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ABSTRACT

High temperature (HT) and water deficit (WD) are frequent environmental constraints restricting plant growth and productivity. These stresses often occur simultaneously in the field, but little is known about their combined impacts on plant growth, development and physiology. We evaluated the responses of 10 *Arabidopsis thaliana* natural accessions to prolonged elevated air temperature (30 °C) and soil WD applied separately or in combination. Plant growth was significantly reduced under both stresses and their combination was even more detrimental to plant performance. The effects of the two stresses were globally additive, but some traits responded specifically to one but not the other stress. Root allocation increased in response to WD, while reproductive allocation, hyponasty and specific leaf area increased under HT. All the traits that varied in response to combined stresses also responded to at least one of them. Tolerance to WD was higher in small-sized accessions under control temperature and HT and in accessions with high biomass allocation to root under control conditions. Accessions that originate from sites with higher temperature have less stomatal density and allocate less biomass to the roots when cultivated under HT. Independence and interaction between stresses as well as the relationships between traits and stress responses are discussed.

Key-words: *Arabidopsis thaliana*; biomass allocation; hyponasty; leaf morphology; multistress; phenology; stomatal density.

INTRODUCTION

High temperature (HT) and water deficit (WD) are two important environmental constraints restricting plant growth and productivity in many areas of the world (Boyer 1982; Ciais *et al*. 2005). Global climate change will presumably increase the occurrence and extend the distribution of these constraints, leading to further reduction of productivity and shifts in biodiversity (Chaves *et al*. 2002; Lobell & Asner 2003; Porter 2005; Thuiller *et al*. 2005; IPCC 2007). The two stresses often occur simultaneously in the field, but little is known about their combined effects on plant growth, development and physiology (Machado & Paulsen 2001; Zhang *et al*. 2008).

Different mechanisms have been identified as ensuring plant survival and growth under elevated temperatures or water shortage. They include long-term evolutionary phenological and morphological adaptations and short-term avoidance or acclimation mechanisms. Even moderate increases in air temperature (Lafta & Lorenzen 1995; Loveys *et al*. 2002) or decreases in soil water availability (Passioura 1996) are responsible for impaired plant growth. Many elementary biological processes and morphological traits underlying plant growth are sensitive to temperature, and their responses repeatedly resemble a bell-shaped curve. As temperature rises above a particular threshold, processes such as net photosynthetic rate are negatively affected (Körner 2006; Sage & Kubien 2007; Parent *et al*. 2010), ultimately leading to a decline in plant performance. Temperature is also the main determinant of plant phenology (Ritchie & NeSmith 1991), and moderate increases in air temperature generally accelerate the rate of developmental processes leading to early flowering in most wild and cultivated species (Johnson & Thornley 1985). Whereas the effects of WD on phenology remain elusive, delayed timing of reproduction is often observed in crop species (McMaster *et al*. 2009). The effects of these stresses also depend on the phenological stage at which they occur (Prasad, Staggenborg & Ristic 2008). For instance, HT has greater impacts on seed yield during the reproductive phase (Jenks & Wood 2010). Therefore, accelerated reproduction in response to HT is generally viewed as an escape mechanism.

HT and WD have contrasted effects on patterns of biomass allocation to organs and tissues. For instance, allocation to roots rapidly increases in response to moderate soil WD (Boyer 1985), whereas leaf relative water content and specific leaf area (SLA) decline in plants subjected to water stress (Poorter *et al*. 2009). Leaf structure is also affected by temperature, but, in contrast to WD, higher temperature often leads to the production of thinner leaves with higher SLA (Boese & Huner 1990; Loveys *et al*. 2002; Luomala *et al*. 2005; Poorter *et al*. 2009). These morphological changes are accompanied by changes in leaf anatomy. Leaves developed under WD have generally smaller cells in the parenchyma and the epidermis (Lecoeur *et al*. 1995) and

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higher stomatal density (Aubert *et al*. 2010; Tisne *et al*. 2010). Wahid *et al*. (2007) reported similar effects of HT and WD on cell density, but limited data are available on changes in leaf anatomy in response to HT.

