Title:

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

Short running title:

Black-Mangroves responses to water limitation

Authors:

Mariana Vargas Cruz[1], Gustavo Maruyama Mori[2], Dong-Ha Oh[3], Maheshi Dassanayake[3], Maria Imaculada Zucchi[4], Rafael Silva Oliveira[1], Anete Pereira de Souza[1].

[1]Department of Plant Biology, Institute of Biology, University of Campinas (Unicamp), Campinas, SP 13083-863, Brazil.

[2]Institute of Biosciences, São Paulo State University (Unesp), São Vicente, SP 11330-900, Brazil.

[3]Department of Biological Sciences, Louisiana State University (LSU), Louisiana, LA 70803, United States.

[4]São Paulo Agency for Agribusiness Technology (APTA), Piracicaba, SP 13400-790, Brazil.

*Author for correspondence. Tel: +55 19 3521 1132 Email: anete@unicamp.br

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

311 (0.168) and %Poly (34.52%). The remaining sites, influenced by the northern branch of the
312 SEC, presented considerably higher levels of diversity (mean π ranging from 0.208 to 0.323;
313 H_E, from 0.195-0.296; H_O, from 0.234-0.328; %Poly 58.31-84,46%), including the PA-arid
314 site, one of the most genetically diverse across the study region. All sampling sites deviated
315 from Hardy-Weinberg equilibrium (HWE), with an excess of heterozygosity in all sites,
316 besides ALC, which showed a small heterozygosity deficit (F_{IS}=0.03). HWE deviation was
317 highest in TMD (F_{IS}=-0.43) but relatively low in all remaining sites, ranging from -0.15
318 (PNB) to +0.03 (ALC).

319 A substantial genetic structure was observed over the Equatorial Brazilian coast, a
320 region subject to highly variable rainfall and riverine freshwater inputs (Figure 1). The genetic
321 diversity, based on 2,297 genome-wide SNPs genotyped in 57 individuals, was organized into
322 four distinct genetic clusters (K=4) (Figure 4a, Supplementary Figure S3a-b). The greatest
323 genetic divergences (F_{ST}>0.46; Nei's distance>0.255) were observed between individuals
324 from TMD and those from all other sites (Figure 4b-c, Supplementary Table S1). The TMD
325 site also presented the highest number of private alleles (pA=46). Most remarkable was the
326 divergence between individuals from PA-arid and its adjacent site, PA-humid (F_{ST}=0.25;
327 Nei's distance=0.181). This divergence was greater than the observed divergence of PA-arid
328 from PRC individuals (Supplementary Table S1), located approximately 900 km distant from
329 each other (F_{ST}=0.23; Nei's distance=0.177), on the semi-arid coast of Brazil. The divergent
330 gene pool of the PA-arid population was also evident when these samples were excluded from
331 structure analyses. The most likely number of ancestral populations dropped from four to
332 three (Figure 4c-d, Supplementary Figure S3c-d), and the overall structure remained
333 unchanged (Figure 4d). At a finer scale, individuals from PA-humid and MRJ sites, on the
334 Amazon Macrotidal Mangrove Coast (AMMC), seemed to be derived from the same ancestral

