Title:

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

Short running title:

Black-Mangroves responses to water limitation

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Abstract

Environmental variation along the geographical space can shape populations by natural selection. In the context of global warming, accompanied by substantial changes in 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*). Fifty-seven trees were sampled in seven sites differing markedly on precipitation regime and riverine freshwater inputs. Using 2,297 genome-wide single nucleotide polymorphic markers, we found signatures of natural selection by the genotype association with the precipitation of the warmest quarter and the annual precipitation. We also found candidate loci for selection, based on statistical deviations from neutral expectations of interpopulation differentiation. Most candidate loci present within coding sequences were functionally associated with central aspects of drought-tolerance or plant response to drought. Complementarily, our results strongly suggest the occurrence of a rapid evolution of a population in response to sudden and persistent limitation in plant access to soil water, following a road construction in 1974. Observations supporting rapid evolution included reduction in tree size and changes in the genetic profile and in transcripts expression levels associated with increased drought-tolerance, through accumulation of osmoprotectants and antioxidants, biosynthesis of plant cuticle, proteins protection against stress-induced degradation, stomatal closure, photorespiration and photosynthesis. We describe a major role of spatial heterogeneity in freshwater availability in the specialization of the typically tropical tree, *A. germinans*.

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

1. Introduction

Natural ecosystems are characterized by wide environmental heterogeneity over the geographical space. These spatial variations in several abiotic conditions can shape populations specializations, particularly in widespread species, through changes in genotypes and phenotypes frequencies (Kawecki & Ebert, 2004). Environmental gradients are, therefore, natural laboratories for the study of environmental selection (De Frenne et al., 2013). As sessile organisms, plants are incapable of escaping from unfavorable conditions and, therefore, 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 nutrients availability. Among the various environmental factors that determine adaptive phenotypic and genotypic diversity in plants, freshwater availability has a prominent role (Choat et al., 2018; Phillips et al., 2010). Accordingly, populational differentiation in drought-tolerance has been widely identified in various studies, providing valuable insights on 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). Understanding the role of selection by heterogeneity in freshwater availability in plants diversification is increasingly relevant, as predictions indicate that we will face a warmer future accompanied by marked changes in rainfall and higher frequency of extreme climatic events in several regions worldwide (Dai, 2011; IPCC, 2014; Rodell et al., 2018). The combination of high atmospheric temperature with reduced rainfall and air

humidity represent a major threat to plants, especially tree species and forest ecosystems they form (Allen, Breshears, & McDowell, 2015; Allen et al., 2010; Asner et al., 2016; Choat et al., 2012; McDowell & Allen, 2015). These changes influence major components of resource-use in plants: it reduces the soil water potential, which limits the water and nutrients supply to leaves, and raises the air vapor pressure deficit (VPD), increasing 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), 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 a great interest in understanding genotypic and phenotypic basis of trees resistance to drought, as these mechanisms are key to improve predictions of environmental consequences of extreme events and to elaborate 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, McDowell, Allen, & Anderson-Teixeira, 2015; Brodribb, Holbrook, Edwards, & Gutiérrez, 2003; Hacke, Sperry, Wheeler, & Castro, 2006; Phillips et al., 2010; 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 disproportionally to 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). The species is particularly suitable for the study of mechanisms involved in trees adaptation to drought, since it is the most widespread mangrove in the Atlantic East-Pacific biogeographic region (Ellison, Farnsworth, & Merkt,

1999), belonging to the most tolerant mangrove genus to limiting conditions for water acquisition, as drought, freezing and salinity (Pranchai et al., 2017; Reef & Lovelock, 2015; Stuart, Choat, Martin, Holbrook, & Ball, 2007). These trees occur naturally under highly variable soil water potential and air VPD, caused by daily and seasonal fluctuations in temperature, air humidity, freshwater inputs and soil salinity (Tomlinson, 1986). In tropical arid zones or during dry seasons, VPD and soil salinity can reach extreme levels, hindering the maintenance of water and ion homeostasis and thus limiting carbon gain and plant growth (Clough, Sim, Inlet, Bay, & Rivers, 1989; Lin & Sternberg, 1992).

We hypothesized that spatial heterogeneity in freshwater availability shapes adaptive variation in allele frequencies and in expression profiles of transcripts associated with the response to and tolerance of limiting freshwater in tropical trees. We sampled *A. germinans* individuals along a wide longitudinal range in an equatorial region (from 0°43'12" S to 8°31'48" S of latitude), showing narrow spatial heterogeneity in temperature and solar radiation, 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: (1) based on the identification of loci deviating from neutral models of interpopulational genetic variation (F_{ST} outlier tests) and (2) 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 highly contrasting soil freshwater availability and salinity 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 suggesting the rapid evolution of a population, with changes in phenotype and in the genetic profile, despite clear possibility of gene flow. 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 area

Sampling sites were located over more than 1,800 km of the north-northeast Brazilian coastline, between 0.724° S and 8.526° S of latitude, along a spatial gradient in freshwater availability (Table 1, Figure 1). We classified this area into three distinguishable regions, based on rainfall regimes and riverine freshwater inputs: (1) the Amazon Macrotidal Mangrove Coast (AMMC), the world's largest continuous mangrove belt (Nascimento Jr., Souza-Filho, Proisy, Lucas, & Rosenqvist, 2013), which has a mean annual precipitation above 2,000 mm yr^{-1} and is influenced by the mouth of the Amazon River; (2) mangroves of Northeast Brazil, which show limited forest development (Schaeffer-Novelli, Cintrón-Molero, Adaime, & de Camargo, 1990) and is characterized by the lack of riverine freshwater inputs and a mean annual precipitation below 2,000 mm yr^{-1}, with pronounced and long dry seasons, and less than 30 mm of precipitation in the driest quarter; and (3) a region influenced by the southward-flowing branch of the South Equatorial coastal current (SEC), characterized by

reduced riverine freshwater inputs, mean annual precipitation below 2,000 mm yr^{-1}, but less pronounced dry season, showing more than 100 mm of precipitation in the driest quarter.

In the AMMC, two adjacent sites require a more detailed description, both of which are located in the peninsula of Ajuruteua, state of Pará, between the Maiaú and Caeté estuaries (Figure 2). These sites were previously covered by a preserved mangrove forest (Cohen & Lara, 2003) and were divided by the construction of a road, in 1974. The hydrology part of the forest was dramatically changed, no longer being influenced by the Caeté River. Instead, it started flooding exclusively during the highest spring tides of the Maiaú River. These changes resulted in the local forest dieback and subsequent recolonization, mainly by *A. germinans*. Soil pore water salinity accumulated to extremely high levels (100 ppt at a 50-cm depth), and the air surface temperature frequently exceeds 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 to the former tall morphology (up to 30 m in height), still observed on surrounding areas, where the hydrology remained unaltered (Menezes, Berger, & Mehlig, 2008) (Figure 2). Throughout this work, we refer to the western side of the road as ‘PA-arid’ and to the eastern side as ‘PA-humid’.

2.2 DNA extraction and sequencing

Leaves from 57 adult *A. germinans* trees were sampled in 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 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 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 (Supplementary Figure S1).

2.3 SNP detection and genotyping

Genotyping-by-sequencing used custom scripts (SNPsaurus, LLC) to create a reference catalog of abundant reads. Read mapping to the catalog allowed two mismatches. Biallelic loci present in at least 10% of samples were selected for the following 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 8x and a minor allele frequency ≥0.05 (Supplementary Figure S1). 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.

2.4 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), loci presenting private alleles (pA), observed (H_O) and expected (H_E) heterozygosities, using poppr 2.8.2 (Kamvar, Tabima,

& Grünwald, 2014), and the percentage of polymorphic loci (%Poly), using adegenet 2.1.1 (Jombart & Ahmed, 2011). We also estimated the pairwise genetic differentiation among populations (F_{ST}), the inbreeding coefficient (F_{IS}) and its 95% confidence interval through 1000 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, the discriminant analysis of principal components (DAPC) (Jombart, Devillard, & Balloux, 2010), and ADMIXTURE 1.3.0 (Alexander, Novembre, & Lange, 2009). For DAPC, we considered numbers of groups from 1 to 50 and the Bayesian information criteria to determine the number of groups (K) and used the optim.a.score function to avoid overfitting during discrimination steps. 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). Environmental data consisting of bioclimatic and oceanographic variables were retrieved from the public databases WorldClim (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005) (15 precipitation, radiation and air vapor pressure layers) and Marspec (Sbrocco & Barber, 2013) (four sea surface salinity and temperature layers). To minimize the interdependence of variables, we used a correlation threshold of 0.8 (Supplementary Figure S2). Because the environmental data obtained for PA-arid could not be distinguished from those obtained for

PA-humid with the highest-resolution WorldClim and Marspec layers, despite their clear abiotic divergence (Figure 2), we excluded PA-arid individuals in LEA analysis.

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, which are solely based on deviations from neutral expectations of allele frequency distributions, regardless of environmental variation data across sampling sites (F_{ST} outlier tests). With Pcadapt 2.0 (Luu, Bazin, & Blum, 2016), population structure is determined by a principal component analysis. A disproportional relation to this structure (FDR < 0.05) is 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 F_{ST} and H_E to describe the neutral distribution under an island migration model. Hence, we detected loci with excessively high or low F_{ST}. Because Lositan may show partially divergent results among independent simulations, we only considered candidate loci conservatively identified in three independent simulations assuming an infinite allele model of mutation, with a confidence interval of 0.99 and a FDR < 0.05, using the neutral mean F_{ST} and forcing the mean F_{ST} options.

Demographic histories can affect the statistical power of tests of selection (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 the distinct methods used for two datasets: (1) the entire genotypic dataset, including PA-arid individuals, using Lositan and Pcadapt consensus candidates and (2) a subset of genotypic data with environmental information, which excluded the PA-arid samples, using Lositan, Pcadapt and LEA consensus candidates (Supplementary Figure S1).

2.5 Functional annotation of candidate loci putatively under natural selection

We functionally annotated candidate loci through the reciprocal nucleotide alignment between nextRAD sequences and the reference transcriptome 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 (Supplementary Figure S1).

2.6 Plant material for transcriptome assembly and differential expression analysis

On August 16th, 2013, three similarly sized seedlings of *A. germinans* with 5-9 stem nodes were collected from the PA-arid and PA-humid sites, separated by the Bragança-Ajuruteua road (Figure 2), and transplanted with the surrounding soil into 3.0-L pots. For acclimation in a homogeneous environment, pots were placed in open-air and naturally shaded conditions and were watered daily after the sunset, with 300 mL of tap water. Plants were harvested at noon, after 72 hours of acclimation, washed with water and split into roots, stems and leaves with a sterile blade to be stored in RNAlater (Ambion Inc., Austin, TX, USA) and transported to the laboratory for RNA extraction (Figure 3).

2.7 RNA extraction, cDNA library preparation and RNA sequencing

RNA extraction was performed according to Oliveira, Viana, Reátegui, & Vincentz (2015). To assess purity, integrity and concentration, we used 1% agarose gel electrophoresis and a NanoVue spectrophotometer (GE Healthcare Life Sciences, Buckinghamshire, UK). Subsequently, cDNA-enriched libraries were constructed using TruSeq RNA Sample Preparation kits (Illumina Inc., California, USA). Libraries qualities were assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, California, USA) 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.).

2.8 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 (KAAS) (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 TransDecoder (http://transdecoder.sf.net) with default parameters. Redundant transcripts were detected using Cd-hit-est 4.6.1 (W. 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 non-coding transcripts from each Cd-hit-est clusters. Functional categories were assigned to putative coding sequences using the *Arabidopsis thaliana* association file from the Gene Ontology Consortium website (Blake et al., 2015) (Supplementary Figure S1).

2.9 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-Ajuruteua road (Figures 2 and 3) with the EdgeR Bioconductor package (Robinson, McCarthy, & Smyth, 2010) at a FDR <0.05. Gene Ontology (GO) term enrichment analyses were performed using the goseq R Bioconductor package (Young, Wakefield, Smyth, & Oshlack, 2010), which takes the length bias into account, with the default Wallenius approximation method and a p-value cutoff set to <0.05 (Supplementary Figure S1).

3. Results

3.1 Population genetics analyses of **A. germinans** *along the equatorial coast of Brazil*

Population genetics statistics are shown in Table 2. The lowest levels of genetic diversity were observed in the TMD site, as estimated by the mean π (0.123), H_O (0.130), H_E

(0.168) and %Poly (34.52%). The remaining sites, influenced by the northern branch of the SEC, presented considerably higher levels of diversity (mean π ranging from 0.208 to 0.323; H_E, from 0.195-0.296; H_O, from 0.234-0.328; %Poly 58.31-84,46%), including the PA-arid site, 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, besides ALC, which showed a small heterozygosity deficit (F_{IS}=0.03). HWE deviation was highest in TMD (F_{IS}=-0.43) but relatively low in all remaining sites, ranging from -0.15 (PNB) to +0.03 (ALC).

