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Dissecting the genetic basis of drought escape across multiple traits in colonizing *Arabidopsis thaliana* lineages

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Summary

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- Drought response in plants is complex, involving integration across a range of physiological processes. However, our knowledge of how different mechanisms of drought response are linked at the genetic level is limited.
- We investigated multi-trait adaptation in *Arabidopsis thaliana* from the Cape Verde Islands (CVI). Using a high-throughput phenotyping platform that minimizes spatial heterogeneity, we measured variation in rosette area, growth rate, leaf color, water use efficiency (WUE), and stomatal patterning under precisely controlled water conditions.
- Relative to the Moroccan outgroup, CVI populations evolved earlier flowering, a smaller rosette size with faster growth, and reduced WUE, consistent with drought escape adaptation. Genome-wide association mapping revealed evidence for pleiotropy involving *MPK12* (WUE, rosette area, growth rate, and leaf color), *NHL26* (WUE and leaf color), *SUVH4* (stomatal patterning, rosette area, and leaf color), and *FRI* (flowering time, WUE, and leaf color), along with an enrichment of signals in ABA response.
- This study advances our knowledge of the genetic mechanisms driving plant adaptation to a novel precipitation environment. By identifying key genetic components and their contributions to multi-trait adaptation, our findings offer insights into how plants respond to environmental challenges and contribute to predicting plant responses to future climate change.

Introduction

Plants are directly exposed to environmental stress and must evolve mechanisms to overcome such challenges (Chaves *et al.*, 2003; Lawlor, 2013). Among these stresses, water availability stands out as a critical factor that limits plant growth and productivity (Boyer, 1982) and profoundly influences development, morphology, and physiology (Stebbins, 1952; Bohnert *et al.*, 1995). In diverse environments, populations exhibit a broad spectrum of phenotypic variation, which is essential for survival and adaptation. This variation often arises from phenotypic plasticity, the ability of a single genotype to exhibit different phenotypes in response to environmental changes (Sultan, 2000; Gratani, 2014; Josephs, 2018). Plasticity enables plants to rapidly adjust to fluctuating conditions, such as changes in water availability (Pigliucci, 2005). However, the underlying genetic architecture plays a pivotal role in shaping long-term evolutionary responses to stress. To optimize growth and fitness under water-limited conditions, plants have evolved three primary

strategies: drought tolerance, drought avoidance, and drought escape (Ludlow, 1989). Drought tolerance maximizes water use efficiency (WUE) by balancing carbon assimilation and water loss via transpiration (Scott, 2000). Drought avoidance enhances water uptake through the development of extensive root systems and minimizes water loss by reducing transpiration (Levitt, 1972). By contrast, drought escape relies on accelerated flowering to complete the life cycle before the onset of severe drought (McKay *et al.*, 2003; Sherrard & Maherali, 2006). These strategies, involving intricate control of physiological processes, and morphological and developmental traits, reflect a dynamic interplay between phenotypic plasticity and the genetic networks that govern plant adaptation.

One critical aspect of this genetic interplay is pleiotropy, where a single gene influences multiple traits, linking diverse responses within a shared genetic framework (Paaby & Rockman, 2013). For instance, traits associated with drought escape, such as accelerated flowering, may be genetically correlated with traits like growth rate or WUE, reflecting trade-offs or coordinated

adaptations to water stress (Lande, 1980; Mackay & Anholt, 2024). Depending on the nature of these genetic linkages, pleiotropy can either facilitate adaptation through synergistic effects where traits align to meet environmental demands or constrain it when antagonistic effects cause conflicts between traits (Zhang, 2023). Understanding how pleiotropy shapes these coordinated responses is crucial for predicting plant evolutionary trajectories under changing climates.

Among the strategies plants use to cope with water stress, plants that rely on drought escape prioritize rapid growth and reproduction, emphasizing the need to balance immediate survival with overall fitness (Ludlow, 1989). The ability of plants to respond to drought conditions relies on a complex genetic network that integrates hormonal signaling pathways, including abscisic acid (ABA), ethylene, and auxin, which coordinate key physiological and developmental processes (Raghavendra *et al.*, 2010; Peleg & Blumwald, 2011). ABA plays a central role in drought responses by activating cascades of drought-responsive genes that trigger morphological and physiological changes, such as stomatal closure and reduction in leaf size, resulting in an overall enhancement in WUE (Abe *et al.*, 2003; Umezawa *et al.*, 2010). However, these responses are shaped by contrasting selection pressures and physiological constraints, resulting in trade-offs between growth and water conservation. For instance, pleiotropic effects can link genes regulating stomatal traits and WUE to flowering time and biomass allocation, reflecting the interconnected nature of adaptive traits (Verslues *et al.*, 2006; Tuteja, 2007). These responses reveal the trait complexity and the trade-offs that plants face to balance growth and survival. Understanding these complex genetic relationships and trade-offs is crucial for unraveling the genetic basis that underpins plant adaptation to drought, the sophisticated strategies plants use to cope with arid conditions, and the co-evolution of such traits in response to environmental pressures.

Natural populations of *Arabidopsis thaliana*, the major molecular model organism for eudicots, thrive in diverse habitats with considerable variation in water availability and other environmental factors (Alonso-Blanco & Koornneef, 2000; Koornneef *et al.*, 2004; Mitchell-Olds & Schmitt, 2006; Verslues & Juenger, 2011; Weigel, 2012; Assmann, 2013). Early research on *A. thaliana* emphasized the significance of natural variation within the species as a valuable tool for understanding the connection between molecular perturbations and phenotypic changes (Somerville & Koornneef, 2002). Moreover, *A. thaliana* has served as a useful model for investigating the molecular genetic basis of complex traits (Mitchell-Olds & Schmitt, 2006). Local adaptation to different environmental conditions, particularly precipitation and humidity, has influenced allele frequency distributions across its European range (Fournier-Level *et al.*, 2011; Hancock *et al.*, 2011; Lasky *et al.*, 2012; Exposito-Alonso *et al.*, 2018; Ferrero-Serrano & Assmann, 2019). Studies focusing on local population sampling have proven particularly powerful for uncovering the genetic basis of adaptation to environmental variation, offering valuable insights into evolutionary processes at fine scales (Brachi *et al.*, 2011; Josephs *et al.*, 2017). These approaches highlight

how local populations can be used to disentangle the molecular and genetic factors underlying plant adaptation to diverse and challenging environments.

A single *A. thaliana* accession, Cvi-0, was collected in the Cape Verde Islands (CVI) 42 yr ago (Lobin, 1983) and has been studied intensively due to its divergence from other well-studied accessions across various traits, such as WUE, stomatal behavior, flooding tolerance, and flowering time (Alonso-Blanco *et al.*, 1998; McKay *et al.*, 2003; Juenger *et al.*, 2005; Christman *et al.*, 2008; Xu & Zhou, 2008; Monda *et al.*, 2011; Vashisht *et al.*, 2011; Kenney *et al.*, 2014). Mapping studies using recombinant inbred lines derived from crosses between Cvi-0 and Ler-0 or Col-0 have identified quantitative trait loci (QTL) associated with these divergent traits, providing valuable insights into the genetic basis of adaptive variation and revealing complex interactions between genetic and environmental factors (Alonso-Blanco *et al.*, 1998; Juenger *et al.*, 2005; Tisé *et al.*, 2013; Marchadier *et al.*, 2019).

More recently, we collected new population samples of *A. thaliana* from the islands of Santo Antão and Fogo (Fulgione *et al.*, 2022). As is common for endemic plant species in Cape Verde, *A. thaliana* is found on rocky outcrops where moisture is provided mainly via humid trade winds (Brochmann *et al.*, 1997; Elfarargi *et al.*, 2023). Sequencing and population genetic analysis revealed that the two CVI island populations experienced strong colonization bottlenecks, which removed nearly all pre-existing genetic variation. Consequently, genetic variation segregating within each island population is highly distinct, both between islands and in comparison to mainland populations (Fulgione *et al.*, 2022). Notably, the vast majority of genetic variants (99.9%) observed in these populations are attributed to novel mutations that arose post-colonization, highlighting the role of recent mutations in shaping genetic diversity in these isolated populations (Fulgione *et al.*, 2022).

In the ecological context of CVI, where growing seasons are short and plants rely heavily on moisture provided by humid trade-winds rather than direct rainfall (Brochmann *et al.*, 1997), selection appears to have favored a drought-escape strategy. CVI populations exhibit more open stomata, enabling higher rates of photosynthesis and faster growth to facilitate drought escape (Fulgione *et al.*, 2022; Elfarargi *et al.*, 2023). The high relative humidity in this environment may mitigate the physiological trade-off between photosynthesis and transpiration, thereby allowing plants to optimize carbon gain without experiencing excessive water loss (Elfarargi *et al.*, 2023). However, localized variation in microclimatic conditions, including temperature and humidity, may impose heterogeneous selection pressures, potentially maintaining alleles such as *MPK12* at intermediate frequencies (Elfarargi *et al.*, 2023). This highlights the role of both spatial and temporal environmental heterogeneity in maintaining functional genetic variation, illustrating how natural selection acts on trait evolution to align with variable local environmental pressures (Fulgione *et al.*, 2022; Elfarargi *et al.*, 2023).