335 population. This population, in turn, diverged from the population that may have given rise to
336 individuals from ALC, PNB, and PRC sites, in Northeast Brazil (Figure 4a-b).
337
338 ***3.2*** **De novo** ***assembly and annotation of the*** **Avicennia germinans** ***reference***
339 ***transcriptome***
340 We used RNA-Seq to sequence and *de novo* assemble a reference transcriptome from
341 leaves, stems and roots of *A. germinans* seedlings, providing a functional context for this
342 study. A total of 249,875,572 high-quality-filtered, paired-end, 72-bp reads, representing
343 78.25% of the raw data was used in the assembly. The reference transcriptome comprised
344 47,821 contigs, after removal of misassembled, redundant and contaminant sequences.
345 Putative ORFs were identified in 29,854 contigs, subsequently annotated as putative protein-
346 coding transcripts. The remaining 17,967 contigs were classified as putative non-coding
347 transcripts. A detailed characterization of contigs can be found in Supplementary Table S2.
348 Raw reads were mapped back to the reference transcriptome, with over 82% uniquely
349 mapped to a single transcript and only 1.31% mapped to more than one transcript
350 (Supplementary Table S3). We found 91.74% of the plant universal orthologs from the
351 BUSCO database (Simão, Waterhouse, Ioannidis, Kriventseva, & Zdobnov, 2015)
352 represented in the reference transcriptome (Supplementary Table S3). We also found from
353 20,529 to 31,348 putative orthologous sequences between the *A. germinans* transcriptome and
354 four other publicly available transcriptomes derived from the genus *Avicennia* L.
355 (Acanthaceae) (Supplementary Table S4).
356 As expected, most putative protein-coding transcripts of the reference transcriptome
357 could be annotated using relevant databases (92.6%), whereas only a few putative non-coding
358 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

551 north-south divergence. The closely related species, *Avicennia marina*, has the ability of
552 directly absorbing rainwater through its leaves, presenting substantial growth spurts following
553 precipitation events (Steppe et al., 2018). This mechanism has been observed in plants from
554 dry lands (Breshears et al., 2008), cloud forests (Eller, Lima, & Oliveira, 2013) and in
555 conifers (Mayr et al., 2014), and might also be present in *A. germinans*. Complementarily,
556 both aridity and salinity enhance the leaf water storage for transient growth under favorable
557 conditions in *A. marina*, requiring the use of alternative water sources to that supplied by
558 roots (Nguyen et al., 2017). Therefore, our results suggest that the more even distribution of
559 rainfall throughout the year in the TMD site likely alleviates water-stress in *A. germinans*,
560 whereas more limited rainfall in the warmest quarter of northern regions reduces opportunities
561 for rehydration via foliar water uptake (Figure 1), potentially favoring traits, which increase
562 drought-tolerance. It is plausible that this environmental filter contributes to the genetic
563 divergence observed between TMD and remaining populations (Figure 4). This hypothesis is
564 corroborated by the detection of 21 loci candidate for selection correlated to the spatial
565 variation in the precipitation of the warmest quarter over the study area (Figure 1). Although
566 seven out of these loci were poorly characterized, hampering inferences about their functional
567 relevance in the environmental context, we were able to associate 11 candidate loci with
568 biological processes influenced by drought, as photosynthesis, cell wall metabolism, cell
569 elongation, plant growth, protein protection from stress-induced degradation and regulation of
570 abscisic acid signaling (Figure 5, Table 3). The adaptive importance of freshwater limitation
571 in tropical trees was also suggested in the mangrove, *A. schaueriana* (M. V. Cruz et al.,
572 2018), and in tropical forests and savannas (Ciemer et al., 2019), for which it was similarly
573 suggested that an environmental filtering mechanism driven by high rainfall variability
574 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*