A substantial genetic structure was observed over the Equatorial Brazilian coast, a region subject to highly variable rainfall and riverine freshwater inputs (Figure 1). The genetic diversity, based on 2,297 genome-wide SNPs genotyped in 57 individuals, was organized into four distinct genetic clusters (K=4) (Figure 4a, Supplementary Figure 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 4b-c, Supplementary Table S1). The TMD site also presented the highest number of private alleles (pA=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 greater than the observed divergence of PA-arid from PRC individuals (Supplementary Table S1), located approximately 900 km distant from each other (F_{ST}=0.23; Nei's distance=0.177), on the semi-arid 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 three (Figure 4c-d, Supplementary Figure S3c-d), and the overall structure remained unchanged (Figure 4d). At a finer scale, individuals from PA-humid and MRJ sites, on the Amazon Macrotidal Mangrove Coast (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 ALC, PNB, and PRC sites, in Northeast Brazil (Figure 4a-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 leaves, stems and roots of *A. germinans* seedlings, providing 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 was used in the assembly. The reference transcriptome comprised 47,821 contigs, after removal of misassembled, redundant and contaminant sequences. Putative ORFs were identified in 29,854 contigs, subsequently annotated as putative protein-coding transcripts. The remaining 17,967 contigs were classified as putative non-coding transcripts. A detailed characterization of contigs can be found in Supplementary 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 (Supplementary 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 (Supplementary 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) (Supplementary 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 non-coding transcripts could be annotated (33.6%) (Supplementary Figure S5). We found 2,207 putative

coding transcripts (7.4%) and 11,925 putative non-coding 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 (Figure 4a-b). Fifty-six putative outlier loci were consistently identified by two F_{ST} 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* (Supplementary Figure 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, further 153 candidate loci for selection were identified by G-E correlation and two F_{ST} outlier approaches. Out 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* (Supplementary 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 (Figure 5, Table 3). A complete characterization of candidate loci within putative coding sequences is available in Supplementary Table S5.

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 3). Most DETs were detected in roots (2,337) and in stems (1,383), followed by leaves (361) (Supplementary Figure 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 DET showing a significantly higher 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%) (Supplementary 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 and surface temperature (Lara & Cohen, 2006; 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 non-model species, including mangroves (Figure 6).

3.4.1. Photosynthesis

Mangroves have aerial, photosynthesizing roots, which contribute to carbon gain and enable root respiration in anaerobic soils, using both atmospheric and photosynthetically regenerated oxygen (Kitaya et al., 2002). Transcripts associated with photosynthesis were enriched in roots from the DET-Arid set. These transcripts included putative enzymes required for chloroplast biogenesis or development, such as HEAT SHOCK PROTEIN 90-5 (HSP90-5) (Oh, Yeung, Babaei-Rad, & Zhao, 2014), the THYLAKOID FORMATION 1

(THF1) (Q. Wang et al., 2004), RNA POLYMERASE SIGMA SUBUNIT 2 (SIGB) (Shirano et al., 2000) and TRANSLOCASE SUBUNIT SECA1 (SECA1), which is also involved in acclimation to fluctuating light (Liu et al., 2010). Additionally, transcripts supposedly encoding several subunits of the photosynthetic apparatus and light-harvesting complexes were identified in the DET-Arid set. These DETs included photosystem II (PSII) subunits PSBX, PSBO-2, PSBQ-2, PSBR, PSBP-1, PSBY, PSII REACTION CENTER W (PSBW) and CHLOROPHYLL A-B BINDING (PSBS); photosystem I (PSI) subunits PSAD-2, PSAE-2, PSAF, PSAG, PSAH-2, PSAL, PSAO, and PSI REACTION CENTER PSI-N (PSAN); ATP SYNTHASE DELTA-SUBUNIT (ATPD); all subunits from the PSII light-harvesting complex (LHCA1-5) and most subunits from PSI (LHCB1-6). The DET-Arid set of roots also included transcripts associated with the C3 carbon fixation pathway, for instance, GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE, subunits A and B (GAPA1, GAPB) and their activator, THIOREDOXIN F-TYPE 1 (TRXF1) (Marri et al., 2009); two CALVIN CYCLE PROTEINS (CP12-2, CP12-3), PHOSPHOENOLPYRUVATE CARBOXYLASE 4 (PPC4), PYRUVATE ORTHOPHOSPHATE DIKINASE (PPDK), RIBULOSE BISPHOSPHATE CARBOXYLASE SMALL CHAIN 1A (RBCS1A) and its activator, RUBISCO ACTIVASE (RCA); and two FRUCTOSE-1,6-BISPHOSPHATASES (CFBP1, CYFBP). Additionally, transcripts similar to chlorophyll biosynthesis enzymes were enriched in the DET-Arid set, as ISOPENTENYL DIPHOSPHATE ISOMERASE 1 (IPP1), MAGNESIUM-CHELATASE 12 (CHLI2) and the subunit CHLH (GUN5), DICARBOXYLATE DIIRON PROTEIN (CRD1), the PROTOCHLOROPHYLLIDE OXIDOREDUCTASE C (PORC) and GLUTAMYL-TRNA REDUCTASE (HEMA1).

3.4.2. Response to light

The DET-Arid set was enriched in transcripts associated with the response to light but not directly associated with photosynthesis. These included transcripts associated with light acclimation, similar to PSII dephosphorylating PROTEIN PHOSPHATASE 2C (PBCP) (Samol et al., 2012) and RNA POLYMERASE SIGMA FACTOR A (SIGA), which are essential for maintaining electron flow and photosynthetic efficiency under changing light conditions (Privat, Hakimi, Buhot, Favory, & Lerbs-Mache, 2003). The DET-Arid set also included putative light-signaling genes and photoreceptors, as the blue light photoreceptors PHOTOTROPINS 1 and 2 (PHOT1, PHOT2), CRYPTOCHROME 1 (CRY1) and the red/far-red photoreceptors PHYTOCHROME E and A (PHYE, PHYA). 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. For instance, B-BOX DOMAIN PROTEIN 24 (STO), CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) and SPA PROTEINS (SPA2, SPA4) (Crocco et al., 2015; Holtkotte, Ponnu, Ahmad, & Hoecker, 2017; Indorf, Cordero, Neuhaus, & Rodríguez-Franco, 2007; Pacín, Semmoloni, Legris, Finlayson, & Casal, 2016; Pedmale et al., 2016). Furthermore, we identified DETs similar to the low light-induced transcription factors HOMEOBOX PROTEIN 1 and 52 (HB1, HB52), regulatory of developmental processes (Henriksson et al., 2005); the PSEUDO-RESPONSE REGULATOR 5 (PRR5) (Takase, Mizoguchi, Kozuka, & Tsukaya, 2013), which regulates growth in the shade avoidance response; and a transcript associated with chloroplast accumulation upon low blue light, J-DOMAIN PROTEIN REQUIRED FOR CHLOROPLAST ACCUMULATION RESPONSE 1 (JAC1) (Suetsugu, Kagawa, Wada, &

Corporation, 2005). Remarkably, in the DET-Arid set, we detected putative proteins required during sugar starvation induced by dark, namely, THIAMIN DIPHOSPHATE-BINDING FOLD PROTEIN (THDP-binding), 2-OXOACID DEHYDROGENASES ACYLTRANSFERASE (BCE2), GLUTAMINE-DEPENDENT ASPARAGINE SYNTHASE 1 (DIN6) and TRANSKETOLASE (DIN4) (Fujiki, Ito, Itoh, Nishida, & Watanabe, 2002; Fujiki, Ito, Nishida, & Watanabe, 2000, 2001).

3.4.3. Response to water deprivation and response to salt

The DET-Arid sets of roots and stems were 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 example, various transcripts associated with the positive regulation of abscisic acid (ABA)-dependent stomatal closure, such as LIPASE/LIPOOXYGENASE PLAT/LH2 (PLAT1) (Hyun et al., 2014); THIAZOLE BIOSYNTHETIC ENZYME (THI1) (C.-L. Li et al., 2016); CBL-INTERACTING PROTEIN KINASE 1 (CIPK1) (D'Angelo et al., 2006); ZEAXANTHIN EPOXIDASE (ZEP) (Park et al., 2008); the CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) (Moazzam-Jazi, Ghasemi, Seyedi, & Niknam, 2018); FORMS APLOID AND BINUCLEATE CELLS 1B and 1C (FAB1B, FAB1C) (Bak et al., 2013); and PHOSPHOLIPASE D DELTA (PLDDELTA) (Katagiri, Takahashi, & Shinozaki, 2001; Uraji et al., 2012). Additionally, in the DET-Arid set, we detected putative ion transporters, as SLAC1 HOMOLOGUE 3 (SLAH3), essential for efficient stomatal closure and opening and induced by drought stress (A. Zhang et al., 2016); SODIUM:HYDROGEN ANTIPORTER 1 (NHD1), which protects chloroplasts from deleterious Na^+ concentrations (Müller et al., 2014); and Na^+/Ca^{2+} EXCHANGER (NCL), involved in Ca^{2+} homeostasis under abiotic stress (P. Wang et al., 2012). Complementarily, in

the DET-Arid set, we found transcripts associated with biosynthesis and accumulation of oligosaccharides and increasing tolerance of osmotic stress, as GALACTINOL SYNTHASE 1 (GolS1) (A. Nishizawa, Yabuta, & Shigeoka, 2008) and RAFFINOSE SYNTHASE (DIN10) (Taji et al., 2002). Transcripts induced by drought associated with epicuticular wax biosynthesis and transport were also among the DET-Arid. For example, transcripts similar to MYB DOMAIN PROTEIN 94 (MYB94), a key activator of wax biosynthesis genes (S. B. Lee & Suh, 2015); to the fatty acid hydroxylase ECERIFERUM1 (CER1) (Bourdenx et al., 2011), activated by MYB94; WHITE-BROWN COMPLEX HOMOLOG PROTEIN 11 (WBC11), required for exporting wax and cutin monomers (Panikashvili et al., 2007); and to LIPID TRANSFER PROTEIN 4 (LTP4), involved in wax or cutin deposition in cell walls (Chae et al., 2010). Additionally, in the DET-Arid set, we detected several transcripts associated with reactive oxygen species (ROS) dissipation for the control of cell damage caused by drought, high light or salt stress, including putative COPPER/ZINC SUPEROXIDE DISMUTASES 1 and 2 (CSD1, CDS2) (Attia, Arnaud, Karray, & Lachaâl, 2008), CHLOROPLASTIC DROUGHT-INDUCED STRESS PROTEIN OF 32 kDa (CDSP32) (Broin, Cuiné, Eymery, & Rey, 2002) and MAP KINASE 6 (MAPK6) (Teige et al., 2004).

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 chaperones that modulate thermotolerance, such as the heat-shock proteins (HSPs) HSP17.4 (Wehmeyer, Hernandez, Finkelstein, & Vierling, 1996), HSP17.6 (Sun, Bernard, Cotte, Montagu, & Verbruggen, 2001), HSP18.2 (Lim et al., 2006), HSP70 (S. Lee et al., 2009), HSP90.1 (Cha et al., 2013) and HSP101 (Queitsch, Hong, Vierling, & Lindquist, 2000), five HSP20-LIKE

CHAPERONE PROTEINS (AT1G52560, AT1G53540, AT2G27140, AT2G29500, AT5G51440) (Helm, Schmeits, & Vierling, 1995; Ayako Nishizawa et al., 2006), UNIVERSAL STRESS PROTEIN (AT3G53990) (Jung et al., 2015), the chloroplast chaperone CASEIN LYTIC PROTEINASE B3 (CLPB3) (Myouga, Motohashi, Kuromori, Nagata, & Shinozaki, 2006), and co-chaperones, as ROTAMASE FKBP 1 and 2 (ROF1 and ROF2) (Meiri & Breiman, 2009; Meiri et al., 2010). Additionally, we identified transcripts associated with heat-shock response initiation and putative components of thermomemory, as HEAT SHOCK TRANSCRIPTION FACTOR A2 (AT2G26150) and HEAT-STRESS-ASSOCIATED 32 (AT4G21320), required for long-term maintenance of acquired thermotolerance (Charng et al., 2006).

3.4.5. Photorespiration

In the DET-Arid set of roots, we detected enrichment of putative genes associated with photorespiration similar to enzymes participating in transamination, namely. PYRIMIDINE 4 (PYD4), ALANINE:GLYOXYLATE AMINOTRANSFERASE (SGAT) and ALANINE-2-OXOGLUTARATE AMINOTRANSFERASE 2 (GGT2) (Liepman & Olsen, 2001); similar to PEROXISOMAL NAD-MALATE DEHYDROGENASE 1 (PMDH1), which is required for maintaining photosynthesis under photorespiratory conditions and for carbon flow during photorespiration (Cousins, Walker, Pracharoenwattana, Smith, & Badger, 2011); and D-GLYCERATE 3-KINASE (GLYK), which catalyzes the concluding reaction of photorespiration (Boldt et al., 2005).

4. Discussion

Our study suggests a major role of variation in freshwater availability driving adaptive molecular responses in the typically tropical and widespread tree. Gradual changes in freshwater inputs from both precipitation and rivers along a spatial gradient (Figure 1) seem to play an important role in the specialization of individuals of *Avicennia germinans*, via natural selection. Limited gene flow, likely caused by geographical distance and the flow direction of superficial sea currents, may have facilitated the accumulation of adaptive differences. Additionally, we described how drastic and persistent restriction in soil freshwater availability caused the evolution of phenotype, gene pool and gene expression profile of a recently founded population of *A. germinans*, without depletion in genetic diversity and despite clear possibility of gene flow with an adjacent, unchanged population.

