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Genetic variability and plasticity of plant allometry

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Abstract
1. The metabolic scaling theory (MST) predicts quasi-universal trait–size relationships in plants, characterised by a unique allometric exponent within and across large taxonomic scales. However, recent studies have identified variability in allometric relationships, without a clear understanding of the modulating role played by genetic variation and environment.
2. Here, we investigated (1) the allometric relationships for two central traits of MST, namely total leaf area and plant growth rate, in the model species *Arabidopsis thaliana*, (2) the variability of plant allometries between genotypes and (3) the plastic responses of plant allometries under water deficit, high temperature and their interaction.
3. Using a population of 120 genotypes, we found that intraspecific allometries adhered on average with MST predictions. However, a broad variability but a moderate plasticity in the allometric exponents was observed across genotypes and environments. Allometric exponents were impacted significantly, yet weakly, by water deficit, but not by high temperature. Moreover, genotypes that deviated from MST predictions exhibited more plasticity in trait–size relationships than genotypes that followed MST predictions.
4. Our study suggests that plant allometry is genetically variable and might be related to different adaptive strategies to cope with stressing conditions. Thus, our results highlights the need of assessing trait–size relationships within species to understand the mechanisms of plant adaptation to contrasted environments.

KEYWORDS
Arabidopsis thaliana, growth rate, intraspecific trait variability, high temperature, plasticity, metabolic scaling theory, water stress

1 | INTRODUCTION

One of the main consequences of a change in organism size is the modification of the proportional ratio between surface and volume. Indeed, the surface-to-volume ratio decreases with body size, which has profound implications for organismal resource use, growth, development, reproduction and survival (Lindmark et al., 2019;

Malerba et al., 2017; Malerba & Marshall, 2019; Ohlberger, 2013). In plants, individual size varies tremendously over the course of ontogeny, between genotypes and species, as well as in response to environmental variation. However, understanding the adaptive significance of plant size variation continues to be puzzling because it remains difficult to tease apart the different sources of phenotypic variance (ontogenic, genetic and environmental) in natural settings,

and because the links between plant size and the environment remain unclear. For instance, large individuals are supposed to lose more water by transpiration, which might be beneficial for thermoregulation under high temperature, but at the same time detrimental under water deficit (Chaves et al., 2003; Vile et al., 2012). Yet, both stresses often co-occur along geographic gradients. Taking advantage of intraspecific trait variability to determine how trait–size relationships vary depending on genotypes and environments is critical to assess the adaptive value of size variation. Accordingly, we tested here the variability of trait–size relationships in 120 plant genotypes grown under highly controlled conditions of water deficit and high temperature.

Allometric relationships are defined, in a broad sense, as the covariation between the biomass of an organism and its morpho-physiological properties (Niklas, 1994). For both plants and animals, allometric models take the form of a power law function $Y = \alpha M^{\beta}$, where Y is a morphological or physiological trait that can be predicted by the individual mass M, a constant α and the allometric exponent β (Huxley, 1924; Niklas, 1994). Since the integration of phenotypic traits with biomass is intrinsically multiplicative (Kerkhoff & Enquist, 2009), allometric relations are generally studied on logarithmic scales where the exponent becomes the slope of the function $\log_{10}(Y) = \log_{10}(\alpha) + \beta \times \log_{10}(M)$. The slope of trait–size relationships, β (called allometric exponent), estimates the relative trait value when scaled to individual size, such as relative growth rate and relative surface-to-biomass ratio (Huxley & Teissier, 1936). In 1932, Kleiber first elaborated that energy consumption, or metabolic rate, varies with body mass with an allometric exponent β of ¾ in all animal species (Kleiber, 1932). In plants, metabolic scaling relationships are also characterised by power laws where the metabolic rate is expected to scale isometrically (i.e. with exponent $\beta = 1$) with total leaf area and total leaf biomass, and allometrically (i.e. with exponent $\beta < 1$) with the biomass of the plant.

Scaling equations form the mathematical foundation of the metabolic scaling theory (MST), which invokes the geometry of branching vascular networks as the main determinant of a series of allometric relationships between organismal morphology, physiology and biomass (Enquist, Kerkhoff, et al., 2007; Price et al., 2010; Price & Enquist, 2006, 2007; West et al., 1997). Consistent with Kleiber's principle, MST predicts that the allometric exponent β is ¾ for many plant and animal traits such as metabolic rate, growth rate and total leaf area (Brown et al., 2004; Enquist, Allen, et al., 2007; Savage et al., 2004). The theoretical expectation of MST assumes that natural selection has universally tended to optimise vascular networks to maximally supply resources to the body but yet, at the same time, minimise the energy required for resource distribution (West et al., 1997, 1999b). However, tests of MST focused primarily on the interspecific scale, comparing a wide range of highly contrasting species sizes. While this body of work has revealed the global patterns of covariation that generally confirm the predictions of MST (Enquist, Allen, et al., 2007; Enquist, Kerkhoff, et al., 2007; Niklas & Enquist, 2001), large interspecific datasets are often limited to examine subtle variation in allometric relationships

(Coomes, 2006; Enquist & Bentley, 2012; Killen et al., 2010; Price et al., 2010; Russo et al., 2007). Moreover, there could be slope differences and nonlinearity within species that cannot be detectable across species.