The effects of WD, particularly osmotic stresses or watering deprivation, and HT, particularly short periods of acute heat stress, have been mostly analysed separately. There is, however, strong evidence that HT and WD interact to influence plant functioning (Rizhsky, Liang & Mittler 2002; Rizhsky *et al*. 2004). For instance, WD induces stomatal closure and reduces transpiration fluxes (Hsaio 1973). This in turn can cause an increase in leaf temperature by reducing transpirational cooling (Cook, Dixon & Leopold 1964), and potentially enhances plant susceptibility to higher air temperature. Increase in leaf temperature can also raise plant water loss through transpiration (Lafta & Lorenzen 1995), and decrease root growth (Kuroyanagi & Paulsen 1988), thus increasing plant susceptibility to water shortage. By contrast, changes in leaf orientation in response to elevated temperature (Fu & Ehleringer 1989) such as hyponasty (Koini *et al*. 2009; Van Zanten *et al*. 2009) modify the leaf energy balance and could contribute to water saving by limiting rises in leaf temperature and evaporative demand. Hyponasty could also increase water consumption if associated with increased transpiration. Lastly, effects of HT on growth could lead to reduced leaf area, limiting plant water losses and thus mitigating the effects of WD.

In the face of the multiplicity of interacting, sometimes opposite effects between these two stresses, it appears difficult to predict plant responses to combined HT and WD. The aim of this study was therefore to evaluate the responses to both isolated and combined HT and WD in natural accessions of the model plant *Arabidopsis thaliana*. The following questions were addressed: (1) How do HT and WD interact on traits related to plant growth, morphology and development and to what extent do their combined effects differ from those of isolated stresses? (2) Is the variability of responses to isolated and combined HT and WD related to the climatic conditions at the accessions collection sites? (3) To what extent are these responses related to trait values exhibited in control conditions? A set of 10 *Arabidopsis* accessions spanning nearly the entirety of the latitudinal range of this species was selected to identify common responses and explore the natural variation of *Arabidopsis* tolerance to both stresses. Controlled environmental conditions were applied in full factorial experiments and maintained constant from the seedling to the reproductive stage. Control air temperature (CT) was set to 20 °C, as in most experimental studies (Balasubramanian *et al*. 2006; Saidi, Finka & Goloubinoff 2011), whereas HT was set to 30 °C. This HT level has been identified to be the basal thermotolerance, that is the highest temperature tolerated by a plant that has never encountered previous HT, of the *Arabidopsis* accession Col-0 (Ludwig-Muller, Krishna & Forreiter 2000). Soil WD was maintained constant at a level previously shown to significantly decrease leaf water potential and impair plant growth, resulting in reduced plant size of Col-0 by half (Aguirrezábal *et al*. 2006).

MATERIALS AND METHODS

Plant material and growth conditions

Ten accessions of *A. thaliana* were grown in one to three independent experiments depending on the accession (Table 1). Seeds of all genotypes were stored at 4 °C in the dark ensuring stratification. Five seeds from each genotype were directly sown at the soil surface in 225 mL culture pots filled with a mixture (1:1, v : v) of loamy soil and organic compost (Neuhaus N2). Pots were damped with sprayed deionized water three times a day and placed in two controlled growth chambers in darkness (20 °C, 65% air relative humidity) until germination. After germination, plants were cultivated with a daily cycle of 12 h light supplied from a bank of HQi lamps which provided 175 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) at plant height.