In this study, we measured eight phenotypic traits in response to precisely controlled drought stress in an expanded sampling of the Santo Antão natural population, where the Cvi-0 accession

originated. Compared to our earlier work (Elfarargi *et al.*, 2023), we extended this study to incorporate Fogo and Moroccan populations while also examining a broader set of phenotypic traits, enabling a broader comparison of phenotypic and genetic variation across islands and the mainland. We found that for several traits, phenotype distributions for the CVI islands are significantly shifted compared to Morocco, which represents the closest outgroup population. We further conducted genome-wide association (GWA) mapping using univariate and multivariate genome-wide association studies (GWAS) models to dissect the genetic basis of phenotypic adaptation underlying drought stress in the CVI *A. thaliana* populations and to compare them to the Morocco *A. thaliana* population. This analysis revealed a moderately complex genetic architecture marked by several major effect variants. Notably, some loci identified through GWAS co-localized across traits, suggesting pleiotropic effects and their involvement in multiple drought-responsive pathways. Overall, these findings enrich our understanding of the genetic complexities of plant adaptation, reflecting the critical trade-offs plants maintain for their survival in arid environments and underlying the importance of understanding genetic correlations to predict future evolutionary dynamics under changing climatic conditions.

Materials and Methods

Study populations

In this study, we used a total of 350 accessions of *Arabidopsis thaliana* (L.) Heynh. from the CVI and Morocco to investigate genetic and phenotypic variation associated with drought adaptation (Fig. 1a; Supporting Information Table S1). Our sampling included 189 accessions (Fulgione *et al.*, 2022) along with 15 newly collected accessions from 26 locations in Santo Antão (Fig. 1b). Additionally, we examined 146 accessions collected across 18 locations in Fogo (Fulgione *et al.*, 2022) and 61 Moroccan accessions (Durvasula *et al.*, 2017) as an outgroup population.

We generated maps to visualize geographic sampling locations using R v.3.4.4 (R Development Core Team, 2008), and employed the GGMAP (Kahle & Wickham, 2013) and GGLOT2 (Wickham, 2016) libraries for plotting.

Whole-Genome Sequencing

We sequenced 15 new Santo Antão accessions using Illumina HiSeq3000 machines. Genomic DNA was extracted using the DNeasy Plant Mini kits (Qiagen Sciences, Germantown, MD, USA), fragmented using sonication (Covaris S2, Covaris LLC, Woburn, MA, USA), and libraries were prepared with Illumina TruSeq DNA sample prep kits (Illumina, San Diego, CA, USA) and NEBNext Ultra II FS DNA Library Prep Kit (New England Biolabs, Ipswich, MA). Libraries were sequenced with 2× 100–150 bp paired-end reads. We assessed DNA quality and quantity via capillary electrophoresis (TapeStation; Agilent Technologies, San Diego, CA) and fluorometry (Qubit and Nanodrop; Thermo Fisher Scientific, Waltham, MA, USA).

Variant identification and genotyping

Here, we called variants from the newly sequenced 15 Santo Antão accessions together with the previously released whole-genome short-read data for 189 Santo Antão accessions (ENA: PRJEB39079 (ERP122550)) (Fulgione *et al.*, 2022). We aligned the raw Illumina sequence for the whole dataset to the Arabidopsis TAIR10 reference genome. Further, we used SHORE (Ossowski *et al.*, 2008) to identify SNP variants following a pipeline described here (https://github.com/HancockLab/SNP_calling_Arabidopsis). Furthermore, the obtained variant call format file was filtered to minimize variant calling bias and to retain only high-quality variants: (1) retain only bi-allelic variants; (2) convert heterozygous sites to missing data to mask possible false positives; (3) retain variants with coverage greater than 3 and base quality greater than 25. This resulted in a total of 20 582 SNPs for Santo Antão, 15 123 SNPs for Fogo, and 611 818 SNPs for Morocco.

Population structure analysis

In this analysis, we used 350 accessions from CVI and 61 accessions from Morocco. We analyzed population structure by first pruning SNPs for linkage disequilibrium using PLINK v1.9 (Purcell *et al.*, 2007). SNPs with pairwise correlation coefficients (r^2) > 0.1 were removed within 50 kb sliding windows with a 10 bp step size. Additionally, we excluded variants with missing genotype data by setting the --geno parameter to 0. We conducted a principal component analysis (PCA) to summarize genetic variation patterns among populations using the --pca function in PLINK v1.9.

Climatic data and variable selection

To identify the environmental drivers of genetic variation across Santo Antão, Fogo, and Moroccan populations, we compiled an initial dataset of 27 climatic variables (Table S2) for original collection sites. This set included 19 bioclimatic variables and monthly water vapor pressure, which were obtained from the WorldClim 2.1 database (Fick & Hijmans, 2017) (<https://worldclim.org/data/worldclim21.html>) at a 30 arc-second resolution (*c.* 1 km²). Data for the global aridity index, mean annual actual evapotranspiration, and global reference evapotranspiration were sourced from the CGIAR-CSI database (<https://csidinfo.wordpress.com/category/data/>; Table S2). Finally, four variables representing the annual range of monthly near-surface relative humidity, mean monthly climate moisture index, potential evapotranspiration, and accumulated rainfall during the growing season were retrieved from the CHELSA database (<https://chelsa-climate.org/>; Table S2).

To identify a minimal, non-redundant set of climatic variables that best explain the observed genomic variation, we implemented a three-step selection procedure. First, to mitigate multicollinearity, we iteratively removed variables from highly correlated pairs (Pearson's $|r| \geq 0.75$), retaining the variable with the lower mean correlation against all other predictors. The remaining

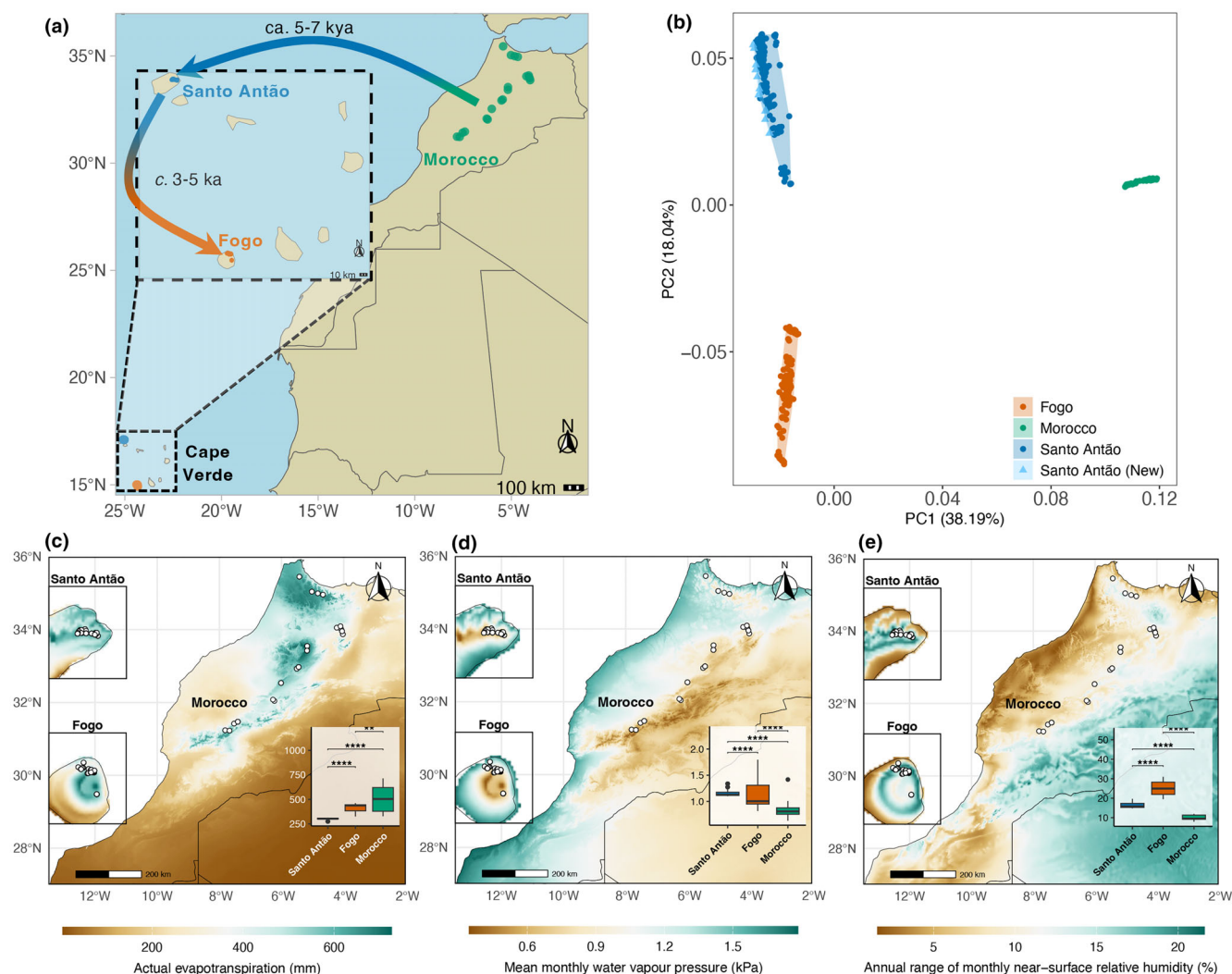


Fig. 1 Geographical distribution and genetic structure of the Cape Verde Islands (CVI) and Morocco. (a) Geographic locations of populations of *Arabidopsis thaliana* in CVI (Santo Antão and Fogo) and the outgroup panel of Moroccan *A. thaliana* accessions. (b) Principal component analysis (PCA) of genome-wide single nucleotide polymorphisms (SNPs) between CVI (Santo Antão and Fogo) and Moroccan *A. thaliana* populations. (c–e) Climatic niche differentiation among the three populations for three key variables identified by forward selection and variance inflation factor (VIF) analysis. The maps show spatial variation at the sampling localities for (c) Mean annual actual evapotranspiration (mm), (d) Mean monthly water vapor pressure (kPa), and (e) Annual range of monthly near-surface relative humidity (%). Main maps correspond to Morocco, with insets for Santo Antão and Fogo. Inset boxplots quantify the significant differences in climatic values, where the center line represents the median, the box edges represent the 25th and 75th percentiles, the whiskers extend to 1.5 times the interquartile range, and individual points represent outliers. Significant differences are denoted by **, $P \leq 0.01$; ****, $P \leq 0.0001$ from pairwise Mann–Whitney–Wilcoxon tests.

variables were then assessed using the variance inflation factor (VIF), and we continued to remove predictors until all remaining variables exhibited a VIF below 10. Finally, from this filtered set, we performed forward selection on a redundancy analysis (RDA) model using the ‘ordiR2step’ function in the R package VEGAN v. 2.6-8 (Oksanen *et al.*, 2020). Starting from a null model conditioned on population structure, this procedure added variables that significantly increased the model’s adjusted R^2 ($P < 0.05$, based on 1000 permutations). This selection process yielded three key explanatory variables: mean annual actual evapotranspiration, mean monthly water vapor pressure, and annual range of monthly near-surface relative humidity (Fig. 1c–e; Table S3).

