Running head: Adaptive homeostasis in Arabidopsis drought response

Genetic variation associated with plastic and homeostatic growth responses to drought in Arabidopsis

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One-sentence summary: Information on phenotype, genotype, and transcript abundance is integrated to identify both plasticity and homeostasis genes and processes associated with local adaptation to drought stress in Arabidopsis accessions of the Iberian Peninsula.

Total word count (excluding summary, references and legends):	6,479	Number of figures	5
		SI Figure files	1
Introduction:	521	SI Table files	1
Materials & Methods:	1,804	SI Documents	1
Results:	1,458		
Discussion:	2,696		

Summary

- Natural genetic variation influences plant responses to environmental stressors. However, the extent to which such variation underlies plastic versus homeostatic response phenotypes has been little studied.
- We quantified the extent of drought-induced changes in leaf area in a set of Iberian *Arabidopsis* accessions and then performed association studies correlating variation in this phenotype with genomic and transcriptomic variation.
- Drought-induced plastic reductions in relative leaf area typified accessions originating from productive environments. In contrast, homeostasis in relative leaf area typified accessions originating from unproductive environments. Genome-Wide Association Studies (GWAS), Transcriptome Wide Association Studies (TWAS), and expression GWAS (eGWAS) highlighted the importance of auxin-related processes and, in particular, the potential role of the *SMALL AUXIN UP RNA 26 (SAUR26)* gene in conferring leaf area plasticity. Homeostatic responses in relative leaf area were associated with a diverse gene set and positively associated with a higher intrinsic water use efficiency (WUE_i) as confirmed in a TWAS metanalysis of previously published $\delta^{13}C$ measurements.
- We have identified not only candidate “plasticity genes” but also candidate “homeostasis genes” controlling leaf area. Our results demonstrate the value of a combined GWAS, TWAS, and eGWAS approach to identify mechanisms underlying phenotypic responses to stress.

Keywords: *Arabidopsis thaliana* (Arabidopsis), drought, eGWAS, GWAS, plasticity, transcriptome, TWAS, WUE_i .

INTRODUCTION

Earth is undergoing a climate change scenario resulting in extreme weather events that are increasingly frequent and variable (Easterling *et al.*, 2000; Meehl and Tebaldi, 2004; Schär *et al.*, 2004). These changing conditions are creating opportunities for some species to spread beyond their native ranges (Chen *et al.*, 2011) while posing a challenge to species that are currently adapted to their local environment (Holt, 1990). As sessile organisms, plants complete their life cycle in their local environment and consequently are experiencing climate conditions of increasing variability and severity. Changes in the environment during the lifetime of individual plants typically induce plastic responses in life-history traits (Bradshaw, 1965; West-Eberhard, 2003). However, simply because a given trait is plastic does not mean that this plasticity is adaptive (Ghalambor *et al.*, 2007). For example, growth plasticity may arise from an unpredictable resource limitation such as insufficient water, mineral nutrients, or light (Dorn *et al.*, 2000; van Kleunen and Fischer, 2005; Forsman, 2015). In situations where plasticity derives from resource limitation and is maladaptive or neutral for a given trait, homeostasis or lack of plasticity may, in turn, be adaptive. However, while there has been much discussion in the ecological literature about “plasticity genes” (Bradshaw, 1965; West-Eberhard, 2003; Nilson & Assmann, 2010), relatively little attention has been paid to the identification of potential “homeostasis genes.”

Drought is among the strongest factors driving adaptation in plants (Siepielski *et al.*, 2017; Exposito-Alonso *et al.*, 2019). Since climate projections predict precipitation extremes to become more severe and unpredictable (Donat *et al.*, 2016), we can expect that drought episodes will become progressively more difficult for plants to adapt to. For this reason, understanding the genetic basis and adaptation potential of phenotypic traits and their plastic or homeostatic responses to drought is paramount for ameliorating climate change impacts in both natural and managed ecosystems.

The Iberian Peninsula is a drought-prone region particularly vulnerable to climate change effects on precipitation (Vicente-Serrano, 2006a; Vicente-Serrano, 2006b; Coll *et al.*, 2016; Páscoa *et al.*, 2017). The Iberian *Arabidopsis* population is particularly valuable because its accessions originate from a broad range of environments (Lobo *et al.*, 2001; Rey Benayas and Scheiner, 2002), and previous studies of this population have provided considerable insight into *Arabidopsis* evolution and development (Píco *et al.*, 2008; Montesinos *et al.*, 2009; Gomaa *et al.*, 2011; Méndez-Vigo *et al.*, 2011; Montesinos-Navarro *et al.*, 2011; Píco, 2012; Manzano-Piedras *et al.*, 2014; Wolfe and Tonsor, 2014; Vidigal *et al.*, 2016; Exposito-Alonso *et al.*, 2018a; Marcer *et al.*, 2018).

In the present study, we utilize GWAS, Transcriptome-Wide Association Studies (TWAS), and Expression Genome-Wide Association Studies (eGWAS) to identify natural genetic and transcript abundance variation underlying both plastic and homeostatic leaf area responses to drought in Iberian *Arabidopsis* accessions. Leaf area is a vital trait in relation to growth: leaf area production determines the total amount of light intercepted by plants, carbon assimilation, and the extent of evaporative water loss. We uncover an adaptive homeostatic growth strategy in accessions from less productive environments, dissect its genetic basis, and conclude that phenotypic homeostasis is under genetic control and of evolutionary importance, just as previously argued for plasticity.

MATERIALS AND METHODS

Plant materials and phenotyping

Seeds for 43 Iberian accessions (Table P1 for the list of accessions) were cold-stratified for four days at 4°C to synchronize germination. Plants were grown in 10 cm square pots containing Metro-mix 360 potting mixture (Sun Gro Horticulture Canada Ltd) in a greenhouse with an average daytime 500 $\mu\text{mol m}^{-2}\text{s}^{-1}$ photosynthetic photon flux density (PPFD) (8h light: 16h dark, with light supplied as natural daylight supplemented with 1000W metal halide lamps) and average day/night temperatures of 22 °C /20°C.

For each Iberian accession, we grew one plant per pot, with five replicates for each of control and drought treatments (10 plants for each individual accession), for a total of 430 plants (43 accessions $\times$ five control $\times$ five droughted plants). Both control and drought treatment were well-watered for the first 28 days, maintaining the soil relative water content at field capacity as measured by monitoring pot weight daily and adding water when necessary. After 28 days, plants in the drought treatment were allowed to dry down and then were rewatered to soil saturation once every ten days for a total of 14 drought cycles until the conclusion of the experiment 175 days after seeds were initially sown. Here, as in the natural environments that we sought to mimic, the soil water content was not adjusted based on the size of individual plants. Our protocol simulated the natural recurring cycle of rainfall and drought. Such cycles are often used in studies of natural variation (i.e., Exposito-Alonso *et al.*, 2018b; Exposito-Alonso *et al.*, 2019) as well as in field trials on crop cultivars (IRRI, 2021). In contrast, the well-watered treatment was maintained near field capacity throughout the experiment. Plants were spatially randomized within the greenhouse every week throughout the

experiment. At the end of the experiment, nine accessions within the drought treatment with mortality rates higher than 40% (i.e., at least two of the five replicates; Table P1) were excluded from downstream analyses.

Photographs of rosettes were taken at the time of flowering and subsequently analyzed using ImageJ (<http://imagej.nih.gov/ij/>) to determine the projected leaf area for each accession under both well-watered and drought treatments (Fig. S1; Table P1).

Determination of relative leaf area and plasticity

Relative leaf area (rLA) is defined here as the leaf area (LA) at the time of flowering for each accession under each experimental condition (either well-watered or drought) relative to all the accessions under the same condition. The relative leaf area of a given accession under well-watered conditions ($rLA_{i \text{ (well-watered)}}$) is thus calculated as:

$$rLA_{i \text{ (well-watered)}} = \frac{LA_{i \text{ (well-watered)}}}{LA_{m \text{ (well-watered)}}$$

where $LA_{i \text{ (well-watered)}}$ is the leaf area of any individual accession and $LA_{m \text{ (well-watered)}}$ is the average of the leaf area for all accessions grown under the same conditions. Similarly, the relative leaf area under drought ($rLA_{i \text{ (drought)}}$) for each accession is calculated as:

$$rLA_{i \text{ (drought)}} = \frac{LA_{i \text{ (drought)}}}{LA_{m \text{ (drought)}}$$

where $LA_{i \text{ (drought)}}$ is the leaf area of any individual accession under drought and $LA_{m \text{ (drought)}}$ is the average value for all accessions when grown under drought. A high rLA reflects greater vegetative growth of any given accession relative to the set of accessions utilized in this study. The use of rLA, rather than actual values, was essential to allow a normalized comparison of the leaf area of each accession between the watering regimes. Reaction norms based on rLA were employed to quantify plastic changes in relative leaf area between the two treatments (well-watered vs. drought). Leaf area plasticity indexes were calculated based on an established definition (Weijschedé *et al.*, 2006). The leaf area plasticity index to drought (PI) was calculated for each accession as:

$$PI = rLA_{i \text{ (drought)}} - rLA_{i \text{ (well-watered)}}$$

A positive leaf area plasticity index does not necessarily imply leaf area for that accession was higher under drought, but rather that under drought, a given accession displays a higher rLA relative to the rest of accessions in the study.