599 The PA-arid population of *A. germinans*, was originated after 1974, when the
600 construction of the Bragança-Ajuruteua road altered the hydrology of part of the mangrove
601 forest in the AMMC region (Figure 2) (Cohen & Lara, 2003). This sudden environmental
602 change caused a large dieback of the mangrove vegetation. Gradually, the impacted area was
603 recolonized, mainly by *A. germinans*. We estimate that the approximately 40 years since this
604 event occurred, represent from 4 to 40 reproductive cycles of *A. germinans*, based on previous
605 studies of closely related *Avicennia* sp. (Almahasheer, Duarte, & Irigoien, 2016; M. V. Cruz
606 et al., 2018; Polidoro et al., 2010). Recolonizing individuals of PA-arid started presenting a
607 dwarf, shrub architecture, very distinct from the former tall, arboreal architecture, still
608 observed in surrounding areas where the hydrology remained unchanged (Figure 2) (Pranchai
609 et al., 2017). This observation suggests that limitation in plant access to soil water favored
610 smaller tree sizes, one of the most integrative characteristic of drought resistance (Bennett et
611 al., 2015; Corlett, 2016; Naidoo, 2006; Rowland et al., 2015). On the one hand, we cannot
612 determine how much of the difference in tree size is inherited or determined by phenotypic
613 plasticity. On the other hand, our results revealed that a substantial and rapid change in the
614 gene pool of the PA-arid population occurred without reduction in levels of overall genetic
615 diversity, estimated by various parameters (Figure 2, Figure 4a-b, Table 2). The presence of
616 high levels of genome-wide genetic variation, simultaneously with strong selection on
617 individual traits may be a signature of substantial multivariate genetic constraints (B. Walsh
618 & Blows, 2009). Interestingly, this divergence in allele frequencies was observed despite
619 clear possibility of gene flow, given the geographical proximity (<10.0 m) of the PA-arid site
620 to well preserved populations. Consistent with an adaptive response to selection by limited
621 access to soil freshwater, F_{ST} outlier tests detected eight candidate loci within transcripts
622 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).

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Conflicts of interest

The authors have no conflict of interest to disclose.

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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

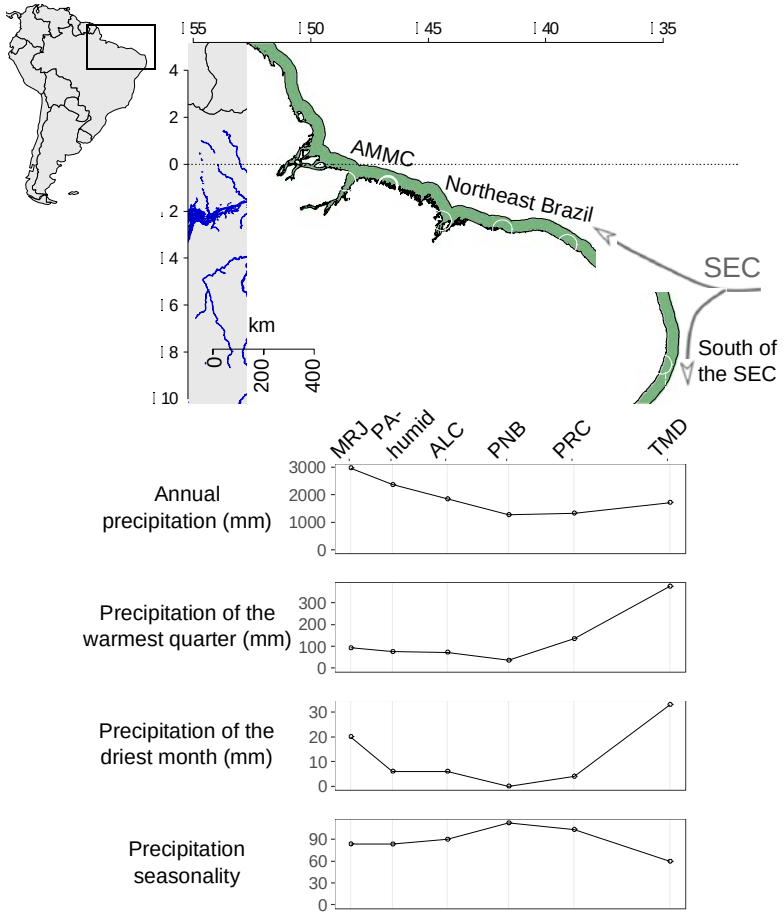

Figure 1. *Avicennia germinans* sampling sites along a low-latitude salinity and precipitation gradient. Black points represent sampling sites, green area represents the occurrence of the species, and blue areas represent ponds and rivers. *Top:* Geographical location of the study area and sampling sites.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