An example of the limitations of interspecific patterns is that they remain unconnected from the primary assumption that there is stabilising selection for a constant allometric exponent of ¾ (West et al., 1997, 1999b), and selection acts within populations, not between species. Allometric studies traditionally use a trait average per species, despite natural selection requiring genetic variability within species to operate (Careau & Glazier, 2022). Recent intraspecific studies in plants show that growth rate scales on average with a slope of ¾ among natural ecotypes of *Arabidopsis thaliana* (Vasseur et al., 2012, 2018, 2019) as well as in wild tomatoes (Muir & Thomas-Huebner, 2015). This suggests that the allometry of metabolism and growth rate results from strong biophysical constraints operating independently of taxonomic scale and, as a result, broadly supports the predictions of MST. However, these studies also reveal that the allometric exponent varied significantly around ¾ due to adaptation of the genotypes to different environments. Previous work indicates that variation in allometric exponents is genetically associated with different carbon and water use strategies (Vasseur et al., 2012, 2014), as well as reproductive output and resistance to stress (Muir & Thomas-Huebner, 2015; Vasseur et al., 2018). Therefore, and by contrast with MST predictions, these studies suggest that selective pressures in contrasting environments maintain diversity in trait:size ratio, which translates into allometric variation within species. Thus, a key question in allometry research is whether adaptation to contrasting environments is the result of, or is dependent on, substantial variation in trait–size relationships. To address this question, we assessed here the role of genetic variability—that is, phenotypic differences among genotypes in a single environment—as well as phenotypic plasticity—that is, phenotypic differences of a single genotype between environments—on the variation of plant allometry.

Water deficit (WD) and high temperature (HT) are the main abiotic stresses impairing plant growth and productivity. Plant size plays a prominent role in the regulation of water and carbon fluxes to adapt to these stresses (Vasseur et al., 2014). For instance, total leaf area is a major determinant of plant photosynthesis and transpiration that control water use efficiency. Moreover, plant size is strongly impacted by WD and HT, and sometimes in opposite direction: HT can promote larger leaves to improve leaf cooling through transpiration while WD can induce a decrease in leaf size to avoid water loss. Yet, the combined effects of WD and HT on trait:size relationships remain unclear. Here, we used a high-throughput phenotyping platform, PHENOPSIS (Granier et al., 2006), to examine intraspecific variation in allometric relationships in the model species *A. thaliana*. We focused on detailing the allometry of two central traits of MST: total leaf area and plant growth rate, measured in 120 contrasted genotypes grown under four combinations of water availability and temperature. Comparing the growth rate–plant biomass and the total leaf

area–plant biomass allometric relationships across genotypes enabled us to: (i) test if intraspecific trait–size relationships follow the 3/4 allometric slope observed across species, (ii) evaluate the genetic variability of allometric coefficients between genotypes and (iii) determine whether allometric relationships are plastic to water deficit and/or high temperature.

2 | MATERIALS AND METHODS

2.1 | Plant material

We used a population of 120 recombinant inbred (RI) lines previously generated from a reciprocal cross between two parental *A. thaliana* accessions: Landsberg *erecta* (Ler) and Cape Verde Islands (Cvi) (Alonso-Blanco et al., 1998). This population was chosen because Ler and Cvi carry different alleles at strong pleiotropic QTLs (Fu et al., 2009) and because these two accessions exhibit contrasted responses to high temperature and water deficit (Vile et al., 2012).

2.2 | Experimental design

We performed four consecutive experiments (experiment1, experiment2, experiment3, experiment4) in the PHENOPSIS platform (Granier et al., 2006; Vasseur et al., 2014) to examine the effects of water deficit (WD), high temperature (HT) and their interaction on plant metabolism, physiology and performance. Genetic analyses of the phenotypic traits measured have been previously published (Vasseur et al., 2012, 2014). Experiment 1 was performed under control temperature x well-watered (CT×WW) conditions, experiment 2 was performed under control temperature×water deficit (CT×WD), experiment 3 was performed under high temperature×well-watered (HT×WW) and experiment 4 was performed under high temperature×water deficit (HT×WD).