146

147 **Genome-wide association study**

148 Genome-Wide Association Studies (GWAS) identify genetic variants associated with natural
149 variation in a phenotypic trait of interest. We used a GWAS approach to identify polymorphisms
150 associated with natural variation in rLA and its plasticity. The online tool GWAPP
151 (<http://gwas.gmi.oeaw.ac.at/>) was employed using a linear regression model (Seren *et al.*, 2012). The
152 linear regression model does not address confounding effects of population stratification, family
153 structure, and cryptic relatedness (Price *et al.*, 2010); however, those issues are minimized by our
154 choice of a regional (Iberian) population (Frachon *et al.*, 2018; Tabas-Madrid *et al.*, 2018; Frachon
155 *et al.*, 2019). Nonetheless, we demonstrate that the candidate SNPs discussed here were also
156 significant after the application of a mixed model approach implemented either using GWAPP (Seren
157 *et al.*, 2012) or GWAS-Flow (Freudenthal *et al.*, 2019) (Fig. S2; Tables G1-G6), providing further
158 confidence in our results.

159 We filtered out low-frequency variants (minor allele frequency (MAF) < 10%) to focus on
160 variants less likely to be undergoing negative selection or to be spurious SNPs from sequencing
161 errors. We obtained gene model annotation from the TAIR11 genome release. We defined the
162 ‘consensus’ transcript variant as the variant producing the longest transcript. We predicted the effect
163 of every SNP using SnpEff (Cingolani *et al.*, 2012). To focus on SNPs in gene transcriptional units,
164 we subsetted the set of variants included in the untranslated regions at the 5’ and 3’ ends and the open
165 reading frames, as defined in Araport11 (Cheng *et al.*, 2017), with the potential for affecting gene
166 expression and protein composition. We also included the promoter region, defined as 5 kb upstream
167 of the most distal transcription start site. We imposed a stringent arbitrary significance threshold
168 corresponding to a score of 4 ($P < 0.0001$; Fig. S3).

169 Using the results from GWAS obtained from the association with rLA, we obtained reaction
170 norms that would reflect plastic or homeostatic patterns of genome-wide associations of these traits
171 in response to drought. The association between a phenotype and any given SNP was designated as
172 homeostatic if found significantly associated with that trait under both conditions (well-watered and
173 drought).

174

Transcriptome-wide association

Natural variation in gene expression is an important mechanism of local adaptation (Des Marais *et al.*, 2012; Lasky *et al.*, 2014). Transcriptome-Wide Association Studies (TWAS) identify variation in transcript abundance associated with observed variation in phenotypic traits (Gusev *et al.*, 2016; Kremling *et al.*, 2019). TWAS analysis does not suffer from the limitations imposed by false non-causal synthetic associations due to linkage disequilibrium (Li *et al.*, 2021) typical of GWAS (Korte and Farlow 2013). Also, TWAS evaluates continuous variation in transcript abundance rather than the presence/absence of SNPs with (potentially) additive effects on a continuous phenotype. We retrieved the transcriptome data from rosette leaves of 727 Arabidopsis accessions from the GEO dataset with accession number GSE80744 and SRA study SRP074107 (Kawakatsu *et al.*, 2016) and subsetted the 35 Iberian accessions that overlapped with the 43 Iberian accessions used in our study. Data wrangling was conducted utilizing the dplyr (Wickham *et al.*, 2015) and tidyr (Wickham and Henry, 2018) packages. Spearman's rank correlation coefficient between individual phenotypic values (relative leaf area (rLA_i), plasticity index (PI), and intrinsic water use efficiency (WUE_i) from Dittberner *et al.* (2018; Tables P1 and P2), and individual transcript abundance values were calculated using the correlation function of the Hmisc package (Harrell Jr and Dupont, 2019). The stronger the association of the two variables, the closer the Pearson correlation coefficient, r_s , will be to either +1 or -1. We imposed a threshold based on this correlation coefficient ($0.4 \geq |r_s| \leq 4.0$). We considered this threshold to be stringent after inspection of the distribution of the results (Fig. S3). We include the r_s and corresponding *P*-values in Tables T1 and T2.

Using the results from TWAS, we derived reaction norms to reflect homeostatic patterns of transcriptome-wide associations of rLA in response to drought. Any given transcript was designated homeostatic if found significantly associated with rLA (either positively or negatively; $|r_s| \geq 4.0$) under both conditions (well-watered and drought).

eGWAS

Natural variation in transcript abundance can also be used to identify candidate SNPs (expression SNPs or eSNPs) that control transcript abundance in an Expression Genome-Wide Association Study (eGWAS). eGWAS was conducted by compiling the transcript abundance values of a given gene, extracting transcript abundance for that particular gene in the 727 Arabidopsis accessions from the GEO dataset with accession number GSE80744 and SRA study SRP074107 (Kawakatsu *et al.*, 2016),

then subsetting the 665 accessions included in the 1001 Genomes project (The 1001 Genomes Consortium, 2016). Then, the online tool GWAPP (<http://gwas.gmi.oeaw.ac.at/>) was employed using a linear regression model (Seren *et al.*, 2012) to identify candidate regulatory variants. Regulatory variants are either cis or trans-acting, depending on the location of the genetic variant. cis-eSNPs were defined as those located within the ORF, UTRs, or the promoter region of the regulated gene. Trans-eSNPs were defined as those located within the ORF, UTRs, or promoter region of a gene different from the gene transcriptionally regulated by that genetic variant.

Meta-analysis of 189 Iberian Arabidopsis accessions

AraCLIM (<https://github.com/CLIMtools/AraCLIM>) is a database that describes the local environment of the Arabidopsis accessions sequenced as part of the 1001 Genomes Project (Ferrero-Serrano and Assmann, 2019). We obtained from AraCLIM the values for MOD17A2 (Net Primary Productivity in the spring season) (Heinsch *et al.*, 2003; Running and Zhao, 2015) at the collection site of each accession and assessed correlation with rLA.

WUE_i is defined as the ratio between instantaneous carbon gain and transpirational water loss, typically calculated per unit of leaf area (Condon *et al.*, 2002). To integrate the information from previously published studies on the Iberian Arabidopsis population, we retrieved available data on natural variation in intrinsic water use efficiency (WUE_i, $\delta^{13}\text{C}$), previously recorded by Dittberner *et al.* (2018), from PhenoCLIM (<https://github.com/CLIMtools/PhenoCLIM>) (Ferrero-Serrano and Assmann, 2019). During photosynthesis, plants discriminate against $^{13}\text{CO}_2$ in the reaction center of *Ribulose 1, 5-biphosphate carboxylase/oxidase* (*RuBisCO*). As stomata close, minimizing water loss in response to drought, the discrimination against $^{13}\text{CO}_2$ decreases because the internal CO_2 concentration (C_i) becomes unavoidably reduced. As a result of their reduced C_i , plants with greater WUE_i experience lower CO_2 concentrations at the sites of carboxylation (C_c) and have less negative $\delta^{13}\text{C}$ values.

Analyses

All statistical analyses and figures were produced using R (Team, 2013). The code and data to reproduce the analysis, figures, and tables are available at <https://github.com/AssmannLab> and Zenodo. Gene Ontology (GO) biological process analysis (Tables GO1-GO10) was performed using the open-source software ShinyGO v0.61: <http://bioinformatics.sdstate.edu/go60/> (Ge *et al.*, 2020)

with the following settings: the search species was “*Arabidopsis thaliana*,” the P -value cutoff (FDR) was 0.05, and the number of most significant terms to show was ten. To replicate these results, the lists of genes that were input into these analyses based on significance thresholds can be selected from Tables G1-G6, T1, T2. Each analytical technique used in this study has assumptions and limitations, which we discuss in Document S1.

RESULTS

Natural variation in leaf area and plasticity in response to long-term cyclical drought

To quantify phenotypic variation in Iberian *Arabidopsis* accessions exposed to drought, we conducted a common garden experiment (Fig. 1a). We used image analysis to derive rosette leaf area under well-watered or drought conditions (Figs. 1b, S1; Table P1). As expected, we observed a significant ($P < 0.001$) reduction in leaf area resulting from drought in most genotypes, as evident from the phenotypic reaction norms. In contrast, a small number of accessions exhibited constant or increased leaf area under drought (Fig. 1b; Table P1), demonstrating that a reduction in leaf area in drought is not inescapable. Based on these data, we calculated the relative leaf area (rLA) of each accession under both well-watered and drought conditions (see Materials & Methods; Fig. 1c; Table P1). We used rLA rather than actual leaf area to allow comparison of the leaf area of each accession relative to the study population for each watering regime. We also show that the plasticity index derived from the difference in rLA between conditions (i.e. reflecting the slopes of the reaction norms in Fig. 1c; Table P1) is proportional to the fold change in leaf area under drought relative to the well-watered condition (Fig. S4).

We explored the relationship between rLA in the absence of drought and its plastic response (plasticity index; PI) to drought. Figure 1d shows that accessions with higher rLA displayed greater plastic reductions in rLA when subjected to drought (negative leaf area plasticity). On the contrary, accessions with lower rLA under well-watered conditions displayed homeostatic phenotypic responses to drought (Fig. 1d). Despite this overall trend, we also observed individual accessions with high rLA under well-watered conditions and positive plasticity indexes under drought (green datapoints in Fig. 1d and Fig. 1e). Conversely, a few accessions displayed low rLA and negative leaf area plasticity indexes (red datapoints in Fig. 1d and Fig. 1e).