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

The genetic structure inferred by genome-wide SNP loci (Figure 4) suggested the importance of both neutral and non-neutral environmental drivers of variation. Remarkable divergence was observed between samples influenced by the northern and southern branches of the South Equatorial sea current (SEC), corroborating previous results found for various coastal trees using putatively neutral molecular markers (Francisco, Mori, Alves, Tambarussi, & Souza, 2018; Mori, Zucchi, & Souza, 2015; Takayama, Tateishi, Murata, & Kajita, 2008). The bifurcated flow of coastal currents provides a neutral explanation to the north-south divergence, due to restrictions in the dispersal of buoyant propagules, which likely facilitate the accumulation of random north-south genetic divergences (Figure 4). 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, non-neutral role in shaping this

north-south divergence. The closely related species, *Avicennia marina*, has the ability of directly absorbing rainwater through its leaves, presenting substantial growth spurts following precipitation events (Steppe et al., 2018). This mechanism has been observed in plants from dry lands (Breshears et al., 2008), cloud forests (Eller, Lima, & Oliveira, 2013) and in conifers (Mayr et al., 2014), and might also be present in *A. germinans*. Complementarily, both aridity and salinity enhance the leaf water storage for transient growth under favorable conditions in *A. marina*, requiring the use of alternative water sources to that supplied by roots (Nguyen et al., 2017). Therefore, our results suggest that the more even distribution of rainfall throughout the year in the TMD site likely alleviates water-stress in *A. germinans*, whereas more limited rainfall in the warmest quarter of northern regions reduces opportunities for rehydration via foliar water uptake (Figure 1), potentially favoring traits, which increase drought-tolerance. It is plausible that this environmental filter contributes to the genetic divergence observed between TMD and remaining populations (Figure 4). This hypothesis is corroborated by the detection of 21 loci candidate for selection correlated to the spatial variation in the precipitation of the warmest quarter over the study area (Figure 1). Although seven out of these loci were poorly characterized, hampering inferences about their functional relevance in the environmental context, we were able to associate 11 candidate loci with biological processes influenced by drought, as photosynthesis, cell wall metabolism, cell elongation, plant growth, protein protection from stress-induced degradation and regulation of abscisic acid signaling (Figure 5, Table 3). The adaptive importance of freshwater limitation in tropical trees was also suggested in the mangrove, *A. schaueriana* (M. V. Cruz et al., 2018), and in tropical forests and savannas (Ciemer et al., 2019), for which it was similarly suggested that an environmental filtering mechanism driven by high rainfall variability favored the survival of more drought resistant taxa.

Although *Avicennia* propagules can remain viable for long periods and present transoceanic dispersal (Mori, Zucchi, Sampaio, & Souza, 2015), at a finer scale, we observed a genetic divergence between sites in the AMMC region (MRJ and PA-humid) and samples from Northeast Brazilian mangroves (ALC, PNB and PRC) (Figure 4). 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, reduced rainfall and the lack of riverine freshwater inputs in Northeast Brazil could limit plants access to soil freshwater, due to increased soil salinity, potentially contributing to the local specialization of individuals. Even though we do not have soil salinity data to perform direct G-E association tests for selection, we were able to detect two loci correlated with the variation in total annual precipitation: one associated with a poorly characterized protein kinase and the other, with an RNA hydrolase, also correlated with heterogeneity in mean sea surface salinity (Supplementary Table S5). Given the unclear functional relevance of these putative adaptive loci in the environmental context, we recommend future efforts to analyze the role of other neutral and non-neutral variables, as soil salinity or the demographic history of *A. germinans*, to find additional explanations for the genetic divergence observed between AMMC and Northeast Brazilian mangroves (Figure 4). Candidate loci detected in our study play a significant role in drought adaptations, but their molecular functions need to be further characterized in plants adapted to physiological drought. These proteins may not get 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*, was 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 2) (Cohen & Lara, 2003). This sudden environmental change caused a large dieback of the mangrove vegetation. Gradually, the impacted area was recolonized, mainly by *A. germinans*. We estimate that the approximately 40 years since this event occurred, represent from 4 to 40 reproductive cycles of *A. germinans*, based on previous studies of closely related *Avicennia* sp. (Almahasheer, Duarte, & Irigoien, 2016; M. V. Cruz et al., 2018; Polidoro et al., 2010). Recolonizing individuals of PA-arid started presenting a dwarf, shrub architecture, very distinct from the former tall, arboreal architecture, still observed in surrounding areas where the hydrology remained unchanged (Figure 2) (Pranchai et al., 2017). This observation suggests that limitation in plant access to soil water favored smaller tree sizes, one of the most integrative characteristic of drought resistance (Bennett et al., 2015; Corlett, 2016; Naidoo, 2006; Rowland et al., 2015). On the one hand, we cannot determine how much of the difference in tree size is inherited or determined by phenotypic plasticity. On the other hand, our results revealed that a substantial and rapid change in the gene pool of the PA-arid population occurred without reduction in levels of overall genetic diversity, estimated by various parameters (Figure 2, Figure 4a-b, Table 2). The presence of high levels of genome-wide genetic variation, simultaneously with strong selection on individual traits may be a signature of substantial multivariate genetic constraints (B. Walsh & Blows, 2009). Interestingly, this divergence in allele frequencies was observed despite clear possibility of gene flow, given the geographical proximity (<10.0 m) of the PA-arid site to well preserved populations. Consistent with an adaptive response to selection by limited access to soil freshwater, F_{ST} outlier tests detected eight candidate loci within transcripts associated with functions as suppression of cell elongation, wood and xylem tracheids

formation, photosynthetic machinery biogenesis and repair, regulation of adaptation to stress and of protection from stress-induced protein degradation (Table 3, Figure 5).

Besides genotypic changes, we also identified transcripts expression differences between seedlings from PA-arid and PA-humid sites (Table 1, Figure 6) after three days acclimatization in pots under shaded, well-watered conditions (Figure 3). 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). Increased transcripts expression, mainly in roots of PA-arid samples, were associated with biological processes previously identified to be involved in central aspects of drought-tolerance in model and non-model plants (Ding et al., 2013; Fan et al., 2018; C. Zhang et al., 2015). For instance, we found transcripts associated with ABA-mediated stomatal closure, a well-known mechanism for maintaining water status under drought, which severely compromises growth (Murata, Mori, & Munemasa, 2015). Stomatal closure reduces the CO_2:O_2 ratio in mesophyll cells, increasing photorespiration, also an enriched process in DET-Arid. Although photorespiration decreases photosynthesis efficiency, it plays an essential role in protecting the photosynthetic machinery from damage caused by excessive photochemical energy (Kozaki & Takeba, 1996; Wingler, Lea, Quick, & Leegood, 2000). Conversely, we also observed an enrichment of genes involved in photosynthesis, shade avoidance and response to low light intensity. Being sessile under extremely arid conditions, PA-arid plants might need to rapidly adjust photosynthesis gene expression to intermittent freshwater availability (Chaves, Flexas, & Pinheiro, 2009; Urban, Aarrouf, & Bidel, 2017). The increased freshwater availability and decreased solar irradiance under experimental conditions, compared to source-site (Figure 3), likely required that PA-arid individuals broadly adjust their photosynthetic machinery. Additionally, we

observed several putative HSPs and heat-shock factors among DET-Arid, contributing to the protection of proteins and membranes against water-stress-induced changes (Al-Whaibi, 2011). Various transcripts associated with epicuticular wax and cutin synthesis, export and deposition also showed higher expression in PA-arid than in PA-humid seedlings, likely reflecting an increased protection of cells against detrimental effects of drought by the plant cuticle of PA-arid plants (Aharoni et al., 2004; Javelle, Vernoud, Rogowsky, & Ingram, 2011). Complementarily, we found transcripts associated with the accumulation of raffinose among DET-Arid, functioning as osmoprotectants and antioxidants under drought and osmotic stress and maintaining cell turgor (ElSayed, Rafudeen, & Golldack, 2014). Overall, our comparative transcriptomic analyses suggest that PA-arid plants deal better with heat, UV, salt and drought than PA-humid plants, through changes in key regulatory mechanisms, which increase abiotic stress tolerance. These strategies may have enhanced the relative capacity of PA-arid seedlings to grow under high leaf water-deficit status and infrequent freshwater inputs.

We acknowledge that we cannot disentangle effects from genetic and epigenetic divergences underlying the differential transcripts expression observed. Nevertheless, both genetic and epigenetic changes might contribute to the observed trait divergence. Epigenetic changes can emerge faster than adaptive genetic changes, playing an important role, especially in early adaptive process (Kenkel & Matz, 2016; Pavey, Nosil, & Rogers, 2010). Although the mechanisms are not entirely clear, our results suggest, through independent approaches, that a rapid evolution of this population occurred, with phenotypic, genetic and gene expression changes. The desertification of the PA-arid site represents a drastic and persistent environmental change, analogous to a climate change, freshwater exclusion

experiment for mangroves of the tropical Brazilian coastline, in which limited access to soil freshwater likely 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).

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 harbor sufficient diversity to enable species persistence through natural selection of drought-resistant plants, as observed in the PA-arid site. Recolonizing individuals in this location share alleles with individuals from distant areas, as the PRC site (Figure 4a), located over 1,200 km away, in Northeast Brazil. Therefore, our results suggest that forests from Northeastern Brazilian mangroves contributed as source of adaptive variation through sea-dispersed propagules in the recolonization of the PA-arid site, additional to the selection on seed-bank or on migrants from closer areas.

Our findings indicated limited gene flow between populations influenced by northern and southern branches of the SEC (Figure 4), suggesting the contribution of neutral forces, but also of environmental selection by variation in precipitation of the warmest quarter. Given the substantial north-south genetic divergence, we recommend that northern and southern populations should be treated as two independently evolving management units (Moritz, 1999). In the context of an increasingly drying climate, reforestation plans for populations of

A. germinans located south of the SEC should consider the use of mixed stocks of seedlings (Moritz, 1999), to introduce genetic variation associated with increased drought-tolerance in Northeaster mangroves, while also maintaining local alleles, possibly associated with site-specific environmental characteristics.

4.4 Concluding remarks

We provide novel insights into the consequences of limited access to soil freshwater to the variation in allele frequencies, gene expression and phenotypes of a dominant tropical tree. Research on the genomic basis of tree adaptation are 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. Advances in the understanding of the genomic basis of drought-tolerance in tropical trees can support effective protection plans and mitigating 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).

Acknowledgments

We acknowledge Ilmarina Menezes for assistance with fieldwork. M.V.C. and G.M.M. received fellowships from the São Paulo Research Foundation (FAPESP 2013/26793-7, 2013/08086-1, 2014/22821-9). M.V.C. received a fellowship from the Coordination for the Improvement of Higher Education Personnel (CAPES 99999.008084/2015-07, 88887.177158/2018-00). G.M.M. received a grant from the Brazilian

National Council for Scientific and Technological Development (CNPq 448286/2014-9). A.P.S. received a research fellowship from CNPq (309661/2014-5).

Conflicts of interest

The authors have no conflict of interest to disclose.

6. References

Aharoni, A., Dixit, S., Jetter, R., Thoenes, E., Arkel, G. van, & Pereira, A. (2004). The SHINE Clade of AP2 Domain Transcription Factors Activates Wax Biosynthesis, Alters Cuticle Properties, and Confers Drought Tolerance when Overexpressed in *Arabidopsis*. *The Plant Cell Online*, *16*(9), 2463–2480. doi:10.1105/tpc.104.022897

Akman, M., Carlson, J. E., Holsinger, K. E., & Latimer, A. M. (2016). Transcriptome sequencing reveals population differentiation in gene expression linked to functional traits and environmental gradients in the South African shrub Protea repens. *New Phytologist*, *210*(1), 295–309. doi:10.1111/nph.13761

Al-Whaibi, M. H. (2011). Plant heat-shock proteins□: A mini review. *Journal of King Saud University - Science*, *23*(2), 139–150. doi:10.1016/j.jksus.2010.06.022

Alexander, D. H., Novembre, J., & Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome Research*, *19*(9), 1655–1664. doi:10.1101/gr.094052.109

Allen, C. D., Breshears, D. D., & McDowell, N. G. (2015). On underestimation of global vulnerability to tree mortality and forest die-off from hotter drought in the Anthropocene. *Ecosphere*, *6*(8), 1–55. doi:10.1890/ES15-00203.1

Allen, C. D., Macalady, A. K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., … Cobb, N. (2010). A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management*, *259*(4), 660–684. doi:10.1016/j.foreco.2009.09.001

Almahasheer, H., Duarte, C. M., & Irigoien, X. (2016). Phenology and Growth dynamics of Avicennia marina in the Central Red Sea. *Scientific Reports*, *6*, 37785. doi:10.1038/srep37785

Alvares, C. A., Stape, J. L., Sentelhas, P. C., Gonçalves, J. L. de M., & Sparovek, G. (2013). Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift*, *22*(6), 711–728. doi:10.1127/0941-2948/2013/0507

Amorim, M. E., Schoener, T. W., Ramalho, G. C. C. S., Lins, A. C. R., Piovia-Scottd, J., & Brandão, R. A. (2017). Lizards on newly created islands independently and rapidly adapt in morphology and diet. *Proceedings of the National Academy of Sciences*, *114*(33), 8812–8816. doi:10.1073/pnas.1709080114

Antao, T., Lopes, A., Lopes, R. J., Beja-Pereira, A., & Luikart, G. (2008). LOSITAN: A workbench to detect molecular adaptation based on a F st -outlier method. *BMC Bioinformatics*, *9*(1), 1–5. doi:10.1186/1471-2105-9-323

Aranda, I., Cano, F. J., Gascó, A., Cochard, H., Nardini, A., Mancha, J. A., … Sánchez-

gómez, D. (2014). Variation in photosynthetic performance and hydraulic architecture across European beech (Fagus sylvatica L .) populations supports the case for local adaptation to water stress. *Tree Physiology*, *35*(1), 34–46. doi:10.1093/treephys/tpu101

Asner, G. P., Brodrick, P. G., Anderson, C. B., Vaughn, N., Knapp, D. E., & Martin, R. E. (2016). Progressive forest canopy water loss during the 2012 – 2015 California drought. *Proceedings of the National Academy of Sciences*, *113*(2), 249–255. doi:10.1073/pnas.1523397113

Attia, H., Arnaud, N., Karray, N., & Lachaâl, M. (2008). Long-term effects of mild salt stress on growth, ion accumulation and superoxide dismutase expression of *Arabidopsis* rosette leaves. *Physiologia Plantarum*, *132*(3), 293–305. doi:10.1111/j.1399-3054.2007.01009.x

Bak, G., Lee, E.-J., Lee, Y., Kato, M., Segami, S., Sze, H., … Lee, Y. (2013). Rapid Structural Changes and Acidification of Guard Cell Vacuoles during Stomatal Closure Require Phosphatidylinositol 3,5-Bisphosphate. *The Plant Cell*, *25*(6), 2202–2216. doi:10.1105/tpc.113.110411