The PHENOPSIS platform allows automated rosette area measurements of 504 potted plants under highly controlled environmental conditions (Granier et al., 2006). In each experiment, we phenotyped the parental accessions (Ler and Cvi; $n = 8$ replicates) and the 120 RI lines ($n = 4$). Seeds of all lines were stored at 4°C. Five seeds from each genotype were directly sown at the soil surface in 225mL culture pots filled with a mixture (1:1, v:v) of loamy soil and organic compost (Neuhaus N2), and stratified at 10°C for 3 days. Soil water content was controlled before sowing to estimate the amount of dry soil and water in each pot. Pots were damped with sprayed deionised water three times a day in darkness (20°C, 85% air relative humidity) until germination. Between germination and the emergence of the first two true leaves, plants were cultivated at 20°C with a daily cycle of 12 h light supplied from a bank of HQi lamps which provided 190 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) at plant height. Air water vapour pressure deficit (VPD$_{air}$) was maintained constant at 0.5–0.6 kPa and soil moisture at 0.35 g H$_2$0 g^{-1} dry soil. WD and HT treatments were applied after the emergence of the first two true leaves, avoiding early-growth effects. CT was set to 20/17°C day/night, while HT was set to 30/25°C. HT has been identified to be the basal thermotolerance, that is, the highest temperature tolerated by *A. thaliana* when plants have never encountered previous HT (Ludwig-Muller et al., 2000). Soil water content was maintained at 0.35 and 0.20 g H20 g^{-1} dry soil with a modified one-tenth strength Hoagland solution in the WW and WD treatments respectively. This WD level has been shown to significantly decrease leaf water potential and impair plant growth (Aguirrezábal et al., 2006). Each pot was automatically adjusted to reach the target soil water content by weighing and watering once a day. PPFD was maintained at 190 µmol m^{-2} s^{-1} and VPD$_{air}$ was maintained at 0.7–0.8 kPa. All detailed meteorological data, including daily soil water content, air temperature and VPD$_{air}$, are available in the PHENOPSIS database (Fabre et al., 2011) (http://bioweb.supagro.inra.fr/phenopsis/).

2.3 | Trait measurements

At flowering (first flower open), each rosette was cut, separated from roots and flowering stem and wrapped in moist paper and kept at 4°C overnight in darkness. After complete rehydration, leaf blades were weighed (saturated fresh weight, mg), then separated from the petioles and scanned for area measurements. Total leaf area (TLA, mm^2) was determined with ImageJ (Rasband, 2011). Leaf blades and petioles were then oven-dried at 65°C for 96h. Total leaf blade dry weight and petiole dry weight were measured separately, and plant vegetative dry mass at reproduction (mg) was calculated as the sum of dry mass of leaf petioles and leaf blades. Leaf dry mass per area (LMA, g m^{-2}) was calculated as the ratio of total leaf blade dry weight and TLA.

PHENOPSIS allows the daily acquisition of zenithal images of the plants (Sony SSC-DC393P camera). The projected leaf area of the rosette (RA, mm^2) was determined every 2–3 days with ImageJ. A sigmoid curve was fitted for each plant using the four-parameter logistic model:

$$RA = a / (1 + \exp([d - d_0] / b), \tag{1}$$

where d is the number of days after the emergence of the first two true leaves, a is the maximum vegetative rosette area, d_0 is the time when $a/2$ leaf area has expanded and b is related to the maximum rate of leaf production. The maximum rate of rosette expansion (R_{max}, mm^2 day^{-1}) was calculated from the first derivative of the logistic model at d_0 as $R_{max} = a/(4b)$. We then calculated maximum absolute growth rate (g dry mass day^{-1}) as the product of R_{max} and LMA (Vasseur et al., 2012, 2014). Phenotypic data, including rosette pictures, are stored in the PHENOPSIS database (http://bioweb.supagro.inra.fr/phenopsis/).

Trait values are available as supplementary information attached to the paper (Table S1), as well as in DOI Digital Repository: https://doi.org/10.57745/FRJELS (Vile et al., 2023).

2.4 | Statistical analyses.

In each environmental condition, we estimated β_{tot}: the allometric slopes for total leaf area and plant growth rate measured by fitting linear regressions across all genotypes, following the equation $\log_{10}(Y) = \log_{10}(\alpha) + \beta_{tot} \times \log_{10}(M)$, where M is the vegetative dry mass. Linear regressions were fitted with a standard major axis (SMA) method. Differences of β_{tot} between treatments were tested by pairwise SMA comparisons. In addition, we tested for nonlinear allometric relationships following previous methodologies (Kolokotrones et al., 2010). We fitted nonlinear quadratic models using two-order polynomial functions such as $\log_{10}(Y) = \log_{10}(b_0) + b_1 \log_{10}(M) + b_2 (\log_{10}(M))^2$. The 'local' allometric slope, β_{loc}, can be calculated for any individual plant of biomass M, as the derivative of the quadratic function, such as $\beta_{loc} = b_1 + 2b_2 \log_{10}(M)$.

All analyses were performed in R 3.6.3 (R Core Team, 2014). We used Akaike information criterion (AIC) to compare the goodness of fit between SMA regressions, linear models and quadratic models. 95% confidence intervals (CIs) of the regression coefficients were obtained with the *sma* function (Warton et al., 2012) and *confint* function in R. The effects of water stress and high temperature, and their interaction, on the coefficients of the allometric equations were estimated with two-way ANOVAs. We used a significance threshold of 0.01. We used the *lsmeans()* function to calculate mean genotypic values.

3 | RESULTS

3.1 | Allometric relationships across *A. thaliana* genotypes support MST predictions.