Soil WD and HT treatments were applied to half of the pots after emergence of the first two true leaves (stage 1.02 in Boyes *et al*. 2001) ensuring a good establishment of the seedlings. In the first growth chamber, CT was set to 20/17 °C day/night, while HT treatment was set to 30/25 °C in the second one. Air relative humidity was adjusted to 65% under CT and 85% under HT in order to maintain equal water vapour pressure deficit (VPD) at 0.9 kPa. This was set up in order to avoid the confounding effect of temperature on transpiration through increased VPD. Soil water content was controlled before sowing to estimate the amount of dry soil and water in each pot. Subsequent changes in pot weight were due to changes in water status. Soil water content was maintained at 0.35 and 0.20 g H_2O g^{-1} dry soil with a modified one-tenth strength Hoagland solution (Hoagland & Arnon 1950) in the well-watered (WW) and WD treatments, respectively. The field capacity of the substrate was 0.78 g H_2O g^{-1} dry soil (Granier *et al*. 2006); therefore, the WW and WD treatments represented 45 and 25% of the soil field capacity, respectively. Pot weight was precisely adjusted to reach the target soil water content by weighing and watering each individual pot every Monday, Wednesday and Friday. Other days, a standard volume of nutrient solution amounting to the mean volume of previously weighed water applications for each treatment was added to the plants without weighing the pots.

Three consecutive experiments were carried out following the same experimental procedure (see Table 1). In experiments 1 and 2, only one plant per pot was maintained until first silique shattering, while one to three plants were maintained until inflorescence emergence in experiment 3 for photosynthesis measurements and abscisic acid (ABA) content determination.

Measurement of plant traits

During the course of plant development, the following stages were scored: germination, cotyledons fully opened, two rosette leaves >1 mm, inflorescence emergence, first flower open and first silique shattered (stages 0.7, 1.0, 1.02, 5.01, 6.00 and 8.00 of Boyes *et al*. (2001), respectively). Leaf

Table 1. Origin of the accessions studied and climate at the collection sites

Accession	Latitude (°N)	Longitude (°E)	Country	Mean autumn-spring precipitations (mm)	Mean autumn-spring temperature (°C)	Diurnal temperature range	Relative humidity	Exp. 1	Exp. 2	Exp. 3
Cvi-0	15	–23.4	Cape Verde Island	0.36	21.8	5.52	74.4		X	X
Mt-0	32.6	22.8	Libya	52.2	15.2	7.57	62.0		X	X
Ct-1	37	15	Italy	61.19	12.3	8.73	73.5		X	X
Sha	38	68	Tadjikistan	53.1	8.2	13.16	59.5	X	X	X
Bay-0	49		Germany	29	2.99	8.58	77.0	X		
An-1	51.2	4.4	Belgium	64.2	5.96	7.74	82.2		X	X
Col-0	53	10	Poland	52.9	3.96	7.59	86.3	X	X	X
Ler	53	16	Poland	38.2	2.6	7.63	83.1	X	X	X
Lc-0	58	–5	UK	161.0	4.1	5.83	88.5		X	X
Est-1	59	28	Russia	48.7	–2.1	7.68	84.3		X	X

X indicates the experiments in which accessions were studied.

number was determined for each plant at each precise adjustment of soil water content, that is three times a week, only in experiments 2 and 3.

Dynamics of leaf production

For each plant in experiment 2, a sigmoid curve was fitted to the relationship between total number of rosette leaves (*LN*) and time from stage 1.02 to stage 8.00 by the following four-parameter logistic model:

$$LN = \frac{a}{1 + e\left(-\frac{(d - d_0)}{b}\right)} \quad (1)$$

where d is the number of days after stage 1.02, a is the maximum vegetative leaf number, d_0 is the time when $a/2$ leaves have developed and b is the inverse of slope factor which refers to the steepness of the curve, and is thus a parameter related to the maximum rate of leaf production. In order to standardize between genotypes, we used an estimate of leaf production duration (days) as $d_0 - b\ \ln(0.05/0.95)$, that is the time period for vegetative leaf number to increase from 5 to 95% maximum number. The maximum rate of leaf production (R_{max}, leaf d^{-1}) was calculated from the first derivative of the logistic model at d_0 as $R_{max} = a/(4b)$.

In experiment 3, since leaf emergence rate is maximal and nearly constant between stage 1.02 and stage 5.01, R_{max} was fairly well estimated by the slope of the relationship between LN and time during this period. R_{max} varied across genotypes and treatments with highly reproducible results between experiments ($r = 0.85$, $P < 0.001$). Most of the plants survived the HT and WD treatments, and reached the reproductive stage. Only a few plants did not survive the combined HT × WD treatment.