Bang, W. Y., Hata, A., Jeong, I. S., Umeda, T., Masuda, T., Chen, J., … Bahk, J. D. (2009). AtObgC, a plant ortholog of bacterial Obg, is a chloroplast-targeting GTPase essential for early embryogenesis. *Plant Molecular Biology*, *71*(4–5), 379–390. doi:10.1007/s11103-009-9529-3

Bartlett, M. K., Scoffoni, C., & Sack, L. (2012). The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: A global meta-analysis. *Ecology Letters*, *15*, 393–405. doi:10.1111/j.1461-0248.2012.01751.x

Beaumont, M. A., & Nichols, R. A. (1996). Evaluating Loci for Use in the Genetic Analysis of Population Structure. *Proceedings of the Royal Society B: Biological Sciences*, *263*(1377), 1619–1626. doi:10.1098/rspb.1996.0237

Bennett, A. C., McDowell, N. G., Allen, C. D., & Anderson-Teixeira, K. J. (2015). Larger trees suffer most during drought in forests worldwide. *Nature Plants*, *1*, 1–5. doi:10.1038/NPLANTS.2015.139

Berardini, T. Z., Reiser, L., Li, D., Mezheritsky, Y., Muller, R., Strait, E., & Huala, E. (2015). The Arabidopsis Information Resource: Making and Mining the "Gold Standard" Annotated Reference Plant Genome. *Genesis*, *53*, 474–485. doi:10.1002/dvg.22877

Blake, J. A., Christie, K. R., Dolan, M. E., Drabkin, H. J., Hill, D. P., Ni, L., … Westerfeld, M. (2015). Gene ontology consortium: Going forward. *Nucleic Acids Research*, *43*, D1049–D1056. doi:10.1093/nar/gku1179

Bohne, A. V., Schwenkert, S., Grimm, B., & Nickelsen, J. (2016). Roles of Tetratricopeptide Repeat Proteins in Biogenesis of the Photosynthetic Apparatus. *International Review of Cell and Molecular Biology*, *324*, 187–227. doi:10.1016/bs.ircmb.2016.01.005

Boldt, R., Edner, C., Kolukisaoglu, Ü., Hagemann, M., Weckwerth, W., Wienkoop, S., … Bauwe, H. (2005). Prosthesis Retention and Effective Use of Denture Adhesive in Complete Denture Therapy. *The Plant Cell*, *17*(8), 2413–2420. doi:10.1105/tpc.105.033993.enzyme

Bourdenx, B., Bernard, A., Domergue, F., Pascal, S., Leger, A., Roby, D., … Joubes, J. (2011). Overexpression of *Arabidopsis* ECERIFERUM1 Promotes Wax Very-Long-Chain Alkane Biosynthesis and Influences Plant Response to Biotic and Abiotic Stresses. *Plant Physiology*, *156*(1), 29–45. doi:10.1104/pp.111.172320

Breshears, D. D., McDowell, N. G., Goddard, K. L., Dayem, K. E., Martens, S. N., Meyer, C. W., & Brown, K. M. (2008). Foliar absorption of intercepted rainfall improves woody plant water status most during drought. *Ecology*, *89*(1), 41–47. doi:10.1890/07-0437.1

Brodribb, T. J., Holbrook, N. M., Edwards, E. J., & Gutiérrez, M. V. (2003). Relations

between stomatal closure, leaf turgor and xylem vulnerability in eight tropical dry forest trees. *Plant, Cell and Environment*, *26*(3), 443–450. doi:10.1046/j.1365-3040.2003.00975.x

Broin, M., Cuiné, S., Eymery, F., & Rey, P. (2002). The plastidic 2-cysteine peroxiredoxin is a target for a thioredoxin involved in the protection of the photosynthetic apparatus against oxidative damage. *The Plant Cell*, *14*(6), 1417–32. doi:10.1105/tpc.001644.been

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., & Madden, T. L. (2009). BLAST+: architecture and applications. *BMC Bioinformatics*, *10*, 421. doi:10.1186/1471-2105-10-421

Cha, J. Y., Ahn, G., Kim, J. Y., Kang, S. Bin, Kim, M. R., Su'udi, M., … Son, D. (2013). Structural and functional differences of cytosolic 90-kDa heat-shock proteins (Hsp90s) in *Arabidopsis thaliana*. *Plant Physiology and Biochemistry*, *70*, 368–373. doi:10.1016/j.plaphy.2013.05.039

Chae, K., Gonong, B. J., Kim, S. C., Kieslich, C. A., Morikis, D., Balasubramanian, S., & Lord, E. M. (2010). A multifaceted study of stigma/style cysteine-rich adhesin (SCA)-like *Arabidopsis* lipid transfer proteins (LTPs) suggests diversified roles for these LTPs in plant growth and reproduction. *Journal of Experimental Botany*, *61*(15), 4277–4290. doi:10.1093/jxb/erq228

Charng, Y. -y., Liu, H. -c., Liu, N. -y., Chi, W. -t., Wang, C. -n., Chang, S. -h., & Wang, T. -t. (2006). A Heat-Inducible Transcription Factor, HsfA2, Is Required for Extension of Acquired Thermotolerance in *Arabidopsis*. *Plant Physiology*, *143*(1), 251–262. doi:10.1104/pp.106.091322

Choat, B., Brodribb, T. J., Brodersen, C. R., Duursma, R. A., López, R., & Medlyn, B. E. (2018). Triggers of tree mortality under drought. *Nature*, *558*(7711), 531–539. doi:10.1038/s41586-018-0240-x

Choat, B., Jansen, S., Brodribb, T. J., Cochard, H., Delzon, S., Bhaskar, R., … Zanne, A. E. (2012). Global convergence in the vulnerability of forests to drought. *Nature*, *491*(7426), 752–755. doi:10.1038/nature11688

Ciemer, C., Boers, N., Hirota, M., Kurths, J., Müller-hansen, F., Oliveira, R. S., & Winkelmann, R. (2019). Higher resilience to climatic disturbances in tropical vegetation exposed to more variable rainfall. *Nature Geoscience*, *12*, 174–179. doi:10.1038/s41561-019-0312-z

Clough, B. F., Sim, R. G., Inlet, T., Bay, M., & Rivers, I. (1989). Oecologia of mangroves in response to salinity and vapour pressure deficit. *Oecologia*, (459), 38–44.

Cohen, M. C. L., & Lara, R. J. (2003). Temporal changes of mangrove vegetation boundaries in Amazonia: Application of GIS and remote sensing techniques. *Wetlands Ecology and Management*, *11*(4), 223–231. doi:10.1023/A:1025007331075

Corlett, R. T. (2016). The Impacts of Droughts in Tropical Forests. *Trends in Plant Science*, *21*(7), 584–593. doi:10.1016/j.tplants.2016.02.003

Correll, M. J., Coveney, K. M., Raines, S. V., Mullen, J. L., Hangarter, R. P., & Kiss, J. Z. (2003). Phytochromes play a role in phototropism and gravitropism in *Arabidopsis* roots. *Advances in Space Research*, *31*(10), 2203–2210. doi:10.1016/S0273-1177(03)00245-X

Cousins, A. B., Walker, B. J., Pracharoenwattana, I., Smith, S. M., & Badger, M. R. (2011). Peroxisomal hydroxypyruvate reductase is not essential for photorespiration in *Arabidopsis* but its absence causes an increase in the stoichiometry of photorespiratory CO2 release. *Photosynthesis Research*, *108*(2–3), 91–100. doi:10.1007/s11120-011-9651-3

Crocco, C. D., Locascio, A., Escudero, C. M., Alabadí, D., Blázquez, M. A., & Botto, J. F.

(2015). The transcriptional regulator BBX24 impairs della activity to promote shade avoidance in *Arabidopsis thaliana*. *Nature Communications*, *6*. doi:10.1038/ncomms7202

Cruz, M. V., Mori, G. M., Müller, C. S., Cristina, C., Oh, D., Dassanayake, M., … Silva, R. (2018). Local adaptation insights from genomics and ecophysiology of a neotropical mangrove. *Biorxiv*, 1–43. doi:10.1101/378612

Cruz, M. V., Mori, G. M., Oh, D., Dassanayake, M., Zucchi, M. I., Oliveira, R. S., & Souza, A. P. (2019). Data from: Molecular responses to freshwater limitation in the mangrove tree Avicennia germinans (Acanthaceae). *Dryad Digital Repository*. doi:doi:10.5061/dryad.h11t255

D'Angelo, C., Weinl, S., Batistic, O., Pandey, G. K., Cheong, Y. H., Schültke, S., … Kudla, J. (2006). Alternative complex formation of the Ca^{2+}-regulated protein kinase CIPK1 controls abscisic acid-dependent and independent stress responses in *Arabidopsis*. *Plant Journal*, *48*(6), 857–872. doi:10.1111/j.1365-313X.2006.02921.x

da Costa, C. L., Galbraith, D., Almeida, S., Tanaka Portela, B. T., da Costa, M., de Athaydes Silva Junior, J., … Meir, P. (2010). Effect of seven years of experimental drought on the aboveground biomass storage of an eastern Amazonian rainforest. *New Phytologist*, *187*, 579–591. doi:10.1111/j.1469-8137.2010.03309.x

Dai, A. (2011). Drought under global warming: A review. *Wiley Interdisciplinary Reviews: Climate Change*, *2*(1), 45–65. doi:10.1002/wcc.81

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., … Durbin, R. (2011). The variant call format and VCFtools. *Bioinformatics*, *27*(15), 2156–2158. doi:10.1093/bioinformatics/btr330

Daniel, B., Pavkov-Keller, T., Steiner, B., Dordic, A., Gutmann, A., Nidetzky, B., … Macheroux, P. (2015). Oxidation of monolignols by members of the berberine bridge enzyme family suggests a role in plant cell wall metabolism. *Journal of Biological Chemistry*, *290*(30), 18770–18781. doi:10.1074/jbc.M115.659631

De Frenne, P., Graae, B. J., Rodríguez-Sánchez, F., Kolb, A., Chabrerie, O., Decocq, G., … Verheyen, K. (2013). Latitudinal gradients as natural laboratories to infer species' responses to temperature. *Journal of Ecology*, *101*(3), 784–795. doi:10.1111/1365-2745.12074

Ding, Y., Liu, N., Virlouvet, L., Riethoven, J. J., Fromm, M., & Avramova, Z. (2013). Four distinct types of dehydration stress memory genes in *Arabidopsis thaliana*. *BMC Plant Biology*, *13*(1). doi:10.1186/1471-2229-13-229

Donihue, C. M., Herrel, A., Fabre, A. C., Kamath, A., Geneva, A. J., Schoener, T. W., … Losos, J. B. (2018). Hurricane-induced selection on the morphology of an island lizard. *Nature*, *560*(7716), 88–91. doi:10.1038/s41586-018-0352-3

Donovan, L. A., Ludwig, F., Rosenthal, D. M., Rieseberg, L. H., & Dudley, S. A. (2009). Phenotypic selection on leaf ecophysiological traits in Helianthus. *New Phytologist*, *183*(3), 868–879. doi:10.1111/j.1469-8137.2009.02916.x.

Eller, C. B., Lima, A. L., & Oliveira, R. S. (2013). Foliar uptake of fog water and transport belowground alleviates drought effects in the cloud forest tree species, Drimys brasiliensis (Winteraceae). *New Phytologist*, *199*(1), 151–162. doi:10.1111/nph.12248

Ellison, A. M., Farnsworth, E. J., & Merkt, R. E. (1999). Origins of mangrove ecosystems and the mangrove biodiversity anomaly. *Global Ecology and Biogeography*, *8*(2), 95–115. doi:10.1046/j.1466-822X.1999.00126.x

ElSayed, A. I., Rafudeen, M. S., & Golldack, D. (2014). Physiological aspects of raffinose family oligosaccharides in plants: Protection against abiotic stress. *Plant Biology*, *16*(1),

1–8. doi:10.1111/plb.12053

Etterson, J. R. (2004). Evolutionary potential of Chamaecrista fasciculata in relation to climate change. I . Clinal patterns of selection along and environmental gradient in the great plains. *Evolution*, *58*(7), 1446–1458. doi:10.1111/j.0014-3820.2004.tb01726.x

Fan, L., Wang, G., Hu, W., Pantha, P., Tran, K., Zhang, H., … Qiu, Q. (2018). Plant Physiology and Biochemistry Transcriptomic view of survival during early seedling growth of the extremophyte Haloxylon ammodendron. *Plant Physiology and Biochemistry*, *132*(9), 475–489. doi:10.1016/j.plaphy.2018.09.024

Finn, R. D., Bateman, A., Clements, J., Coggill, P., Eberhardt, R. Y., Eddy, S. R., … Punta, M. (2014). Pfam: The protein families database. *Nucleic Acids Research*, *42*(D1), 222–230. doi:10.1093/nar/gkt1223

Francisco, P. M., Mori, G. M., Alves, F. M., Tambarussi, E., & Souza, A. P. De. (2018). Population genetic structure, introgression, and hybridization in the genus Rhizophora along the Brazilian coast. *Ecology and Evolution*, *8*(6), 3491–3504. doi:10.1002/ece3.3900

Frichot, E., & François, O. (2015). LEA: An R package for landscape and ecological association studies. *Methods in Ecology and Evolution*, *6*(8), 925–929. doi:10.1111/2041-210X.12382

Frydman, J. (2001). Folding of newkly translated proteins in vivo: the role of molecular chaperones. *Annual Review of Biochemistry*, *701*(1), 603–647.