Vegetative dry mass, total leaf area and growth rate were highly variable across genotypes and environments (Tables S1 and S2). For instance, vegetative dry mass spanned three orders of magnitude in each condition (Table S1). As expected, the three traits significantly decreased under HT and WD, as well as under their combination

($p < 0.01$; Table S2). Population-level allometric exponents (β_{tot}) of total leaf area and growth rate were estimated with SMA regressions, using vegetative dry mass as the explanatory variable. Consistent with MST predictions, results indicated that under control conditions (CT×WW), the population-level allometric exponent, β_{tot}, was 0.78 (95% CI = [0.76–0.79]) for total leaf area and 0.75 (95% CI = [0.73–0.78]) for growth rate (Table 1). For total leaf area, β_{tot} increased to 0.85 in response to high temperature ($p < 0.01$; Table S1), but it was not significantly impacted by WD (Table 1 and Table S1). For growth rate, β_{tot} did not differ significantly among any conditions ($P > 0.01$ for all pairwise comparisons; Table 3).

3.2 | Plant allometry is strongly variable between genotypes

We noticed variable degrees of curvilinearity in the allometric relationships in the log–log scale (Figure 1), which indicated that genotypes exhibited variable slopes β. Accordingly, quadratic models often fitted better than SMA regressions and linear models (Table S3). Based on AICs, quadratic models improved model fitting for six out of eight relationships (Table S3), that is, all except total leaf area under HT×WD and growth rate under CT×WD. From the

TABLE 1 Linear regressions between vegetative dry mass and traits Y. Traits Y are total leaf area (mm²) and plant growth rate (mg day⁻¹). Data were $\log_{10}$-transformed. α_{tot} and β_{tot} are the intercept and slope, respectively, of the standard major axis (SMA) regressions ($\log_{10}(Y) = \log_{10}(\alpha) + \beta_{tot} \times \log_{10}(M)$), performed across all genotypes in each condition (CT, control temperature; HT, high temperature; WW, well-watered; WD, water deficit). 95% confidence intervals are in brackets.

Trait Y	Condition	α_{tot}	β_{tot}
Total leaf area	CT×WW	1.89 [1.86; 1.91]	0.78 [0.76; 0.79]
	CT×WD	1.68 [1.65; 1.71]	0.80 [0.78; 0.82]
	HT×WW	1.83 [1.81; 1.84]	0.85 [0.84; 0.87]
	HT×WD	1.69 [1.68; 1.71]	0.85 [0.83; 0.86]
Growth rate	CT×WW	−0.80 [−0.83; −0.76]	0.75 [0.73; 0.78]
	CT×WD	−1.03 [−1.06; −0.99]	0.78 [0.75; 0.8]
	HT×WW	−0.91 [−0.95; −0.88]	0.74 [0.71; 0.77]
	HT×WD	−1.13 [−1.16; −1.09]	0.74 [0.70; 0.78]