While the severity of drought depends on multiple environmental parameters, its physiological response is very characteristic. It results in stomatal closure and a reduction in CO_2

assimilation, as reflected in decreases in Net Primary Productivity (NPP), an index that, therefore, can be used as a reliable drought indicator (Huang *et al.*, 2016). We determined that the plastic leaf area responses we observed in our study were typical of accessions from more productive habitats as reflected as Net Primary Productivity in spring (NPP spring; Fig. 2a). In contrast, homeostatic leaf area responses were typical of accessions from less productive habitats (Fig. 2a).

Genetic variants significantly associated with leaf area plasticity were identified by GWAS (Fig. 2b; Table G3). Gene ontology (GO) analysis of functions associated with the genes harboring the variants identified by GWAS revealed an enrichment in auxin-related processes (Table GO1). Among the variants identified from GWAS (Fig. 2b; Table G3) as associated with leaf area plasticity, we highlight two co-varying SNPs in the 5' and 3' UTR that comprise a haplotype of *SMALL AUXIN UP RNA 26* (*SAUR26*, AT3G03850) (Fig. 2c). These variants were identified by GWA analysis using a linear model (Fig. 2b; Table G3). To confirm that identification of these candidates was not an artifact due to population structure, we verified that these variants were also identified using two different analyses that correct for population structure in the sample: Accelerated Mixed Model using GWAPP and an alternative Mixed Model approach, GWAS-Flow (these two SNPs are among the green datapoints in Fig. S2; see Table G3).

We additionally found from TWA analysis that the transcript levels of *SAUR26* are also correlated with natural variation in leaf area plasticity in response to drought (Tables T1, T2). eGWAS confirmed the cis-regulatory effect of these genetic variants (cis-regulation; Fig. 2d; Table G6), consistent with the correlation between the genetic variation in *SAUR26*, its transcript abundance, and leaf area plasticity.

Figure 2e illustrates the haplotypic differences in leaf area plasticity for *SAUR26* in our study. Accessions harboring the minor *SAUR26* haplotype display negative plasticity in rLA, while the major haplotype is associated with homeostatic and positive plasticity responses in rLA (Fig. 2e; Wilcoxon *P*-value < 0.001). These haplotypic differences were also associated with the transcript levels of *SAUR26* (Fig. 2f), which we confirmed not only in the group of accessions included in our study (Wilcoxon *P*-value < 0.001) but also in the much larger group of 189 Iberian accessions included within the 1001 Genomes Project (Fig. 2f; Wilcoxon *P*-value < 0.001). Both the genetic variation defined by this haplotype in *SAUR26* and the resulting *SAUR26* transcript abundance are associated with the interplay between plasticity and homeostasis that we determine in our study (Fig.

2e, f). These results highlight *SAUR26* as a candidate regulator of leaf area plasticity in response to drought (Figs. 2g, h).

In our GWA analysis on rLA (Fig. S5a, Table G1, G2), no SNPs were associated with rLA under both well-watered and drought conditions, i.e., no SNPs were associated with homeostasis and thus would be depicted as horizontal lines in Fig. S5a. The results from TWA analysis were strikingly different, identifying not only transcripts associated with leaf area plasticity (Fig. S5b) but also transcripts associated with leaf area homeostasis (Fig. 3; Fig. S5b). Regarding homeostasis, four transcripts are positively correlated with rLA under both well-watered and drought conditions: *SOFL2*, *CPN60B*, *AT5G39471*, and *CKL8* (Fig. 3a). Accessions with higher transcript abundance of these genes display a higher rLA under both well-watered and drought conditions (Fig. 3b-e; note the similarity of the regression lines). Conversely, we identified four genes negatively correlated with rLA: *RHF1A*, *AT5G60160*, *AT4G15260*, and *AT5G16800* (Fig. 3f). Accessions with a higher transcript abundance of these genes display lower rLA under both well-watered and drought conditions (Fig. 3g-j; note the similarity of the regression lines).

Trade-off between plasticity and intrinsic water use efficiency

It was not surprising to find a trade-off between growth and drought resistance among the accessions in our study (Fig. 1d), as this is a well-established phenomenon (Zhang *et al.*, 2020). Accordingly, we hypothesized that those accessions that displayed a homeostatic response to drought would exhibit an increased intrinsic water-use efficiency (WUE_i), minimizing water loss. Given that $\delta^{13}C$ reflects intrinsic water use efficiency (WUE_i) (Juenger *et al.*, 2005; Easlon *et al.*, 2014), we analyzed the $\delta^{13}C$ values recorded by Dittberner *et al.* (2018), available for 21 of the accessions used in our study. The results confirm a trade-off between plasticity and WUE_i (Fig. 4a). Accessions that exhibit strong negative plasticity following drought (negative leaf area plasticity indices) have a lower WUE_i in the absence of drought (more negative $\delta^{13}C$ values). Those accessions that maintain or increase leaf area in response to drought (positive leaf area plasticity indices) display a higher WUE_i in the absence of drought (more positive $\delta^{13}C$ values).

GWA analysis failed to identify any genetic variants associated with the observed phenotypic trade-off between intrinsic WUE_i and leaf area plasticity (Fig. 4b; Table G3, G4), i.e., no datapoints in the upper right quadrant of Fig. 4b. However, TWA analysis (Fig. 4c) provided insight into this trade-off. Red datapoints in Fig. 4c are transcripts with greater abundance in those negatively plastic

and less water-use efficient accessions depicted in red in Fig. 4a; GO analysis revealed that these were enriched in processes involved in RNA modification (Table GO2); we chose the putative pseudouridine synthase *CBF5* (*AT3G57150*) as an example (Figs. 4d, e). Conversely, green points in Fig. 4c represent transcripts with greater abundance in the homeostatic and water-use efficient accessions depicted in green in Fig. 1d. We chose *CYCLIC NUCLEOTIDE-GATED CHANNEL 16* (*AT3G48010*; *CNGC16*) as an example (Figs. 4f, g).

We determined that in our study, accessions that in response to drought displayed either increased or homeostatic leaf area plasticity also had higher WUE_i in the absence of drought (Fig. 4a). We then conducted a meta-analysis on the 95 Iberian accessions with published $\delta^{13}C$ information (Dittberner *et al.*, 2018). GWA analysis of these accessions rendered a distinctive peak in a single gene, identifying a haplotype consisting of eight co-varying SNPs in *ClpX3* (*AT1G33360*; Fig. 5a; Table G5), an ATP-dependent Clp protease subunit that plays essential roles in chloroplast development (Kim *et al.*, 2009). Consistent with this result but more informative was the TWA analysis of WUE_i in the 88 Iberian accessions with both published transcript abundance and $\delta^{13}C$ information (Fig. 5b): we uncovered a distinctive enrichment in photosynthesis-related genes (Fig. 5c; Table GO3) among transcripts that are upregulated among accessions with high WUE_i (more positive $\delta^{13}C$ values; Fig. 5b; Fig. S6; Table T1, T2). We highlight the positive relationships with WUE_i uncovered for the transcript abundance of *RuBisCO small subunit 2B* (*AT5G38420*; *RBCS2B*; Fig. 5d) and the positive relationship between the transcript abundance of *SAUR26* and WUE_i (Fig. 5e), in concordance with other findings in our study.

DISCUSSION

Identifying candidate regulators of plasticity and homeostasis

An adaptive trade-off between growth and drought resistance in plants is well-established (Zhang *et al.*, 2020). In productive environments, accessions with higher rLA in the absence of drought may have an advantage in competing for aboveground resources relative to smaller individuals, as proposed by Tilman (1988). On the other hand, plants adapted to unproductive environments typically fix less carbon, have higher WUE_i , and display reduced vegetative growth, even when grown in rich environments (Parsons, 1968; Chapin, 1991); however, these plants are typically stress-tolerant (Billings and Mooney, 1968; Grime, 1977). In our study, accessions typical of more productive areas

display strong and negative plastic responses to drought (Fig. 2a). Conversely, accessions with homeostatic or even positive plasticity in rLA in response to drought typify less productive areas (Fig. 2a). Here, we sought to identify the genetic basis of these contrasting ecological strategies.

Our main interest was not to identify natural variation associated with well-watered or drought phenotypes, but rather natural variation associated with the degree of plasticity evoked in response to drought, i.e., natural variation associated with reaction norms, rather than with phenotypes in one environment or the other (well-watered or drought). In exploring the genetic basis of plasticity, we identified 31 genes corresponding to the top 50 candidate SNPs with the strongest association with the leaf area plasticity index (Figure 2b; Table 1; Table G3). To the best of our knowledge, 20 out of these 31 genes have been previously characterized, and many of these are related to auxin regulation and leaf expansion. This enrichment in auxin-related processes was confirmed by GO analysis (Table GO1). Notably, when we compare the candidates resulting from GWA analysis on rLA vs. leaf area plasticity, only eight candidate SNPs overlapped between both traits (green datapoints in Figure S7a), and none of these eight SNPs are in genes related to auxin. In other words, the auxin-related candidate genes are related to *plasticity* in this trait and not to the trait itself.

The variant with the strongest association with the plastic response to drought was the auxin efflux carrier family protein *PIN8* (*AT5G15100*). Among the associated SNPs, we also found two co-varying SNPs in *AUXIN RESPONSE FACTOR 16* (*ARF16*; *AT4G30080*), two co-varying SNPs in *SMALL AUXIN UP RNA 26* (*SAUR26*; *AT3G03850*); two variants in *AGAMOUS-like 15* (*AT5G13790*; *AGL15*), a gene that negatively regulates auxin signaling in Arabidopsis (Zheng *et al.*, 2016); and three variants in *LONESOME HIGHWAY LIKE 1* (*LL2*; *AT2G31280*), an atypical basic helix-loop-helix (bHLH) transcription factor that regulates early xylem development downstream of auxin (Ohashi-Ito *et al.*, 2013).

