of limited freshwater. *Avicennia germinans* individuals were sampled along a wide longitudinal range in the equatorial Brazilian coastline, an area showing narrow spatial heterogeneity in temperature seasonality but encompassing high variation in the intensity and duration of the dry season, in annual precipitation levels and in riverine freshwater input, which influence levels of soil salinity (Figure 1). We used the Nextera-tagmented reductively amplified DNA (nextRAD) sequencing approach for the identification and genotyping of genome-wide single nucleotide polymorphisms (SNPs). We analyzed the organization of the genetic structure and performed statistical tests to identify candidate loci for selection. To minimize false positives in the detection of candidate loci (Lotterhos & Whitlock, 2015), we used distinct approaches: (a) based on the identification of loci deviating from neutral models of interpopulational genetic variation (FST outlier tests) and (b) based on direct genetic-environment (G–E) association tests. RNA sequencing (RNA-Seq) was used to assemble and characterize the transcriptome of the species, providing a functional basis for the annotation of candidate loci for selection. Additionally, we examined the role of molecular adaptation to extreme soil freshwater limitation via differential gene expression analysis between samples from adjacent sites differing in tidal inundation frequency (Lara & Cohen, 2006; Pranchai et al., 2017), acclimated in pots under homogeneous, watered conditions. Our results provide converging signs of adaptive responses of *A. germinans* to the environmental heterogeneity in freshwater availability, including a case that suggests the rapid evolution of a population, with changes in phenotype and in the genetic profile. We highlight the relevance of environmental heterogeneity in freshwater availability as a key selective pressure in tropical tree species.

2 | MATERIALS AND METHODS

2.1 | Study system

As with any mangrove tree, *Avicennia germinans* L. (L.) naturally experiences daily and seasonal fluctuations in temperature, air humidity, freshwater inputs and soil salinity (Tomlinson, 1986). However, this species is particularly suitable for the study of the mechanisms underlying tree adaptation to drought since it belongs to the mangrove genus most tolerant to conditions limiting water acquisition, such as drought, freezing and salinity (Pranchai et al., 2017; Reef & Lovelock, 2015; Stuart, Choat, Martin, Holbrook, & Ball, 2007). Moreover, it presents a generalist insect pollination system (Nadia, Menezes, & Machado, 2013), and although it is predominantly outcrossing, it also supports self-fertilization (Mori, Zucchi, & Souza, 2015; Nettel-Hernanz, Dodd, Ochoa-Zavala, Tovilla-Hernández, & Días-Gallegos, 2013). Additionally, *A. germinans* reproduces by producing cryptoviviparous, buoyant, and salt-tolerant propagules,

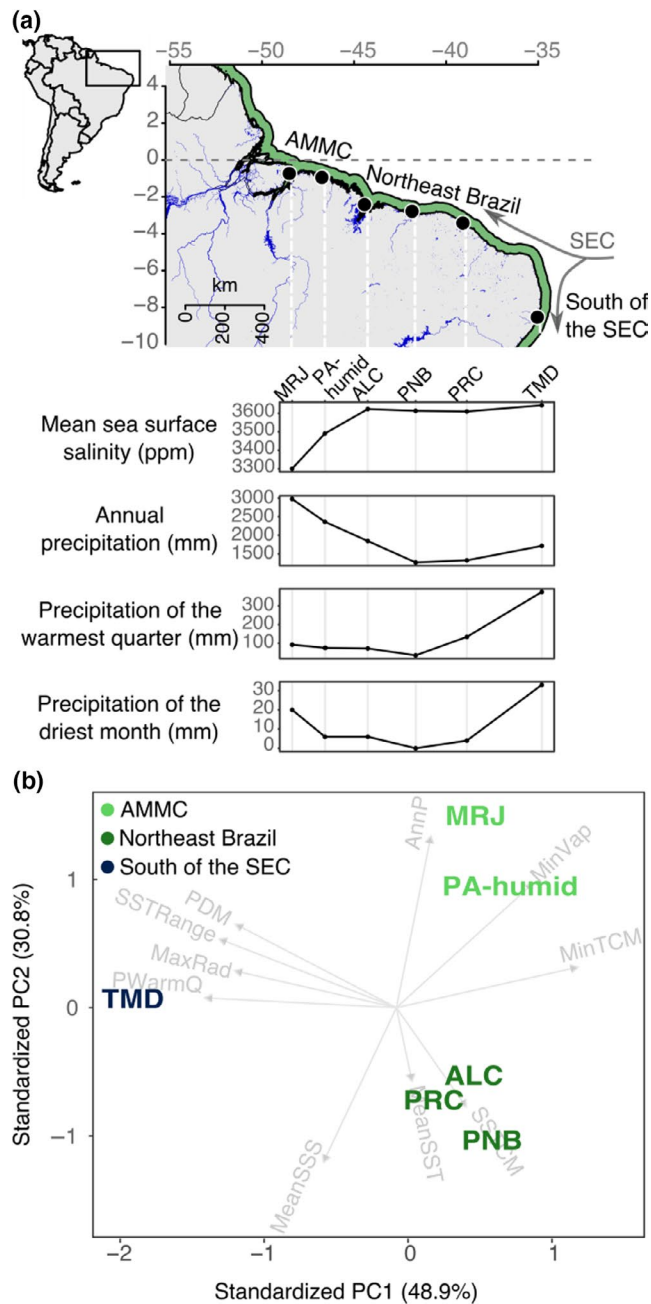


FIGURE 1 *Avicennia germinans* sampling sites along a low-latitude salinity and precipitation gradient. (a) Top: Geographical location of the study area and sampling sites. Black points represent sampling sites, green areas represent the occurrence of the species, and blue areas represent ponds and rivers. Bottom: Environmental variation based on sea surface salinity and precipitation variables across sampling sites (SOURCE: WORLDCLIM and MARSPEC). AMMC: Amazon Macro-tidal Mangrove Coast; SEC: South Equatorial Current. (b) Bidimensional projection of sampling sites based on a principal component analysis. PC1 and PC2 retained 79.7% of the variance of noncollinear environmental variables (eigenvectors). AnnP: annual precipitation; MinVap: minimum air water vapour pressure; MinTCM: minimum temperature of the coldest month; SSTCM: sea surface temperature of the coldest month; MeanSST: mean sea surface temperature; MeanSSS: mean sea surface salinity; PWarmQ: precipitation of the warmest quarter; MaxRad: maximum solar radiation; SSTRange: sea surface temperature range; PDM: precipitation of the driest month [Colour figure can be viewed at wileyonlinelibrary.com]

WORLDCLIM (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005; 15 precipitation, radiation and air vapour pressure layers) and MARSPEC (Sbrocco & Barber, 2013; four sea surface salinity and temperature layers). To minimize the collinearity of variables, we used a correlation threshold of 0.8 (Figure S1). We classified the study area into three distinguishable regions based on a principal component analysis of the environmental data set (Figure 1b): (a) the Amazon Macro-tidal Mangrove Coast (AMMC), the world's largest continuous mangrove belt (Nascimento, Souza-Filho, Proisy, Lucas, & Rosenqvist, 2013), which has a mean annual precipitation above 2,000 mm/year and is influenced by the mouth of the Amazon River; (b) mangroves of Northeast Brazil, which show limited forest development (Schaeffer-Novelli et al., 1990) and are characterized by a lack of riverine freshwater inputs and a mean annual precipitation below 2,000 mm/year, with pronounced and long dry seasons and less than 30 mm of precipitation in the driest quarter; and (c) a region influenced by the southward-flowing branch of the South Equatorial coastal current (South of the SEC), characterized by

TABLE 1 Characterization and geographic location of *Avicennia germinans* sampling sites

Sampling site ID	Climate classification ^a	Latitude; longitude	Number of samples	
			nextRAD	RNA-Seq
MRJ	Am	00.72 S; 48.49 W	8	—
PA-arid ^b	Am	00.90 S; 46.69 W	9	3
PA-humid	Am	00.94 S; 46.72 W	9	3
ALC	Aw	02.41 S; 44.41 W	5	—
PNB	Aw	02.78 S; 41.82 W	9	—
PRC	Aw	03.41 S; 39.06 W	7	—
TMD	As	08.53 S; 35.01 W	10	—

^aKöppen-Geiger climate classification system (Alvares, Stape, Sentelhas, Gonçalves, & Sparovek, 2013). Am, tropical monsoon climate; Aw, Tropical wet savanna climate; As, Tropical dry savanna climate.