Phenoscope drought experiment and phenotyping

We conducted phenotypic measurements using the high-throughput Phenoscope platform (<https://phenoscope.versailles.inra.fr/>) as previously described (Tisné *et al.*, 2013). In this platform, pots are automatically and constantly rotated to eliminate the effects of micro-environmental heterogeneity and watering is adjusted multiple times daily to maintain highly controlled soil water content, enabling experiments that would be impractical using manual methods (Tisné *et al.*, 2013). A total of 359 accessions of *Arabidopsis thaliana* were included: 152 from Santo Antão, 146 from Fogo (Fulgione *et al.*, 2022), and 61 from

Morocco (Brennan *et al.*, 2014). Additionally, we included two well-characterized accessions, Cvi-0 and Col-0, as control lines, each with 35 replicates distributed across the experimental design. Plants were grown under controlled environmental conditions (8 h : 16 h, day : night, 21°C : 17°C, 65% relative humidity, and 230 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity). Two watering conditions were implemented: a well-watered (WW) condition where pots were maintained at 60% of maximum soil water content (SWC; 4.6 g H_2O per g dry soil), and a water deficit (WD) condition where pots were maintained at 25% SWC (1.4 g H_2O per g dry soil). For each accession, two biological replicates were phenotyped under each of the two watering treatments (WW and WD), resulting in four plants per accession in total. The Phenoscope setup, WUE measurements, and sample collection protocols can be found here (Elfarargi *et al.*, 2023). The image-based traits were defined as follows: projected rosette area (PRA), hue and saturation (converted from rosette RGB color components), compactnessPC (ratio of PRA (P) to convex hull area (C)) were extracted at final timepoint (31 Days After Sowing (DAS)). Relative Expansion Rate (RER) was integrated from PRA over time window 18 DAS to 31 DAS.

We measured leaf stomatal density and stomatal pore width primarily in the Santo Antão population. We additionally measured stomatal traits for a small subset of Moroccan accessions ($n = 22$) to provide an outgroup comparison; however, the limited Moroccan sample size precluded GWA analyses for stomatal traits in this population. Given the labor-intensive nature of this microscopy-based method, we prioritized analyses of stomatal traits in the Santo Antão population, where our previous study identified substantial genetic variation associated with stomatal conductance and water use efficiency (Elfarargi *et al.*, 2023). We adapted a previously published protocol, 'Microscopy-Based Stomata Analyses' (Eisele *et al.*, 2016), using a small piece of Scotch tape. The tape was applied firmly onto the abaxial surface of a detached, fully developed leaf per accession and per treatment of each genotype. Then, we gently peeled off the tape from the leaf surface, not tearing it. We transferred the tape immediately to a Petri dish containing 4% formaldehyde solution for 10–20 min at room temperature to fix the status of stomatal guard cells and prevent their responses to subsequent signals. Next, we put the samples (tapes with epidermal peels) into a clean tissue and added 50 μl of 1 mg ml^{-1} propidium iodide solution to each sample and incubated them for 5–10 min in the dark. Then we placed the tape on a microscope slide and gently pressed it to remove any air bubbles. As a final step, we examined for stomatal densities and stomatal apertures using confocal images of epidermal peels taken with a confocal microscope (LSM 700, ZEISS). The images were captured and analyzed using IMAGEJ software (<https://imagej.nih.gov/ij/>). The measurements were performed across several days by the end of the Phenoscope experiment (29–32 DAS) around midday.

To dissect the components of the drought escape strategy, we selected traits with known relevance to plant morphology, growth, and physiology. Morphological and growth-related traits included PRA, compactness, and RER, which reflect vegetative development and biomass accumulation. To assess physiological adjustments in water management, we measured WUE (via $\delta^{13}\text{C}$) and

stomatal-related traits (SD, stomatal pore width (SPW)). Stomatal traits (density and pore width), quantified primarily in the Santo Antão population, directly relate to gas exchange and transpiration control. We also investigated leaf color traits, hue and saturation, which represent complex proxies for pigment content. Hue values, in particular, have been found to correlate with Chl content under certain conditions and may provide indirect insights into photosystem II photochemical yield (Fragó *et al.*, 2018). Finally, we integrated flowering time data collected on the same panel in a Cape Verde simulated environment (Fulgione *et al.*, 2022), as early flowering is a key component of the drought escape strategy. Together, these traits form an integrative framework to investigate the genetic basis of adaptation to drought.

Phenotype data analysis

We assessed differences in phenotype distributions using both parametric and non-parametric tests. Wilcoxon rank sum tests were performed with the *wilcox.test* function from the GGPUBR package (Kassambara, 2020) to evaluate pairwise comparisons. To analyze the fixed effects of treatment, geographic region, and their interaction (Population-by-Treatment interaction) on phenotypic traits, we employed linear mixed models (LMMs) implemented in the LME4 package in R (Bates *et al.*, 2014). For each phenotype, we fit the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} represents the phenotypic value, μ is the overall mean, α_i is the effect of treatment, β_j is the effect of geographic region, γ_{ij} is the interaction between treatment and region, and ε_{ijk} is the residuals. The Moroccan population and the well-watered/Moroccan environment (WW/MOR_ENV) were set as the reference levels for the models. To account for false positives arising from multiple testing across all traits and model terms, we implemented the Benjamini-Yekutieli procedure to adjust P -values using a False Discovery Rate (FDR) threshold of 0.05 (*p.adjust* function, R base). Additionally, for visualizing phenotypic plasticity, we performed post-hoc pairwise comparisons of the treatment effect within each population using the EMMEANS package (Lenth & Piaskowski, 2025).

To further assess standing genetic variation for phenotypic plasticity within each population, we conducted within-population analyses of Genotype (G), Environment (E), and Genotype-by-Environment ($G \times E$) interactions. For each trait within each population, we fit a linear model consisting of the main effects of Genotype and Treatment, and their interaction. The significance of the $G \times E$ interaction term was evaluated using standard ANOVA to determine the presence of segregating genetic variation for plasticity. Because tissue from biological replicates was pooled before the $\delta^{13}\text{C}$ assay to ensure sufficient mass, within-population $G \times E$ interaction terms could not be statistically modeled for WUE due to a lack of information about residual variance. Therefore, to evaluate standing variation and potential evolutionary constraints on plasticity across all traits (including WUE), we also calculated cross-environment genotype mean correlations.

Univariate and multivariate GWAS We performed GWA analyses to identify genetic variants linked to drought-related traits, using the average trait value across WW and WD conditions, and the drought response (calculated as the difference between conditions: WW–WD) as inputs. First, we filtered the SNP data to retain only biallelic variants. Variants were filtered based on read coverage ($DP \geq 3$) and genotype quality ($GQ \geq 25$), followed by applying a minor allele frequency ($MAF \geq 5\%$) cutoff to exclude rare variants. Subsequently, we carried out the association analyses between genomic variants and studied traits for each of the island populations (Santo Antão and Fogo) as well as the Moroccan outgroup population using the univariate linear mixed model implemented in GEMMA (Zhou & Stephens, 2012), adjusting for population structure by incorporating a kinship matrix as a random effect. These univariate analyses were performed separately for well-watered and drought conditions as well as the average for each trait across both conditions and the drought response (difference between conditions: WW–WD). In the Santo Antão population, where we had population-level data for stomatal traits (WUE, SD, and SPW), we also applied a multivariate linear mixed model (mvLMM) in GEMMA (Zhou & Stephens, 2014) to examine these traits as a group because they are related and may thus have a shared genetic basis.

Following Shim *et al.* (2015), we calculated the proportion of variance explained (PVE) by each SNP based on GEMMA outputs. The PVE was computed using the following equation:

$$PVE = \frac{2\hat{\beta}^2 MAF(1-MAF)}{2\hat{\beta}^2 MAF(1-MAF) + \left(SE(\hat{\beta}) \right)^2 2NMAF(1-MAF)}$$

where $\hat{\beta}$ is the effect size estimate, $SE(\hat{\beta})$ is the standard error of effect size for the variant, MAF is the minor allele frequency for the variant, and N is the sample size.

Genetic correlations were calculated in R using the *cor.test* function to assess the correlations between the effect size estimate ($\hat{\beta}$) for each trait in the average trait value and in response to drought. To ensure consistency and comparability with genetic patterns, phenotypic correlation analyses were conducted after accounting for population structure. We used the *cor.test* function in R to evaluate relationships between phenotypes in the average trait value and in response to drought. The significance of the correlations was evaluated with the *t*-test implemented in the *cor.test* function.