Among the candidates that are not formally included in the GO category “response to auxin,” we found six co-varying SNPs in the abscisic acid receptors *PYR1-like 11* (*PYL11*; *AT5G45860*) and *PYR1-like 12* (*PYL12*; *AT5G45870*). ABA and auxin pathways interact in the regulation of growth (Emenecker & Strader 2020), and also in the regulation of plasma membrane H⁺ ATPases (of which there is one member in the list, *H⁺ ATPase 2* (*AHA2*; *AT4G30190*)) during cell expansion (Spartz *et al.*, 2014) and stomatal control (Wong *et al.*, 2021). We also found four co-varying SNPs in the *Basic helix-loop-helix (bHLH) DNA-binding family protein MYC3* (*AT5G46760*), a known regulator of glucosinolate biosynthesis (Schweizer *et al.*, 2013). Auxin negatively regulates glucosinolate levels,

which appears to integrate growth and stomatal regulation during drought (Salehin *et al.*, 2019). GO biological process analysis on positively correlated candidates resulting from TWAS analysis on leaf area plasticity index for drought also revealed an enrichment of glucosinolate biosynthesis genes (Table GO4).

We found that among the auxin-related variants associated with leaf area plasticity (Fig. 2b), SNPs in *SAUR26* were of particular interest. The majority of characterized *SAUR* genes are positive regulators of plant growth (Spartz *et al.*, 2014; Li *et al.*, 2015; Stortenbeker and Bemer, 2019). The two associated variants in *SAUR26* were found in the 5' and 3' UTR, respectively (Fig. 2c), regions of the transcript that typically regulate gene expression. Indeed, we found the transcript levels of *SAUR26* to be associated with the genetic variation in *SAUR26* itself (cis-regulation; Fig. 2d), which we confirmed both in the group of accessions included in this study, and importantly, in the larger group of 189 Iberian accessions in the 1001 Genomes Project (Fig. 2f). The minor haplotype is associated with negative plasticity in leaf area in response to drought. Conversely, the major haplotype, associated with higher transcript abundance of *SAUR26*, is associated with leaf area homeostasis and positive plastic responses (Fig. 2e).

Natural variation in transcript abundance of *SAUR26* explained 18% of the observed plastic response in leaf area to drought in our study (Fig. 2g, h). The importance of *SAUR26* is supported by previous work that revealed natural genetic variation in *SAUR26* associated with growth thermo-responsiveness of the rosette compactness index (Wang *et al.*, 2019), and cis-elements in the 5' upstream region or the 3'UTR region of *SAUR26* that were responsible for this response (Wang *et al.* 2021). This conclusion is also supported by our meta-analysis of the larger Iberian population with transcriptome data, which also identified *SAUR26* transcript abundance as positively correlated with higher WUE_i (Fig. 5b, e), along with other related auxin-related transcripts such as *AUXIN RESISTANT 3* (*AXR3*, also known as *IAA17*, *ATIG04250*; Fig. S6a) (Leyser *et al.*, 1996) and *SMALL AUXIN UPREGULATED 15* (*SAUR15*, *AT4G38850*; Fig. S6b) (Oh *et al.*, 2014) (Fig. 5b). As we uncover in this study, both transcript abundance and natural genetic variation in *SAUR26* cis-regulatory elements are associated with the interplay between plasticity and homeostasis in rLA following drought stress (Fig. 2, Tables G3, T1, T2).

Interestingly, the minor haplotype frequency for this *SAUR26* haplotypic variant in our study sample (minor haplotypic frequency, MHF = 38.09%) is similar to the frequencies we find in the 189 accessions within the Iberian population (MHF = 35.98%). However, the minor haplotype in the

Iberian population becomes the haplotype in higher frequency (64.14%) in the global 1001 Genomes collection. It has been shown that Iberian *Arabidopsis* populations locally adapted to drought conditions in this region out-perform accessions from wetter areas of Northern Europe when both are exposed to drought (Exposito-Alonso *et al.*, 2019). Following this, given that the major haplotype in our study is associated with homeostatic responses to drought (Fig. 2e) and that its frequency becomes reduced outside the drought-prone Iberian region, we can speculate that this *SAUR26* haplotypic variant is under selection in the Iberian population, conferring an adaptive advantage to the water-limited conditions of the region.

We did not identify any SNPs associated with rLA under both well-watered and drought conditions. Therefore, the genetic basis of growth homeostasis under drought could not be defined in our study using GWAS (Fig. S5a). On the other hand, the reaction norms based on the strength of association of transcript abundance with rLA, i.e., TWAS, identify four transcripts that are positively correlated with rLA under both well-watered and drought conditions (Fig. 3a-e). This means that accessions with greater transcript abundance for those genes display higher rLA under both well-watered and drought conditions. Conversely, we identified four transcripts negatively correlated with rLA such that accessions with a higher transcript abundance of those genes display lower rLA under both well-watered and drought conditions (Fig. 3f-j). Therefore, these eight genes are strong functional candidates for the homeostatic regulation of rLA.

Of the eight resultant candidate genes, most have not been characterized, and for those that have, their function is not necessarily known to be involved in growth processes regulating leaf area, suggesting that further research is needed regarding the functional roles of these genes and how they contribute to homeostasis.

Trade-off between relative leaf area (rLA), plasticity and WUE_i

Plant species with low relative growth rates exhibit high WUE_i (Angert *et al.*, 2009; Kimball *et al.*, 2012). We can hypothesize that those plants with lower growth rates will have better drought resistance, consequently displaying growth homeostasis. These observations are both consistent with results from classic ecological studies and confirmed in crop cultivars (Kasuga *et al.*, 1999; Ferrero-Serrano and Assmann, 2016; Ferrero-Serrano *et al.*, 2018). In our study, accessions with lower rLA and homeostatic responses to drought display a greater abundance of transcripts encoding functions related to ion transport (yellow datapoints in Fig. S7b; Tables T1, T2, GO5), which could be related

to osmoregulation and stomatal control of transpiration. Consistent with this argument, the homeostatic response in rLA in response to prolonged drought that we observe in our study correlates positively with a high predicted WUE_i recorded by Dittberner *et al.* (2018) for the same accessions (Fig. 4a).

We then studied the genetic basis of this trade-off between rLA, plasticity, and WUE_i . There were no SNPs associated with both the leaf area plasticity index and WUE_i (Fig. 4b). However, when we compare the candidates obtained from TWA analysis, we identify transcripts associated with both low WUE_i and a negative plastic response in leaf area following sustained drought (Fig. 4c). Conversely, we identify a set of transcripts that is significantly associated with both leaf area homeostasis (more positive leaf area plasticity index) and increased WUE_i (Fig. 4c). To exemplify these opposed responses to drought, given the enrichment in RNA modification and ion transport genes that we found in the transcripts regulating the trade-off between relative leaf area and plasticity (Fig S7b; Tables T1, T2), we highlight two of the genes identified by TWAS, with opposite correlations between transcript abundance and relative leaf area plasticity (Figs. 4d, e vs. Figs 4f, g): (*CBF5*; *AT3G57150*) and *CYCLIC NUCLEOTIDE GATED CHANNEL 16* (*CNGC16*; *AT3G48010*).

CBF5 encodes a protein with homology to a category of pseudouridine synthases, and its knockout in Arabidopsis is lethal (Lemontova *et al.*, 2007). Originally only non-coding RNAs, particularly rRNAs, were thought to be pseudouridylated, but it is now recognized that mRNAs are also a target for pseudouridylation. In both yeast and human cells, this post-transcriptional modification is induced by heat shock, with consequent impacts on splicing, mRNA stability, and translation (Karijolic *et al.*, 2015). We observe that accessions with higher transcript abundance of *CBF5* display both strong negative plastic responses in rLA in response to drought (Fig. 4d) and reduced WUE_i (Fig. 4e). Our observations suggest that stress-induced pseudouridylation may also occur in plants, with functional consequences for drought tolerance; other types of mRNA modifications in plants have been correlated with increased mRNA stability and translation under salinity stress (Kramer *et al.*, 2020) and improved heat tolerance (Tang *et al.*, 2020).

Conversely, transcript abundance of *CNGC16* is associated with homeostatic responses in rLA and increased WUE_i . CNGCs are a superfamily of ion channels permeable to divalent and monovalent cations (Köhler *et al.*, 1999; DeFalco *et al.*, 2016). CNGCs like *CNGC5* and *CNGC6* have been reported to be the basis of a cyclic GMP (cGMP)-activated nonselective Ca^{2+} -permeable cation channel activity in the plasma membrane of Arabidopsis guard cells (Wang *et al.*, 2013)

associated with stomatal closure. *CNGC16* is also critical for reproductive success following drought stress (Tunc-Ozdemir *et al.*, 2013). Consistent with these functional roles, in our study, higher transcript abundance of *CNGC16* is found in accessions with positive leaf area plasticity indexes under drought (leaf area homeostasis; Fig. 4f); and with high WUE_i (Fig. 4g).

Given the increased plastic responses in leaf area found in accessions with lower WUE_i, we next conducted a meta-analysis on the larger set of 95 Iberian accessions with $\delta^{13}\text{C}$ information from a previous study focused on the global Arabidopsis population (Dittberner *et al.*, 2018). We identified a distinctive peak consisting of eight co-varying SNPs in a single gene, *ClpX3* (*AT1G33360*; Fig. 5a), an ATP-dependent Clp protease subunit (Nishimura and van Wijk, 2015). Null mutants in a different chloroplast protease subunit, *ClpR*, display a remarkable loss of chlorophyll (95 to 98% reduction) and photosynthetic capacity, turning entirely white when grown under high light (Kim *et al.*, 2009).