^bSamples from a forest flooded exclusively during spring tides (Lara & Cohen, 2006).

which enables long-distance and even transoceanic dispersal (Mori, Zucchi, Sampaio, & Souza, 2015; Tomlinson, 1986). This set of characteristics contributes to making *A. germinans* one of the most widespread mangroves in the Atlantic East-Pacific biogeographic region (Ellison, Farnsworth, & Merkt, 1999).

2.2 | Study area

Sampling sites were located over more than 1,800 km of the north-east Brazilian coastline, between 0.724°S and 8.526°S latitude and along a spatial gradient in freshwater availability (Table 1; Figure 1a). We obtained environmental data for each sampling site, consisting of 21 bioclimatic and oceanographic variables from the public databases

the presence of scattered estuaries, with a mean annual precipitation of approximately 1,000 mm/year and a mild dry season with more than 100 mm of precipitation in the driest quarter.

In the AMMC, we visited two adjacent sites (which require a more detailed description), both located on the peninsula of Ajuruteua, state of Pará, Brazil (Figure 2a). The peninsula was covered by a well-preserved, daily-flooded mangrove forest (Cohen & Lara, 2003) until it was divided by the construction of a road (Figure 2b) in 1974. The hydrology of the area was then dramatically changed, and a part of the forest started flooding exclusively during the highest spring tides of only one out of the two estuaries surrounding the peninsula (Figure 2a). The changed hydrology resulted in forest dieback and subsequent recolonization of the degraded area, mainly by *A. germinans*. Pore water salinity accumulated to extremely high levels (100 ppt at 50 cm depth), and the air surface temperature frequently exceeded 40°C (Vogt et al., 2014). These environmental features contributed to the dwarfism of recolonizing individuals (Cohen & Lara, 2003; Pranchai et al., 2017), whose shrub architecture (up to 2.0 m in height) contrasts the former tall morphology (up to 30 m in height), which is still observed in surrounding areas where the hydrology remained unaltered (Menezes, Berger, & Mehlig, 2008; Figure 2b). Throughout this work, we refer to the western side of the road as “PA-arid” and the eastern side as “PA-humid”.

2.3 | Population genetics

2.3.1 | DNA extraction and sequencing

Leaves from 57 adult *A. germinans* trees were sampled from seven distinct sites (Table 1) and stored in bags with silica gel. DNA extraction was performed using the DNeasy Plant Mini Kit (Qiagen) and NucleoSpin Plant II (Macherey Nagel). DNA quality and quantity were assessed using 1% agarose gel electrophoresis and the QuantiFluor dsDNA system in a Quantus fluorometer (Promega). NextRAD libraries were constructed by SNPsaurus (SNPsaurus, LLC; Russello, Waterhouse, Etter, & Johnson, 2015). Genomic DNA fragmentation and short-adapter ligation were performed with Nextera reagent (Illumina, Inc.), followed by amplification, in which one of the primers matched the adapter and extended it by nine arbitrary nucleotides of DNA. Thus, amplicons were fixed at the selective end, and their lengths depended on the initial fragmentation, leading to consistent genotyping of amplified loci. Subsequently, nextRAD libraries were sequenced in a HiSeq 2500 (Illumina, Inc) with 100 bp single-end chemistry (Figure S2).

2.3.2 | SNP detection and genotyping

Genotyping-by-sequencing used custom scripts from our sequencing service facility, SNPsaurus LLC, to create a reference catalogue of abundant reads. Read mapping to the catalogue allowed two

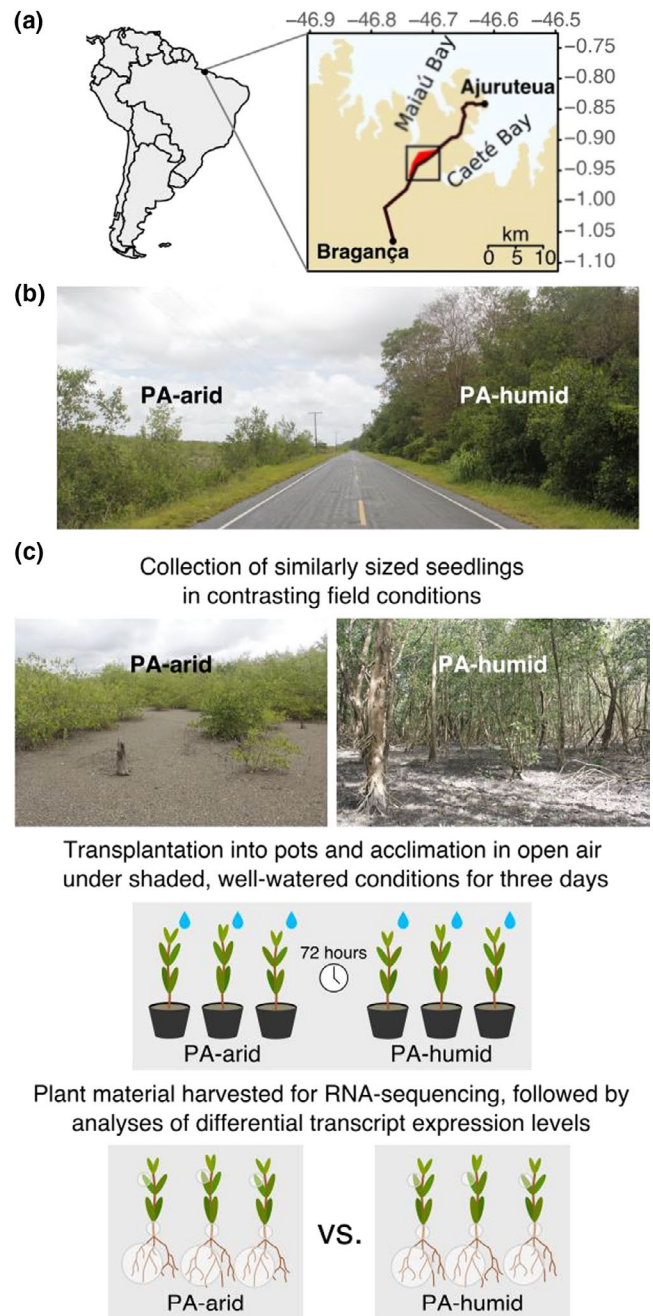


FIGURE 2 PA-arid and PA-humid sampling sites and RNA sequencing (RNA-Seq) experimental design. (a) Geographical location of sites, highlighted by a square (red coloured area is the PA-arid site). (b) Photograph of a section of the Bragança-Ajuruteua road (Pará, Brazil) along which severe changes in hydrology altered the mangrove community and tree morphology. (c) RNA-Seq experimental design. Photographs: G.M. Mori and M.V. Cruz [Colour figure can be viewed at wileyonlinelibrary.com]

mismatches. Biallelic loci present in at least 10% of samples were selected for the subsequent steps. Using vcftools 0.1.12b (Danecek et al., 2011), we selected high-quality sequences (Phred score >30), allowing a maximum of 65% missing data and one SNP per sequence, requiring a minimum coverage of 8× and a minor allele frequency ≥ 0.05 (Figure S2). To reduce false SNP detection rates due to

paralogy or low-quality genotype calls, we used a maximum read of 56, resulting from the product of the average read depth and 1.5 standard deviation of the mean.

2.3.3 | Genetic diversity analysis and identification of candidate SNP loci associated with water-stress tolerance

For each sampling site, we estimated the genetic diversity using per-site nucleotide diversity (π), calculated using *vcftools* (Danecek et al., 2011); we identified private alleles (pA), observed (H_O) and expected (H_E) heterozygosities using *POPPR* 2.8.2 (Kamvar, Tabima, & Grünwald, 2014); and we determined the percentage of polymorphic loci (%Poly) using *ADEGENET* 2.1.1 (Jombart & Ahmed, 2011). We also estimated pairwise genetic differentiation (FST), the inbreeding coefficient (FIS) and its 95% confidence interval through 1,000 bootstrap resampling over all SNP loci using *HIERFSTAT* 0.04-22 (Goudet, 2005). The genetic structure of *A. germinans* was described by a multivariate model-free method, a discriminant analysis of principal components (Jombart, Devillard, & Balloux, 2010), and *ADMIXTURE* 1.3.0 (Alexander, Novembre, & Lange, 2009). We considered numbers of groups from 1 to 50 and the Bayesian information criteria to determine the number of groups (K). The *OPTIM.A.SCORE* function was used to avoid overfitting during discrimination steps (Jombart, 2008). For *ADMIXTURE* analysis, we performed three separate runs for values of K varying from 1 to 15 using the block-relaxation method for point estimation. Computing was terminated when estimates increased by less than 0.0001. The lowest level of cross-validation error indicated the most likely K -value.