After initial mapping using the LMM, we applied a local score approach to the output of *P*-values provided by LMM and mvLMM in GEMMA to improve precision and detection power. This local score approach, which takes LD into account when estimating associations, increases the power of detecting significant genomic regions associated with trait variation and is subsequently converted into scores (Bonhomme *et al.*, 2019). The local score statistic is calculated by integrating association scores over genomic regions and finding the maximum of a Lindley process over the sequence of these scores (Bonhomme *et al.*, 2019). Scripts used to calculate the Lindley scores are available here (<https://forge-dga.jouy.inra.fr/documents/940>).

Inference of genetic architecture To infer the genetic architecture of drought-related traits, we used the Bayesian Sparse Linear Mixed Model implemented in GEMMA (Zhou & Stephens, 2012). This polygenic model accommodates both large- and small-effect loci and accounts for relatedness among individuals by including a genomic kinship matrix as a random effect. Furthermore, the approach accounts for the LD between variants by inferring locus effect sizes (β) while controlling for other variants included in the model. Using this approach, we modeled two effect hyperparameters: a basal effect (β), which captures small-effect loci contributing to the studied trait, and an additional effect (γ), which captures a subset of loci with the most potent effects. To estimate the effects of all variants, the sparse effect size for each locus was calculated by multiplying (β) by (γ). We used the 1% variants (> 99% quantile) distribution of locus effects to identify which genes had the highest sparse effects on the studied trait.

Candidate loci identification and GO enrichment analysis To identify candidate loci associated with drought-related traits, we integrated results from the GWAS models described above. For each trait and condition within each population, GWAS results were ranked according to their respective association metrics. These ranks were subsequently transformed using the harmonic mean rank, ensuring contributions from all GWAS models were equally weighted. SNP variants within the top 5% of the harmonic rank distribution were selected as significant candidates. From this subset, we highlighted the most relevant candidate genes associated with average traits and drought response traits across populations. For downstream analyses, gene annotations corresponding to the top-ranked SNPs were retrieved using the TAIR10 GFF3 gene annotation file processed with SNPEff (Cingolani *et al.*, 2012). Gene ontology (GO) enrichment analysis was performed to determine if specific biological processes were overrepresented among the candidate loci. GO enrichment analysis was conducted using the ShinyGO tool (Ge *et al.*, 2020).

Results

Genomic and climatic divergence between CVI and Moroccan populations

Since our study contains newly collected accessions relative to previous studies we conducted on the CVI population (Fulgione *et al.*, 2022; Elfarargi *et al.*, 2023), we initially examined the genetic relatedness and climatic niches of the expanded 350-accession panel (Fig. 1a; Table S1). Analysis of population structure using PCA (Fig. 1b) reveals distinct clustering of Santo Antão and Fogo populations relative to the Moroccan population. These findings are consistent with previous results, which indicated that the two islands diverged without any subsequent admixture between them (Fulgione *et al.*, 2022). The climatic conditions of the CVI and Moroccan collection sites are distinguished by significant differences in both terrestrial and atmospheric water dynamics. The CVI populations experience

Table 1 Key to phenotypic traits.

Trait ID	Trait description	Unit
WUE	Water Use Efficiency	%
PRA	Projected Rosette Area	cm ²
Compactness	CompactnessPC – ratio between PRA and its convex hull area	NA
RER	Relative Rosette Expansion Rate	d ⁻¹
Hue	Rosette average color hue	%
Saturation	Rosette average color saturation	%
SD	Stomatal Density	1 mm ⁻²
SPW	Stomatal Pore Width	mm

pronounced terrestrial aridity, as indicated by significantly lower actual evapotranspiration compared to the Moroccan sites (Fig. 1c). By contrast, the atmosphere in the CVI is characterized by significantly higher mean water vapor pressure, reflecting greater overall moisture content (Fig. 1d). Furthermore, this atmospheric moisture in the CVI is subject to greater seasonal fluctuation, demonstrated by a significantly larger annual range of relative humidity (Fig. 1e). These differences highlight a key environmental pressure in CVI: extreme soil aridity, where the most consistent source of moisture derives from the elevated atmospheric humidity.

Variation in drought response within CVI and compared to Morocco

We investigated phenotypic variation and plasticity for a range of traits (Table 1) in response to moderate drought stress in the CVI islands and the nearest outgroup *A. thaliana* population from Morocco. Traits were assayed under tightly controlled conditions utilizing the Phenoscope, a high-throughput phenotyping platform (Tisné *et al.*, 2013).

We conducted ANOVAs to assess differences between populations in average traits and in the response to drought. Both CVI populations exhibited lower WUE, smaller PRA, reduced compactness, higher RER, and lower hue than the Moroccan population (Fig. 2; Table S4). All traits except hue shifted significantly overall in response to drought, highlighting phenotypic plasticity in CVI populations under water-limited conditions. We found that drought had a notable impact on leaf growth (Fig. S1), stomata-related traits, and leaf color-related traits in Santo Antão and Fogo populations compared to Moroccan *A. thaliana* populations (Fig. 2). Under WD conditions, CVI plants exhibited reduced rosette size, diminished growth rates, and changes in leaf color-related traits compared to WW conditions (Fig. 2a–f). WUE was significantly lower in the Santo Antão *A. thaliana* population compared to the Moroccan population under both WW conditions and drought conditions (Fig. 2a). WUE in Fogo was higher than in Santo Antão but lower than in the Moroccan population. Furthermore, we integrated data from a previous study (Fulgione *et al.*, 2022) for flowering time, a key life-history trait associated with the drought escape strategy. This analysis revealed a strong population-by-environment interaction when plants were grown under simulated Moroccan (MOR_ENV) and

Cape Verde Islands (CVI_ENV) conditions (Fig. 2g). The Moroccan population exhibited significant phenotypic plasticity, with flowering time substantially delayed when plants were grown under the simulated CVI conditions. By contrast, both the Santo Antão and Fogo populations displayed canalized flowering times, maintaining their rapid life cycle with no significant change between the two simulated environments. These findings are consistent with adaptive responses to localized water regimes, suggesting a complex interplay of genetic and environmental factors driving these differences.

To delve deeper into the physiological mechanisms underlying the lower WUE in Santo Antão, we examined variation in stomatal density and SPW in a subset of the Santo Antão and Moroccan population samples (Fig. 2h–i). Stomatal density was similar in the two populations in both WW and drought conditions, and only the Moroccan population exhibited a significant shift in response to drought. Although mean SPW was higher in Santo Antão relative to Morocco, the difference was not significant, and again only the Moroccan population exhibited a significant decrease in response to drought.

To dissect the sources of phenotypic variation, we used LMMs to assess the main effects of population, environment, and the population-by-environment interaction. Our analysis revealed significant main effects of population for most traits. For instance, in the reference WW condition, both Santo Antão and Fogo populations had a significantly smaller PRA than the Moroccan population ($\beta_{\text{Santo Antão}} = -2.39$, $P_{\text{adj}} < 0.001$; $\beta_{\text{Fogo}} = -1.98$, $P_{\text{adj}} < 0.001$; Table S4). The main effect of treatment was also significant for nearly all traits (Fig. S2; Table S4); for instance, there was a strong overall reduction in PRA under drought ($\beta_{\text{Tt}} = -4.14$, $P_{\text{adj}} < 0.001$), confirming that the stress treatment induced strong phenotypic plasticity across the experiment. Additionally, we detected a significant population-by-environment interaction for several traits, including WUE, PRA, RER, saturation, and flowering time, indicating that the magnitude of phenotypic plasticity was not uniform across the three populations (Table S4). This was most pronounced for flowering time, where the Fogo population's response to the simulated CVI environment was dramatically different from that of the Moroccan population ($\beta_{\text{population-by-environment, Fogo}} = -18.29$, $P_{\text{adj}} < 0.001$). Similarly, a significant population-by-environment interaction for saturation was observed for both CVI populations (e.g. $\beta_{\text{population-by-environment, Fogo}} = 3.47$, $P_{\text{adj}} < 0.001$), showing that their leaf color response to drought differed significantly from that of Morocco. These results reveal that the island populations have evolved a fixed 'drought escape' strategy, which contrasts sharply with the more plastic, environmentally responsive strategy of the Moroccan population.

We next determined whether there is standing variation in plasticity within each population. To do this, we conducted within-population analyses testing for Genotype (G), Environment (E), and Genotype-by-Environment (G × E) interactions (Table S5). We detected significant G × E interactions for several key traits across the islands, including PRA ($P = 0.027$) and stomatal density ($P < 0.001$) in Santo Antão, as well as leaf saturation ($P < 0.001$) and flowering time ($P < 0.001$) in Fogo. This