TWA analysis of WUE_i determined from $\delta^{13}\text{C}$ values in the 88 Iberian accessions with both $\delta^{13}\text{C}$ information (Dittberner *et al.*, 2018) and published transcript abundance (Kawakatsu *et al.*, 2016) further supported the relationship with photosynthesis. Our TWAS on WUE_i in the larger Iberian population identified 31 out of the 45 candidate genes for WUE_i that we also identified in the subset of 20 Iberian accessions in our experimental study for which published $\delta^{13}\text{C}$ data were available (Table T1 and T2). Gene Ontology (GO) biological process analysis of the resultant 45 candidates positively correlated with $\delta^{13}\text{C}$ uncovered an unequivocal enrichment in photosynthesis-related genes for transcripts that are upregulated in accessions with high WUE_i (less negative $\delta^{13}\text{C}$ values; Figs. 5b, 5c; Table GO3). Among the candidates from our TWA analysis that are positively related to WUE_i was *RuBisCO small subunit 2B* (*AT5G38420*; *RBCS2B*; Fig. 5d). An upregulation in *RuBisCO* may compensate for suboptimal CO₂ concentrations at the sites of carboxylation arising from reduced stomatal apertures in plants from drought-prone environments. We illustrate eight additional genes with transcript abundances that were positively associated with WUE_i in Fig. S6. Among these are *NADH dehydrogenase-like complex M* (*NdhM*, *AT4G37925*; Fig. S6c), required for *NdH* complex assembly (Ifuku *et al.*, 2011), and *High cyclic electron flow 1* (*HCEF1*; *AT3G54050*; Fig. S6d) (Livingston *et al.*, 2010), both regulators of cyclic electron flow around PSI (Yamori *et al.*, 2011), *ATP-Synthase δ -Subunit* (*ATPD*, *AT4G09650*; Fig. S6e) (Maiwald *et al.*, 2003), *Photosystem I reaction center subunit PSI-N* (*PSAN*, *AT5G64040*; Fig. S6f) (Haldrup *et al.*, 1999), and *Photosystem II subunit O-2* (*PSBO2*; *AT3G50820*; Fig. S6g) (Murakami *et al.*, 2005). Also, the candidate with the highest association score is a member of the plasma membrane intrinsic protein

(PIP) subfamily of aquaporins (*PIP1;5*; *AT4G23400*; Fig. S6h). PIPs regulate water flux in guard cells as well as membrane permeability to CO₂ in the mesophyll, with an important effect on mesophyll conductance and CO₂ concentration at the sites of carboxylation (C_c) (Groszmann *et al.*, 2017). Thus, *PIP1;5* is also a candidate regulator of photosynthetic rates. As discussed before, *SAUR26* is also among these candidates (Fig. 5b, e), increasing our confidence in the role of this gene as a regulator of plastic responses to drought (Fig. 2).

Among the candidates that are negatively related to high WUE_i there is an enrichment in carbohydrate transport-related processes (Table GO6). We can speculate that this enrichment reflects the transport of carbohydrates produced during photosynthesis to promote plant growth. This would be concordant with the trade-off between growth and WUE_i that we observe in our study.

Conclusions

The adaptive role of plasticity has been at the center of a long-standing debate. Much work and discussion have focused on the principle that if indeed plasticity is adaptive, it may be under genetic control (Bradshaw, 1965; Via *et al.*, 1995); however, few relevant genes have been identified. Here we propose that not only plasticity but also homeostasis is under genetic control and subject to natural selection. This concept is supported by our dissection of the genetic basis of both plasticity and homeostasis in leaf area following drought, which identifies specific genes implicated in each process.

Knowledge from natural populations of how plants adapt to their environment can be incorporated in breeding and biotechnological approaches to crop improvement. In this study, we observe a trade-off between growth and drought response. A similar trade-off between stress tolerance and productivity threatens food security. It is noteworthy that we also found accessions that did not display this trade-off, e.g., those accessions marked in green in Fig. 1d,e. These accessions may be of particular interest from an agronomic perspective.

The concerted use of TWAS and GWAS increases the power to link genes to phenotypes (Kremling *et al.*, 2019). A similarly integrative approach has identified water use efficiency traits in sorghum (Ferguson *et al.*, 2020). Here, this approach has yielded insights into the genetic basis of response to resource limitation, identifying both candidate “plasticity genes” and candidate “homeostasis genes” for further study. In particular, the identification of potential homeostatic mechanisms has important implications for maintaining crop yield in the face of climate change.

Results arising from diverse genome-wide analyses in both model and crop species (Schindele et al., 2020) can now be used to inform powerful new CRISPR-based approaches for gene editing and modulation of transcript abundance toward crop improvement (Abudayyeh et al., 2017).

Supporting information

The following supplemental materials are available

Figure S1. Rosette leaf area observations at the time of flowering (Table P1).

Figure S2. The use of different GWAS methods improves confidence in candidates for leaf area plasticity and WUE_i.

Figure S3. Histograms of the distribution of r_s and scores obtained from GWA and TWA analyses of the traits discussed in this study illustrate the stringent significance thresholds imposed in this study.

Figure S4. The plasticity index that we calculated is predictive of the fold change in leaf area under drought relative to well-watered conditions.

Figure S5. Genotypic and transcriptomic reaction norms based the strength of association of individual SNPs and transcripts highlight the variation of these associations depending on water availability.

Figure S6. Illustration of transcript variants that regulate intrinsic water use efficiency in Iberian Arabidopsis accessions.

Figure S7. TWAS identifies the genetic basis of the trade-off between relative leaf area (rLA) and its plastic response to drought.

Table P1. Leaf area measurements recorded in this experiment (well-watered & drought). Calculated relative leaf area (rLA) and plasticity, water use efficiency (WUE_i, $\delta^{13}\text{C}$) values recorded by Dittberner *et al.* (2018) and MOD17A2 net primary productivity) information obtained from AraCLIM (Ferrero-Serrano & Assmann, 2019) for the accessions included in our study.

Table P2. Water use efficiency (WUE_i, $\delta^{13}\text{C}$) values recorded by Dittberner *et al.* (2018) for 95 Iberian Arabidopsis accessions.

Table G1. Candidates obtained from GWAS on relative leaf area (rLA) under well-watered conditions. Only associations with a score ≥ 4 are included here. For a raw, unfiltered, as well as for a fully filtered and annotated (see Materials & Methods) version of this GWAS output, please visit the data repository.

574 **Table G2.** Candidates obtained from GWAS on relative leaf area (rLA) under drought. Only
575 associations with a score ≥ 4 are included here. For a raw, unfiltered, as well as for a fully filtered
576 and annotated (see Materials & Methods) version of this GWAS output, please visit the data
577 repository.

578 **Table G3.** Candidates obtained from GWAS on leaf area plasticity. Only associations with a score $\geq$
579 4 are included here. For a raw, unfiltered, as well as for a fully filtered and annotated (see Materials
580 & Methods) version of this GWAS output, please visit the data repository.

581 **Table G4.** Candidates obtained from GWAS on water use efficiency (WUEi, $\delta^{13}\text{C}$) values recorded
582 by Dittberner *et al.* (2018) for the accessions included in our study. Only associations with a score $\geq$
583 4 are included here. For a raw, unfiltered, as well as for a fully filtered and annotated (see Materials
584 & Methods) version of this GWAS output, please visit the data repository.

585 **Table G5.** Candidates obtained from GWAS on water use efficiency (WUEi, $\delta^{13}\text{C}$) values recorded
586 by Dittberner *et al.* (2018) for 95 Iberian Arabidopsis accessions. For a raw, unfiltered, as well as for
587 a fully filtered and annotated (see Materials & Methods) version of this GWAS output, please visit
588 the data repository.

589 **Table G6.** Candidates obtained from eGWAS on the natural variation in transcript abundance of
590 SAUR26 extracted from Kawakatsu *et al.* (2016). For a raw, unfiltered, as well as for a fully filtered
591 and annotated (see Materials & Methods) version of this GWAS output, please visit the data
592 repository.

593 **Table T1.** Results from TWAS (r_s).

594 **Table T2.** Results from TWAS (P -value).

595 **Table GO1.** GO analysis on the 31 genes corresponding to the top 50 candidate SNPs resulting from
596 GWAS analysis on leaf area plasticity index for drought.

597 **Table GO2.** GO analysis on the 58 genes identified from TWAS to exhibit transcript abundances
598 that are negatively correlated with leaf area plasticity index for drought, and negatively correlated
599 with water use efficiency determined from ($\delta^{13}\text{C}$) values from accessions overlapping with our sample
600 (red datapoints in 4C).

601 **Table GO3.** GO analysis on the 45 genes identified from TWAS to exhibit transcript abundances
602 that are positively correlated with water use efficiency determined from ($\delta^{13}\text{C}$) values in 88 Iberian
603 Arabidopsis accessions (datapoints with $r_s \geq 0.4$ in Figure 5b).

604 **Table GO4.** GO analysis on the 494 genes identified from TWAS to exhibit transcript abundances
 605 that are positively correlated with leaf area plasticity index for drought (green and grey datapoints
 606 with $r_s \geq 0.4$ in the y-axis representing leaf area plasticity index in Figure 4c).