Fujiki, Y., Ito, M., Itoh, T., Nishida, I., & Watanabe, A. (2002). Activation of the promoters of *Arabidopsis* genes for the branched-chain α-keto acid dehydrogenase complex in transgenic tobacco BY-2 cells under sugar starvation. *Plant and Cell Physiology*, *43*(3), 275–280. doi:10.1093/pcp/pcf032

Fujiki, Y., Ito, M., Nishida, I., & Watanabe, A. (2000). Multiple signaling pathways in gene expression during sugar starvation. Pharmacological analysis of din gene expression in suspension-cultured cells of *Arabidopsis*. *Plant Physiology*, *124*(3), 1139–1148. doi:10.1104/pp.124.3.1139

Fujiki, Y., Ito, M., Nishida, I., & Watanabe, A. (2001). Leucine and its keto acid enhance the coordinated expression of genes for branched-chain amino acid catabolism in *Arabidopsis* under sugar starvation. *FEBS Letters*, *499*(1–2), 161–165. doi:10.1016/S0014-5793(01)02536-4

Goudet, J. (2005). Hierfstat, a package for R to compute and test hierarchical F -statistics. *Molecular Ecology Notes*, *5*(1), 184–186. doi:10.1111/j.1471-8278

Gould, B. A., Chen, Y., & Lowry, D. B. (2018). Gene Regulatory Divergence Between Locally Adapted Ecotypes in Their Native Habitats. *Molecular Ecology*, *27*(21), 4174–4188. doi:10.1111/mec.14852

Hacke, U. G., Sperry, J. S., Wheeler, J. K., & Castro, L. (2006). Scaling of angiosperm xylem structure with safety and efficiency. *Tree Physiology*, *26*(6), 689–701. doi:10.1093/treephys/26.6.689

Helm, K. W., Schmeits, J., & Vierling, E. (1995). An Endomembrane-Localized Small Heat-Shock Protein from Ara bidopsis tha bana '. *Plant Physiology*, *107*(1 995), 287–288. doi:10.1104/pp.107.1.287

Henriksson, E., Olsson, A. S. B., Johannesson, H., Johansson, H., Hanson, J., Engstro, P., … Soderman, E. (2005). Homeodomain leucine zipper class I genes in *Arabidopsis*. Expression patterns and phylogenetic relationships. *Plant Physiology*, *139*(1), 509–518. doi:10.1104/pp.105.063461

Heschel, M. S., & Riginos, C. (2005). Mechanisms of selection for drought stress tolerance

and avoidance in *Impatiens capensis* (Balsaminaceae). *American Journal of Botany*, *92*(1), 37–44. doi:10.3732/ajb.92.1.37

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, *25*(15), 1965–1978. doi:10.1002/joc.1276

Holliday, J. A., Aitken, S. N., Cooke, J. E. K., Fady, B., González-Martínez, S. C., Heuertz, M., … Plomion, C. (2017). Advances in ecological genomics in forest trees and applications to genetic resources conservation and breeding. *Molecular Ecology*, 706–717. doi:10.1111/mec.13963

Holtkotte, X., Ponnu, J., Ahmad, M., & Hoecker, U. (2017). The blue light-induced interaction of cryptochrome 1 with COP1 requires SPA proteins during *Arabidopsis* light signaling. *PLoS Genetics*, *13*(10), 1–15. doi:10.1371/journal.pgen.1007044

Hussey, S. G., Mizrachi, E., Spokevicius, A. V., Bossinger, G., Berger, D. K., & Myburg, A. A. (2011). SND2, a NAC transcription factor gene, regulates genes involved in secondary cell wall development in *Arabidopsis* fibres and increases fibre cell area in *Eucalyptus*. *BMC Plant Biology*, *11*, 1–17. doi:10.1186/1471-2229-11-173

Hyun, T. K., Van Der Graaff, E., Albacete, A., Eom, S. H., Großkinsky, D. K., Böhm, H., … Roitsch, T. (2014). The *Arabidopsis* PLAT domain protein 1 is critically involved in abiotic stress tolerance. *PLoS ONE*, *9*(11). doi:10.1371/journal.pone.0112946

Indorf, M., Cordero, J., Neuhaus, G., & Rodríguez-Franco, M. (2007). Salt tolerance (STO), a stress-related protein, has a major role in light signalling. *Plant Journal*, *51*(4), 563–574. doi:10.1111/j.1365-313X.2007.03162.x

IPCC. (2014). *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. (CoreWritingTeam, R. K. Pachauri, & L. A. Meyer, Eds.), *Cambridge University Press* (Cambridge). Geneva, Switzerland: IPCC.

Javelle, M., Vernoud, V., Rogowsky, P. M., & Ingram, G. C. (2011). Epidermis: The formation and functions of a fundamental plant tissue. *New Phytologist*, *189*(1), 17–39. doi:10.1111/j.1469-8137.2010.03514.x

Jombart, T., & Ahmed, I. (2011). adegenet 1 .3-1: new tools for the analysis of genome-wide SNP data. *Bioinformatics*, *27*(21), 3070–3071. doi:10.1093/bioinformatics/btr521

Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics*, *11*(1), 94. doi:10.1186/1471-2156-11-94

Jung, Y. J., Melencion, S. M. B., Lee, E. S., Park, J. H., Alinapon, C. V., Oh, H. T., … Lee, S. Y. (2015). Universal Stress Protein Exhibits a Redox-Dependent Chaperone Function in *Arabidopsis* and Enhances Plant Tolerance to Heat Shock and Oxidative Stress. *Frontiers in Plant Science*, *6*, 1–11. doi:10.3389/fpls.2015.01141

Kamvar, Z. N., Tabima, J. F., & Grünwald, N. J. (2014). Poppr: an R package for genetic analysis of populations with clonal , partially clonal , and / or sexual reproduction. *PeerJ*, *2:e281*. doi:10.7717/peerj.281

Katagiri, T., Takahashi, S., & Shinozaki, K. (2001). Involvement of a novel *Arabidopsis* phospholipase D, AtPLDδ, in dehydration-inducible accumulation of phosphatidic acid in stress signalling. *Plant Journal*, *26*(6), 595–605. doi:10.1046/j.1365-313X.2001.01060.x

Kawecki, T. J., & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters*, *7*(12), 1225–1241. doi:10.1111/j.1461-0248.2004.00684.x

Keller, S. R., Soolanayakanahally, R. Y., Guy, R. D., Silim, S. N., Olson, M. S., & Tiffin, P.

(2011). Climate-driven local adaptation of ecophysiology and phenology in balsam poplar, Populus balsamifera L. (Salicaceae). *American Journal of Botany*, *98*(1), 99–108. doi:10.3732/ajb.1000317

Kenkel, C. D., & Matz, M. V. (2016). Gene expression plasticity as a mechanism of coral adaptation to a variable environment. *Nature Ecology & Evolution*, *1*, 059667. doi:10.1038/s41559-016-0014 |

Kitaya, Y., Yabuki, K., Kiyota, M., Tani, A., Hirano, T., & Aiga, I. (2002). Gas exchange and oxygen concentration in pneumatophores and prop roots of four mangrove species. *Trees*, *16*, 155–158. doi:10.1007/s00468-002-0167-5

Kozaki, A., & Takeba, G. (1996). Photorespiration protects C3 plants from photooxidation. *Nature*, *384*, 557–560. doi:10.1038/384557a0

Langmead, B., Trapnell, C., Pop, M., & Salzberg, S. (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol.*, *10*(3), R25. doi:10.1186/gb-2009-10-3-r25

Lara, R. J., & Cohen, M. C. L. (2006). Sediment porewater salinity, inundation frequency and mangrove vegetation height in Bragança, North Brazil: An ecohydrology-based empirical model. *Wetlands Ecology and Management*, *14*(4), 349–358. doi:10.1007/s11273-005-4991-4

Lawlor, D. W. (2002). Limitation to photosynthesis in water-stressed leaves: Stomata vs. Metabolism and the role of ATP. *Annals of Botany*, *89*(SPEC. ISS.), 871–885. doi:10.1093/aob/mcf110

Lee, S. B., & Suh, M. C. (2015). Cuticular wax biosynthesis is up-regulated by the MYB94 transcription factor in *Arabidopsis*. *Plant and Cell Physiology*, *56*(1), 48–60. doi:10.1093/pcp/pcu142

Lee, S., Lee, D. W., Lee, Y., Mayer, U., Stierhof, Y.-D., Lee, S., … Hwang, I. (2009). Heat Shock Protein Cognate 70-4 and an E3 Ubiquitin Ligase, CHIP, Mediate Plastid-Destined Precursor Degradation through the Ubiquitin-26S Proteasome System in *Arabidopsis*. *The Plant Cell*, *21*(12), 3984–4001. doi:10.1105/tpc.109.071548

Li, C.-L., Wang, M., Wu, X.-M., Chen, D.-H., Lv, H.-J., Shen, J.-L., … Zhang, W. (2016). THI1, a Thiamine Thiazole Synthase, Interacts with Ca^{2+} -Dependent Protein Kinase CPK33 and Modulates the S-Type Anion Channels and Stomatal Closure in Arabidopsis. *Plant Physiology*, *170*(2), 1090–1104. doi:10.1104/pp.15.01649

Li, W., & Godzik, A. (2006). Cd-hit: A fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics*, *22*(13), 1658–1659. doi:10.1093/bioinformatics/btl158

Liepman, A. H., & Olsen, L. J. (2001). Peroxisomal alanine: Glyoxylate aminotransferase (AGT1) is a photorespiratory enzyme with multiple substrates in *Arabidopsis thaliana*. *Plant Journal*, *25*(5), 487–498. doi:10.1046/j.1365-313x.2001.00961.x

Lim, C. J., Yang, K. A., Hong, J. K., Yun, J. S. C. • D.-J., Hong, J. C., Chung, W. S., … Lim, C. O. (2006). Gene expression profiles during heat acclimation in *Arabidopsis thaliana* suspension-culture cells. *Journal of Plant Research*, *119*, 373–383. doi:10.1007/s10265-006-0285-z

Lin, G., & Sternberg, L. da S. L. (1992). Comparative study of water uptake and photosynthetic gas exchange between scrub and fringe red mangroves, Rhizophora mangle L. *Oecologia*, *90*(3), 399–403. doi:10.1007/BF00317697

Liu, D., Gong, Q., Ma, Y., Li, P., Li, J., Yang, S., … Wang, N. N. (2010). CpSecA, a thylakoid protein translocase subunit, is essential for photosynthetic development in *Arabidopsis*. *Journal of Experimental Botany*, *61*(6), 1655–1669.

doi:10.1093/jxb/erq033

Lorenzo, L. de, Merchan, F., Laporte, P., Thompson, R., Clarke, J., Sousa, C., & Crespi, M. (2009). A Novel Plant Leucine-Rich Repeat Receptor Kinase Regulates the Response of Medicago truncatula Roots to Salt Stress. *The Plant Cell*, *21*(1), 668–680. doi:10.1105/tpc.108.059576

Lotterhos, K. E., & Whitlock, M. C. (2015). The relative power of genome scans to detect local adaptation depends on sampling design and statistical method. *Molecular Ecology*, *24*(5), 1031–1046. doi:10.1111/mec.13100

Luu, K., Bazin, E., & Blum, M. G. B. (2016). pcadapt: An R package to perform genome scans for selection based on principal component analysis. *Molecular Ecology Resources*, *33*, 67–77. doi:10.1111/1755-0998.12592

Manfield, I. W., Devlin, P. F., Jen, C.-H., Westhead, D. R., & Gilmartin, P. M. (2006). Conservation, Convergence, and Divergence of Light-Responsive, Circadian-Regulated, and Tissue-Specific Expression Patterns during Evolution of the *Arabidopsis* GATA Gene Family. *Plant Physiology*, *143*(2), 941–958. doi:10.1104/pp.106.090761

Marri, L., Zaffagnini, M., Collin, V., Issakidis-Bourguet, E., Lemaire, S. D., Pupillo, P., … Trost, P. (2009). Prompt and easy activation by specific thioredoxins of calvin cycle enzymes of *Arabidopsis thaliana* associated in the GAPDH/CP12/PRK supramolecular complex. *Molecular Plant*, *2*(2), 259–269. doi:10.1093/mp/ssn061

Mayr, S., Schmid, P., Laur, J., Rosner, S., Charra-vaskou, K., Dämon, B., & Hacke, U. G. (2014). Uptake of Water via Branches Helps Timberline Conifers Refill Embolized Xylem in Late Winter. *Plant Physiology*, *164*(4), 1731–1740. doi:10.1104/pp.114.236646

McAdam, S. A. M., & Brodribb, T. J. (2015). The Evolution of Mechanisms Driving the Stomatal Response to Vapor Pressure Deficit. *Plant Physiology*, *167*(3), 833–843. doi:10.1104/pp.114.252940

McDonough, M. A., Kadirvelraj, R., Harris, P., Poulsen, J. C. N., & Larsen, S. (2004). Rhamnogalacturonan lyase reveals a unique three-domain modular structure for polysaccharide lyase family 4. *FEBS Letters*, *565*(1–3), 188–194. doi:10.1016/j.febslet.2004.03.094

McDowell, N. G., & Allen, C. D. (2015). Darcy's law predicts widespread forest mortality under climate warming. *Nature Climate Change*, *5*(7), 669–672. doi:10.1038/nclimate2641

McRae, G. J. (1980). A Simple Procedure for Calculating Atmospheric Water Vapor Concentration. *Journal of the Air Pollution Control Association*, *30*(4), 394–394. doi:10.1080/00022470.1980.10464362

Meiri, D., & Breiman, A. (2009). *Arabidopsis* ROF1 (FKBP62) modulates thermotolerance by interacting with HSP90.1 and affecting the accumulation of HsfA2-regulated sHSPs. *Plant Journal*, *59*(3), 387–399. doi:10.1111/j.1365-313X.2009.03878.x

Meiri, D., Tazat, K., Cohen-Peer, R., Farchi-Pisanty, O., Aviezer-Hagai, K., Avni, A., & Breiman, A. (2010). Involvement of *Arabidopsis* ROF2 (FKBP65) in thermotolerance. *Plant Molecular Biology*, *72*(1–2), 191–203. doi:10.1007/s11103-009-9561-3