Whole plant and leaf traits

In experiment 2, 20 d after germination, tip height, total length and blade length of the youngest fully expanded leaf were measured on each plant with a digital calliper as described in Hopkins, Schmitt & Stinchcombe (2008). At this time, plants had six to 14 leaves depending on the genotype, and inflorescence had not emerged. Measurements were taken in randomized order between 2 and 4 h after lights went on in the chambers to avoid any effects associated with time of the day like endogenous rhythms. The proportion of leaf composed of blade was estimated by the blade ratio, the blade length divided by total leaf length. Leaf insertion angle (degree) was calculated as θ = arcsine (leaf tip height/leaf length).

Plants were harvested at stage 8.00, in the morning and after irrigation. Rosettes were cut, inflorescences were detached from the rosettes and their fresh weights (FWs) (milligrams) were determined immediately. Leaf blades were separated from the rosette, and FWs of the sixth and ninth leaves were determined. Mean leaf thickness (LT) of

these two leaves was determined with a linear variable displacement transducer (Solartron) connected to a multimeter and previously calibrated with 5 μm accuracy. Depending on the size of the leaf, LT was measured on 6 to 10 points per leaf blade, avoiding the mid-vein. All blades were then stuck on a sheet of paper, arranged by order of emergence on the rosette, and the sheet of paper was scanned for area measurements. Additionally, a transparent imprint of the adaxial epidermis of the sixth leaf was obtained by drying off a varnish coat spread on the surface of the leaf. Imprint was peeled off and then stuck on microscope slides with one-sided adhesive for further measurements. Roots were carefully extracted from the soil and gently washed in deionized water. Leaf blades, petioles, reproductive structures and roots were then separately oven-dried at 65 °C for at least 3 d, and dry masses were determined. Rosette area (cm^2) was determined as the sum of individual leaf blade areas measured on the scans with an image analysis software (Bioscan-Optimas 4.10, Edmond, WA, USA). From these measurements, leaf dry matter content (LDMC, the ratio of dry mass to fresh mass, mg g^{-1}) and SLA (the ratio of leaf area to leaf dry mass, m^2 kg^{-1}) were calculated at the rosette and leaf (for leaves 6 and 9) levels. Biomass allocation was assessed by the ratios of above-ground vegetative, reproductive and below-ground dry masses to total plant dry mass. Root-to-shoot ratio was calculated as the ratio of root to vegetative above-ground masses.

Leaf epidermal anatomy

Epidermal imprints of the sixth leaves were placed under a microscope (Leitz DM RB; Leica, Wetzlar, Germany) coupled to an image analyser. Mean cell and stomatal densities were determined by counting the number of cells and stomata in two 0.12 mm^2 zones in the middle part of the leaf blade distributed on both sides of the mid-vein halfway from the margins. Stomatal index was calculated as $100 \times$ stomatal number/(stomatal number + stomatal number $\times$ 2 + epidermal cell number).

Net photosynthetic rate

Net photosynthetic rate was measured using a single leaf chamber designed for *Arabidopsis* connected to an infrared gas analyser system (CIRAS 2, PP Systems, Amesbury, MA, USA) in experiment 3. Carbon fluxes were determined at steady state (approximately 15 min after light was switched on) under control temperature (20 °C) and HT (30 °C) but only in WW conditions, and under ambient CO_2 (390 ppm) and light intensity (175 μmol m^{-2} s^{-1} PPFD). Photosynthesis was measured on two to 15 plants at bolting on An-1, Col-0, Cvi-0, Ler, Mt-0 and Sha.

Leaf ABA content

Leaf ABA (ng g^{-1} FW) was determined by radioimmunoassay (Quarrie *et al*. 1988) as previously described (Barrieu & Simonneau 2000). Leaf samples were ground finely under liquid nitrogen, placed in distilled water (5 mL *per* mg FW) and immediately warmed at 70 °C for 5 min before shaking at 4 °C overnight. Extracts were then centrifuged at 16 000 *g* for 10 min at 4 °C; the supernatant was conserved at –20 °C and used for radioimmunoassay.