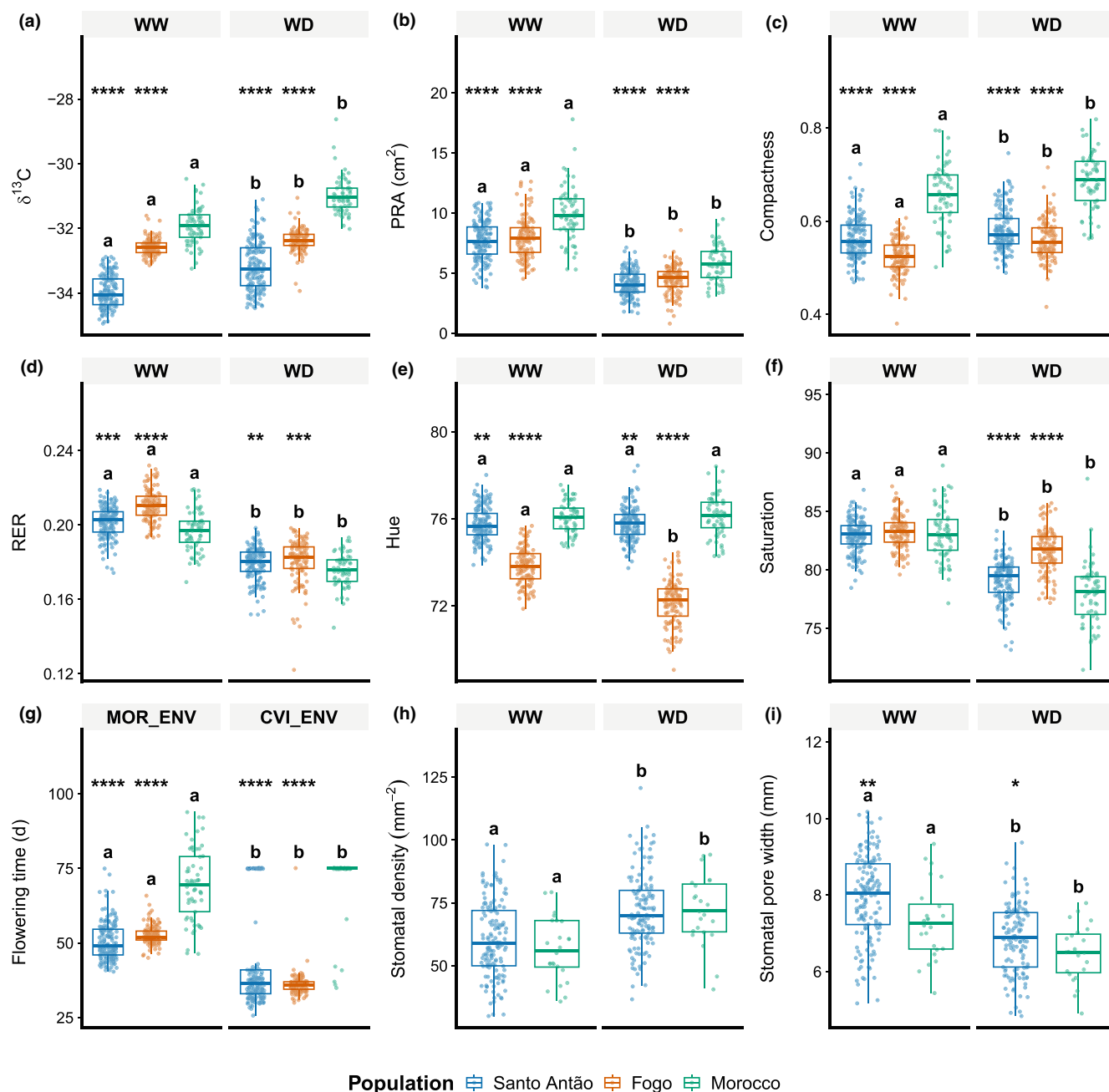


Fig. 2 Phenotypic variation in morphological and physiological traits for Santo Antão and Fogo *Arabidopsis thaliana* populations relative to the Moroccan population. The line in the center of the boxplots represents the median, the box edges represent the 25th and 75th percentiles (lower and upper bound, respectively), and the whiskers represent the 95% CI. Traits were measured under well-watered (WW) and water-deficient (WD) conditions (a–f, h, i), while flowering time (g) from our previous study (Fulgione *et al.*, 2022) was assessed under simulated Moroccan (MOR_ENV) and Cape Verde Islands (CVI_ENV) environmental conditions. (a) Water use efficiency (WUE) is measured as carbon isotope discrimination ($\delta^{13}\text{C}$), and the carbon isotope ratio is expressed per mil, ‰. (b) Projected rosette area (PRA). (c) Compactness (ratio of PRA (P) to surface of convex hull area (C)). (d) Relative expansion rate (RER), the relative growth rate of PRA; integrated over the 18–31 Days After Sowing (DAS) time window. (e, f) Hue is the tint of the rosette color, and saturation represents color intensity. (g) Flowering time. (h) Stomatal density (i) and stomatal pore width. Statistical significance was determined through the Mann–Whitney–Wilcoxon test, using the Moroccan population as a reference. Asterisks represent the significance of the difference from the Moroccan population, with *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ****, $P \leq 0.0001$. Different letters (a, b) indicate significant differences between the two environmental conditions (WW vs WD and MOR_ENV vs CVI_ENV) for each population ($P \leq 0.05$).

confirms the presence of segregating genetic variation that affects drought plasticity within CVI. However, alongside these interactions, we observed universally strong main effects of Genotype

across nearly all traits ($P < 0.001$). A strong Genotype main effect implies a positive genetic correlation across environments, meaning a genotype's relative performance in WW conditions

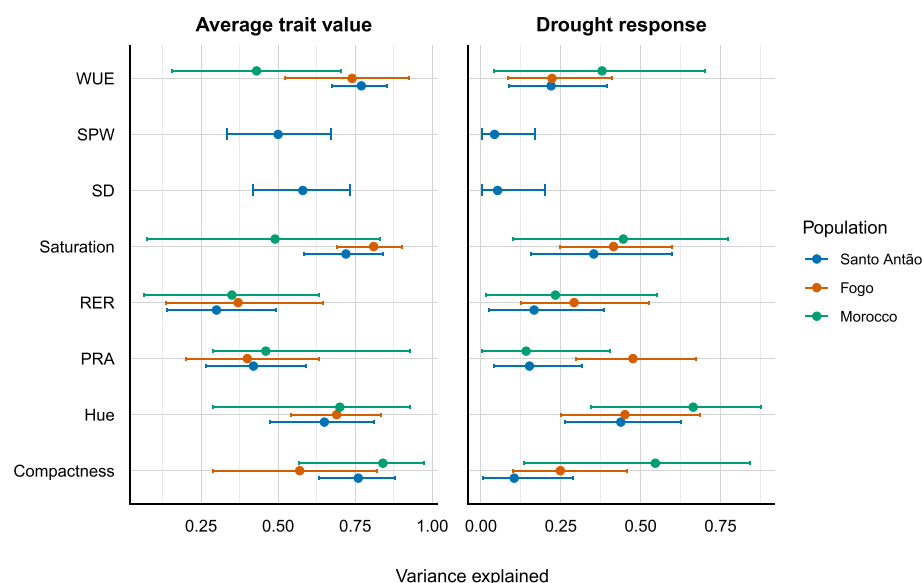


Fig. 3 Dot chart shows estimates and confidence intervals for the proportion of variance explained by sequence polymorphisms in the average trait (left) and the drought response (right) in Santo Antão, Fogo, and Morocco *Arabidopsis thaliana* populations. Error bars represent equal-tailed posterior probability intervals (95% ETPPIs). PRA, projected rosette area; RER, relative expansion rate; SD, stomatal density; SPW, stomatal pore width; WUE, water use efficiency.

largely predicts its performance under drought. To assess the potential strength of this evolutionary constraint on plasticity, we calculated the genotype mean correlations across the two environments. We found highly significant cross-environment correlations for nearly all traits within the island populations (Table S5). For example, within Santo Antão, strong positive correlations across environments were observed for WUE ($r = 0.84$, $P < 0.001$), compactness ($r = 0.80$, $P < 0.001$), and flowering time ($r = 0.73$, $P < 0.001$). Similarly, Fogo exhibited strong cross-environment constraints for WUE ($r = 0.65$, $P < 0.001$) and leaf hue ($r = 0.72$, $P < 0.001$). Overall, these findings indicate that while there is standing genetic variation for how these plants respond to drought, their evolutionary potential is restricted. The strong genetic link between environments limits the ability of the island populations to independently fine-tune their drought responses without also altering their baseline growth.

We next estimated trait heritability in each population and condition. Broad-sense heritabilities (H^2), estimated based on repeatability across replicates, indicated moderate to high heritability for most traits across treatment conditions (Table S6). These findings highlight the genetic basis of key drought-related traits and reveal substantial variability in their genetic architecture across populations and environmental conditions.

Average traits and drought response traits

To better understand phenotypic variation across populations and conditions, we examined variation in average trait values and drought responses. Average traits provide insights into baseline performance and constitutive genetic effects, while drought responses capture phenotypic plasticity and condition-specific adaptations to WD. This distinction allows us to evaluate not only overall trait variation in separate conditions but also the degree of plasticity expressed in response to drought stress. Analyzing these complementary aspects may enhance our ability to

disentangle genetic mechanisms driving phenotypic shifts under contrasting conditions and strengthen the detection of loci involved in adaptive responses.

We next quantified the PVE by sequence polymorphisms for average traits and drought responses across populations to assess the relative contribution of genetic effects. Results revealed substantial variation in the PVE across traits and populations (Fig. 3). Average traits consistently exhibited high PVE (Fig. 3), particularly compactness in Morocco (0.84, 95% CI 0.57–0.97) and WUE in Santo Antão (0.77, 95% CI 0.67–0.85) and Fogo (0.74, 95% CI 0.52–0.92), highlighting strong genetic effects segregating within these populations. Drought responses showed moderate PVE, reflecting greater plasticity (Fig. 3). Notably, hue exhibited significant PVE in Santo Antão (0.44, 95% CI 0.26–0.63) and Fogo (0.45, 95% CI 0.25–0.68), while Morocco's hue trait had the highest PVE (0.66, 95% CI 0.34–0.88) under drought. Overall, these findings demonstrate that while average traits are largely driven by genetic effects, drought responses also exhibit measurable genetic variation, albeit shaped by greater plasticity. This dual approach of examining baseline performance and plastic responses provides a framework for identifying genetic loci underpinning drought adaptation.