To detect signatures of natural selection, we used three distinct methods. Loci that were highly correlated with the environmental structure (false discovery rate (FDR) < 0.05) were detected using the R package *LEA* (Frichot & François, 2015), which is based on analyses of genetic structure and ecological association (G-E association tests). In these analyses, we used noncollinear environmental bioclimatic and oceanographic variables (Figure S1) retrieved from the public databases *WORLDCLIM* (Hijmans et al., 2005; precipitation, radiation and air vapour pressure layers) and *MARSPEC* (Sbrocco & Barber, 2013; sea surface salinity and temperature layers). Since the environmental divergence between PA-arid and PA-humid is evident (Figure 2) but undistinguishable based on the highest-resolution environmental data sets, we excluded PA-arid samples from G-E association tests.

To overcome limitations in the acquisition of environmental data for the PA-arid site, two other methods were used to detect candidate loci for selection. These methods are based solely on deviations from neutral expectations of allele frequency distributions, regardless of environmental variation data across sampling sites (FST outlier tests). With *PCADAPT* 2.0 (Luu, Bazin, & Blum, 2016), population structure was determined by a principal component analysis. A disproportional relation to this structure (FDR < 0.05) was indicative of

selection. With *LOSITAN* (Antao, Lopes, Lopes, Beja-Pereira, & Luikart, 2008), which uses the *FDIST2* method (Beaumont & Nichols, 1996), we assessed the relationship between FST and H_E to describe the neutral distribution under an island migration model. Hence, we detected loci with excessively high or low FST. Because *Lositan* may show partially divergent results among independent simulations, we only considered candidate loci conservatively identified in three independent *Lositan* simulations assuming an infinite allele model of mutation, with a confidence interval of 0.99 and an FDR < 0.05, using the neutral mean FST and forcing the mean FST options.

Demographic histories can affect the statistical power of selection tests (Lotterhos & Whitlock, 2015); thus, type I and type II errors are frequently associated with these approaches (Narum & Hess, 2011). We minimized the potential for false discovery by identifying consensus candidate loci for selection among distinct methods used for two data sets: (a) the entire genotypic data set, including PA-arid individuals, using *LOSITAN* and *PCADAPT* consensus candidates and (b) a subset of genotypic data, which excluded the PA-arid samples due to absence of environmental data, using *LOSITAN*, *PCADAPT* and *LEA* consensus candidates (Figure S2).

2.3.4 | Functional annotation of candidate loci putatively under natural selection

A functional annotation of candidate loci was obtained by reciprocal nucleotide alignment between *nextRAD* sequences and the reference transcriptome, which was also characterized in the present study. *BLAST* + 2.2.31 (Camacho et al., 2009) was used with a threshold of at least 50 aligned nucleotides, a maximum of one mismatch and no gaps (Figure S2).

2.4 | Transcriptomics

2.4.1 | Plant material for transcriptome assembly and differential expression analysis

On August 16, 2013, three similarly sized *A. germinans* seedlings with 5–9 stem nodes were collected from the PA-arid and PA-humid sites, separated by the Bragança-Ajuruteua road, and transplanted with the surrounding soil into 3.0 L pots (Figure 2c). Based on previous observations of other *Avicennia* species (Almahasheer, Duarte, & Irigoien, 2016; Duarte, Thampanya, Terrados, Geertz-Hansen, & Fortes, 1999), we estimate that all samples were 3.5 to 11 months old. For acclimation in a homogeneous environment, pots were placed in open-air and naturally shaded conditions and were watered daily with 300 ml of tap water after sunset. Plants were harvested at noon, after 72 hr of acclimation; washed with water; and split into roots, stems and leaves with a sterile blade to be stored in *RNAlater* (Ambion Inc.) and transported to the laboratory for RNA extraction.

2.4.2 | RNA extraction, cDNA library preparation and RNA sequencing

RNA extraction was performed according to Oliveira, Viana, Reátegui, and Vincentz (2015). To assess purity, integrity and concentration, we used 1% agarose gel electrophoresis and a NanoVue spectrophotometer (GE Healthcare Life Sciences). Subsequently, cDNA-enriched libraries were constructed using TruSeq RNA sample preparation kits (Illumina Inc.). Library qualities were assessed using an AGILENT 2100 BIOANALYZER (Agilent Technologies), and concentrations were quantified using quantitative real-time PCR (qPCR) with a Sequencing Library qPCR Quantification kit (Illumina Inc.). Sequencing was performed with two 36-cycle TruSeq SBS paired-end kits (Illumina Inc.) on a Genome Analyzer IIx platform (Illumina Inc.).

2.4.3 | Transcriptome assembly and functional annotation of transcripts

Raw data were filtered by quality using Phred > 20 for 70% of the read length, and adapters were trimmed using NGS QC Toolkit 2.3 (Patel & Jain, 2012). Filtered reads were de novo assembled into transcripts using the CLC Genomics Workbench (<https://www.qiagenbioinformatics.com/>). The distance between paired-end reads was set to 300–500 bp, the k-mer size was set to 45 bp, and the remaining default parameters were not changed.

Reads were mapped to the transcriptome using Bowtie1 (Langmead, Trapnell, Pop, & Salzberg, 2009), and contiguous sequences (contigs) without read-mapping support were removed from the assembly. For transcript annotation, we used BLAST + 2.2.31 (Camacho et al., 2009) with an e-value < 1e-10, using reference sequences from manually curated databases as the National Center for Biotechnology Information (NCBI) RefSeq protein and RefSeq RNA (O'Leary et al., 2016) and representative proteins and cDNA from The Arabidopsis Information Resource (TAIR; Berardini et al., 2015). We removed putative contaminant contigs from the assembly, which did not match plant sequences but showed high similarity to nonplant sequences from the NCBI's RefSeq database. Sequences were assigned to the Kyoto Encyclopedia of Genes and Genomes (KEGG) orthology (KO) identifiers using the KEGG Automatic Annotation Server (Moriya, Itoh, Okuda, Yoshizawa, & Kanehisa, 2007). Protein family domains were searched using the Pfam database and the HMMER3 alignment tool (Finn et al., 2014).

We identified putative open reading frames (ORFs) using the program TRANSDCODER (<http://transdecoder.sf.net>) with default parameters. Redundant transcripts were detected using CD-HIT-EST 4.6.1 (Li & Godzik, 2006) in the local alignment mode, with 95% identity and 70% coverage of the shortest sequence thresholds. To minimize redundancy, in the final assembly, we retained only sequences with the longest putative ORF and the longest putative noncoding transcripts from each CD-HIT-EST cluster. Functional categories were assigned to

putative coding sequences using the *Arabidopsis thaliana* association file from the Gene Ontology Consortium website (Blake et al., 2015; Figure S2).

2.4.4 | Analysis of differentially expressed transcripts (DET)

Counts of reads mapped to assembled transcripts per sequenced sample were used as input files in DET analyses. Reads that mapped to multiple transcripts were excluded. The count matrix was normalized and used to detect transcripts with significant differential expression between PA-arid and PA-humid samples from the Bragança-Ajurateua road (Figure 2) with the EdgeR Bioconductor package (Robinson, McCarthy, & Smyth, 2010) at an FDR < 0.05. Gene Ontology (GO) term enrichment analyses were performed using the goseq R Bioconductor package, which takes the length bias into account, with the default approximation method and a *p*-value cutoff set to < 0.05 (Young, Wakefield, Smyth, & Oshlack, 2010; Figure S2).

3 | RESULTS

3.1 | Population genetics analyses of *Avicennia germinans* along the equatorial coast of Brazil

Genetic diversity indices are shown in Table 2. The lowest levels of genetic variation were observed at the TMD site. The remaining sites, influenced by the northern branch of the SEC, presented considerably higher levels of diversity, including the PA-arid site, which was one of the most genetically diverse across the study region. All sampling sites deviated from Hardy-Weinberg equilibrium (HWE), with an excess of heterozygosity in all sites, except for ALC, which showed a small heterozygosity deficit (FIS = 0.03). HWE deviation was highest in TMD (FIS = -0.43) but relatively low at all remaining sites, ranging from -0.15 (PNB) to +0.03 (ALC).