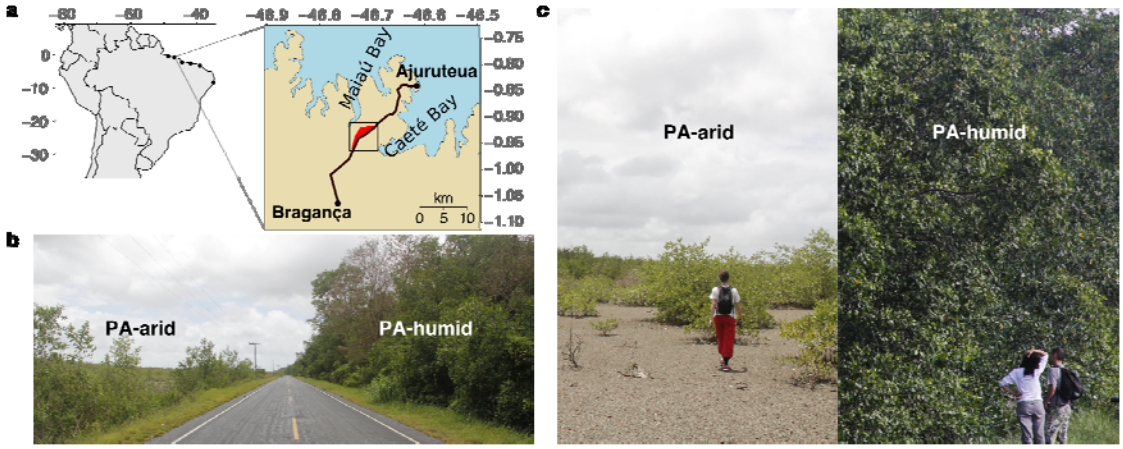

Figure 2. PA-arid and PA-humid sampling sites. (a) Geographical location of sites, highlighted by a square (red colored area: PA-arid); (b) photograph of a section of the Bragança-Ajuruteua road (Pará, Brazil) along which severe changes in hydrology altered the mangrove community and tree morphology;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