Meteorological data at the geographical origin of the accessions

Meteorological data (temperature, precipitation, relative humidity, diurnal temperature range) at the geographical origin of the accessions were extracted from high-resolution gridded datasets of climate data (New *et al*. 2002). Mean monthly parameters were calculated for the main period of vegetative growth of *A. thaliana* from September to May (Hoffmann 2002).

Data analysis

Statistical significance of trait variation was tested by three-way multivariate and univariate analyses of variance (MANOVA and ANOVA) with genotype, soil water content and air temperature as fixed factors. Post hoc comparison between treatments was performed with Kruskal–Wallis non-parametric test. Principal component analyses (PCAs) were performed to study the relationships between the traits and the effects of the temperature and soil water treatments. PCAs were performed on data from the experiment where higher number of both traits and genotypes were studied (experiment 2) and on standardized mean trait values by genotype and treatment ($n = 36$) because traits were measured in very different units. Between- and within-treatment PCAs were performed on mean trait values to test for differences between treatments and focus on genotypic effects, respectively (Chessel, Dufour & Thioulouse 2004). The null hypothesis that there is no difference between treatments was tested with a randomization test (*randtest.between* in the R/*ade4* package). The procedure checks that the observed value of the between/total inertia ratio is higher than expected under the null hypothesis. The distribution of the between/total inertia ratio is obtained by permuting the rows of the data frame, that is means per genotype and treatment ($n = 999$) and thus changing assignment to treatment group. Response ratios (R) between treated (T) and control (C) groups were calculated as $R_{T/C}$ = mean trait $value_T$/mean trait $value_C$ to quantify the effects of the treatments for each genotype. Five values of response ratios were calculated to obtain the response to WD according to the control conditions (WD-20 °C/WW-20 °C), the response to WD at HT (WD-30 °C/WW-30 °C), the response to HT in WW conditions (WW-30 °C/WW-20 °C), the response to HT in WD conditions (WD-30 °C/WD-20 °C) and the response to the combination of HT and WD compared to the control conditions (WD-30 °C/WW-20 °C). The response ratio quantifies the proportionate change that results from an experimental

manipulation (Hedges, Gurevitch & Curtis 1999). Response ratios were log-transformed in the statistical analyses. We tested the significance of the relationships between traits, response ratios, coordinates of the genotypes of the PCA axes and climatic descriptors with correlation coefficients. All statistical tests were performed using R v.2.10 (R Development Core Team 2009).

RESULTS

Analysis of multiple plant traits reveals significant genotype by environment effects but predominant additive effects of HT and WD

ANOVAs explained from 25 to 85% of the total variance of 16 functional traits related to plant growth, structure and physiology, and the MANOVA explained 58% of the total variance in the multivariate dataset (Table 2). Across traits, there was a highly significant genotypic variability among accessions (18% of variance explained in the MANOVA; from 4 to 47% of variance explained across traits). Additionally, strong genotype by environment (soil water content, temperature or both) interactions were detected for all traits as indicated by highly significant first- and second-order interaction terms, highlighting the large natural phenotypic variability in the responses to both isolated and combined HT and WD. While significant for most of the traits, the effect of WD was not significant at the multivariate level. Interestingly, lack of significant interaction between water regime and temperature at the multivariate level and for most of the traits was indicative of prevailing additive effects of WD and HT (Table 2).

A PCA was performed in order to explore the multivariate pattern of effects of both isolated and combined HT and WD on the studied traits. First, second and third principal components (PC) explained 45, 25 and 9% of the total variance, respectively (Fig. 1; see Supporting Information Table S1 for variable loadings). Size-related traits contributed most to PC1 which opposed large plants with numerous vegetative leaves and high rate of leaf production to plants that had high reproductive mass allocation and thinner, more erect leaves with high SLA (Fig. 1a). Biomass allocation to the roots, epidermal cell density and stomatal density closely and negatively correlated with PC2. LDMC contributed less to this axis but contributed to most of the variation on third axis.