A substantial genetic structure was observed over the Equatorial Brazilian coast (Figure 3). The genetic diversity, based on 2,297 genome-wide SNPs genotyped in 57 individuals, was organized into four distinct genetic clusters ($K = 4$; Figure 3a and S3a–b). The greatest genetic divergences ($F_{ST} > 0.46$; Nei's distance > 0.255) were observed between individuals from TMD and those from all other sites (Figure 3b–c; Table S1). The TMD site also presented the highest *p*A (46). Most remarkable was the divergence between individuals from PA-arid and its adjacent site PA-humid ($F_{ST} = 0.25$; Nei's distance = 0.181). This divergence was even greater than the observed divergence of PA-arid from PRC individuals (Table S1), located approximately 900 km from each other ($F_{ST} = 0.23$; Nei's distance = 0.177) on the semiarid coast of Brazil. The divergent gene pool of the PA-arid population was also evident when these samples were excluded from structure analyses. The most likely number of ancestral populations dropped from four to

TABLE 2 Genetic diversity statistics, based on analyses of 2,297 genome-wide polymorphic loci detected in 57 individuals sampled in seven sampling sites along the equatorial Atlantic coastline of South America

Sampling site	π (mean)	π (SE)	H_E	H_O	pA	%Poly	FIS	Low level (95% CI)	High level (95% CI)
MRJ	0.264	0.206	0.244	0.292	1	72.70	-0.136	-0.154	-0.102
PA-arid	0.317	0.189	0.295	0.326	1	83.76	-0.050	-0.067	-0.022
PA-humid	0.301	0.186	0.280	0.328	0	84.46	-0.113	-0.132	-0.084
ALC	0.282	0.214	0.249	0.269	0	69.83	0.030	0.009	0.070
PNB	0.208	0.210	0.195	0.234	0	58.21	-0.154	-0.180	-0.111
PRC	0.323	0.193	0.296	0.327	1	82.15	-0.035	-0.053	-0.002
TMD	0.123	0.192	0.130	0.168	46	34.52	-0.426	-0.452	-0.376

Abbreviations: π , nucleotide diversity; H_E , expected heterozygosity; H_O , observed heterozygosity; pA, private alleles; %Poly, percentage of polymorphic loci; FIS, inbreeding coefficient.

three (Figure 3c–d and S3c–d), and the overall structure remained unchanged (Figure 3d). At a finer scale, individuals from the PA-humid and MRJ sites on the AMMC seemed to be derived from the same ancestral population. This population, in turn, diverged from the population that may have given rise to individuals from the ALC, PNB, and PRC sites in Northeast Brazil (Figure 3a–b).

3.2 | De novo assembly and annotation of the *Avicennia germinans* reference transcriptome

We used RNA-Seq to sequence and de novo assemble a reference transcriptome from the leaves, stems and roots of *A. germinans* seedlings to provide a functional context for this study. A total of 249,875,572 high-quality-filtered, paired-end, 72 bp reads representing 78.25% of the raw data were used in the assembly. The reference transcriptome comprised 47,821 contigs after removing misassembled, redundant and contaminant sequences. Putative ORFs were identified in 29,854 contigs and subsequently annotated as putative protein-coding transcripts. The remaining 17,967 contigs were classified as putative noncoding transcripts. A detailed characterization of contigs can be found in Table S2.

Raw reads were mapped back to the reference transcriptome, with over 82% uniquely mapped to a single transcript and only 1.31% mapped to more than one transcript (Table S3). We found 91.74% of the plant universal orthologs from the BUSCO database (Simão, Waterhouse, Ioannidis, Kriventseva, & Zdobnov, 2015) represented in the reference transcriptome (Table S3). We also found from 20,529 to 31,348 putative orthologous sequences between the *A. germinans* transcriptome and four other publicly available transcriptomes derived from the genus *Avicennia* L. (Acanthaceae; Table S4).

As expected, most putative protein-coding transcripts of the reference transcriptome could be annotated using relevant databases (92.6%), whereas only a few putative noncoding transcripts could be annotated (33.6%; Figure S4). We found 2,207 putative coding transcripts (7.4%) and 11,925 putative noncoding transcripts (66.4%) unique to *A. germinans*, which may represent lineage-specific sequences.

3.3 | Detection of candidate loci responding to environmental selection

Genome-wide signatures of selection were detected from genotypic data retrieved from samples from all seven sites of collection. A total of 56 putative outlier loci were consistently identified by two FST outlier methods, solely based on deviations from neutral expectations of the distribution of genetic diversity. Eleven of these loci aligned to sequences in the reference transcriptome, of which eight were highly similar to proteins associated with the response or tolerance to drought in *A. thaliana* or *Sesamum indicum* (Figures 4a and S5).

The exclusion of PA-arid samples was necessary for G-E association tests due to limitations in the resolution of MARSPEC and WORLDCLIM environmental layers. From the remaining subset of 48 individuals from six sampling sites, 153 candidate loci for selection were identified by G-E correlation and two FST outlier approaches (Figure 4b). Of these candidate loci, 24 aligned to the reference transcriptome, of which 20 were putative protein-coding showing high similarity to gene models from *A. thaliana* or *S. indicum* (Figure S5).

Among all candidate loci for selection detected along the sampling region, we found 14 loci associated with plant growth and development, wood formation, cell wall metabolism, biogenesis of the photosynthetic apparatus, abiotic stress perception and response and protein protection from stress-induced aggregation, among other processes (Table 3; Figure 4a).

3.4 | Differential transcript expression analysis

Transcriptome sequencing of seedlings grown under contrasting field conditions revealed significant expression differences in 2,454 transcripts, despite previous acclimatization under homogeneous shaded, well-watered conditions (Figure 2c). Most DETs were detected in roots (2,337) and stems (1,383), followed by leaves (361; Figures 5 and S6). We refer to DETs that showed higher expression levels in samples from the PA-arid site than in samples from the PA-humid site as “DET-Arid” and to DETs showing a significantly higher

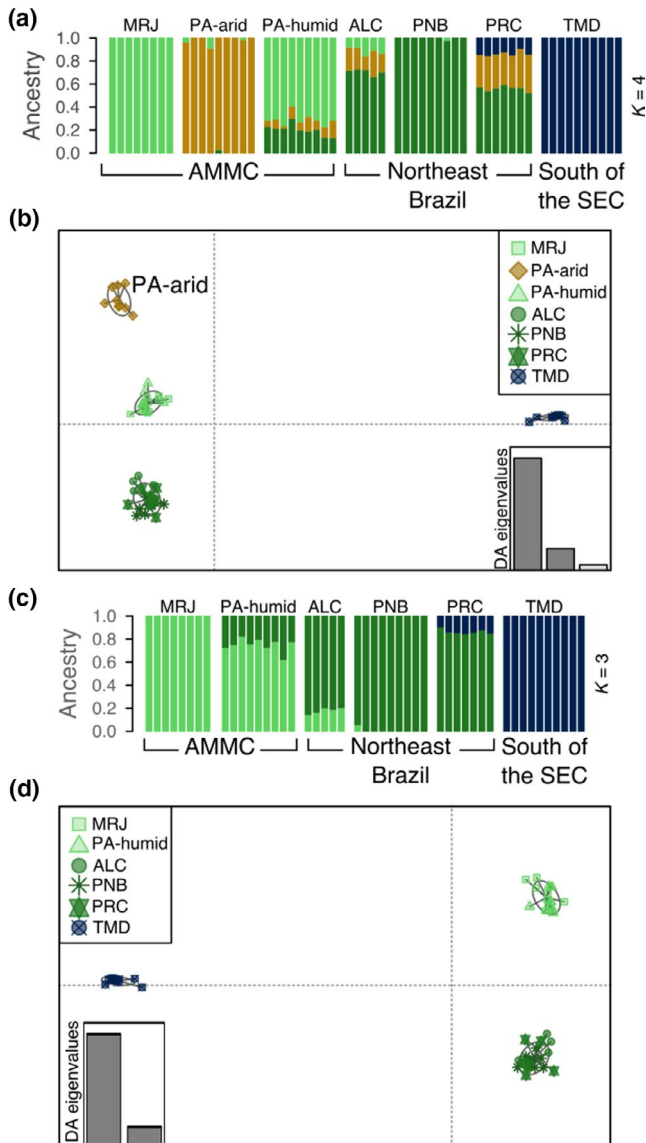


FIGURE 3 Genetic structure inferred from genome-wide single nucleotide polymorphisms (SNPs) detected in *Avicennia germinans*. (a) Attribution of ancestry implemented in the program ADMIXTURE 1.3.0 for all sampled individuals; stacked bars represent individuals, and each colour represents one ancestral cluster ($K = 4$). (b) Scatterplot of the first two principal components of the multivariate discriminant analysis of principal components (DAPC) of total genetic variance; all sampled individuals are represented as points; distinct symbols indicate sampling sites. (c) Attribution of ancestry (using ADMIXTURE 1.3.0) for all sampled individuals, excluding PA-arid samples; stacked bars represent individuals, and each colour represents one ancestral cluster ($K = 3$). (d) Scatterplot of the first two principal components of the DAPC of total genetic variance; sampled individuals, except for individuals sampled in the PA-arid site, are represented as points; and distinct symbols indicate sampling sites [Colour figure can be viewed at wileyonlinelibrary.com]