Phenotypic and genetic correlations between drought-related traits

A shared genetic basis of traits, that is, pleiotropy, could act to restrict or promote adaptation. To investigate the extent of correlations between pairs of traits and evidence for a shared genetic basis, we estimated both genetic (Figs 4, S2) and phenotypic (Fig. S3) correlations for average traits and drought responses, accounting for population structure. Overall, we found significant congruence between phenotypic and genetic correlations across drought-related traits in Santo Antão, Fogo, and Morocco (Figs 4, S4). Similarity between phenotypic and genotypic correlations was especially strong for the traits PRA, RER, WUE, and

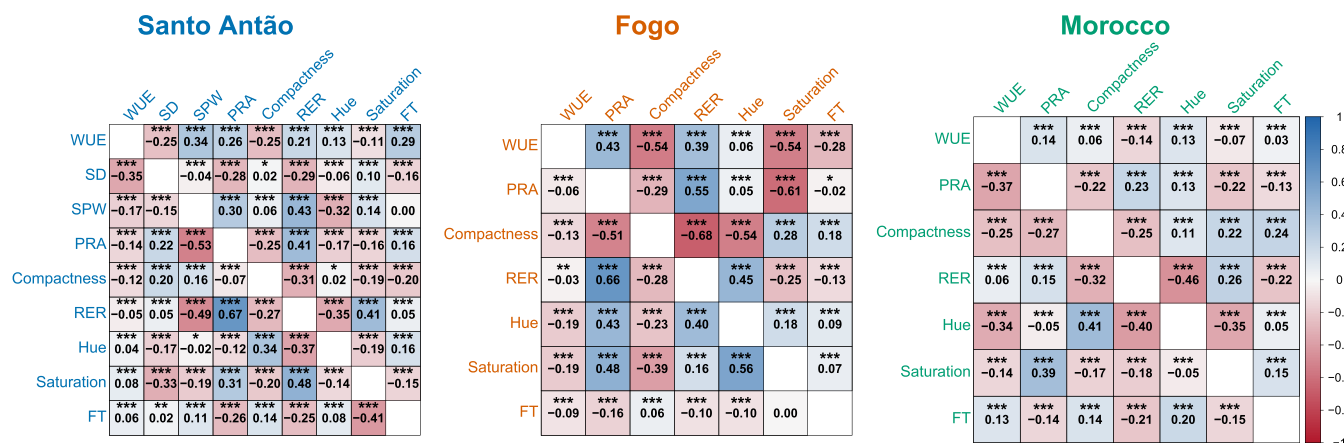


Fig. 4 Pairwise Pearson genetic correlations among traits for the Santo Antão, Fogo, and Morocco *Arabidopsis thaliana* populations. Each panel shows correlations between single nucleotide polymorphism (SNP) effects for the average trait value (lower-left triangle) and for the drought response (upper-right triangle). The color spectrum, dark red to dark blue, represents highly negative to highly positive correlations, and the number represents the correlation values. Significance levels are indicated by asterisks (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

flowering time, implying that in these cases, there is likely a strong shared genetic foundation for these drought-related traits. Such strong genetic-phenotypic correlations indicate that the ability of plants to fine-tune their growth patterns and WUE in response to drought is governed by underlying pleiotropic functions. This tight trait coordination underscores the biological importance of understanding the genetic basis of phenotypic traits, particularly in the context of environmental adaptations.

Genetic basis of drought response in natural populations

To identify specific loci underlying average traits and drought-response traits, we conducted univariate (LMM) and multivariate (mvLMM) GWAS enhanced by a local score approach (Bonhomme *et al.*, 2019). By employing these strategies, we identified significant associations across the genome. Fig. 5 highlights the most relevant candidate genes associated with average traits and drought response traits in the three populations. Individual GWAS results are provided in Figs S5–S11 and Table S7.

Our GWAS on the Santo Antão population identified several potential candidate genes associated with both response variables for rosette growth (average trait values and drought responses), including those shown to be involved in auxin signaling (*TPST*), starch turnover (*DPE2*), cuticle formation (*ABCG5*), wax ester accumulation (*WSD1-like*), and stress-responsive signaling (*MPK12*, *DREB2B*, *ATJ3*). Additional published evidence supports the role of these loci in drought response. *TPST* mutants exhibit a small rosette phenotype, early senescence, and a reduced number of flowers and siliques (Komori *et al.*, 2009). Similarly, *DPE2* mutants show reduced leaf and rosette area under drought conditions with respect to wild-type, and they also display altered transpiration phenotypes, suggesting that its central role in carbohydrate metabolism influences both growth and water regulation under stress (Massonnet *et al.*, 2015; Westgeest *et al.*, 2023). *ABCG5* is crucial for cuticle formation, which helps in retaining water and supporting plant growth under stress (Lee *et al.*, 2021).

Additionally, *WSD1* plays a role in accumulating wax esters, which are vital for reducing water loss (Patwari *et al.*, 2019). *DREB2B*, which is induced by drought stress, regulates the transcription of drought-responsive genes through binding to the DRE/CRT sequence (Liu *et al.*, 2019). This gene is known for its role in enhancing drought tolerance by regulating stress-responsive gene expression (Nakashima *et al.*, 2000; Sakuma *et al.*, 2006). *ATJ3*, a member of the HSP40 family, contributes to heat stress tolerance by interacting with HSP70-4 and participating in heat stress granules (Wang *et al.*, 2021). These findings highlight potential interplay between metabolic pathways and drought adaptation.

Loci identified in the Fogo population exhibited distinct genetic associations compared to those in the Santo Antão population, which aligns with their early separation and lack of shared genetic variation. In Fogo, loci associated with average trait values and drought responses for rosette growth included *DOF5.8*, *UGT79B2*, *PIF1*, *FEY*, *PHO1-H10*, and *AAP1*. *DOF5.8*, a transcription factor, regulates the *ANAC069* gene, which is involved in integrating auxin and salt signals for seed germination (He *et al.*, 2015). *UGT79B2*, induced by various abiotic stresses, enhances plant tolerance by modulating anthocyanin accumulation (Li *et al.*, 2017). *PIF1* influences ABA biosynthesis and signaling component genes (Oh *et al.*, 2007), while *FEY* is implicated in leaf positioning and meristem maintenance (Callos *et al.*, 1994). *PHO1-H10*, involved in phosphate homeostasis, is upregulated by ABA, suggesting its role in drought response through modulating phosphate availability (Ribot *et al.*, 2008). *AAP1*, whose expression is induced by ABA, may play a role in amino acid transport under drought stress (Wang *et al.*, 2017). While the specific candidate genes differ between the islands, they frequently belong to similar functional pathways (e.g. ABA signaling, stress response, and growth regulation), suggesting that while they are genetically divergent, their adaptive strategies have converged on common mechanisms.

Regarding the leaf color traits, which are proxies for pigment content, our analysis also revealed a significant genetic association.

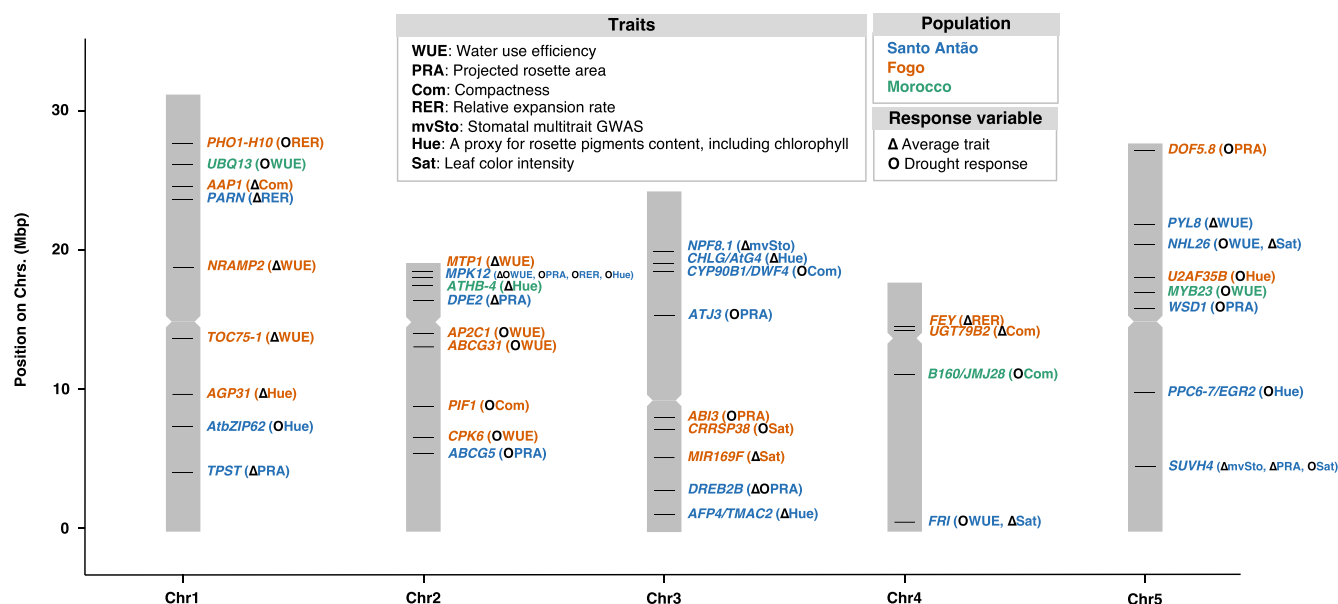


Fig. 5 Genomic associations across eight traits in response to drought identified by genome-wide association study (GWAS). Associations shown include single nucleotide polymorphisms (SNPs) linked to 39 candidate genes, selected based on their harmonic mean rank and functional relevance to drought-related traits (see the [Materials and Methods](#) section). Traits (see abbreviations) were analyzed across Santo Antão, Fogo, and Morocco *Arabidopsis thaliana* populations (distinguished by color) using two response variables (represented by different shapes: triangles for the trait average across well-watered (WW) and water deficit (WD); circles for the trait difference between WW and WD, representing the drought response). Detailed annotations of all SNPs are available in Supporting Information Table S7.