Menezes, M. P. M. de, Berger, U., & Mehlig, U. (2008). Mangrove vegetation in Amazonia: a review of studies from the coast of Pará and Maranhão States, north Brazil. *Acta Amazonica*, *38*(3), 403–420. doi:10.1590/S0044-59672008000300004

Moazzam-Jazi, M., Ghasemi, S., Seyedi, S. M., & Niknam, V. (2018). COP1 plays a prominent role in drought stress tolerance in *Arabidopsis* and Pea. *Plant Physiology and Biochemistry*, *130*(July), 678–691. doi:10.1016/j.plaphy.2018.08.015

Moran, E. V., Hartig, F., & Bell, D. M. (2016). Intraspecific trait variation across scales: Implications for understanding global change responses. *Global Change Biology*, *22*(1), 137–150. doi:10.1111/gcb.13000

Mori, G. M., Zucchi, M. I., Sampaio, I., & Souza, A. P. (2015). Species distribution and introgressive hybridization of two Avicennia species from the Western Hemisphere unveiled by phylogeographic patterns. *BMC Evolutionary Biology*, *15*(1), 1–15. doi:10.1186/s12862-015-0343-z

Mori, G. M., Zucchi, M. I., & Souza, A. P. (2015). Multiple-geographic-scale genetic structure of two mangrove tree species: the roles of mating system, hybridization, limited dispersal and extrinsic factors. *Plos One*, *10*, e0118710. doi:10.1371/journal.pone.0118710

Moritz, C. (1999). Conservation units and translocations: Strategies for conserving evolutionary processes. *Hereditas*, *130*(3), 217–228. doi:10.1111/j.1601-5223.1999.00217.x

Moriya, Y., Itoh, M., Okuda, S., Yoshizawa, A. C., & Kanehisa, M. (2007). KAAS: An automatic genome annotation and pathway reconstruction server. *Nucleic Acids Research*, *35*, 182–185. doi:10.1093/nar/gkm321

Müller, M., Kunz, H. H., Schroeder, J. I., Kemp, G., Young, H. S., & Ekkehard Neuhaus, H. (2014). Decreased capacity for sodium export out of *Arabidopsis* chloroplasts impairs salt tolerance, photosynthesis and plant performance. *Plant Journal*, *78*(4), 646–658. doi:10.1111/tpj.12501

Murata, Y., Mori, I. C., & Munemasa, S. (2015). Diverse Stomatal Signaling and the Signal Integration Mechanism. *Annual Review of Plant Biology*, *66*(1), 369–392. doi:10.1146/annurev-arplant-043014-114707

Myouga, F., Motohashi, R., Kuromori, T., Nagata, N., & Shinozaki, K. (2006). An *Arabidopsis* chloroplast-targeted Hsp101 homologue, APG6, has an essential role in chloroplast development as well as heat-stress response. *Plant Journal*, *48*(2), 249–260. doi:10.1111/j.1365-313X.2006.02873.x

Naidoo, G. (2006). Factors contributing to dwarfing in the mangrove Avicennia marina. *Annals of Botany*, *97*, 1095–1101. doi:10.1093/aob/mcl064

Narum, S. R., & Hess, J. E. (2011). Comparison of FST outlier tests for SNP loci under selection. *Molecular Ecology Resources*, *11*(SUPPL. 1), 184–194. doi:10.1111/j.1755-0998.2011.02987.x

Nascimento Jr., W. R., Souza-Filho, P. W. M., Proisy, C., Lucas, R. M., & Rosenqvist, A. (2013). Mapping changes in the largest continuous Amazonian mangrove belt using object-based classification of multisensor satellite imagery. *Estuarine, Coastal and Shelf Science*, *117*, 83–93. doi:10.1016/j.ecss.2012.10.005

Nguyen, H. T., Meir, P., Sack, L., Evans, J. R., Oliveira, R. S., & Ball, M. C. (2017). Leaf water storage increases with salinity and aridity in the mangrove Avicennia marina: integration of leaf structure , osmotic adjustment and access to multiple water sources. *Plant, Cell and Environment*, *40*(8), 1576–1591. doi:10.1111/pce.12962

Nishizawa, A., Yabuta, Y., & Shigeoka, S. (2008). Galactinol and Raffinose Constitute a Novel Function to Protect Plants from Oxidative Damage. *Plant Physiology*, *147*(3), 1251–1263. doi:10.1104/pp.108.122465

Nishizawa, Ayako, Yabuta, Y., Yoshida, E., Maruta, T., Yoshimura, K., & Shigeoka, S. (2006). *Arabidopsis* heat shock transcription factor A2 as a key regulator in response to several types of environmental stress. *Plant Journal*, *48*(4), 535–547. doi:10.1111/j.1365-313X.2006.02889.x

Novick, K. A., Ficklin, D. L., Stoy, P. C., Williams, C. A., Bohrer, G., Oishi, A. C., … Phillips, R. P. (2016). The increasing importance of atmospheric demand for ecosystem water and carbon fluxes. *Nature Climate Change*, *6*(11), 1023–1027. doi:10.1038/nclimate3114

O'Leary, N. A., Wright, M. W., Brister, J. R., Ciufo, S., Haddad, D., McVeigh, R., … Pruitt, K. D. (2016). Reference sequence (RefSeq) database at NCBI: Current status, taxonomic expansion, and functional annotation. *Nucleic Acids Research*, *44*, D733–D745. doi:10.1093/nar/gkv1189

Oh, S. E., Yeung, C., Babaei-Rad, R., & Zhao, R. (2014). Cosuppression of the chloroplast localized molecular chaperone HSP90.5 impairs plant development and chloroplast biogenesis in *Arabidopsis*. *BMC Research Notes*, *7*(1), 1–15. doi:10.1186/1756-0500-7-643

Ohgishi, M., Saji, K., Okada, K., & Sakai, T. (2004). Functional analysis of each blue light receptor, cry1, cry2, phot1, and phot2, by using combinatorial multiple mutants in *Arabidopsis*. *Proceedings of the National Academy of Sciences*, *101*(8), 2223–2228. doi:10.1073/pnas.0305984101

Oliveira, R. R., Viana, A. J. C., Reátegui, A. C. E., & Vincentz, M. G. A. (2015). An efficient method for simultaneous extraction of high-quality RNA and DNA from various plant tissues. *Genetics and Molecular Research*, *14*(4), 18828–18838. doi:10.4238/2015.December.28.32

Osland, M. J., Gabler, C. A., Grace, J. B., Day, R. H., Mccoy, M. L., Mcleod, J. L., … Hartley, S. B. (2018). Climate and plant controls on soil organic matter in coastal wetlands. *Global Change Biology*, (February). doi:10.1111/gcb.14376

Pacín, M., Semmoloni, M., Legris, M., Finlayson, S. A., & Casal, J. J. (2016). Convergence of CONSTITUTIVE PHOTOMORPHOGENESIS 1 and PHYTOCHROME INTERACTING FACTOR signalling during shade avoidance. *New Phytologist*, *211*(3), 967–979. doi:10.1111/nph.13965

Pagnussat, G. C., Yu, H.-J., Ngo, Q. A., Rajani, S., Mayalagu, S., Johnson, C. S., … Sundaresan, V. (2005). Genetic and molecular identification of genes required for female gametophyte development and function in *Arabidopsis*. *Development*, *132*(3), 603–614. doi:10.1242/dev.01595

Panikashvili, D., Savaldi-Goldstein, S., Mandel, T., Yifhar, T., Franke, R. B., Hofer, R., … Aharoni, A. (2007). The *Arabidopsis* DESPERADO/AtWBC11 transporter is required for cutin and wax secretion. *Plant Physiology*, *145*(4), 1345–1360. doi:10.1104/pp.107.105676

Park, H. Y., Seok, H. Y., Park, B. K., Kim, S. H., Goh, C. H., Lee, B. ha, … Moon, Y. H. (2008). Overexpression of *Arabidopsis* ZEP enhances tolerance to osmotic stress. *Biochemical and Biophysical Research Communications*, *375*(1), 80–85. doi:10.1016/j.bbrc.2008.07.128

Patel, R. K., & Jain, M. (2012). NGS QC toolkit: A toolkit for quality control of next generation sequencing data. *PLoS ONE*, *7*(2), e30619. doi:10.1371/journal.pone.0030619

Pavey, S. A., Nosil, P., & Rogers, S. M. (2010). The role of gene expression in ecological speciation. *Annals of the New York Academy of Sciences*, *1206*, 110–129. doi:10.1111/j.1749-6632.2010.05765.x

Pedmale, U. V., Huang, S. S. C., Zander, M., Cole, B. J., Hetzel, J., Ljung, K., … Chory, J. (2016). Cryptochromes Interact Directly with PIFs to Control Plant Growth in Limiting Blue Light. *Cell*, *164*(1–2), 233–245. doi:10.1016/j.cell.2015.12.018

Pesquet, E., Korolev, A. V., Calder, G., & Lloyd, C. W. (2010). The microtubule-associated protein AtMAP70-5 regulates secondary wall patterning in *Arabidopsis* wood cells. *Current Biology*, *20*(8), 744–749. doi:10.1016/j.cub.2010.02.057

Phillips, O. L., Aragão, L. E. O. C., Lewis, S. L., Fisher, J. B., Lloyd, J., López-González, G., … Vargas, P. N. (2009). Drought Sensitivity of the Amazon Rainforest. *Science*, *323*(5919), 1344–1347. doi:10.1126/science.1164033

Phillips, O. L., Heijden, G. van der, Lewis, S. L., López-González, G., Aragão, L. E. O. C., Lloyd, J., … Vilanova, E. (2010). Drought–mortality relationships for tropical forests. *New Phytologist*, *187*, 631–646. doi:10.1111/j.1469-8137.2010.03359.x Key

Pieslinger, A. M., Hoepflinger, M. C., & Tenhaken, R. (2010). Cloning of glucuronokinase from *Arabidopsis thaliana*, the last missing enzyme of the myo-inositol oxygenase pathway to nucleotide sugars. *Journal of Biological Chemistry*, *285*(5), 2902–2910. doi:10.1074/jbc.M109.069369

Polidoro, B. A., Carpenter, K. E., Collins, L., Duke, N. C., Ellison, A. M., Ellison, J. C., … Yong, J. W. H. (2010). The loss of species: Mangrove extinction risk and geographic areas of global concern. *PLoS ONE*, *5*(4), e10095. doi:10.1371/journal.pone.0010095

Powell, T. L., Wheeler, J. K., de Oliveira, A. A. R., da Costa, A. C. L., Saleska, S. R., Meir, P., & Moorcroft, P. R. (2017). Differences in xylem and leaf hydraulic traits explain differences in drought tolerance among mature Amazon rainforest trees. *Global Change Biology*, *23*(10), 4280–4293. doi:10.1111/gcb.13731

Pranchai, A., Jenke, M., Vogt, J., Grueters, U., Yue, L., Mehlig, U., … Berger, U. (2017). Density-dependent shift from facilitation to competition in a dwarf Avicennia germinans forest. *Wetlands Ecology and Management*, 1–12. doi:10.1007/s11273-017-9561-z

Privat, I., Hakimi, M. A., Buhot, L., Favory, J. J., & Lerbs-Mache, S. (2003). Characterization of *Arabidopsis* plastid sigma-like transcription factors SIG1, SIG2 and SIG3. *Plant Molecular Biology*, *51*(3), 385–399. doi:10.1023/A:1022095017355

Queitsch, C., Hong, S.-W., Vierling, E., & Lindquist, S. (2000). Heat Shock Protein 101 Plays a Crucial Role in Thermotolerance in *Arabidopsis*. *Plant Cell*, *12*(4), 479–492. doi:10.1105/tpc.12.4.479

Ramírez-Valiente, J. A., Deacon, N. J., Etterson, J., Center, A., Sparks, J. P., Sparks, K. L., … Cavender-Bares, J. (2018). Natural selection and neutral evolutionary processes contribute to genetic divergence in leaf traits across a precipitation gradient in the tropical oak Quercus oleoides. *Molecular Ecology*, *27*, 2176–2192. doi:10.1111/mec.14566

Reef, R., & Lovelock, C. E. (2015). Regulation of water balance in Mangroves. *Annals of Botany*, *115*(3), 385–395. doi:10.1093/aob/mcu174

Robinson, M. D., McCarthy, D. J., & Smyth, G. K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*, *26*(1), 139–140. doi:10.1093/bioinformatics/btp616

Rodell, M., Famiglietti, J. S., Wiese, D. N., Reager, J. T., Beaudoing, H. K., Landerer, F. W., & Lo, M. H. (2018). Emerging trends in global freshwater availability. *Nature*, *557*(7707), 651–659. doi:10.1038/s41586-018-0123-1

Rowland, L., Da Costa, A. C. L., Galbraith, D. R., Oliveira, R. S., Binks, O. J., Oliveira, A. A. R., … Meir, P. (2015). Death from drought in tropical forests is triggered by hydraulics not carbon starvation. *Nature*, *528*(7580), 119–122. doi:10.1038/nature15539

Russello, M. A., Waterhouse, M. D., Etter, P. D., & Johnson, E. A. (2015). From promise to practice: pairing non-invasive sampling with genomics in conservation. *PeerJ*, *3*, e1106. doi:10.7717/peerj.1106

Samol, I., Shapiguzov, A., Ingelsson, B., Fucile, G., Crèvecoeur, M., Vener, A. V., … Goldschmidt-Clermont, M. (2012). Identification of a Photosystem II Phosphatase Involved in Light Acclimation in *Arabidopsis*. *The Plant Cell*, *24*(6), 2596–2609. doi:10.1105/tpc.112.095703

Sbrocco, E. J., & Barber, P. H. (2013). MARSPEC: ocean climate layers for marine spatial ecology. *Ecology*, *94*(4), 979–979. doi:10.1890/12-1358.1