607 **Table GO5.** GO analysis on the 28 genes identified from TWAS to exhibit transcript abundances
 608 that are negatively correlated with relative leaf area (rLA) under well-watered conditions, and
 609 positively correlated with leaf area plasticity index for drought (blue datapoints in S7b).

610 **Table GO6.** GO analysis on the 11 genes identified from TWAS to exhibit transcript abundances
 611 that are negatively correlated with water use efficiency determined from ($\delta^{13}\text{C}$) values in 88 Iberian
 612 Arabidopsis accessions (datapoints with $r_s \leq -0.4$ in Figure 5b).

613 **Table GO7.** GO analysis on the 302 genes identified from TWAS to exhibit transcript abundances
 614 that are positively correlated with relative leaf area (rLA) under drought.

615 **Table GO8.** GO analysis on the 134 genes identified from TWAS to exhibit transcript abundances
 616 that are negatively correlated with relative leaf area (rLA) under well-watered conditions (blue and
 617 grey datapoints with $r_s \leq 0.4$ in the x-axis of Figure S7b).

618 **Table GO9.** GO analysis on the 177 genes identified from TWAS to exhibit transcript abundances
 619 that are positively correlated with relative leaf area (rLA) under well-watered conditions (yellow and
 620 grey datapoints with $r_s \geq 0.4$ in the x-axis of Figures S7b representing).

621 **Table GO10.** GO analysis on the 233 genes identified from TWAS to exhibit transcript abundances
 622 that are negatively correlated with relative leaf area (rLA) under drought.

623

624 Tables

625 **Table 1.** List of 31 genes corresponding to the top 50 candidate SNPs with the strongest association
626 with the leaf area plasticity index. Genes encoding proteins with auxin-related functions from GO
627 analysis are bolded; additional genes related to auxin based on literature analysis are underlined.

Chr	Position	MAF (%)	Gene ID	Gene description	Label
5	4892996	38.24	AT5G15100	Auxin efflux carrier family protein	PIN8
3	1787622	26.47	AT3G05970	long-chain acyl-CoA synthetase 6	LACS6
4	14703801	26.47	AT4G30080	auxin response factor 16	ARF16
4	14705049	26.47	AT4G30080	auxin response factor 16	ARF16
5	5238732	23.53	AT5G16030	mental retardation GTPase activating protein	AT5G16030
3	975320	32.35	AT3G03810	O-fucosyltransferase family protein	EDA30
3	970856	20.59	AT3G03800	syntaxin of plants 131	SYPI31
5	15293597	26.47	AT5G38280	PR5-like receptor kinase	PR5K
5	15293610	26.47	AT5G38280	PR5-like receptor kinase	PR5K
5	15293481	41.18	AT5G38280	PR5-like receptor kinase	PR5K
4	14691629	35.29	AT4G30060	Core-2/1-branching beta-1,6-N-acetylglucosaminyltransferase family protein	AT4G30060
5	4659907	35.29	AT5G14450	GDSL-like Lipase/Acylhydrolase superfamily protein	AT5G14450
5	<u>18974632</u>	<u>20.59</u>	<u>AT5G46760</u>	<u>Basic helix-loop-helix (bHLH) DNA-binding family protein</u>	<u>MYC3</u>
5	<u>18974915</u>	<u>20.59</u>	<u>AT5G46760</u>	<u>Basic helix-loop-helix (bHLH) DNA-binding family protein</u>	<u>MYC3</u>
5	<u>18975625</u>	<u>20.59</u>	<u>AT5G46760</u>	<u>Basic helix-loop-helix (bHLH) DNA-binding family protein</u>	<u>MYC3</u>
5	<u>18975826</u>	<u>20.59</u>	<u>AT5G46760</u>	<u>Basic helix-loop-helix (bHLH) DNA-binding family protein</u>	<u>MYC3</u>
3	983189	38.24	AT3G03850	SAUR-like auxin-responsive protein family	SAUR26
3	983517	38.24	AT3G03850	SAUR-like auxin-responsive protein family	SAUR26
3	1798927	29.41	AT3G05990	Leucine-rich repeat (LRR) family protein	AT3G05990
5	4433730	29.41	AT5G13740	zinc induced facilitator 1	ZIF1
5	4433740	29.41	AT5G13740	zinc induced facilitator 1	ZIF1
5	4436472	29.41	AT5G13740	zinc induced facilitator 1	ZIF1
5	4436745	29.41	AT5G13740	zinc induced facilitator 1	ZIF1
5	4442005	29.41	AT5G13760	Plasma-membrane choline transporter family protein	AT5G13760
5	4450177	29.41	AT5G13790	AGAMOUS-like 15	AGL15
5	4450180	29.41	AT5G13790	AGAMOUS-like 15	AGL15
2	13323264	23.53	AT2G31260	autophagy 9	APG9
2	13330862	23.53	AT2G31270	CDT1-like protein A	CDT1A
2	<u>13341047</u>	<u>23.53</u>	<u>AT2G31280</u>	<u>transcription factor bHLH155-like protein</u>	<u>LL2</u>
2	<u>13341186</u>	<u>23.53</u>	<u>AT2G31280</u>	<u>transcription factor bHLH155-like protein</u>	<u>LL2</u>
2	<u>13342866</u>	<u>23.53</u>	<u>AT2G31280</u>	<u>transcription factor bHLH155-like protein</u>	<u>LL2</u>
2	13343940	23.53	AT2G31290	Ubiquitin carboxyl-terminal hydrolase family protein	AT2G31290
2	13345229	23.53	AT2G31290	Ubiquitin carboxyl-terminal hydrolase family protein	AT2G31290
2	13345701	23.53	AT2G31290	Ubiquitin carboxyl-terminal hydrolase family protein	AT2G31290
2	13346191	23.53	AT2G31300	actin-related protein C1B	ARPC1B
2	13358923	23.53	AT2G31320	poly(ADP-ribose) polymerase 2	PARP1
3	22329843	23.53	AT3G60400	Mitochondrial transcription termination factor family protein	SHOT1
4	<u>14775183</u>	<u>32.35</u>	<u>AT4G30190</u>	<u>H⁺-ATPase 2</u>	<u>AHA2</u>
4	6154596	32.35	AT4G09770	TRAF-like family protein	AT4G09770
1	29310771	20.59	AT1G77960	repressor ROX1-like protein	AT1G77960
5	18596240	20.59	AT5G45840	Leucine-rich repeat protein kinase family protein	AT5G45840
5	<u>18604234</u>	<u>20.59</u>	<u>AT5G45860</u>	<u>PYR1-like 11</u>	<u>PYL11</u>
5	<u>18606400</u>	<u>20.59</u>	<u>AT5G45870</u>	<u>PYR1-like 12</u>	<u>PYL12</u>
5	<u>18606524</u>	<u>20.59</u>	<u>AT5G45870</u>	<u>PYR1-like 12</u>	<u>PYL12</u>
5	<u>18606721</u>	<u>20.59</u>	<u>AT5G45870</u>	<u>PYR1-like 12</u>	<u>PYL12</u>
5	<u>18606940</u>	<u>20.59</u>	<u>AT5G45870</u>	<u>PYR1-like 12</u>	<u>PYL12</u>
5	<u>18606953</u>	<u>20.59</u>	<u>AT5G45870</u>	<u>PYR1-like 12</u>	<u>PYL12</u>
5	18613579	20.59	AT5G45890	senescence-associated gene 12	SAG12
5	3708088	20.59	AT5G11550	ARM repeat superfamily protein	AT5G11550
5	4655788	20.59	AT5G14430	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein	AT5G14430

629 Figures

630 **Figure 1. Arabidopsis rosette leaf area observations.** (a) Detail of the common garden experiment,
 631 illustrating the randomization of well-watered and drought treatments, as reflected in soil coloration.
 632 (b) Phenotypic reaction norms illustrating a reduction of leaf area in response to drought in most but
 633 not all accessions. Violin distributions represent probability densities of leaf area (cm²) among the
 634 accessions included in this study. Datapoints depict the mean phenotypic values of individual
 635 accessions, and the reaction norms represent the plastic response in the leaf area of each accession in
 636 response to drought (t-test; P -value < 0.001). (c) Phenotypic reaction norms illustrating plastic and
 637 homeostatic responses in relative leaf area (rLA). Violin distributions represent probability densities
 638 of rLA among the accessions included in this study. Datapoints depict the rLA of individual
 639 accessions, and the reaction norms represent the plastic response in rLA of each accession in response
 640 to drought (t-test; P -value > 0.05). (d) Negative relationship (P -value < 0.05) between leaf area
 641 plasticity index (slope of the reaction norms in 1c) in response to drought (y-axis) and rLA under
 642 well-watered conditions (x-axis). Data were fitted using a linear regression model. Grey shadow
 643 represents the 95% CI of the fit regression line. Four different quadrants are defined by two dashed
 644 lines: the average rLA (x-axis), and $y = 0$, which is the homeostatic point at which a change in water
 645 availability does not affect rLA (i.e. a plasticity index of 0). Data points depict individual accessions
 646 with their respective label. Data points are colored based on in which quadrant they fall (see text).
 647 (e) Images illustrating the plastic and homeostatic responses to drought found among the accessions
 648 included in this study.