Although previous research has indicated that the saturation parameter was less important than hue values in specific experiments (De Vyllder *et al.*, 2012), we discovered an association between average saturation and the *FRIGIDA* (*FRI*) gene, which influences various traits, including flowering and WUE (Lovell *et al.*, 2013). This suggests that *FRI* may have pleiotropic effects on drought adaptation, influencing both leaf color and WUE.

A combined GO enrichment analysis integrating candidate genes identified by both response variables (average trait values and drought responses) revealed significant enrichment for gene sets involved in drought response, providing evidence that the GWAS was detecting true associations (Fig. 6; Table S8). We found that terms related to ‘response to abscisic acid’ were overrepresented for the candidate genes. This was driven by genes such as *ABI1* and *PYL8* (Fig. 6), known ABA receptor components (Belda-Palazon *et al.*, 2018), and *MPK12*, which is implicated in ABA signal transduction, *VQ18*, and *DREB2B*, known for its involvement in drought-responsive pathways (Liu *et al.*, 2019).

Co-localization of GWAS peaks across traits in the Santo Antão population indicates potential pleiotropic effects of some of the identified loci in shaping the drought escape syndrome. We found that *MPK12* not only associates with WUE but also with drought response of PRA, RER, and Hue, reflecting the strong genetic correlations observed among these traits. Crucially, the allelic effects at this locus are directionally aligned as the allele associated with lower WUE simultaneously promotes greater vegetative growth (increased PRA and RER). This suggests that *MPK12* may act as a central regulator, mediating coordinated responses to drought stress across physiological and

morphological traits to facilitate a rapid-growth escape strategy. Furthermore, a locus that includes a premature truncation of *FRI*, a gene traditionally known for its role in flowering time regulation, was associated with WUE and leaf color intensity (saturation) (Figs 5, S11a), indicating a broader adaptive response to drought. Consistent with the phenotypic correlations, the early flowering *FRI* allele is coupled with lower WUE, demonstrating that this pleiotropy actively reinforces the drought escape syndrome by aligning accelerated reproduction with high transpiration. Additionally, *SUVH4*, an epigenetic regulator via H3K9 methylation, was associated with stomatal traits, PRA and leaf color, suggesting a potential link between epigenetic modifications and the physical adaptation to drought. Together, these findings from Santo Antão offer concrete examples of how pleiotropic loci can link a complex, multi-trait response to an environmental pressure by aligning allelic effects in the same adaptive direction, thereby avoiding the evolutionary constraints of antagonistic pleiotropy. The divergence in genetic correlation structures across phenotypes in the three populations (Fig. 4) suggests that the evolution of the drought escape syndrome can capitalize on distinct, population-specific genetic links, though distinguishing the relative contributions of selection on pleiotropic genes vs correlative selection on linked loci across all lineages remains an important direction for future investigation.

Discussion

Here we investigated the phenotypic and genetic basis of drought response in *A. thaliana* populations from the CVI in comparison

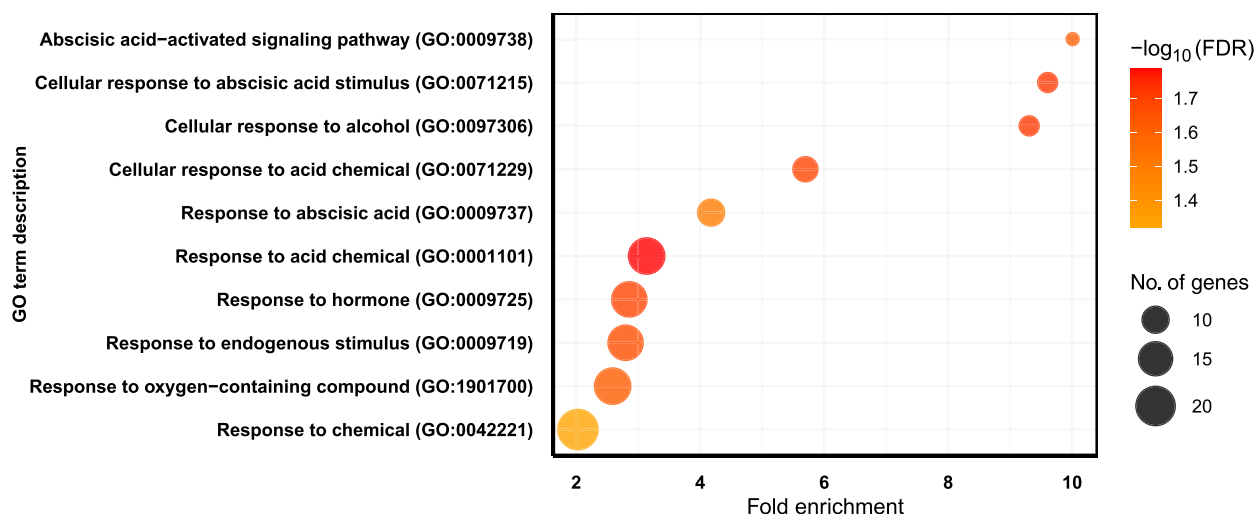


Fig. 6 Gene ontology (GO) term enrichment analysis for candidate genes identified using the two response variables (average trait values and drought responses) across the studied traits in the Santo Antão, Fogo, and Moroccan *Arabidopsis thaliana* populations. The size of the circles indicates the number of genes and the circle color indicates the corresponding P -value for enrichment after false discovery rate (FDR) correction.

to their closest outgroup populations from Morocco. We used a high-throughput phenotyping platform (Phenoscope) to measure key traits under controlled drought conditions and GWAS to connect trait variation with its genetic underpinnings. CVI populations showed significant shifts in the distribution of traits relative to Morocco, including earlier flowering time, lower WUE, smaller rosette size, reduced compactness, and higher relative growth rates. Genetic correlations between traits and congruence in GWAS peaks across some trait pairs provided evidence for pleiotropy. These coordinated changes raise key questions about the nature of drought adaptation. Specifically, how do plants balance the trade-offs between growth and water conservation, and what is the genetic basis of this coordination? We discuss below how the observed trait divergence points to the evolution of a drought escape strategy in the CVI. Furthermore, we explore how this strategy is genetically coordinated, examining the critical role of pleiotropy, the enrichment of ABA-related pathways, and the specific contributions of identified key loci in driving this complex adaptive response.

The observed differences among populations reveal that trait divergence in both CVI populations likely reflects adaptive differentiation driven by long-term exposure to water limitation. Across populations, drought-induced strong phenotypic changes in most traits, consistent with previous evidence for drought-induced shifts in morphology and physiology (McKay *et al.*, 2003; Juenger *et al.*, 2005; Des Marais *et al.*, 2012; Exposito-Alonso *et al.*, 2018). However, population differences in the magnitude and direction of phenotypic shifts highlight the heterogeneous nature of drought adaptation. Importantly, significant population-by-environment interactions for multiple traits (Table S4), including WUE, PRA, and flowering time, highlight that adaptive responses to drought differ among populations. The high population-by-environment for flowering time, where the response of both CVI populations diverged markedly from that of Morocco, suggests that the magnitude of plasticity and

thus adaptive potential is population-specific. These findings align with earlier studies showing that divergence in phenotypic plasticity can drive population-level specialization in drought escape vs avoidance strategies (Des Marais *et al.*, 2013; Ågren *et al.*, 2017). Together, these results underscore that the CVI populations have evolved a drought escape strategy, contrasting with the more environmentally responsive plasticity observed in Morocco. Furthermore, our within-population models (Table S5) detected significant genotype-by-environment ($G \times E$) interactions for traits including rosette size, stomatal density, and flowering time. This confirms that genetic variation for plasticity still exists within CVI. However, this evolutionary flexibility is highly constrained. We found strong positive cross-environment genotype mean correlations for nearly all measured traits, including WUE ($r = 0.65$ – 0.84), compactness, and leaf color (Table S5). A high cross-environment correlation means that a genotype's performance under WW conditions heavily dictates its performance under drought, which restricts the ability to evolve independent trait responses (Arnold *et al.*, 2019; Opedal *et al.*, 2023). Therefore, even though the island populations possess the genetic variation needed to theoretically alter their plasticity, this strong genetic link across environments limits their ability to independently fine-tune their drought responses without simultaneously altering baseline growth. This constraint effectively locks the CVI populations into their fixed drought-escape strategy.

The genetic correlations between traits (Fig. 4) clarify the evolutionary constraints and trade-offs shaping these adaptive strategies. In models of drought adaptation, a fundamental physiological trade-off exists between rapid phenological development (drought escape) and efficient water conservation (drought avoidance) (Geber & Griffen, 2003; McKay *et al.*, 2003). Our data reflect this dynamic in Santo Antão, where early flowering is correlated with lower WUE and reduced rosette compactness. Conversely, Fogo exhibits an inverted correlational structure,

with early flowering associating with a higher WUE drought response and increased compactness. This divergence suggests that while both populations converged on early flowering, their specific genetic architectures and resulting physiological trade-offs differ substantially, likely driven by the distinct *de novo* mutations characterizing each population (Fulgione *et al.*, 2022). Furthermore, across all populations, flowering time exhibits relatively modest genetic correlations with most morphological and physiological traits, contrasting with the highly integrated growth traits (e.g. PRA and RER). This relative phenotypic decoupling points to a modular genetic architecture underlying the drought response. Such modularity implies that natural selection can shift reproductive timing to drive a drought escape strategy without being heavily constrained by antagonistic pleiotropy across the broader physiological network (Agrawal & Stinchcombe, 2008).