expression in samples from the PA-humid than in samples from the PA-arid site as “DET-Humid”.

The functional annotation and subsequent assignment of most putative protein-coding transcripts to GO terms (76.24%; Figure

S4) enabled the assessment of DETs that highlighted key aspects of a differential response of *A. germinans* to contrasting source environments, differing markedly in hydrological regime, soil pore water salinity, solar radiation and surface temperature (Lara & Cohen, 2006; Pranchai et al., 2017; Vogt et al., 2014). We focused this analysis on enriched biological processes previously identified to be involved in the tolerance, resistance or response to osmotic and drought stress in various crops, model and nonmodel species, including mangroves (Figure 5). A detailed characterization of the differentially expressed transcripts belonging to such processes is shown in Table S5. In the following subsections, we summarize these results.

3.4.1 | Photosynthesis

Differential transcript expression analyses revealed that photosynthesis-associated transcripts were enriched, mainly in the roots and stems from the DET-Arid set. These included putative enzymes and genes required for chloroplast biogenesis or development, for chlorophyll biosynthesis, and for the assembly of the photosynthetic apparatus and light-harvesting complexes and those involved in acclimation to fluctuating light. The DET-Arid set of root samples also presented transcripts associated with the C3 carbon fixation pathway, including glyceraldehyde-3-phosphate dehydrogenase subunits, Calvin cycle proteins, and Ribulose biphosphate carboxylase small chain 1A (RBCS1A), which showed a 7.8-fold change relative to the roots of PA-humid samples.

3.4.2 | Response to light

The DET-Arid sets of roots and stems were enriched in transcripts associated with the response to light but not directly associated with photosynthesis. These included transcripts similar to genes and transcription factors involved with light acclimation and essential for maintaining electron flow and photosynthetic efficiency under changing light conditions (Privat, Hakimi, Buhot, Favory, & Lerbs-Mache, 2003). The DET-Arid sets of roots and stems also included putative light-signalling genes and photoreceptors such as phototropins, cryptochromes and phytochromes. These photoreceptors are sensitive to light intensity and control complex light and stress responses, including photoinduced movements as well as growth and development under limiting light (Correll et al., 2003; Ohgishi, Saji, Okada, & Sakai, 2004; Pedmale et al., 2016). Complementarily, we identified transcripts similar to proteins that interact with these photoreceptors in the mediation of shade avoidance and phototropism under low light and to low-light-induced transcription factors, which regulate developmental processes and growth in response to shade avoidance. Moreover, in the DET-Arid sets of roots and stems, we found transcripts associated with chloroplast accumulation upon low blue light and with the response to sugar starvation induced by dark.

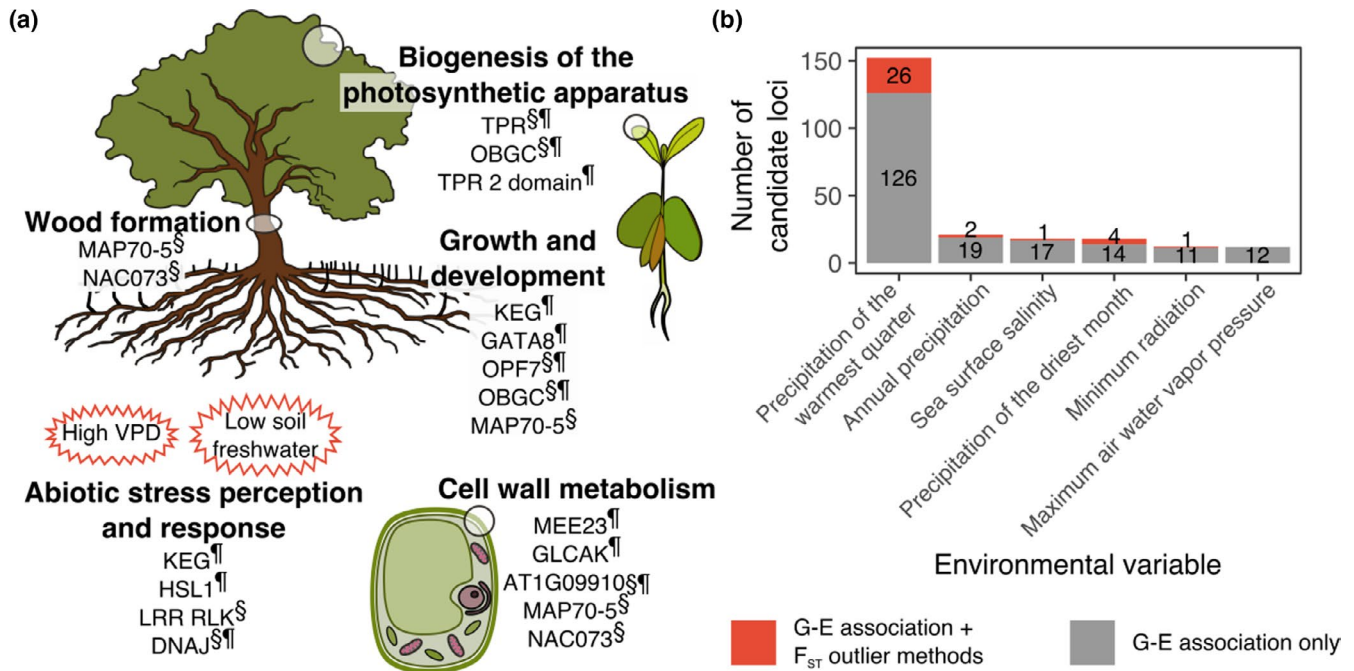


FIGURE 4 Candidate loci for selection detected from *Avicennia germinans* sampled along tropical mangrove forests of the north-northeastern Brazilian coast. (a) Schematic representation of key biological processes associated with candidate loci. *Arabidopsis thaliana* gene symbols listed below biological processes represent the functional annotation of candidate loci present within putative protein-coding regions of the genome. §Labelled gene symbols, candidates obtained using FST outlier tests performed with all sampled individuals, including PA-arid samples. ¶Labelled gene symbols, candidates obtained by combining genetic-environmental association tests and FST outlier approaches, using a subset of samples, without PA-arid ones, for which environmental characterization could not be retrieved from public databases. (b) Number of candidate SNPs associated with noncollinear environmental variables [Colour figure can be viewed at wileyonlinelibrary.com]

3.4.3 | Response to water deprivation and response to salt

The DET-Arid sets of roots and stems were also enriched in transcripts associated with the response to osmotic stress and water deprivation, including putative genes that play relevant roles in drought and salt stress resistance. For instance, we found transcripts associated with abscisic acid (ABA)-dependent stomatal closure, a well-known mechanism for maintaining water status under drought, which severely compromises growth (Murata, Mori, & Munemasa, 2015). Additionally, the DET-Arid sets of roots and stems included transcripts associated with drought-induced ion transporters, which protect chloroplasts from deleterious Na⁺ concentrations (Müller et al., 2014) and are involved in Ca²⁺ homeostasis under abiotic stress. Complementarily, we found in the DET-Arid sets of roots and stems transcripts associated with biosynthesis and accumulation of oligosaccharides, which may act as osmoprotectants, increasing tolerance to osmotic stress (ElSayed, Rafudeen, & Golladack, 2014; Nishizawa, Yabuta, & Shigeoka, 2008; Taji et al., 2002). Additionally, transcripts associated with drought-induced epicuticular wax biosynthesis, transport and deposition in cell walls were among DET-Arid sets of roots and stems. We also detected in these sets of DETs several transcripts associated with reactive oxygen species dissipation for the control of cell damage caused by drought, high light or

salt stress, including copper/zinc superoxide dismutases, a chloroplastic drought-induced stress protein (CDSP32) and MAP KINASE 6 (MAPK6).