649 **Figure 2. *SMALL AUXIN UP RNA 26 (SAUR26, AT3G03850)* is a candidate regulator of**
 650 **Arabidopsis leaf plasticity in response to drought.** (a) Accessions from more productive areas
 651 display stronger negative reductions in leaf area plasticity relative to accessions from less productive
 652 environments. Scatter plot depicting the negative relationship (P -value < 0.05) between leaf area
 653 plasticity index (y-axis) and Net Primary Productivity during the spring season (x-axis). (b)
 654 Manhattan plot representing the strength of association between genetic variants and leaf area
 655 plasticity in response to drought. C. *SAUR26* gene model, including the two co-varying SNPs
 656 highlighted in 2b. Orange block depicts the sole exon, green block the 5' UTR, dark green block the
 657 3' UTR. Labels above indicate the position of the two SNPs that co-vary, forming a haplotype in our
 658 sample. Major/minor allele frequency in our sample, in the 189 accessions from the Iberian Peninsula

659 included in the 1001 Genome Project, and in the whole 1001 Genomes Project sample dataset is
 660 provided. **(d)** Manhattan plot representing the genome-wide strength of association between the
 661 genetic variation present within the 665 accessions from the transcript abundance reference panel
 662 (Kawakatsu *et al.*, 2016) that overlap with the collection of accessions included in the 1001 Genomes
 663 Project, and the variation in the transcript abundance of *SAUR26* (Kawakatsu *et al.*, 2016). The peak
 664 in chromosome 3 represents the cis-genetic variants in *SAUR26* affecting its transcript abundance.
 665 For the Manhattan plots in **2b** and **e**, the score (y-axis) consists of the negative logarithm of the *P*-
 666 value and provides association strength. Each data point represents a SNP with a color corresponding
 667 to the chromosome where the genetic variants are located. The x-axis and the different colors shown
 668 in the SNPs depicted in the Manhattan plots represent the different chromosomes. The higher the
 669 score, the lower the *P*-value, and the stronger the association between genetic variation and leaf area
 670 plasticity or *SAUR26* transcript abundance. **(e)** The major haplotype of *SAUR26* is associated with
 671 homeostatic or positive responses of relative leaf area (rLA) in response to drought. Conversely, the
 672 minor haplotype is associated with plastic responses in rLA in response to drought. Violin plots show
 673 significantly different probability densities of leaf area plasticity in response to drought for the major
 674 and minor allele haplotypes in *SAUR26*. **(f)** The haplotypes in *SAUR26* associated with leaf area
 675 plasticity/homeostasis in response to drought are associated with difference in *SAUR26* gene
 676 expression. Violin plots show significantly different probability densities of *SAUR26* transcript
 677 abundance for the major and minor allele haplotypes in *SAUR26* for both our sample and the entire
 678 Iberian population. **(g)** Scatter plot illustrating the positive relationship between leaf area plasticity
 679 index (y-axis) and *SAUR26* transcript abundance (x-axis). For **2a** and **2g**, the grey shadow represents
 680 the 95% CI of the fit regression line (dark grey). **(h)** Cartoon illustrating the regulatory role of genetic
 681 and transcript variation in *SAUR26* and the response of leaf area to drought.

682 **Figure 3. Transcriptomic reaction norms uncover genetic correlates of Arabidopsis leaf area**
 683 **homeostasis in response to drought.** **(a)** homeostatic reaction norms for transcripts significantly and
 684 positively correlated with relative leaf area (rLA) under both watering regimes. **(b)** to **(e)**. Scatter
 685 plots illustrating positive and homeostatic relationships (*P*-value < 0.05) between rLA under both
 686 well-watered (closed circles) and drought conditions (open circles) conditions (y-axis), and transcript
 687 abundance (FPKM; x-axis) of **(b)** AT5G39471, **(c)** *SOB FIVE-LIKE 2 (SOFL2; AT1G68870)*, **(d)**
 688 *CHAPERONIN 60 BETA (CPN60B; AT1G55490)*, and **(e)** *CASEIN KINASE I-LIKE 8 (CKL8;*
 689 *AT5G43320)*. **(f)** homeostatic reaction norms for transcripts significantly and negatively correlated

with rLA under both watering regimes. (g) to (j) Scatter plots illustrating negative and homeostatic relationships (P -value < 0.05) between rLA under both well-watered (closed circles) and drought conditions (open circles) (y-axis), and transcript abundance (FPKM; x-axis) of (g) AT5G60160, (h) AT4G15260, (i) AT5G16800, and (j) RING-H2 GROUP F1A (RHF1A; AT4G14220). For 3a and 3f, Spearman's rank correlation coefficient (r_s ; y-axis) indicates the correlation between the rLA and the natural variation in transcript abundance of each gene. In figures b to e and g-j, data were fitted using a linear regression model. Also, in panels b-e and g-j, filled circles depict the rLA of accessions grown under well-watered conditions, while empty circles squares depict the rLA of accessions grown under drought.

Figure 4. Arabidopsis accessions that display leaf area homeostasis in response to drought, display higher water use efficiency as defined by $\delta^{13}C$. (a) Scatter plot illustrating the positive relationship (P -value < 0.05) between leaf area homeostasis in response to drought (leaf area plasticity index, y-axis) and water use efficiency (x-axis) among Iberian Arabidopsis accessions used in this study. Data were fitted to a linear regression model. Grey shadow represents the 95% CI of the fit regression line (black). $\delta^{13}C$ data for the accessions were retrieved from Dittberner *et al.* (2018). Four different quadrants are defined by two dashed lines: $y = 0$, which is the homeostatic point at which a change in water availability does not affect relative leaf area (i.e., a plasticity index of 0), and the average intrinsic water use efficiency (WUE_i , $\delta^{13}C$; x-axis). Data points depict individual accessions with their respective labels. (b) The genetic basis of leaf area plasticity in response to drought and water use efficiency. This plot depicts the respective relationship between the GWAS associations found for the leaf area plasticity index in response to drought (y-axis) and those for WUE_i (Dittberner *et al.*, 2018) (x-axis). Data points represent individual SNPs. The y-axis and x-axis depict the extent of the association with, respectively, leaf area plasticity, and WUE_i , as the negative logarithm of the p-value. Dashed lines depict an arbitrary threshold for significance that is consistent across comparisons. (c) Transcriptomic association with plastic leaf area responses to drought and WUE_i . Spearman's rank correlation depicts the relationship between the association of the transcript abundance of each gene in the Arabidopsis genome with leaf area plasticity in response to drought (r_s) on the y-axis and with WUE_i on the x-axis. Dashed lines define an arbitrary threshold to consider transcripts correlated positively ($r_s \geq 0.4$) or negatively ($r_s \leq -0.4$) associated with leaf area plasticity (y-axis) or WUE_i (x-axis). *CBF5* and *CNGC16* exemplify transcripts with opposing relationships to

leaf area plasticity and water use efficiency as shown in 4c. **d** and **e**. Scatter plots depicting the relationship (P -value < 0.05) between leaf area plasticity index in response to drought (**4d**), or water use efficiency (**4e**) on the y-axis, and transcript abundance (FPKM) of *CBF5* on the x-axis. **f** and **g**. Scatter plots depicting the relationship (P -value < 0.05) between (**4f**) leaf area plasticity index in response to drought or (**4g**) water use efficiency (Dittberner *et al.*, 2018) on the y-axis, and transcript abundance (FPKM) of *CYCLIC NUCLEOTIDE-GATED CHANNEL 16 (CNGC16)* on the x-axis. In all panels, data were fitted using a linear regression model. Grey shadow represents the 95% CI of the fit regression line.

Figure 5. Photosynthesis-related transcript variants regulate water use efficiency in Iberian Arabidopsis accessions. **(a)** Manhattan plot representing the genome-wide strength of association between genetic variation and variation in $\delta^{13}\text{C}$ present within 95 Iberian accessions with $\delta^{13}\text{C}$ values available from (Dittberner *et al.*, 2018). The score (y-axis) consists of the negative logarithm of the P -value and provides association strength. **Inset:** Violin plots representing the probability densities of WUE_i for the major and minor allele haplotypes in *ClpX* for the accessions included in 5a. **(b)** T-Manhattan plot representing transcriptome-wide strength of association between variation in transcript abundance (Kawakatsu *et al.*, 2016) and natural variation in $\delta^{13}\text{C}$. This analysis was performed on all Iberian accessions with both transcript abundance data (Kawakatsu *et al.*, 2016) and $\delta^{13}\text{C}$ information (Dittberner *et al.*, 2018) ($n = 88$ accessions). The relationship is defined by the Spearman's rank correlation coefficient (r_s). Dashed lines define an arbitrary threshold for positive ($r_s \geq 0.4$) or negative ($r_s \leq -0.4$) correlation with water-use efficiency (y-axis). Blue labels highlight the transcripts positively associated with higher WUE_i , while red labels highlight those negatively associated with higher WUE_i . **(c)** Hierarchical clustering tree summarizing the correlation among significant GO Biological Process categories resulting from analysis of the positively correlated candidates resulting from TWA analysis of water use efficiency determined from $\delta^{13}\text{C}$ values in 88 Iberian Arabidopsis accessions. Ratios provide the number of genes positively correlated with WUE_i within each GO category relative to the total number of genes within that category. The size of the blue dots increases proportionally with P -value significance. **(d)** Scatter plot depicting the positive relationship (P -value < 0.05) between WUE_i (y-axis) and *RuBisCO SMALL SUBUNIT 2B (AT5G38420; RBCS2B)* transcript abundance (x-axis) for the Iberian accessions included in 6b. **(e)** Scatter plot depicting the positive relationship (P -value < 0.05) between intrinsic water-use efficiency

(WUE_i; y-axis) and *SMALL AUXIN UP RNA 26* (*SAUR26*, AT3G03850) transcript abundance (x-axis) for the Iberian accessions included in 6b. **Inset:** Scatter plot depicting the positive relationship (P -value < 0.05) between intrinsic water-use efficiency (WUE_i; y-axis) and *SMALL AUXIN UP RNA 26* (*SAUR26*, AT3G03850) transcript abundance (x-axis) for the 43 accessions included in our study. For **d**), and **e**), gray shadow represents the 95% CI of the fit regression line (black).