FIGURE 1 Allometric relationships for total leaf area and plant growth rate in each environmental condition. Upper panels: log–log relationships between vegetative dry mass and total leaf area (mm²; (a, c, e and g), and plant growth rate (mg day⁻¹; (b, d, f and h). The 120 dots per condition represent the means of the 120 genotypes ($n = 4$ per genotype), coloured lines are the nonlinear curves estimated by quadratic regressions (CTxWW in light blue, CT×WD in dark blue, HT×WW in orange and HT×WD in red). Black lines represent linear regressions with a slope of 0.75. Lower panels: boxplots representing the scaling exponent of the 120 genotypes for the trait considered, with genotypes ordered by increasing vegetative dry mass in the corresponding condition. Black lines represent a scaling exponent of 0.75. (a) Allometry of total leaf area in control temperature and well-watered condition (CT×WW). (b) Allometry of plant growth rate in CT×WW. (c) Allometry of total leaf area in control temperature and water deficit condition (CT×WD). (d) Allometry of plant growth rate in CT×WD. (e) Allometry of total leaf area in high temperature and well-watered condition (HT×WW). (f) Allometry of plant growth rate in HT×WW. (g) Allometry of total leaf area in high temperature and water deficit condition (HT×WD). (h) Allometry of plant growth rate in HT×WD.
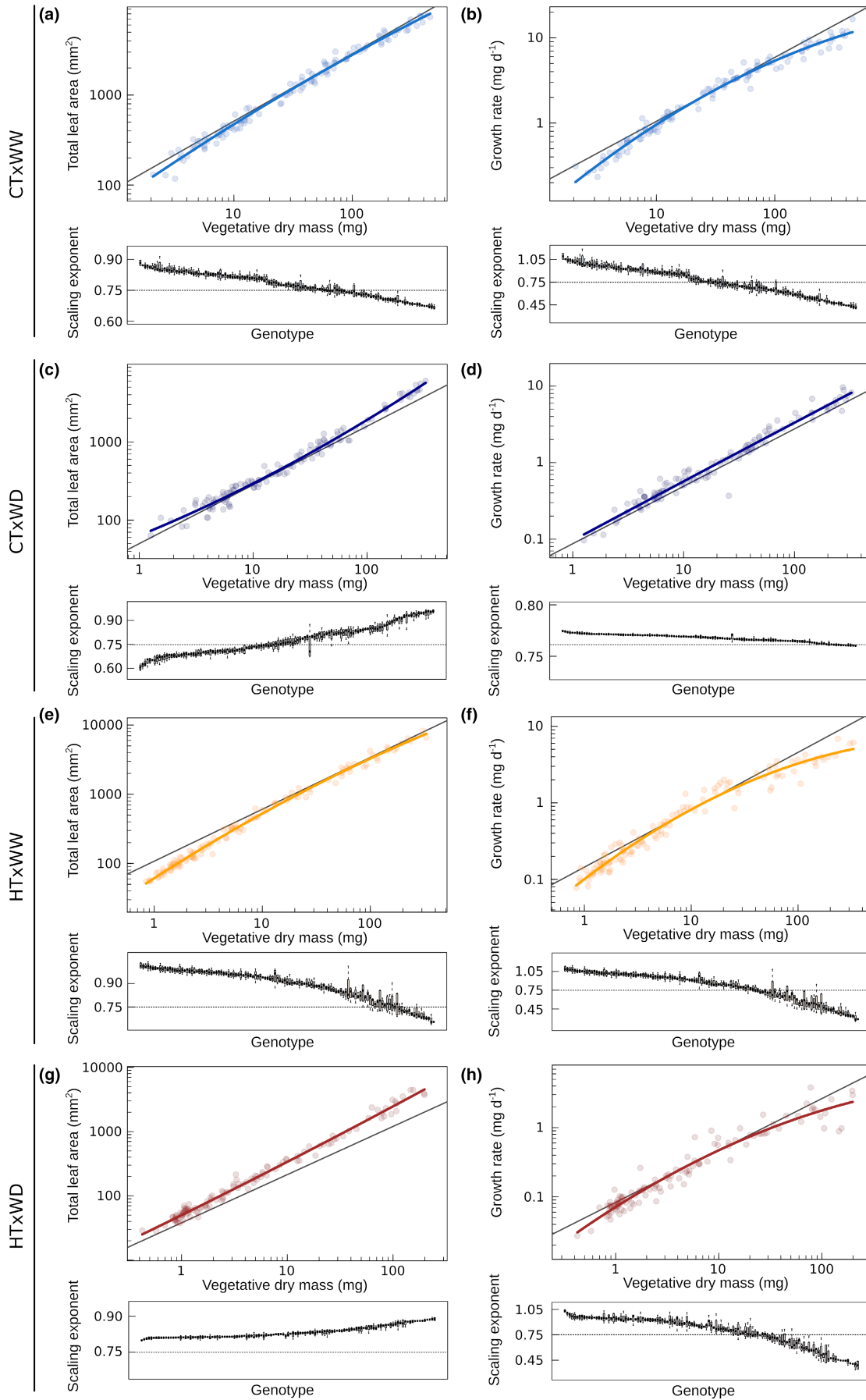
coefficients of the quadratic models (Table 2), we estimated the individual-level allometric exponents, (β_{loc}) as the first derivative of the trait–size allometric relationships. Plotting the distribution of β_{loc} within each condition (Figure 2) revealed that genotypic values of allometric exponents were centred around ¾ for both total leaf area and growth rate. However, there was a high variability in allometric exponents between genotypes within each condition (β_{loc} ranging from 0.10 to 1.75 for total leaf area, and between 0.05 and 1.55 for growth rate; with a significant genotype effect, $p < 0.01$ for both traits).

3.3 | Plant allometry is plastic to water deficit but not to high temperature

The distribution of allometric exponents β_{loc} measured among genotypes and conditions (Figure 2) revealed a strong overlap of exponent values between control and stressing conditions, which suggests that allometry was much more variable between genotypes in a single environment than between environments. To assess quantitatively whether plants modified their allometry in response to isolated or combined water deficit and high temperature, we examined the effects of environmental stresses on the scaling parameters (Tables 3 and 4). Despite small differences, high temperature had significant effects ($p < 0.01$) on the linear allometric slopes (β_{tot}) of total leaf area in both WW and WD conditions (Table 3), while there was no significant difference on the allometric slopes of plant growth rate (Table 3). However, the analysis of the scaling parameters derived from nonlinear regressions revealed that water deficit and high temperature had no significant effect on the intercept of the relationships when applied separately, but the combined stress had a significant negative effect on the intercept of the growth rate–biomass relationship (Table 4). In general, slope-related coefficients, b_1 and b_2, that is, the first- and second-order terms that jointly determine β_{loc}, were significantly impacted by water stress but not by high temperature. Water deficit significantly impacted the allometric coefficient of both total leaf area and growth rate ($p < 0.01$ for b_1 and b_2; Table 4). By contrast, high temperature did not impact the allometric coefficient of either total leaf area and growth rate ($p > 0.01$ for b_1 and b_2; Table 4). In addition, the interaction of high temperature and water deficit was not significant for either traits ($p > 0.01$; Table 4), suggesting that

TABLE 2 Quadratic regressions between vegetative dry mass and traits Y. Traits Y are total leaf area (mm^2) and plant growth rate (mg day^{-1}). b_0, b_1 and b_2 are the intercept, first- and second-order terms, respectively, of the quadratic regressions ($\log_{10}(Y) = \log_{10}(b_0) + b_1 \times \log_{10}(M) + b_2 \times (\log_{10}(M))^2$) performed in each condition (CT, control temperature; HT, high temperature; WW, well-watered; WD, water deficit). 95% confidence intervals are in brackets.