Schaeffer-Novelli, Y., Cintrón-Molero, G., Adaime, R. R., & de Camargo, T. M. (1990). Variability of mangrove ecosystems along the Brazilian coast. *Estuaries*, *13*(2), 204–218. doi:10.1007/BF02689854

Schoener, T. W. (2011). The Newest Synthesis: Understanding the Interplay of Evolutionary and Ecological Dynamics. *Science*, *331*(6016), 426–429. doi:10.1126/science.1193954

Shirano, Y., Shimada, H., Kanamaru, K., Fujiwara, M., Tanaka, K., Takahashi, H., … Shibata, D. (2000). Chloroplast development in *Arabidopsis thaliana* requires the nuclear-encoded transcription factor Sigma B. *FEBS Letters*, *485*(2–3), 178–182. doi:10.1016/S0014-5793(00)02216-X

Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V., & Zdobnov, E. M. (2015). BUSCO: Assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics*, *31*(19), 3210–3212. doi:10.1093/bioinformatics/btv351

Steppe, K., Vandegehuchte, M. W., Van de Wal, B. A. E., Hoste, P., Guyot, A., Lovelock, C. E., & Lockington, D. A. (2018). Direct uptake of canopy rainwater causes turgor-driven growth spurts in the mangrove Avicennia marina. *Tree Physiology*, *38*(7), 979–991. doi:10.1093/treephys/tpy024

Stone, S. L., Williams, L. A., Farmer, L. M., Vierstra, R. D., & Callis, J. (2006). KEEP ON GOING, a RING E3 Ligase Essential for *Arabidopsis* Growth and Development, Is Involved in Abscisic Acid Signaling. *Plant Cell Online*, *18*(12), 3415–3428. doi:10.1105/tpc.106.046532

Stuart, S. A., Choat, B., Martin, K. C., Holbrook, N. M., & Ball, M. C. (2007). The role of freezing in setting the latitudinal limits of mangrove forests. *New Phytologist*, *173*(3), 576–583. doi:10.1111/j.1469-8137.2006.01938.x

Suetsugu, N., Kagawa, T., Wada, M., & Corporation, T. (2005). An Auxilin-Like J-Domain Protein , JAC1 , Regulates Phototropin-Mediated Chloroplast Movement. *Plant Physiology*, *139*(September), 151–162. doi:10.1104/pp.105.067371.flowering

Sun, W., Bernard, C., Cotte, B. van de, Montagu, M. Van, & Verbruggen, N. (2001). At-HSP17.6A, encoding a small heat-shock protein in *Arabidopsis*, can enhance osmotolerance upon overexpression. *The Plant Journal*, *27*(5), 407–415. doi:10.1046/j.1365-313X.2001.01107.x

Taji, T., Ohsumi, C., Iuchi, S., Seki, M., Kasuga, M., Kobayashi, M., … Shinozaki, K. (2002). Important roles of drought- and cold-inducible genes for galactinol synthase in stress tolerance in *Arabidopsis thaliana*. *Plant Journal*, *29*(4), 417–426. doi:10.1046/j.0960-7412.2001.01227.x

Takase, M., Mizoguchi, T., Kozuka, T., & Tsukaya, H. (2013). The unique function of the *Arabidopsis* circadian clock gene PRR5 in the regulation of shade avoidance response. *Plant Signaling and Behavior*, *8*(4). doi:10.4161/psb.23534

Takayama, K., Tateishi, Y., Murata, J., & Kajita, T. (2008). Gene flow and population subdivision in a pantropical plant with sea-drifted seeds *Hibiscus tiliaceus* and its allied species: Evidence from microsatellite analyses. *Molecular Ecology*, *17*(11), 2730–2742. doi:10.1111/j.1365-294X.2008.03799.x

Teige, M., Scheikl, E., Eulgem, T., Dóczi, R., Ichimura, K., Shinozaki, K., … Hirt, H. (2004).

The MKK2 pathway mediates cold and salt stress signaling in *Arabidopsis*. *Molecular Cell*, *15*(1), 141–152. doi:10.1016/j.molcel.2004.06.023

ten Hove, C. A., Bochdanovits, Z., Jansweijer, V. M. A., Koning, F. G., Berke, L., Sanchez-Perez, G. F., … Heidstra, R. (2011). Probing the roles of LRR RLK genes in *Arabidopsis thaliana* roots using a custom T-DNA insertion set. *Plant Molecular Biology*, *76*(1–2), 69–83. doi:10.1007/s11103-011-9769-x

Tomlinson, P. B. (1986). *The Botany of Mangroves*. New York: Cambridge University Press.

Tyree, M. T., & Sperry, J. S. (1989). Vulnerability of Xylem to Cavitation and Embolism. *Annual Review of Plant Physiology and Molecular Biology*, 19–38. doi:10.1146/annurev.pp.40.060189.000315

Uraji, M., Katagiri, T., Okuma, E., Ye, W., Hossain, M. A., Masuda, C., … Murata, Y. (2012). Cooperative Function of PLD and PLD 1 in Abscisic Acid-Induced Stomatal Closure in *Arabidopsis*. *Plant Physiology*, *159*(1), 450–460. doi:10.1104/pp.112.195578

Van der Does, D., Boutrot, F., Engelsdorf, T., Rhodes, J., McKenna, J. F., Vernhettes, S., … Zipfel, C. (2017). The *Arabidopsis* leucine-rich repeat receptor kinase MIK2/LRR-KISS connects cell wall integrity sensing, root growth and response to abiotic and biotic stresses. *PLoS Genetics*, *13*(6), 1–27. doi:10.1371/journal.pgen.1006832

Vogt, J., Lin, Y., Pranchai, A., Frohberg, P., Mehlig, U., & Berger, U. (2014). The importance of conspecific facilitation during recruitment and regeneration: A case study in degraded mangroves. *Basic and Applied Ecology*, *15*(8), 651–660. doi:10.1016/j.baae.2014.09.005

Walsh, B., & Blows, M. W. (2009). Abundant Genetic Variation + Strong Selection = Multivariate Genetic Constraints: A Geometric View of Adaptation. *Annual Review of Ecology, Evolution, and Systematics*, *40*(1), 41–59. doi:10.1146/annurev.ecolsys.110308.120242

Walsh, P., Bursać, D., Law, Y. C., Cyr, D., & Lithgow, T. (2004). The J-protein family: Modulating protein assembly, disassembly and translocation. *EMBO Reports*, *5*(6), 567–571. doi:10.1038/sj.embor.7400172

Wang, P., Li, Z., Wei, J., Zhao, Z., Sun, D., & Cui, S. (2012). A Na^{+}/Ca^{2+}exchanger-like protein (AtNCL) involved in salt stress in Arabidopsis. *Journal of Biological Chemistry*, *287*(53), 44062–44070. doi:10.1074/jbc.M112.351643

Wang, Q., Sullivan, R. W., Kight, A., Henry, R. L., Huang, J., Jones, A. M., & Korth, K. L. (2004). Deletion of the Chloroplast-Localized Thylakoid Formation 1 Gene Product in *Arabidopsis* Leads to Deficient Thylakoid Formation and Variegated Leaves. *Plant Physiology*, *136*(3), 3594–3604. doi:10.1104/pp.104.049841

Wang, S., Chang, Y., & Ellis, B. (2016). Overview of OVATE FAMILY PROTEINS, A Novel Class of Plant-Specific Growth Regulators. *Frontiers in Plant Science*, *7*(March), 1–8. doi:10.3389/fpls.2016.00417

Wang, S., Chang, Y., Guo, J., & Chen, J. G. (2007). *Arabidopsis* Ovate Family Protein 1 is a transcriptional repressor that suppresses cell elongation. *Plant Journal*, *50*(5), 858–872. doi:10.1111/j.1365-313X.2007.03096.x

Wehmeyer, N., Hernandez, L. D., Finkelstein, R. R., & Vierling, E. (1996). Synthesis of Small Heat-Shock Proteins Is Part of the Developmental Program of Late Seed Maturation. *Plant Physiology*, *112*(2), 747–757. doi:10.1104/pp.112.2.747

Wingler, A., Lea, P. J., Quick, W. P., & Leegood, R. C. (2000). Photorespiration: metabolic pathways and their role in stress protection. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *355*, 1517–1529. doi:10.1098/rstb.2000.0712

Wolf, J. B. W., Lindell, J., & Backstrom, N. (2010). Speciation genetics: current status and evolving approaches. *Philosophical Transactions of the Royal Society B: Biological*

Sciences, *365*(1547), 1717–1733. doi:10.1098/rstb.2010.0023

Young, M. D., Wakefield, M. J., Smyth, G. K., & Oshlack, A. (2010). Gene ontology analysis for RNA-seq: accounting for selection bias. *Genome Biology*, *11*(2), R14. doi:10.1186/gb-2010-11-2-r14

Zhang, A., Ren, H.-M., Tan, Y.-Q., Qi, G.-N., Yao, F.-Y., Wu, G.-L., … Wang, Y.-F. (2016). S-Type Anion Channels SLAC1 and SLAH3 Function as Essential Negative Regulators of Inward K^+ Channels and Stomatal Opening in Arabidopsis. *The Plant Cell*, *28*(4), 949–965. doi:10.1105/tpc.15.01050

Zhang, C., Zhang, L., Zhang, S., Zhu, S., Wu, P., Chen, Y., … Wu, G. (2015). Global analysis of gene expression profiles in physic nut (Jatropha curcas L.) seedlings exposed to drought stress. *BMC Plant Biology*, *15*(1), 1–14. doi:10.1186/s12870-014-0397-x

Zhong, R., Lee, C., Zhou, J., McCarthy, R. L., & Ye, Z.-H. (2008). A Battery of Transcription Factors Involved in the Regulation of Secondary Cell Wall Biosynthesis in *Arabidopsis*. *The Plant Cell Online*, *20*(10), 2763–2782. doi:10.1105/tpc.108.061325

Data accessibility

- 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 doi:10.5061/dryad.h11t255 (M. V. Cruz et al., 2019).

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., G.M.M. contributed material/reagents/analytical tools. All authors discussed the results and contributed to the manuscript.

Tables and Figures

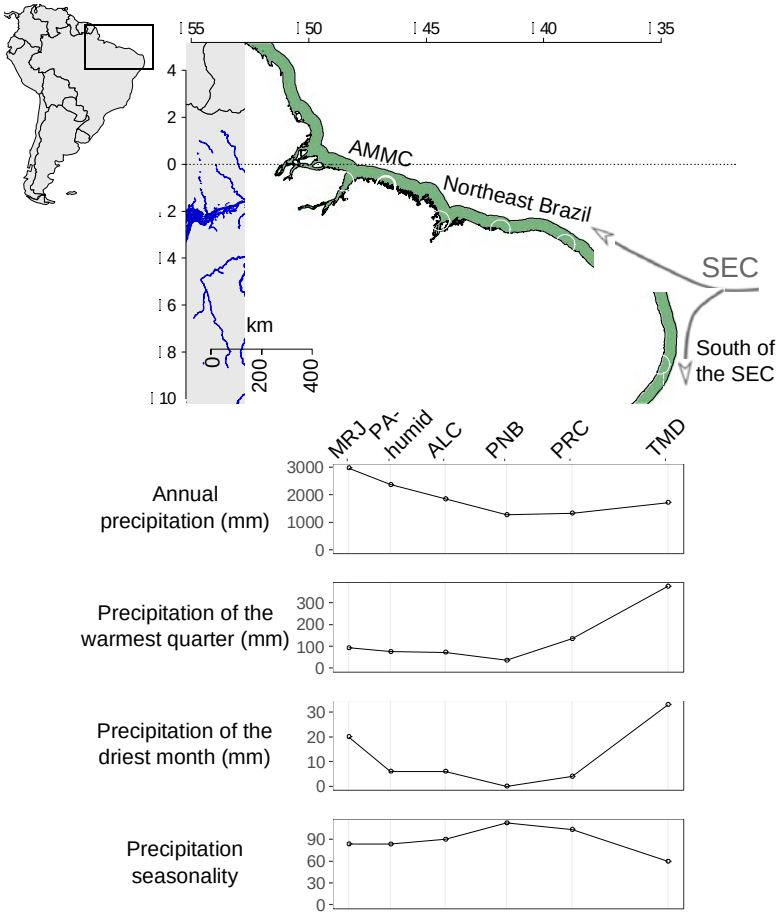

Figure 1. *Avicennia germinans* sampling sites along a low-latitude salinity and precipitation gradient. Black points represent sampling sites, green area represents the occurrence of the species, and blue areas represent ponds and rivers. *Top:* Geographical location of the study area and sampling sites. *Bottom:* Environmental variation across the sampling sites (source: WorldClim). *AMMC*: Amazon Macrotidal Mangrove Coast. *SEC*: South Equatorial Current.

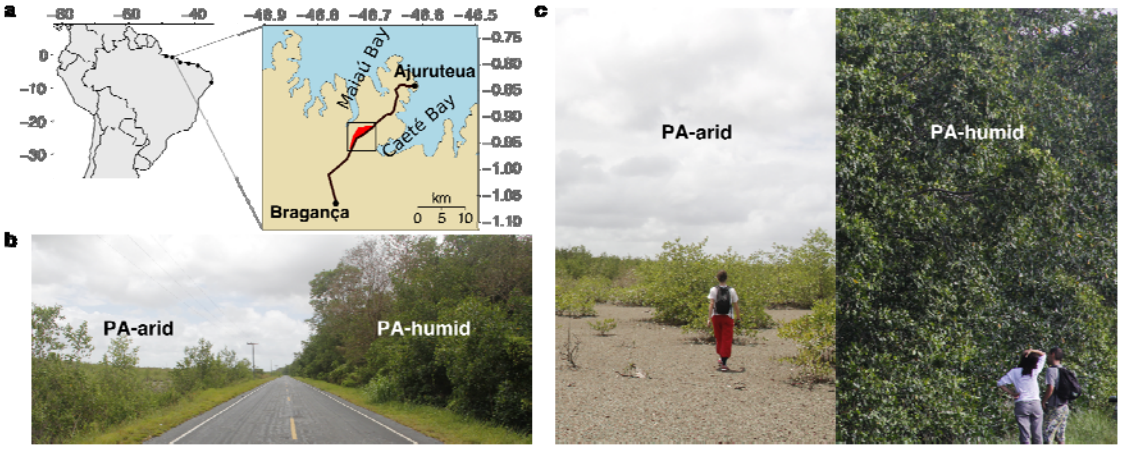

Figure 2. PA-arid and PA-humid sampling sites. (a) Geographical location of sites, highlighted by a square (red colored area: PA-arid); (b) photograph of a section of the Bragança-Ajuruteua road (Pará, Brazil) along which severe changes in hydrology altered the mangrove community and tree morphology; (c) detailed photographs of the PA-arid and PA-humid sites. *Photographs authors:* G.M. Mori and M.V. Cruz.