Projection of the accessions (Fig. 1b) showed that the four temperature-by-soil water treatments were significantly discriminated in the first factorial plane ($P < 0.001$; permutation tests of between-treatments PCA), although the high genotypic variability was distinguishable as indicated by the distance of the accessions from the centroid of each treatment. Along PC1, plants grown under control conditions (20 °C air temperature; 0.35 g H_2O g^{-1} dry soil) were opposed to plants grown under combined HT and WD conditions (30 °C; 0.20 g H_2O g^{-1} dry soil). As indicated by the position of the centroid of each treatment along PC1, all treatments reduced plant performance compared to control conditions, and the combined stress was more detrimental to plants than isolated HT or WD. Isolated HT and WD treatments were significantly separated along PC2, indicating opposite effects of these stresses on traits related to this axis. Specifically, WD led to an increased biomass allocation to roots, a decrease in SLA and higher epidermal cell and stomata densities, whereas HT had opposite effects.

The combination of HT and WD is more detrimental to plant development than isolated effects, but differences between genotypes exist

As shown by the PCA, rosette development dynamics were significantly affected by HT, WD and their combination (Fig. 2; Table 2; Supporting Information Fig. S1). In control conditions, the average of maximum rate of leaf production (R_{max}, leaf d^{-1}) was 0.95 among genotypes and varied significantly from 0.75 in An-1 to 1.08 in Cvi-0 and Mt-0 (Supporting Information Fig. S2). The three treatments significantly reduced R_{max} (Fig. 2a; Table 2). Although the sensitivity of phenology to treatments varied significantly among *Arabidopsis* accessions, WD was, on average, more detrimental for leaf production (23% mean decrease) than HT (16% mean decrease; but see Lc-0 and Sha in Supporting Information Fig. S2a). Combining HT and WD had greater effects (40% mean decrease among genotypes) on R_{max} than isolated treatments (Fig. 2a). The duration of vegetative leaf production, which is highly related to flowering time in *A. thaliana*, also varied widely among accessions from 21 to 63 d in An-1 and Lc-0, respectively (Supporting Information Fig. S2b). Duration of leaf production and flowering time increased or decreased depending on accession and treatment leading to a highly significant second-order interaction term in the ANOVA (Table 2). While not significant in all accessions, WD tended to increase the duration of leaf production either at control or HT (non-significant water regime by temperature interaction in ANOVA; Table 2; Fig. 2b). By contrast, increasing air temperature tended to shorten the life cycle either in WW or WD conditions. As a result of their effects on plant growth dynamics, HT and WD significantly reduced total plant mass in all accessions but Cvi-0 and Lc-0 (Fig. 3; Table 2). On average, HT and WD similarly reduced total dry mass by twofold. Combining HT and WD (HT × WD) reduced plant size more severely than isolated stresses from 55% in An-1 to 91% in Ct-1 (Fig. 3 and 85% mean decrease). In some genotypes, plant dry mass tended to be less affected by isolated or combined HT and WD (An-1, Lc-0), while in others, it was less reduced only under HT (Cvi-0) or WD (Est-1, L*er*). This resulted in weak relationships between response ratios to HT and WD for total dry mass (Supporting Information Fig. S3). However, the response ratio of HT × WD to control conditions ($R_{HT \times WD|C}$) for the total dry mass was close to the sum of the response ratios of WD and HT to control conditions ($R_{WD|C} + R_{HT|C}$) suggesting nearly additive effects. This was true for all accessions except Cvi-0, Lc-0 and Mt-0. These accessions apart, clear additive effects were indicated by a significant relationship between

Table 2. Results of the partitioning of phenotypic variation among the natural genotypes grown in a full factorial design of contrasted soil relative water content (W, 0.35 and 0.20 g H_2O g^{-1} dry soil in well-watered and water deficit treatment, respectively) and air temperature (T, 20 °C and 30 °C in control and high temperature treatment, respectively)