3.4.4 | Response to heat

Transcripts similar to genes that confer tolerance to heat were enriched among the DET-Arid sets of roots and stems. We detected several putative cochaperones and chaperones, which protect proteins from structural degradation (Al-Whaibi, 2011), and transcripts associated with putative components of thermomemory, required for long-term maintenance of acquired thermotolerance (Chang et al., 2006).

3.4.5 | Photorespiration

In the DET-Arid set of roots, we detected an enrichment of putative genes associated with photorespiration, similar to enzymes participating in transamination, required for maintaining photosynthesis under photorespiratory conditions and for carbon flow during photorespiration and which catalyze the concluding reaction of photorespiration.

TABLE 3 Annotation of candidate SNP loci putatively under selection effect identified by two (LOSTAN and PCADAPT) or three (LOSTAN, PCADAPT and LEA) distinct methods from two data sets

Data set	Transcript ID	Similar to ^a	Environmental association ^b	Putative function
1	Ag_23357	AT4G28500, NAC073, NAC domain containing protein 73	NA	Regulation of the biosynthesis of cellulose and hemicellulose in wood fibers and the expression of lignin-polymerizing and signalling genes (Hussey et al., 2011; Zhong, Lee, Zhou, McCarthy, & Ye, 2008)
1	Ag_47094	AT4G17220, MAP70-5, microtubule-associated proteins 70-5	NA	Fundamental for the development of secondary cell wall band patterning in xylem tracheids and for wood formation, playing a role in the anisotropic expansion of cells (Pesquet, Korolev, Calder, & Lloyd, 2010)
1	Ag_26495	AT5G67200, Leucine-rich repeat protein kinase (LRR-RLK) family protein	NA	May play a role in hormones and abiotic stress sensing and signalling (ten Hove et al., 2011; Van der Does et al., 2017) and in regulating adaptation to salt stress (Lorenzo et al., 2009)
1	Ag_43440	Not annotated	NA	Not annotated
1 and 2	Ag_4919	AT1G09910, Rhamnogalacturonate lyase family protein	PWQ (8.31e-05)	Associated with the degradation of the cell wall polysaccharides, and pectin (McDonough, Kadirvelraj, Harris, Poulsen, & Larsen, 2004)
1 and 2	Ag_20543	AT5G18950, Tetraatricopeptide repeat (TPR)-like superfamily protein	PWQ (0.00018)	Associated with photosynthetic machinery biogenesis, stabilization and repair (Bohne, Schwenkert, Grimm, & Nickelsen, 2016)
1 and 2	Ag_5627	PREDICTED: GTP-binding protein OBG, chloroplastic [Sesamum indicum]	PWQ (8.31e-05)	Associated with thylakoid membrane biogenesis and chloroplast protein synthesis and essential for early embryogenesis in response to light stimulus (Bang et al., 2009)
1 and 2	Ag_29619	AT2G18500, OFP7, ovate family protein 7	PWQ (0.00019)	May be involved in various aspects of plant growth and participate in suppressing cell elongation (Wang, Chang, & Ellis, 2016; Wang, Chang, Guo, & Chen, 2007)
1 and 2	Ag_38780	AT2G24395, chaperone protein dnaJ-related	PWQ (8.31e-05)	Regulation of the activity of Hsp70 chaperones (Walsh, Bursac, Law, Cyr, & Lithgow, 2004) and protein protection against stress-induced denaturation (Frydman, 2001)
2	Ag_12760	AT3G54810, GATA8, Plant-specific GATA-type zinc finger transcription factor family protein	PWQ (0.00363); PDM (0.01916)	Associated with the control of plant growth and development (Manfield, Devlin, Jen, Westhead, & Gilmartin, 2006)
2	Ag_135	AT1G28440, HSL1, HAESA-like 1	PWQ (9.09e-05)	The Ag_135 transcript has a kinase domain and is associated with growth and abiotic stress signalling (ten Hove et al., 2011)
2	Ag_15249	AT2G34790, MEE23, FAD-binding Berberine family protein	PWQ (8.31e-05)	Involved in the lignification of the cell wall; mediates the oxidation of monolignols (Daniel et al., 2015) and is required for endosperm development (Pagnussat et al., 2005)
2	Ag_10980	AT5G13530, KEG, keep on going	PWQ (0.00041); PDM (0.02034)	Associated with the control of plant growth and development and critical for seedling regulation of abscisic acid perception and signalling (Stone, Williams, Farmer, Vierstra, & Callis, 2006)
2	Ag_26982	AT3G01640, GLCAK, glucuronokinase G	PWQ (0.00011)	Involved in the biosynthesis of UDP-glucuronic acid, providing nucleotide sugars for the polymerization of cell-wall compounds (Pieslinger, Hoepfner, & Tenhaken, 2010)
2	Ag_11269	AT2G25730, unknown protein	PWQ (0.00016)	The Ag_11269 transcript has a TPR 2 domain and, thus, may be involved in thylakoid membrane biogenesis and with the photosynthetic machinery stabilization and repair (Bohne et al., 2016)
2	Ag_43282	AT1G04300, MUSE13, TRAF-like superfamily protein	PWQ (8.31e-05)	MUSE13 regulates the turnover of nucleotide-binding domain and leucine-rich repeat-containing immune receptors. Loss of MUSE13 and MUSE14 leads to enhanced pathogen resistance, NLR accumulation, and autoimmunity. May regulate ATG6 ubiquitination and formation of autophagosomes (Huang et al., 2016)

(Continues)

TABLE 3 (Continued)

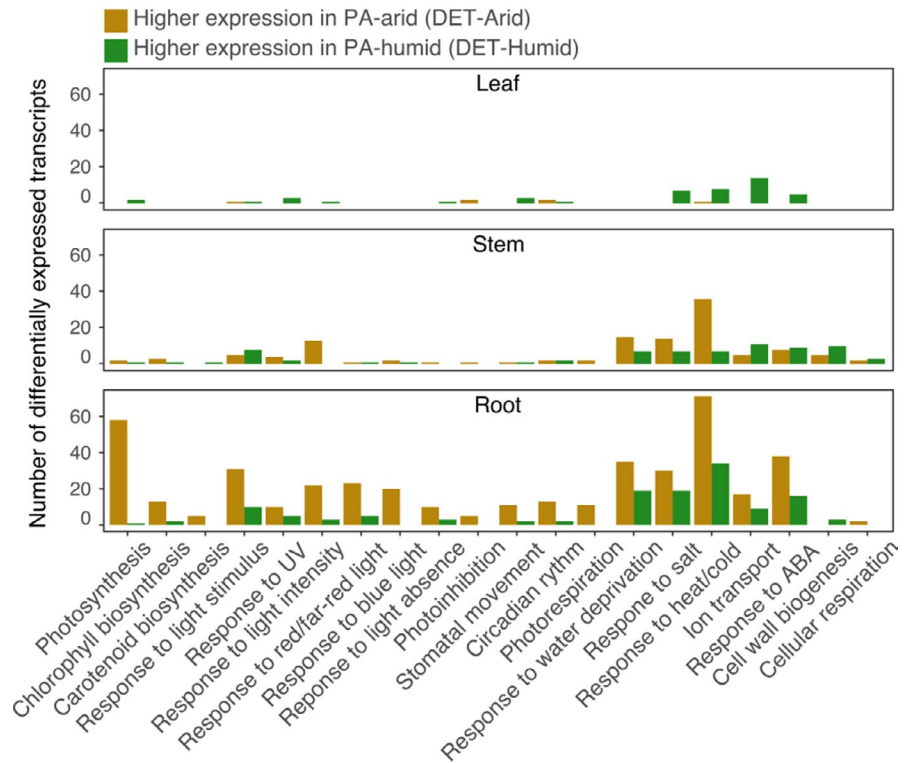
Data set	Transcript ID	Similar to ^a	Environmental association ^b	Putative function
2	Ag_14879	AT2G42520, P-loop containing nucleoside triphosphate hydrolases superfamily protein	PWQ (0.00109)	AT2G42520 has important roles in RNA metabolism such as splicing, RNA transport, ribosome biogenesis, translation and decay and unwinds double-stranded RNA molecules through the hydrolysis of NTP
2	Ag_20289	AT1G48650, DEA(D/H)-box RNA helicase family protein	PDM (0.01222); PANNUAL (5.80E-05); SSS (0.01461); WVPMAX (0.03580); RADMIN (0.03813)	AT1G48650 plays important roles in RNA metabolism such as splicing, transport, ribosome biogenesis, translation and decay; it unwinds double-stranded RNA molecules through the hydrolysis of NTP
2	Ag_24006	AT1G11050, Protein kinase superfamily protein	PWQ (0.00341); PDM (0.00671); PANNUAL (0.01962)	Ag_24006 has a protein kinase domain
2	Ag_22233	AT5G01020 Protein kinase superfamily protein	PWQ (0.00022)	Ag_22233 has a protein kinase domain
2	Ag_43463	AT5G43960, Nuclear transport factor 2 (NTF2) family protein with RNA binding (RRM-RBD-RNP motifs) domain	PWQ (0.00011)	mRNA-binding protein, controls the fate and expression of a transcript
2	Ag_36772	AT2G35680, Phosphotyrosine protein phosphatases superfamily protein	PWQ (0.00026)	AT2G35680 may possess phosphatase activity
2	Ag_13254	AT4G30100, P-loop containing nucleoside triphosphate hydrolases superfamily protein	PWQ (8.31e-05)	AT4G30100 has a hydrolase activity
2	Ag_29952	AT5G11970, Protein of unknown function (DUF3511)	PWQ (8.62e-05)	Unknown function
2	Ag_1345	AT5G55960, unknown protein	PWQ (0.00018)	Unknown function
2	Ag_43837	Not annotated	PWQ (8.62e-05)	Not annotated