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Author contributions

S.M.A & A.F-S. conceived the project, research plans and experimental design. A.F-S. performed the experiments while S.M.A provided guidance throughout the experimental work. A.F-S. analyzed the data and prepared the figures; S.M.A provided supervision with data analysis and figure preparation. S.M.A & A.F-S wrote the manuscript. A.F-S agrees to serve as the author responsible for contact and ensuring communication and assistance with the reproducibility of the results of this study.

Data Availability

The data supporting the results are available as Supporting Information files. The code and data to easily reproduce the analysis, figures, and tables in this manuscript are available at <https://github.com/AssmannLab> and will be uploaded to Zenodo upon acceptance.

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Figure 1. Arabidopsis rosette leaf area observations. (a) Detail of the common garden experiment, illustrating the randomization of well-watered and drought treatments, as reflected in soil coloration. (b) Phenotypic reaction norms illustrating a reduction of leaf area in response to drought in most but not all accessions. Violin distributions represent probability densities of leaf area (cm²) among the accessions included in this study. Datapoints depict the mean phenotypic values of individual accessions, and the reaction norms represent the plastic response in the leaf area of each accession in response to drought (t-test; P -value < 0.001). (c) Phenotypic reaction norms illustrating plastic and homeostatic responses in relative leaf area (rLA). Violin distributions represent probability densities of rLA among the accessions included in this study. Datapoints depict the mean phenotypic values of individual accessions, and the reaction norms represent the plastic response in rLA of each accession in response to drought (t-test; P -value > 0.05). (d) Negative relationship (P -value < 0.05) between leaf area plasticity index (slope of the reaction norms in 1c) in response to drought (y-axis) and rLA under well-watered conditions (x-axis). Data were fitted using a linear regression model. Grey shadow represents the 95% CI of the fit regression line. Four different quadrants are defined by two dashed lines: the average rLA (x-axis), and $y = 0$, which is the homeostatic point at which a change in water availability does not affect rLA (i.e. a plasticity index of 0). Data points depict individual accessions with their respective label. Data points are colored based on in which quadrant they fall (see text). (e) Images illustrating the plastic and homeostatic responses to drought found among the accessions included in this study.

Figure 2. *SMALL AUXIN UP RNA 26 (SAUR26, AT3G03850)* is a candidate regulator of *Arabidopsis* leaf plasticity in response to drought. (a) Accessions from more productive areas display stronger negative reductions in leaf area plasticity relative to accessions from less productive environments. Scatter plot depicting the negative relationship (P -value < 0.05) between leaf area plasticity index (y-axis) and Net Primary Productivity during the spring season (x-axis). (b) Manhattan plot representing the strength of association between genetic variants and leaf area plasticity in response to drought. (c) *SAUR26* gene model, including the two co-varying SNPs highlighted in 2b. Orange block depicts the sole exon, green block the 5' UTR, dark green block the 3' UTR. Labels above indicate the position of the two SNPs that co-vary, forming a haplotype in our sample. Major/minor allele frequency in our sample, in the 189 accessions from the Iberian Peninsula included in the 1001 Genomes Project, and in the whole 1001 Genomes Project sample dataset is provided. (d) Manhattan plot representing the genome-wide strength of association between the genetic variation present within the 665 accessions from the transcript abundance reference panel (Kawakatsu *et al.*, 2016) that overlap with the collection of accessions included in the 1001 Genomes Project, and the variation in the transcript abundance of *SAUR26* (Kawakatsu *et al.*, 2016). The peak in chromosome 3 represents the cis-genetic variants in *SAUR26* affecting its transcript abundance. For the Manhattan plots in 2b and e, the score (y-axis) consists of the negative logarithm of the P -value and provides association strength. Each data point represents a SNP with a color corresponding to the chromosome where the genetic variants are located. The x-axis and the different colors shown in the SNPs depicted in the Manhattan plots represent the different chromosomes. The higher the score, the lower the P -value, and the stronger the association between genetic variation and leaf area plasticity or *SAUR26* transcript abundance. (e) The major haplotype of *SAUR26* is associated with homeostatic or positive responses of relative leaf area (rLA) in response to drought. Violin plots show significantly different probability densities of leaf area plasticity in response to drought for the major and minor allele haplotypes in *SAUR26*. (f) The haplotypes in *SAUR26* associated with leaf area plasticity/homeostasis in response to drought are associated with difference in *SAUR26* gene expression. Violin plots show significantly different probability densities of *SAUR26* transcript abundance for the major and minor allele haplotypes in *SAUR26* for both our sample and the entire Iberian population. (g) Scatter plot illustrating the positive relationship between leaf area plasticity index (y-axis) and *SAUR26* transcript abundance (x-axis). For 2a and 2g, the grey shadow represents the 95% CI of the fit regression line (dark grey). (h) Cartoon illustrating the regulatory role of genetic and transcript variation in *SAUR26* and the response of leaf area to drought.

Figure 3. Transcriptomic reaction norms uncover genetic correlates of Arabidopsis leaf area homeostasis in response to drought. (a) homeostatic reaction norms for transcripts significantly and positively correlated with relative leaf area (rLA) under both watering regimes. (b) to (e). Scatter plots illustrating positive and homeostatic relationships (P -value < 0.05) between rLA under both well-watered (closed circles) and drought conditions (open circles) conditions (y-axis), and transcript abundance (FPKM; x-axis) of (b) *AT5G39471*, (c) *SOB FIVE-LIKE 2* (*SOFL2*; *AT1G68870*), (d) *CHAPERONIN 60 BETA* (*CPN60B*; *AT1G55490*), and (e) *CASEIN KINASE I-LIKE 8* (*CKL8*; *AT5G43320*). (f) homeostatic reaction norms for transcripts significantly and negatively correlated with rLA under both watering regimes. (g) to (j) Scatter plots illustrating negative and homeostatic relationships (P -value < 0.05) between rLA under both well-watered (closed circles) and drought conditions (open circles) (y-axis), and transcript abundance (FPKM; x-axis) of (g) *AT5G60160*, (h) *AT4G15260*, (i) *AT5G16800*, and (j) *RING-H2 GROUP F1A* (*RHF1A*; *AT4G14220*). For 3a and 3f, Spearman's rank correlation coefficient (r_s ; y-axis) indicates the correlation between the rLA and the natural variation in transcript abundance of each gene. In figures b to e and g-j, data were fitted using a linear regression model. Also, in panels b-e and g-j, filled circles depict the rLA of accessions grown under well-watered conditions, while empty circles squares depict the rLA of accessions grown under drought.

Figure 5. Photosynthesis-related transcript variants regulate water use efficiency in Iberian Arabidopsis accessions. (a) Manhattan plot representing the genome-wide strength of association between genetic variation and variation in $\delta^{13}\text{C}$ present within 95 Iberian accessions with $\delta^{13}\text{C}$ values available from (Dittberner *et al.*, 2018). The score (y-axis) consists of the negative logarithm of the P -value and provides association strength. **Inset:** Violin plots representing the probability densities of WUE_i for the major and minor allele haplotypes in *ClpX3* for the accessions included in 5a. (b) T-Manhattan plot representing transcriptome-wide strength of association between variation in transcript abundance (Kawakatsu *et al.*, 2016) and natural variation in $\delta^{13}\text{C}$. This analysis was performed on all Iberian accessions with both transcript abundance data (Kawakatsu *et al.*, 2016) and $\delta^{13}\text{C}$ information (Dittberner *et al.*, 2018) ($n = 88$ accessions). The relationship is defined by the Spearman's rank correlation coefficient (r_s). Dashed lines define an arbitrary threshold for positive ($r_s \geq 0.4$) or negative ($r_s \leq -0.4$) correlation with water-use efficiency (y-axis). Blue labels highlight the transcripts positively associated with higher WUE_i, while red labels highlight those negatively associated with higher WUE_i. (c) Hierarchical clustering tree summarizing the correlation among significant GO Biological Process categories resulting from analysis of the positively correlated candidates resulting from TWA analysis of water use efficiency determined from $\delta^{13}\text{C}$ values in 88 Iberian Arabidopsis accessions. Ratios provide the number of genes positively correlated with WUE_i within each GO category relative to the total number of genes within that category. The size of the blue dots increases proportionally with P -value significance. (d) Scatter plot depicting the positive relationship (P -value < 0.05) between WUE_i (y-axis) and *RuBisCO SMALL SUBUNIT 2B* (*AT5G38420*; *RBCS2B*) transcript abundance (x-axis) for the Iberian accessions included in 6b. (e) Scatter plot depicting the positive relationship (P -value < 0.05) between intrinsic water-use efficiency (WUE_i; y-axis) and *SMALL AUXIN UP RNA 26* (*SAUR26*, *AT3G03850*) transcript abundance (x-axis) for the Iberian accessions included in 6b. **Inset:** Scatter plot depicting the positive relationship (P -value < 0.05) between intrinsic water-use efficiency (WUE_i; y-axis) and *SMALL AUXIN UP RNA 26* (*SAUR26*, *AT3G03850*) transcript abundance (x-axis) for the 43 accessions included in our study. For d), and e), gray shadow represents the 95% CI of the fit regression line (black).