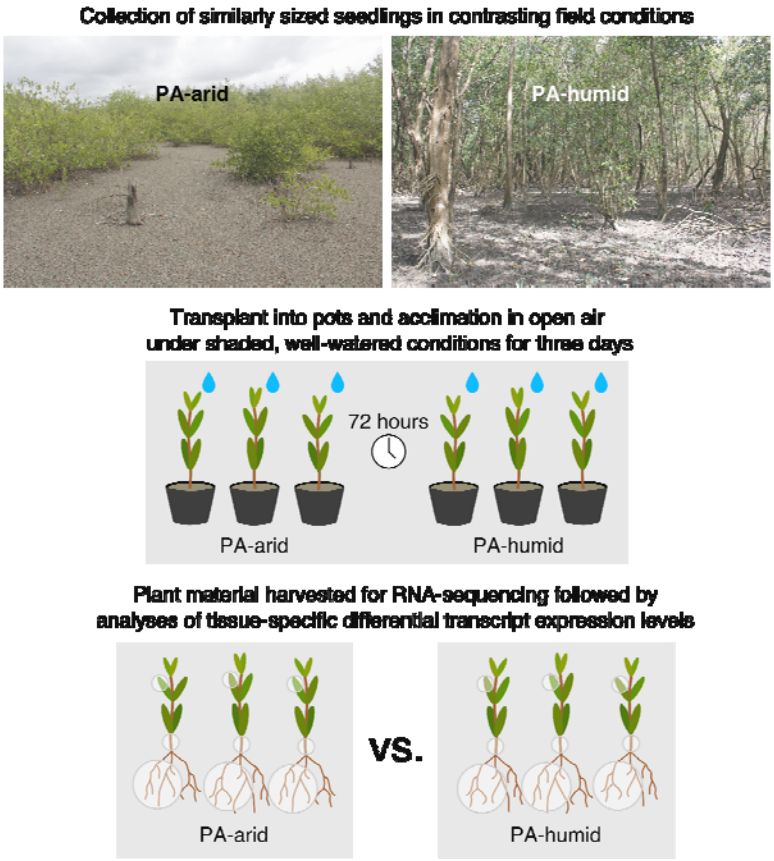

Figure 3. RNA sequencing experimental design. Similarly sized *Avicennia germinans* seedlings naturally grown under adjacent, but contrasting field conditions were collected and transplanted into pots, where they acclimated for 72 hours under homogenous treatment. RNA was extracted from leaves, stems and roots for transcriptome sequencing. The

transcriptome was *de novo* assembled and used as a reference for differential transcript expression analyses.

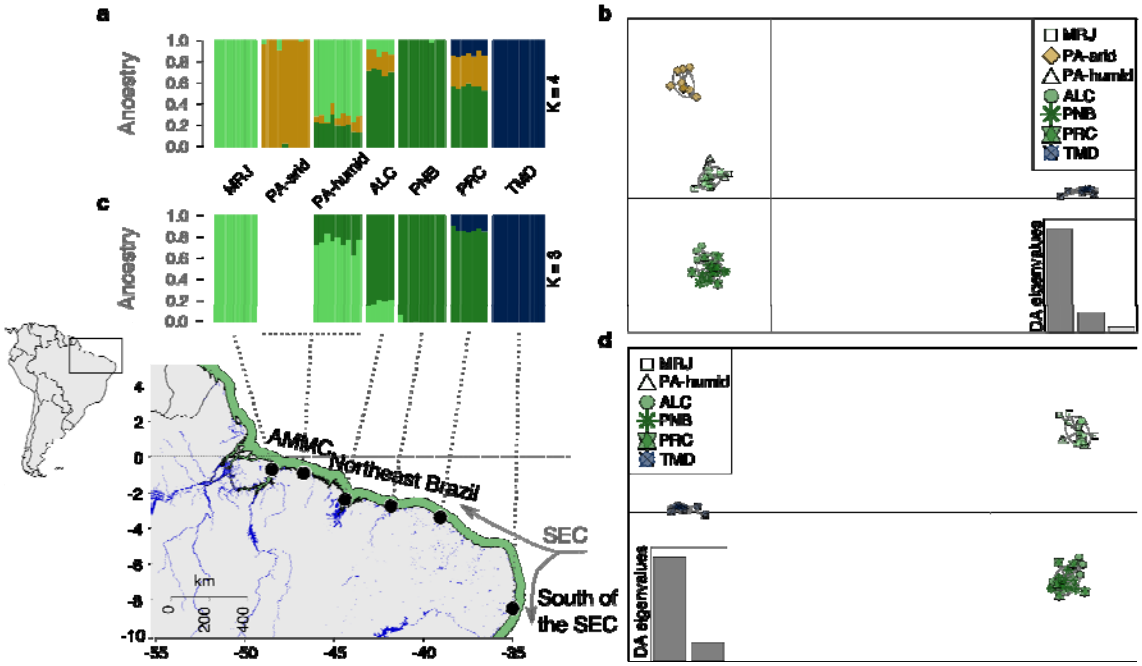

Figure 4. Genetic structure inferred from genome-wide single nucleotide polymorphism (SNP) detected in *Avicennia germinans*. (a) Attribution of ancestry implemented in the program Admixture 1.3.0 for all sampled individuals; stacked bars represent individuals, and each color represents one ancestral 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 color 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; distinct symbols indicate sampling sites.

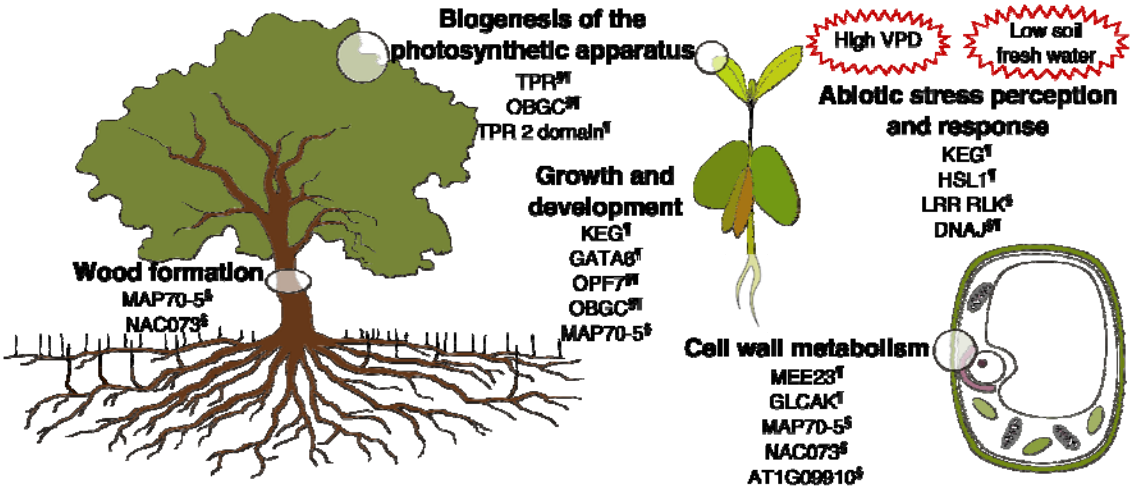

Figure 5. Schematic representation of key biological processes related to physiological drought-tolerance or response associated with candidate loci for selection in *Avicennia germinans* samples along tropical mangrove forests of the north-northeastern Brazilian coast. Candidate loci were detected from two datasets: (1) all sampled individuals, using F_{ST} outlier tests (marked with §) and (2) a subset of individuals, without samples from the PA-arid site, combining genetic-environmental association tests and F_{ST} outlier approaches (marked with ¶).

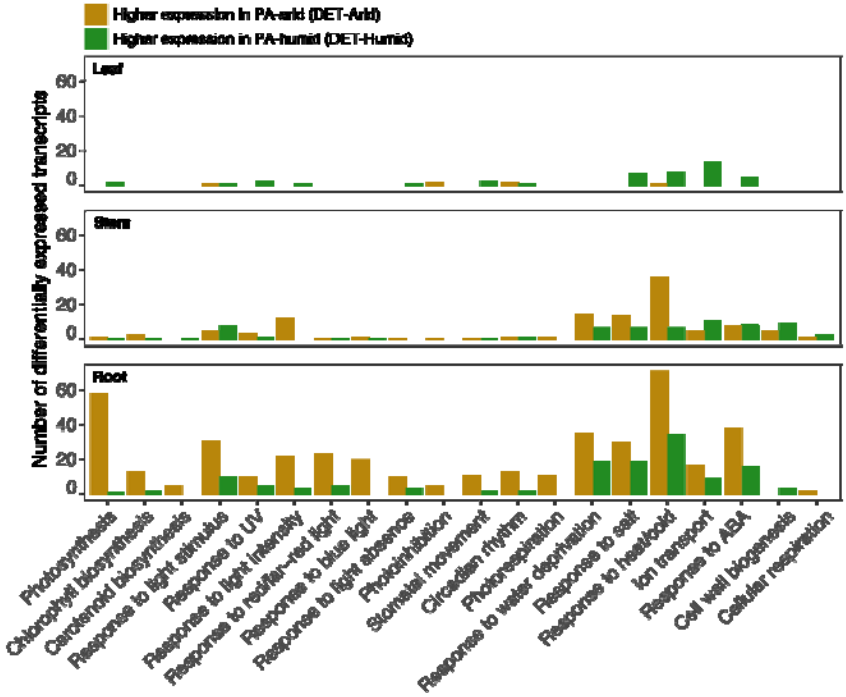

Figure 6. Functional categories of differentially expressed transcripts (DETs) identified between samples grown in the PA-arid and PA-humid sampling sites. Categories were selected based on biological processes previously identified to be involved in the response, acclimation or resistance to water stress in model plants.

Table 1. Characterization and geographic location of *Avicennia germinans* sampling sites.

Sampling site ID	Climate classification†	Latitude; Longitude	Number of samples	
			nextRAD	RNA-Seq
MRJ	Am	00.72 S; 48.49 W	8	-
PA-arid‡	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	-

†Köppen-Geiger climate classification system (Alvares, Stape, Sentelhas, Gonçalves, & Sparovek, 2013). *Am*: Tropical monsoon climate; *Aw*: Tropical wet savanna climate; *As*: Tropical dry savanna climate.
‡Samples from a forest flooded exclusively during spring tides (Lara & Cohen, 2006).

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. π: Nucleotide diversity; H_E: expected heterozygosity; H_O: observed heterozygosity; pA: private alleles; %Poly: percentage of polymorphic loci; F_{IS}: inbreeding coefficient.

Sampling site	π (mean)	π (s.e.)	H_E	H_O	pA	%Poly	F_{IS}	Low Level (95% c.i.)	High Level (95% c.i.)
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

Table 3. Annotation of candidate SNP loci putatively under selection, associated with tolerance of or response to physiological drought. Candidate loci were detected from two datasets: (1) all sampled individuals, including PA-arid samples, using F_{ST} outlier approaches and (2) a subset of samples, excluding individuals from the PA-arid site, combining genetic-environmental association tests and F_{ST} outlier tests.

Dataset	Transcript ID	Similar to†	Environmental association (FDR)‡	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 signaling 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 signaling (ten Hove et al., 2011; Van der Does et al., 2017) and in regulating adaptation to salt stress (Lorenzo et al., 2009)
1	Ag_4919	AT1G09910, Rhamnogalactu-ronate lyase family protein	NA	Associated with the degradation of the cell wall polysaccharides, and pectin (McDonough, Kadirvelraj, Harris, Poulsen, & Larsen, 2004)
1 and 2	Ag_20543	AT5G18950, Tetratricopeptide 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 OBGC, chloroplastic [Sesamum indicum]	PWQ (8.31e-05)	Associated with the 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 (S. Wang, Chang, & Ellis, 2016; S. 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 (P. Walsh, Bursać, Law, Cyr, & Lithgow, 2004) and protein protection from stress-induced denaturation (Frydman, 2001)
2	Ag_12760	AT3G54810, GATA8, Plant-specific GATA-type zinc finger transcription factor family protein	PWQ (0.00363); PSEASON (0.00529); 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 signaling (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 signaling (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, Hoepflinger, & Tenhaken, 2010)
2	Ag_11269	AT2G25730, unknown protein	PWQ (0,00016)	The Ag_11269 transcript has a TPR2 domain and, thus, may be involved in photosynthetic machinery biogenesis, stabilization and repair (Bohne et al., 2016)

†Blastx hit to *Arabidopsis thaliana* or *Sesamum indicum* [when indicated] gene models.
‡(*FDR*): False discovery rate values (Benjamini-Hochberg procedure) for genetic-environment association tests; *NA*: genetic-environment association tests were not available for dataset (1) due to unavailability of environmental data for the PA-arid sites in public databases; *PWQ*: Precipitation of the warmest quarter; *PSEASON*: Precipitation seasonality; *PDM*: Precipitation of the driest month.