In the GWAS analysis, we identified candidate loci involved in ABA signaling and epigenetic processes. The enrichment of candidate genes in the ABA signaling pathway reflects its established role in modulating drought responses, such as stomatal closure, water uptake, and osmotic adjustment (Sharma *et al.*, 2011; Tuberosa, 2012). ABA-related pathways are known to influence physiological processes like stomatal regulation and proline metabolism, both critical for plant resilience to drought (Setter, 2012; Bhargava & Sawant, 2013). Our GWAS findings identified *NPF8.1*, a key gene facilitating ABA uptake into guard cells, which modulates stomatal closure (Tal *et al.*, 2016; Shimizu *et al.*, 2021). This complements prior observations linking ABA signaling to proline accumulation and oxidative stress mitigation (Bhatt *et al.*, 2011; Liang *et al.*, 2013). Additionally, *SUVH4*, influenced by ABA modulation (Zheng *et al.*, 2012), was implicated in multiple drought-related traits, including stomatal density, leaf color, and plant rosette area, suggesting pleiotropic effects. Overall, these results highlight the central role of ABA signaling in coordinating drought-related responses and shaping trait variation across populations, and they reveal the intriguing possibility that epigenetic processes might also contribute to drought response.

The relationship between flowering time and vegetative growth dynamics is well-established (Bac-Molenaar *et al.*, 2016; Hanemian *et al.*, 2020). Our study suggests that pleiotropy may play a role in drought adaptation. Previous studies have demonstrated that correlated physiological traits, such as WUE and flowering time, can influence adaptive processes (McKay *et al.*, 2003; Lovell *et al.*, 2013). Our findings complement these studies by identifying genes like *FRIGIDA* (*FRI*), which may link leaf color traits to adaptive processes such as the transition from vegetative growth to flowering. This pleiotropic link is particularly important for explaining the divergent genetic architectures we observed between the islands. As noted, the correlational structure between early flowering and traits like WUE and compactness differs substantially between Santo Antão and Fogo. This divergence likely reflects the distinct genetic mechanisms underlying early flowering in each island. Whereas early flowering in Santo Antão is driven by a segregating loss-of-function polymorphism in *FRI* (*FRI* 232X), Fogo adapted via a parallel, *de novo* premature truncation in *FLOWERING LOCUS C* (*FLC* 3X) that is fixed within the

population (Fulgione *et al.*, 2022). As a fixed allele, *FLC* 3X does not contribute to the segregating phenotypic variation or the estimated genetic correlations in Fogo. Instead, the remaining phenological variation in this population is governed by secondary, smaller-effect loci that arose more recently (Neto & Hancock, 2023). These secondary loci include genes that are also involved in photoperiod control, light sensing, and epigenetic regulation (e.g. *PAPP2C*, *OXS2*, and *ATX1*), which impose distinct pleiotropic effects (Neto & Hancock, 2023). Consequently, while the segregating *FRI* locus in Santo Antão drives a highly integrated drought-escape syndrome, the fixed *FLC* architecture in Fogo may permit a more modular genetic response. This modularity potentially allows secondary segregating loci to shape a combination of escape and avoidance strategies, conferring a distinct adaptive potential.

Consistent with this highly integrated drought-escape syndrome in Santo Antão, *FRI*, previously associated with reduced time to flowering and increased seed production in CVI environmental conditions (Fulgione *et al.*, 2022), appears to play a broader role in adaptation to drought environments. The presence of the *FRI* 232X variant in Santo Antão suggests it represents an early step in adaptation to the CVI environment, facilitating a drought-escape strategy by accelerating reproduction before water resources become limiting (Fulgione *et al.*, 2022; Neto & Hancock, 2023). Beyond its established role in flowering time regulation, we found *FRI* to be significantly associated with saturation (leaf color intensity) and WUE. The link to saturation likely reflects critical shifts in resource allocation from vegetative growth to reproductive investment, while its association with WUE highlights an optimization of carbon assimilation against water loss. These coordinated pleiotropic effects of *FRI* on traits related to growth, WUE, and reproduction illustrate its integrative role in shaping the escape strategy observed in Santo Antão.

Here, we found evidence that *MPK12* plays a role in multiple drought-related traits, including WUE, stomatal traits, growth, and leaf color. Previous studies have established *MPK12* as a key regulator of stomatal responses to ABA, influencing WUE and guard cell behavior, with the CVI allele altering responses to vapor pressure deficits and ABA-induced inhibition of stomatal opening (Juenger *et al.*, 2005; Jammes *et al.*, 2009; Montillet *et al.*, 2013; Salam *et al.*, 2013; Des Marais *et al.*, 2014; Jakobson *et al.*, 2016). In earlier work on the Santo Antão population, a nonsynonymous variant in *MPK12* (G53R) was shown to drive phenotypic shifts toward higher stomatal conductance and lower WUE (Elfarargi *et al.*, 2023). These shifts supported a drought-escape strategy in Santo Antão, where plants maximize carbon assimilation through open stomata under conditions of high atmospheric humidity (Elfarargi *et al.*, 2023). Incorporating this variant as a covariate in our GWAS model allowed us to uncover additional loci associated with ABA pathways. Notably, *MPK12* was linked to WUE, stomatal traits, growth, and leaf color, indicating its broader pleiotropic role in drought-related adaptation. The ability of populations to adapt rapidly to the extreme climate shifts in CVI likely relies on balancing loci with targeted functions and pleiotropic effects. This trade-off facilitates efficient adaptation for managing complex traits under selection. Our

findings support this hypothesis, with *MPK12* emerging as a central hub in the genetic network underlying drought response.

Despite genetic and allelic heterogeneity between Santo Antão and Fogo populations, our GWAS results reveal a convergence on shared genetic pathways underlying drought responses. While the specific genes identified varied between populations, both islands exhibited enrichment in pathways related to ABA signaling and growth regulation, suggesting that similar physiological processes are being targeted by natural selection. Although the genetic components differ, the underlying functional processes, such as regulating WUE, stomatal behavior, and stress signaling, are remarkably consistent. This suggests that these populations are adapting to drought through distinct genetic mechanisms converging on shared pathways. Such insights deepen our understanding of how distinct populations achieve functional convergence despite genetic divergence, highlighting the complex interplay between local adaptation and shared evolutionary pressures.

While this study highlights promising candidate genes and their associations with drought-related traits, there are several limitations of this study that could be addressed in future work. First, here, we investigated phenotypic and pleiotropic effects of variation that still segregates within each island population. Additionally, the limited sample size of Moroccan individuals constrained GWAS power in that outgroup; a larger sample of Moroccan individuals could enhance statistical power for GWAS. Future work that involves crosses between islands (Neto *et al.*, 2026) or with the Moroccan outgroup could enable inference of such effects. Moreover, functional validation would be required to establish causality with certainty. For example, functional studies using knockout lines, reciprocal transformations, gene expression analyses, and precise genome editing to recapitulate specific sequence variants could enable direct testing of candidate genes we identified as drivers of phenotypic variation. Furthermore, integrating co-expression network analysis could unravel the interplay between loci with targeted and pleiotropic effects. Ultimately, future studies might also extend these findings to crop systems, leveraging the identified genes to improve drought tolerance in agriculturally important species.

This study provides new insights into how plants have evolved to cope with changes in precipitation regimes. It extends our understanding of drought adaptation mechanisms by identifying significant genetic associations linked to key drought-related traits. Further, our findings underscore the potential of pleiotropy to facilitate adaptation. This research paves the way for a deeper understanding of plant responses to abiotic stress and establishes a framework for exploring adaptive mechanisms in both natural populations and agricultural systems.

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Competing interests

None declared.

Author contributions

AFE and AMH conceived and designed the study. OL provided expertise for designing the drought measurement (Phenoscope) experiment. AFE, OL and EG conducted the Phenoscope drought experiment and collected samples for $\delta^{13}\text{C}$ analysis. ND, APMW and members of APMW’s lab performed $\delta^{13}\text{C}$ measurements. AFE prepared data, conducted statistical analyses, and created figures. AFE and AMH analyzed and interpreted the results. AFE, AMH and HD collected plant samples. AFE and AMH wrote the manuscript, with input from all authors. All authors reviewed and approved the final manuscript.

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Data availability

The whole-genome sequencing raw reads for the 15 newly collected *A. thaliana* accessions from Santo Antão have been deposited in the European Nucleotide Archive (ENA) under project ID [PRJEB110651](https://www.ebi.ac.uk/ena/browser/view/PRJEB110651) (ERP191319). Additionally, previously published sequence data were utilized from ENA project ID [PRJEB39079](https://www.ebi.ac.uk/ena/browser/view/PRJEB39079) (ERP122550). All scripts used for data processing and analysis are available at <https://github.com/AhmedElfarargi/CVI-DroughtAdaptation-MultiTraitGWAS.git>.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Images of representative accessions during the drought experiment.

Fig. S2 Phenotypic plasticity of drought-related traits across the populations.

Fig. S3 Pairwise phenotypic correlations among traits across the populations.

Fig. S4 Comparison of genetic and phenotypic correlations across traits.

Fig. S5 Genomic associations for water use efficiency under drought stress.

Fig. S6 Multivariate genomic associations for stomatal traits in Santo Antão.

Fig. S7 Genomic associations for rosette growth traits in Santo Antão.

Fig. S8 Genomic associations for rosette growth traits in Fogo.

Fig. S9 Genomic associations for rosette growth traits in Morocco.

Fig. S10 Genomic associations for leaf color hue under drought stress.

Fig. S11 Genomic associations for leaf color saturation under drought stress.

Table S1 List of *Arabidopsis thaliana* accessions used in this study.

Table S2 Summary of the climatic data used in this study.

Table S3 Best climatic variables explaining genetic variation across the populations.

Table S4 Fixed effects from linear mixed models assessing phenotypic traits.

Table S5 Within-population linear models and cross-environment genotype mean correlations.

Table S6 Summary statistics and broad-sense heritability estimates for measured traits.

Table S7 Summary of significant genome-wide associations across the populations.

Table S8 Gene ontology enrichment analysis for the identified candidate genes.

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