Trait	Genotype (G)	Soil water content (W)	Temperature (T)	W × T	G × W	G × T	G × W × T	R^2 (%)
d.f.	9	1	1	1	9	9	9	
MANOVA	18.1***	0.5	32.7***	0.5	6.7**	3.4	1.9	57.5
Plant growth and final size, and biomass allocation								
R_{max} (leaf d^{-1})[a]	19.5***	31.9***	14.2***	0.0	3.1**	5.2***	0.8	73.9
Growth duration (days)[a]	44.9***	2.6***	8.6***	0.0	2.1	8.9***	6.2***	73.3
Leaf number at flowering (leaf)	46.6***	1.2***	19.1***	0.1	2.1*	6.7***	3.0***	78.7
Total dry mass (mg)	33.4***	18.3***	19.3***	0.0	3.1***	6.3***	3.4***	83.8
Reproductive allocation	31.5***	0.01	16.1***	0.1	2.3	5.3**	4.6*	59.8
Root allocation	10.2***	9.1***	0.5	1.2	6.3*	4.2	4.2	25.6
Leaf allocation	35.6***	1.0**	21.8***	0.3	2.2	7.4***	2.8*	70.8
Root to shoot ratio	19.2***	6.0***	1.1*	0.4	7.3**	4.0	3.8	33.6
Leaf structure, anatomy and physiology								
Leaf insertion angle (°)[a]	4.2***	0.3	69.4***	0.1	0.6	1.8	1.1	76.6
Leaf blade ratio (%)[a]	18.9***	10.5***	29.5***	2.1*	2.1	3.1*	1.5	64.1
Specific leaf area (cm^2 g^{-1})	27.5***	4.0***	18.1***	5.3***	4.7**	3.2*	4.8**	67.6
Leaf dry matter content (mg g^{-1})	14.4***	13.9***	1.7**	6.5***	7.6***	2.3	6.0*	52.4
Leaf thickness (μm)	45.8***	0.9*	10.7***	0.1	1.0	9.0***	2.3	66.4
Cell density (cell mm^{-2})	46.5***	15.2***	6.8***	0.2	6.6***	6.9***	1.3	82.0
Stomatal density (st. mm^{-2})	29.9***	14.9***	24.8***	0.0	4.1***	3.0**	0.9	76.7
Stomatal index (% st. $cell^{-1}$)	7.4**	1.1*	29.3***	0.1	3.6*	11.2***	3.4	52.6
ABA content (ng g^{-1} FW)	9.8	20.2***	8.4***	0.7	17.3*	6.1	14.6*	60.5
Net photosynthetic rate (μmol CO_2 s^{-1} m^{-2})	23.2**	–	23.3***	–	–	1.5	–	48.0

The percentage of variance explained (SS_x/SS_{total}) and the level of significance (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) for each factor and the interactions are indicated. Values are from the full model including all interactions. All non-significant terms are reported, but were removed from the final model. Hypothesis testing was based on Pillai-Bartlett statistic in the multivariate analysis of variance (MANOVA) and on *F*-ratios from type III mean squares for all ANOVAs. R^2 is the proportion of total variance absorbed by the final model. All traits but R_{max} and leaf blade ratio were ln-transformed to fulfil ANOVA requirements. Net photosynthetic rate was not measured in WD conditions.

[a]Data are not available for Bay-0 and traits are not included in the MANOVA.

FW, fresh weight.