Note: (1) All sampled individuals, including PA-arid samples, using FST outlier approaches and (2) a subset of samples, excluding individuals from the PA-arid site, combining genetic-environmental association tests and FST outlier tests.

^aBlastx hit to *Arabidopsis thaliana* or *Sesamum indicum* (when indicated) gene models.

^b(FDR), false discovery rate values (Benjamini-Hochberg procedure) for genetic-environment association tests; NA, genetic-environment association tests were not available for data set (1) due to unavailability of environmental data for the PA-arid sites in public databases; PWQ: precipitation of the warmest quarter; PDM: precipitation of the driest month; PANNUAL: Annual precipitation; SSS: Mean sea surface salinity; WVPMAX: maximum water vapour pressure; RADMIN: Minimum radiation.

FIGURE 5 Functional categories of differentially expressed transcripts (DETs) identified between samples grown in the PA-arid and PA-humid sampling sites. Categories were selected based on biological processes previously identified to be involved in the response, acclimation or resistance to water stress in model plants [Colour figure can be viewed at wileyonlinelibrary.com]



4 | DISCUSSION

Our study suggests that spatial variation in freshwater availability plays an important role in driving adaptation of the typically tropical and widespread tree *Avicennia germinans*. In the following subsections, we describe how variation in oceanographic and bioclimatic variables along the equatorial Brazilian coast seem to influence the organization of genome-wide genetic diversity of the species. Moreover, we show that drastic and persistent restriction in soil freshwater availability probably caused the rapid evolution of the phenotype, gene pool and gene expression profile of a recently founded population of *A. germinans*, without depletion of genetic diversity and despite the clear possibility of gene flow with an adjacent, unchanged population.

4.1 | Gradual environmental variation in freshwater availability may partly explain the organization of non-neutral genetic variation in *A. germinans*

The genetic structure inferred by genome-wide SNPs (Figure 3) suggested the importance of both neutral and nonneutral environmental drivers of variation. The greatest divergence was observed between samples influenced by the northern and southern branches of the South Equatorial sea current (SEC; Figure 1) acting on the Atlantic coast of South America, corroborating previous results found for other coastal trees using putatively neutral molecular markers (Francisco, Mori, Alves, Tambarussi, & de Souza, 2018; Mori, Zucchi, Sampaio, et al., 2015; Takayama, Tateishi, Murata, & Kajita, 2008). The

flow direction of coastal currents provides a neutral explanation for the structure of genome-wide diversity of sea-dispersed species due to restrictions in the dispersal of buoyant propagules, probably facilitating the accumulation of random genetic divergences (Francisco et al., 2018; Mori, Zucchi, & Souza, 2015; Wee et al., 2014; Yan, Duke, & Sun, 2016). Our results also suggest that the lower precipitation of the warmest quarter in northern sites (AMMC and Northeast Brazil regions; Figure 1) may play an additional, nonneutral role in shaping this north-south divergence. We hypothesize that the more even distribution of rainfall throughout the year in the TMD site probably alleviates water stress in *A. germinans*, whereas limited rainfall in the warmest quarter of northern regions increases soil aridity and salinity, reducing opportunities for rehydration and potentially favouring increased drought tolerance. It is plausible that this environmental filter contributes to the genetic divergence observed between TMD and remaining populations (Figure 3). This hypothesis is corroborated by the detection of 26 loci candidate for selection correlated to the spatial variation in the precipitation of the warmest quarter over the study area (Figure 4b). Although most of these loci were poorly characterized, hampering inferences about their functional relevance in the environmental context, we were able to associate 11 candidates with biological processes influenced by drought, such as photosynthesis, cell wall metabolism, cell elongation, plant growth, protein protection from stress-induced degradation and regulation of ABA signalling (Table 3; Figure 4). The adaptive importance of freshwater limitation in tropical trees was also suggested in the mangrove *A. schaueriana* (Cruz et al., 2019) and in tropical forests and savannas (Ciemer et al., 2019), for which it was similarly suggested that an environmental filtering mechanism driven by rainfall variability probably favoured the survival of more drought resistant lineages.

Although *Avicennia* propagules can remain viable for long periods and present transoceanic dispersal (Mori, Zucchi, Sampaio, et al., 2015), at a finer scale, we observed a genetic divergence between sites in the AMMC region (MRJ and PA-humid) and Northeast Brazilian mangroves (ALC, PNB and PRC; Figure 3). The AMMC region shows higher annual precipitation and is more strongly influenced by riverine freshwater inputs than the remaining sites due to its closer proximity to the Amazon River Delta (Figure 1). Conversely, the northeast Brazilian coastline is characterized by reduced rainfall and the lack of riverine freshwater inputs (Figure 1), which limit plant access to soil freshwater due to increased salinity, potentially contributing to the local specialization of individuals. In fact, we were able to detect two loci associated with sea surface salinity data and total annual precipitation based on both FST outlier methods and G-E association tests (Figure 4b). These loci showed similarity to a poorly characterized protein kinase and to an RNA hydrolase (Table 3). Given the unclear functional relevance of these putative adaptive loci in the environmental context, we recommend future efforts to analyze the role of demographic history in *A. germinans* to find additional explanations for the genetic structure observed along the equatorial Brazilian coastline (Figure 3). Additionally, candidate loci detected in our study may play a role in drought adaptation, but their molecular functions need to be further characterized in plants adapted to physiological drought. The functional role of these proteins in drought tolerance may not be highlighted in genetic screenings performed on drought-sensitive model plants distantly related to *A. germinans*.