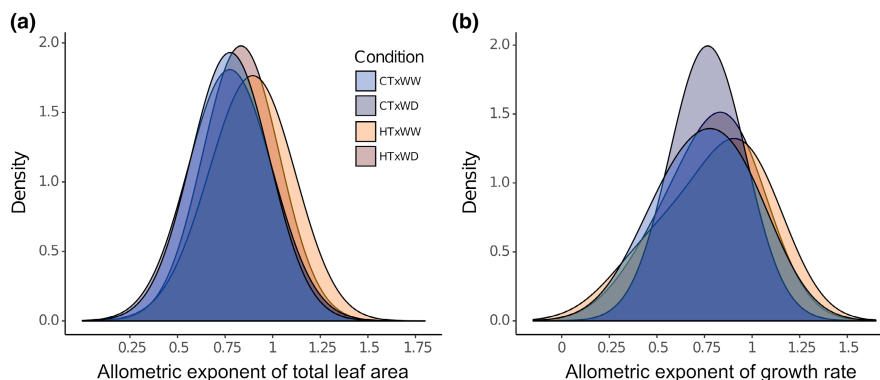|	Condition	b_0	b_1	b_2
Total leaf area	CT×WW	1.82 [1.76; 1.87]	0.90 [0.81; 0.98]	−0.04 [−0.07; −0.01]
	CT×WD	1.81 [1.75; 1.86]	0.59 [0.50; 0.68]	0.07 [0.04; 0.11]
	HT×WW	1.78 [1.77; 1.80]	1.01 [0.96; 1.05]	−0.07 [−0.09; −0.05]
	HT×WD	1.70 [1.68; 1.72]	0.81 [0.76; 0.86]	0.02 [−0.01; 0.04]
Growth rate	CT×WW	−1.07 [−1.13; −1.00]	1.20 [1.10; 1.29]	−0.15 [−0.18; −0.12]
	CT×WD	−1.02 [−1.09; −0.95]	0.77 [0.66; 0.88]	0.00 [−0.04; 0.04]
	HT×WW	−1.00 [−1.04; −0.96]	1.07 [0.99; 1.16]	−0.16 [−0.20; −0.12]
	HT×WD	−1.14 [−1.18; −1.11]	0.94 [0.84; 1.04]	−0.12 [−0.17; −0.07]

FIGURE 2 Distribution of allometric exponents in the four environmental conditions. Distributions of genotypic values ($n = 4$ per genotype) of the individual-based allometric exponent β_{loc}, measured from the derivative of the quadratic regressions. (a) Allometric exponent for total leaf area (mm^2). (b) Allometric exponent for plant growth rate (mg day^{-1}). CT, control temperature; HT, high temperature; WW, well-watered; WD, water deficit.

TABLE 3 Pairwise comparisons of allometric slopes between environmental conditions. *p*-values reported for testing differences in the slope of SMA regressions across genotypes (β_{tot}) between conditions (CT, control temperature; HT, high temperature; WW, well-watered, WD, water deficit).

		CT×WW	CT×WD	HT×WW
Total leaf area	CT×WD	0.049		
	HT×WW	<0.001	<0.001	
	HT×WD	<0.001	0.001	0.606
Growth rate	CT×WD	0.175		
	HT×WW	0.429	0.049	
	HT×WD	0.464	0.076	0.977

only water deficit had a significant effect on trait–size relationships in *A. thaliana*.

We plotted the relationships between plant size in controlled conditions (CT×WW) and the plasticity (measured by the standard deviation) of traits and allometric exponents across environments (Figure 3). We found that intermediate-sized plants were more plastic for traits (Figure 3a) but less plastic for allometric exponents (Figure 3b) than extreme-sized plants (both very small and very large plants). Thus, the genotypes that minimised trait plasticity (essentially the smallest and biggest plants with allometric exponent that deviated from 0.75) were those that maximised allometric plasticity.

4 | DISCUSSION

In plants, the metabolic scaling theory (MST) predicts that the metabolic rate is expected to scale isometrically with total leaf area, total leaf biomass and plant growth rate. In addition, total leaf area and plant growth rate are expected to scale allometrically (with $\beta = 3/4$) with the mass of the plant. Those scaling expectations represent the master equation of the MST (Enquist et al., 1999; West et al., 1997, 1999a, 1999b). In this study, we investigated the allometric relationships of two central traits of the metabolic scaling theory, namely total leaf area and plant growth rate. We specifically examined whether there was substantial variation in allometric relationships across highly diverse genotypes grown in a common environment and measured at the same developmental stage to avoid confounding effect of plant ontogeny. Consistent with previous findings (Kolokotrones et al., 2010; Mori et al., 2010; Muir & Thomas-Huebner, 2015; Vasseur et al., 2012, 2018), we showed that allometric relationships were highly variable among genotypes of *A. thaliana* because of the nonlinearity of trait–size relationships (in log–log scale). By contrast, we showed that allometric relationships were moderately plastic, and they varied more in response to water deficit than to high temperature. Moreover, we showed that allometric plasticity was associated with trait stability, and reciprocally. Below, we discuss the physiological and evolutionary implications of these findings.