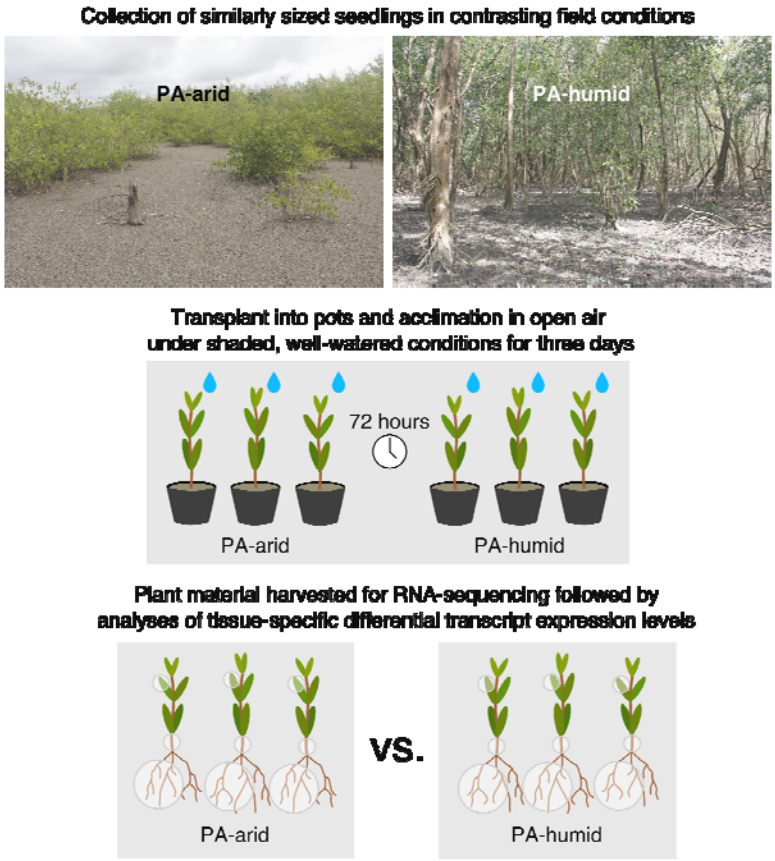

Figure 3. RNA sequencing experimental design. Similarly sized *Avicennia germinans* seedlings naturally grown under adjacent, but contrasting field conditions were collected and transplanted into pots, where they acclimated for 72 hours under homogenous treatment. RNA was extracted from leaves, stems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

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

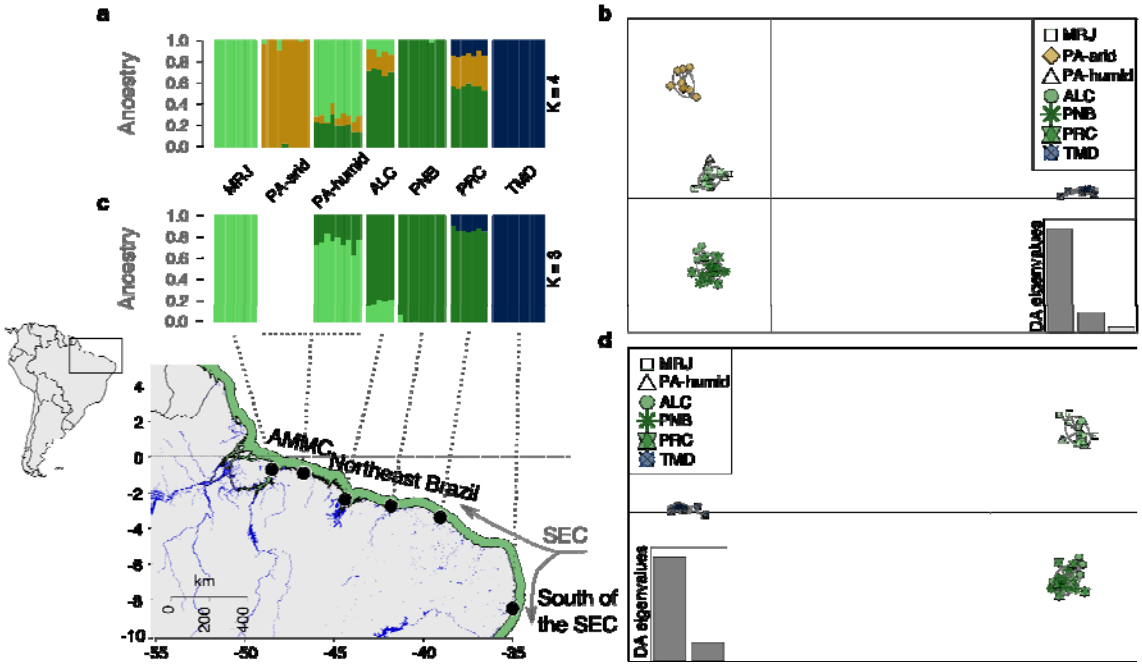

Figure 4. Genetic structure inferred from genome-wide single nucleotide polymorphism (SNP) detected in *Avicennia germinans*. (a) Attribution of ancestry implemented in the program Admixture 1.3.0 for all sampled individuals; stacked bars represent individuals, and each color represents one ancestral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