4.2 | Rapid evolution of *A. germinans* in response to abrupt limitation in access to soil freshwater

The PA-arid population of *A. germinans* originated after 1974, when the construction of the Bragança-Ajuruteua road altered the hydrology of part of the mangrove forest in the AMMC region (Figure 2a–b; Cohen & Lara, 2003). This sudden environmental change caused a large dieback of the mangrove vegetation, followed by a gradual recolonization, mainly by *A. germinans*. We estimate that the time since this event occurred could represent at most 40 reproductive cycles of *A. germinans*, based on previous observations of closely related *Avicennia* spp. (Almahasheer et al., 2016; Cruz et al., 2019), although a conservative estimate could be four generations (Polidoro et al., 2010). The modification of the architecture of recolonizing individuals of PA-arid, from the previous tall/arboreal to the now dwarf/shrub (Figure 2b; Pranchai et al., 2017), suggests that limitation in plant access to soil water favoured smaller tree sizes, one of the most integrative characteristics of drought resistance (Bennett et al., 2015; Corlett, 2016; Rowland et al., 2015). We could not determine how much of the observed difference in tree size is inherited or determined by phenotypic plasticity; however, our results revealed a substantial change in genome-wide allele frequencies of trees, which probably represent samples of the first generations of the recolonizing population (Table 2; Figure 3a). Interestingly, changes in allele frequencies at founding occurred without reductions in levels

of diversity, as estimated by various population genetics parameters (Table 2). Moreover, consistent with an adaptive response to selection and probably driven by restrictions in plant access to soil freshwater, FST outlier tests detected 56 candidate loci, of which eight were present within transcripts associated with functions such as suppression of cell elongation, wood and xylem tracheid formation, photosynthetic machinery biogenesis and repair, regulation of adaptation to stress and protection from stress-induced protein degradation (Table 3; Figure 4a). The simultaneous presence of high levels of diversity in the founding population (Table 2) and strong selection of individual traits is common in natural populations and represents a signature of substantial multivariate genetic constraints (Walsh & Blows, 2009), as expected in this specific case given the magnitude of environmental change.

A surprising aspect of the population genetic analyses, however, was that genome-wide changes in allele frequencies occurred despite expectations of high levels of gene flow, given the proximity (<10.0 m) of contrasting populations and characteristics of the species, which is preferably allogamous, with entomophilous flowers (Mori, Zucchi, & Souza, 2015; Nadia et al., 2013; Nettel-Hernanz et al., 2013). In the absence of a geographic barrier to gene flow, recombination is expected to hinder adaptation unless there is very strong selection or reproductive isolation (Kawecki & Ebert, 2004; Mayr, 1963). As we expect selection to be very strong in this case, due to extreme environmental change, it could be enough to counteract the homogenizing effect of gene flow or even to cause shifts in the timing of flowering (Cho, Yoon, & An, 2017), which may result in reproductive isolation.

In addition to genetic changes, we also identified transcript expression differences between seedlings from PA-arid and PA-humid sites after acclimatization in pots under shaded, well-watered conditions (Figures 2c and 5), indicating an additional molecular basis for phenotypic divergences. Because differential gene expression influences trait variation (Wolf, Lindell, & Backstrom, 2010), it can be substantial between distinct locally adapted populations (Akman, Carlson, Holsinger, & Latimer, 2016; Gould, Chen, & Lowry, 2018). Differential expression was observed mainly in the roots and stems of *A. germinans* seedlings and was functionally associated with differences in freshwater availability between source sites. Mangrove roots are the first organs exposed to increased salinity and water deficits; thus, osmotic stress sensing and signalling to the whole plant are triggered (Chaves, Maroco, & Pereira, 2003; Janiak, Kwaśniewski, & Szarejko, 2016). Moreover, these roots are photosynthesizing, contributing to carbon gain and enabling respiration in anaerobic soils, using both atmospheric and photosynthetically regenerated oxygen (Kitaya et al., 2002). Interestingly, functional categories associated with the detected transcriptome changes in PA-arid seedlings included various biological processes previously identified to be involved in central aspects of osmotic, heat and UV stress in model and nonmodel plants (Ding et al., 2013; Fan et al., 2018; Zhang et al., 2015). Being sessile under extreme environmental conditions, PA-arid plants should need to rapidly adjust gene expression to intermittent freshwater availability (Chaves, Flexas, &

Pinheiro, 2009; Urban, Aarouf, & Bidel, 2017). Thus, the increased freshwater supply and the restriction in solar irradiance, to which samples from the degraded PA-arid site were subject under the experimental conditions (Figure 2c), probably required seedlings to broadly adjust their photosynthetic machinery. Conversely, although environmental conditions were relaxed in the RNA-Seq experiment, we observed several transcripts associated with high temperature, drought and salinity response among DET-Arid, including photorespiration, epicuticular wax and cutin synthesis, export and deposition, accumulation of osmoprotectants and antioxidants and ion homeostasis. By using a common garden to control the environmental variation during 72 hr (Figure 2c), we maximized the chances of finding differences in gene expression caused by inheritable variations at the genetic or epigenetic levels in individuals from different source populations (Hatorangan, Laenen, Steige, Slotte, & Köhler, 2016; Savolainen, Lascoux, & Merilä, 2013). Although we cannot rule out that the observed gene expression differences were caused by epigenetic modifications acquired during germination or growth (i.e., before the experiment) or by maternal inheritance, we expect them to be at least partly inheritable. Thus, we suggest that these findings can potentially represent regulatory mechanisms by which PA-arid plants tolerate a predominantly warm, highly saline and dry environment with extreme UV levels. These strategies may have enhanced the relative capacity of PA-arid seedlings to grow under a high leaf water-deficit status and infrequent freshwater inputs.

We suggest that future efforts to understand the molecular mechanisms of adaptation of the PA-arid population should focus on characterizing epigenetic differences between the PA-arid and PA-humid sites. Epigenetic changes can emerge faster than adaptive genetic changes, also playing an important role in the early adaptive process (Kenkel & Matz, 2016; Pavey, Nosil, & Rogers, 2010). Although the mechanisms are not entirely clear, our results obtained through independent molecular approaches suggest that a rapid evolution of this population occurred with gene expression and genetic changes. The desertification of the PA-arid site represents a drastic and persistent environmental change, analogous to a freshwater exclusion experiment for mangroves of the tropical Brazilian coastline, in which this limitation probably caused the rapid evolution of recolonizing *A. germinans* individuals. Although it is difficult to demonstrate rapid evolution in nature, there is growing empirical evidence that when environmental selection is very intense, evolutionary processes may occur on a very fast time scale (Amorim et al., 2017; Donihue et al., 2018; Schoener, 2011), as observed in this case.

4.3 | Implications for conservation

Wetlands of the northeastern coast of Brazil and the ecosystem services they provide are predicted to be particularly impacted by future reductions in precipitation and freshwater availability (Osland et al., 2018). Despite these threats, the extant gene pool of northern populations of *A. germinans* seems to harbour sufficient diversity

to enable species persistence through natural selection of drought-resistant plants, as observed in the case of PA-arid. Recolonizing individuals in this location share alleles with individuals from distant areas, such as the PRC and ALC sites (Figure 3a) in Northeast Brazil, suggesting that these forests might have contributed as a source of adaptive variation through sea-dispersed propagules in the recolonization of the PA-arid site in addition to the selection of migrants from adjacent areas.

In the context of an increasingly drying climate, reforestation plans for populations of *A. germinans* located south of the SEC should consider using mixed stocks of seedlings (Moritz, 1999) to introduce genetic variation associated with increased drought tolerance present in Northeastern mangroves while also maintaining local alleles, possibly associated with site-specific environmental characteristics.

In conclusion, we provide novel insights into how limited access to soil freshwater can change allele frequencies, gene expression and phenotypes of a dominant tropical tree. These findings are consistent with the predictions of a hypothesis of natural selection, although we acknowledge that other evolutionary processes could also play a role in driving the observed patterns, opening new opportunities for future investigation. Research on the genomic basis of tree adaptation is often limited by difficulties in implementing empirical tests, given their long generation time and the scarcity of basic biological information. In tropical forests, mostly found in developing countries, the lack of resources imposes additional challenges for adaptation studies. Advances in the understanding of the genomic basis of drought tolerance in tropical trees can support effective protection plans and mitigate climate change. As shown in this study, such knowledge can improve predictions of the persistence of the ecosystems they form and services they provide and generate key insight for conservation and management efforts (Holliday et al., 2017; Moran, Hartig, & Bell, 2016).

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AUTHOR CONTRIBUTIONS

A.P.S., G.M.M. and M.V.C. designed the study. M.V.C. and G.M.M. conducted fieldwork, prepared samples for sequencing and wrote the manuscript. M.V.C., G.M.M., R.S.O., M.D. and D.H.O. analyzed the RNA-Seq and nextRAD results. A.P.S., M.I.Z., and G.M.M. contributed material/reagents/analytical tools. All authors discussed the results and contributed to the manuscript.

DATA AVAILABILITY STATEMENT

Gene expression data and transcriptome sequences that support conclusions have been deposited in GenBank with the accession code GSE123659.

SNP genotypes are available at Dryad <https://doi.org/10.5061/dryad.pk0p2nchr> (Cruz et al., 2019).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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