The universality of allometric relationships, specifically the allometric exponent, has been hypothesised from modelling approaches

TABLE 4 Effect of water deficit, high temperature and their interaction on the parameters of the quadratic regressions between vegetative dry mass and traits Y. Traits Y are total leaf area (mm²) and plant growth rate (mg day⁻¹). Data were log₁₀-transformed. b_0, b_1 and b_2 are the intercept, first- and second-order terms respectively. Treatment effects were tested with two-way ANOVAs performed across all genotypes. C, control temperature; WW, well-watered; HT, high temperature; WD, water deficit; NS, non-significant ($p > 0.05$), *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

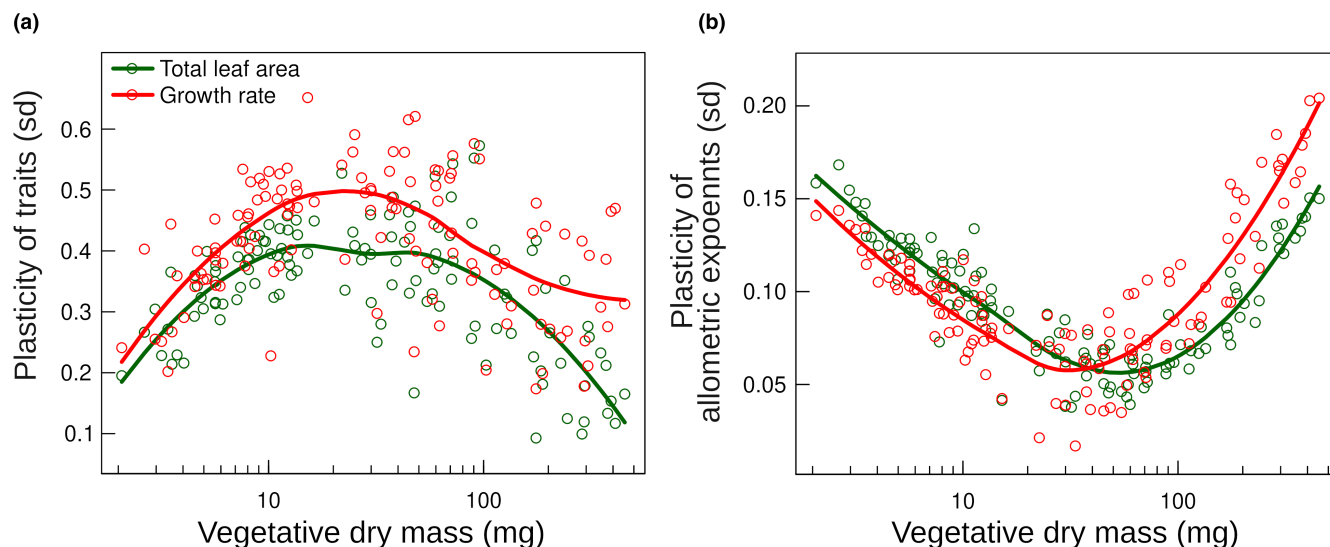|	b_0				b_1				b_2			
	C	xHT	xWD	x(HT*WD)	C	xHT	xWD	x(HT*WD)	C	xHT	xWD	x(HT*WD)
Total leaf area	1.82***	−0.03 NS	−0.01 NS	−0.07 NS	0.90***	0.11*	−0.31***	0.11 NS	−0.04**	−0.03 NS	0.12***	−0.03 NS
Growth rate	−1.07***	0.07 NS	0.05 NS	−0.20**	1.20***	−0.12 NS	−0.42***	0.29*	−0.15***	−0.01 NS	0.15***	−0.11*

FIGURE 3 Relationships between plant size and plasticity. (a) Relationships between plant vegetative dry mass (mg) in controlled conditions (CT×WW) and the plasticity of traits (total leaf area (mm^2) in green and plant growth rate (mg day^{-1}) in red). (b) Relationships between plant vegetative dry mass (mg) in controlled conditions (CT×WW) and the plasticity of allometric exponents for the two studied traits (total leaf area in green and plant growth rate in red). Plasticity was measured as the standard deviation (SD) of all individuals per genotype across the four environments. Loess curves were fitted for each trait and exponent (solid lines).

and large-scale comparisons made across very contrasted plant and animal species (Niklas, 1994; West et al., 1999b). For instance, the mechanistic assumptions behind MST suggest that most organisms must fall along a linear relationship with a slope of ¾ for many morpho-physiological traits (Enquist, Kerkhoff, et al., 2007; Niklas & Enquist, 2001; West et al., 1997, 1999a, 1999b). Inter- and intraspecific comparisons have revealed the global patterns of covariation that generally confirm the predictions of MST in plants and animals (Enquist, Kerkhoff, et al., 2007; Niklas & Enquist, 2001; Vasseur et al., 2012, 2018; West et al., 1997). Also consistent with MST predictions, our results indicated that the allometric exponent measured by linear fitting across all genotypes (β_{tot}) was close to 0.75 in all environmental conditions tested, for both plant growth rate and total leaf area. Other trait–size relationships are also expected to exhibit similar exponent values across species (Savage et al., 2010). Although it is challenging technically, it would be highly informative to explore the intraspecific scaling of physiological traits predicted by MST, such as photosynthesis, transpiration and respiration rates in future experiments.