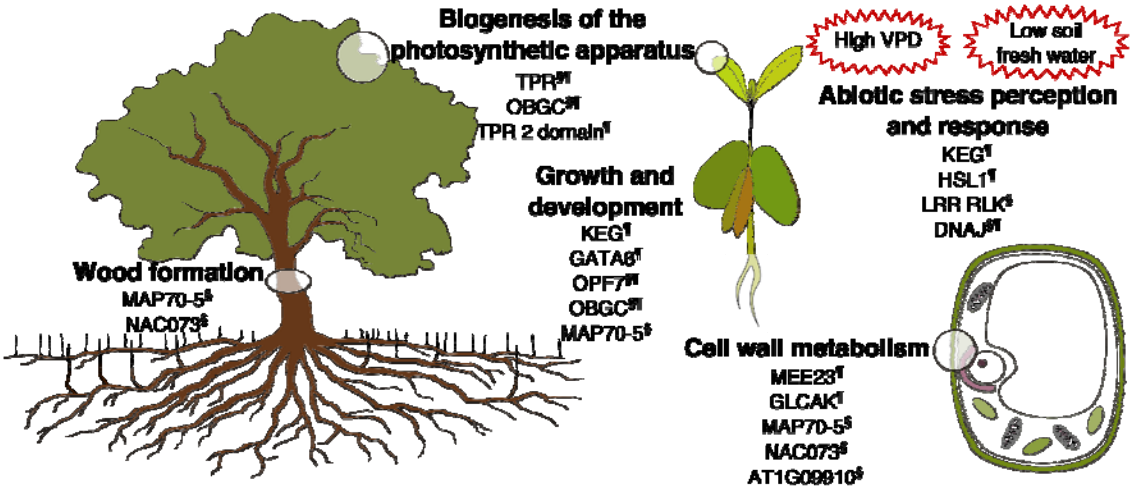

Figure 5. Schematic representation of key biological processes related to physiological drought-tolerance or response associated with candidate loci for selection in *Avicennia germinans* samples along tropical mangrove forests of the north-northeastern Brazilian coast. Candidate loci were detected from 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 ¶).

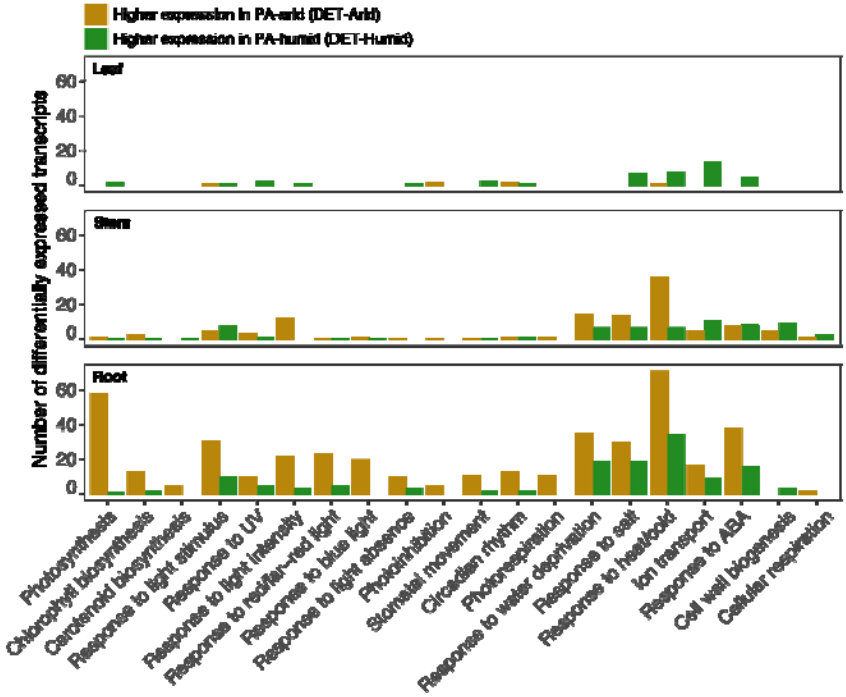

Figure 6. Functional categories of differentially expressed transcripts (DETs) identified between samples grown in the PA-arid and PA-humid sampling sites. Categories were selected based on biological processes previously identified to be involved in the response, acclimation or resistance to water stress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