If many empirical evidences were consistent with MST, the universality of allometric exponent value of ¾ has been strongly debated over the last two decades (Coomes, 2006; Dodds et al., 2001; Glazier, 2005; Kozłowski & Konarzewski, 2004; Russo et al., 2007). For instance, the rate of respiratory metabolism has been found to scale isometrically (i.e. with exponent $\beta \approx 1$) in herbaceous plants and tree seedlings (Reich et al., 2006). Other studies in plants Enquist, Allen, et al. (2007) and mammals (Kolokotrones et al., 2010) showed that respiration rate scaled differently between organisms with small body mass ($\beta \approx 1$) and large body mass ($\beta \approx ¾$). In plants, competition for light resulted in significant variation in allometric relationships in trees (Coomes, 2006; Lines et al., 2012; Russo et al., 2007).

Accordingly, we found that genotypes exhibited highly variable allometric exponent values, which was explained by the nonlinearity in trait–size log-relationships. Moreover, allometric variability in *A. thaliana* has also been shown to be related to genetic differentiation and adaptation along large latitudinal gradients (Vasseur et al., 2018). More specifically, these recent findings suggest that extreme scaling exponents are genetically selected in harsher climatic conditions, such as at the margins of the distribution range of the species (Vasseur et al., 2018). Thus, if the optimisation of the scaling relationships, for instance through the vascular network, might be associated with the optimisation of some fitness components, such as seed production, in non-stressing conditions, scaling deviations could be selected in other, more stressing, conditions, because such deviations could optimise other fitness components such as hydraulic resistance and survival to water deficit. Overall, our findings suggest that MST predictions are valid as traits scaled on average to ¾. However, there were significant deviations towards lower or higher exponents for both traits among genotypes, which mirrored variation in relative growth rate and surface-to-biomass ratio, and might reflect difference in plant's strategies to cope with environmental variability.

Our results indicated that allometric relationships were moderately altered by abiotic stresses. Interestingly, the intermediate-sized genotypes (typically those with an allometric exponent close to 0.75) had contrasted responses to environmental changes compared to very small and very large genotypes (typically those that significantly deviated from 0.75). Indeed, both the small and large plants exhibited low trait plasticity but high allometric plasticity, while intermediate-sized plants exhibited high trait plasticity but low allometric plasticity. Again, this suggests that plants exhibited contrasted strategies to cope with environmental variability depending on their allometric coefficients. Plants that followed MST

predictions (i.e. intermediate-sized plants here) had strongly plastic traits but they did not modify their allometric coefficients. In other words, our findings suggest that intermediate-sized plants responded strongly to stress by modifying their vegetative dry mass and trait value with respect to allometric rules. By contrast, small and large plants that deviated from MST predictions tended to maintain a certain trait homeostasis by strongly modifying their allometric exponent values across environments. A possible explanation is that extreme-sized plants modified their dry mass but not (or less) their traits. In doing so, they modified their trait:size allometric ratio, presumably to maintain trait values under stress close to the values expressed in non-stressing conditions.

Overall, our study highlights the role of allometric models to understand the physiological responses involved in plant adaptation to contrasted environments. In particular, we showed that allometry is more variable between genotypes than between environments. This suggests that natural selection can lead to the genetic differentiation of plant allometries in different environments, but that environmental changes have limited effect on a genetically determined type of allometry. Moreover, plants that significantly deviated from MST predictions were associated with different responses to abiotic stresses: They modified their allometries to minimise trait plasticity. Although this might indicate that deviations from MST are associated with contrasted adaptive strategies, other experiments are needed, in diverse species, to test the generality of this finding. In addition, future studies will also be necessary to investigate the effect of plant allometry for the response to other environmental factors such as nutrient and light limitation.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest. Cyrille Violle is an Associate Editor of Functional Ecology, but took no part in the peer review and decision-making processes for this paper.

DATA AVAILABILITY STATEMENT

Trait values are available as supplementary information attached to the paper, as well as in the DOI Digital Repository (https://doi.org/10.57745/FRJELS). In addition, raw data such as plant pictures are stored in the PHENOPSIS database (http://bioweb.supagro.inra.fr/phenopsis/).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Trait values for all individuals used in the present study. idCondition is the environment in which plants were grown (CT: control temperature, HT: high temperature, WW: well-watered, WD: water deficit). rosetteDM: vegetative dry mass (mg) at flowering, TLA: total leaf area (mm^2), Growth_rate: plant growth rate (mg d^{-1}).

Table S2. Mean trait value and standard deviation (SD) for vegetative dry mass, total leaf area and plant growth rate in the four conditions.

Table S3. Akaike information criterion (AIC) of the different regressions used in the study. SMA: standard major axis (linear regression), linear model and quadratic (nonlinear) model performed on the relationships between vegetative dry mass (mg) and total leaf area (mm^2), as well as between vegetative dry mass and plant growth rate (mg d^{-1}), after log_{10}-transformation. CT: control temperature, HT: high temperature, WW: well-watered, WD: water deficit.