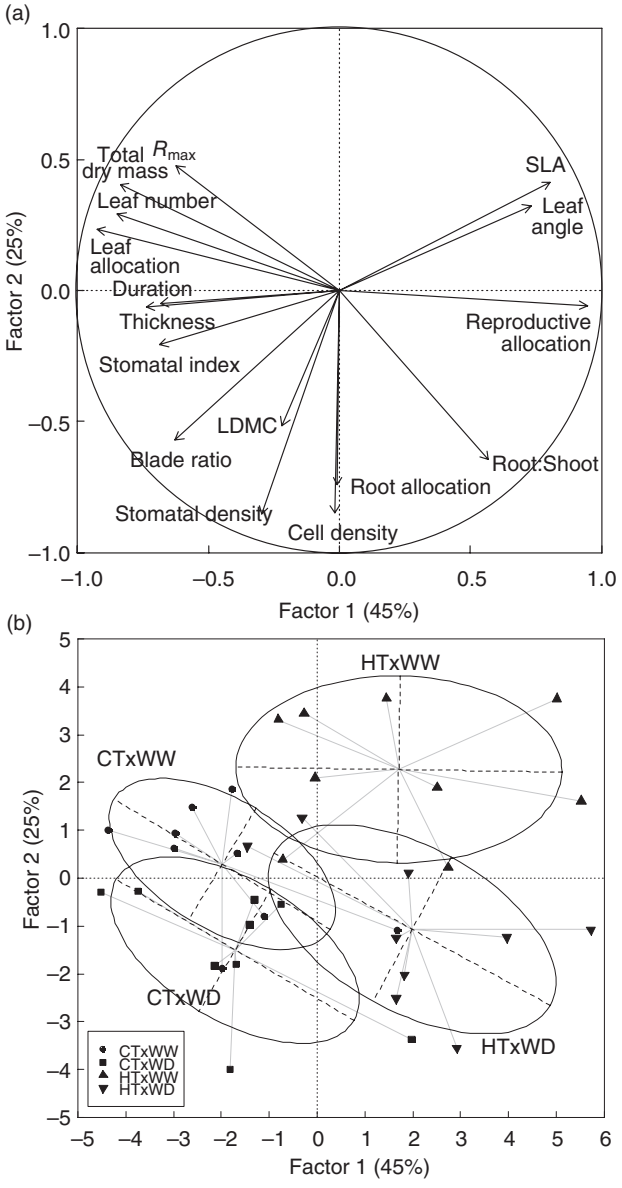

Figure 1. Principal component analysis on traits measured on nine *Arabidopsis* accessions grown under control (CT, 20/17 °C day/night) and high temperature (HT, 30/25 °C day/night), and in well-watered (WW, 0.35 H_2O g^{-1} dry soil) and water deficit (WD, 0.20 H_2O g^{-1} dry soil) conditions. HT and WD treatments were applied after emergence of the first two true leaves and plants were harvested at first visible pod. Only the first two axes are shown. (a) Representation of the variables; LDMC, leaf dry matter content; SLA, specific leaf area. (b) Representation of the accessions with centres of gravity and lines connected to each accession shown for each condition. CT × WW (circles), CT × WD (squares), HT × WW (triangles) and HT × WD (upside-down triangles). Ellipses represent inertia ellipses of each treatment. Each inertia ellipse is centred on the means, its width and height are given by 1.5 times the standard deviation of the coordinates on axes, and the covariance sets the slope of the main axis (Thioulouse *et al*. 1997).

$R_{HT\times WD|C}$ and $R_{WD|C} + R_{HT|C}$ ($r = 0.82$; $P < 0.05$) with a slope not significantly different from one. Compared to other accessions, the growth of Mt-0 was less affected by the combination of HT × WD than by WD only (Fig. 3). To further investigate the genetic variability of responses to HT and WD, we analysed the ranking of the genotypes from the PCA performed on trait values. The rankings were well conserved on PC1 and PC2. The Spearman's coefficients of rank correlation varied from 0.58 to 0.92 (Supporting Information Table S2). This indicated that accessions which exhibited higher value of a trait compared to other accessions in control conditions conserved this advantage when stressed.

Biomass allocation to roots increases under WD and reproductive allocation increases at HT

Biomass allocation also changed at the whole plant and leaf levels in response to isolated and combined WD and HT (Table 2; Fig. 3). Interestingly, at the whole-plant level, WD and HT had different effects on allocation to roots and to reproductive structures. WD resulted in a significant increase in biomass allocation to roots, but reproductive allocation did not change significantly (Fig. 4a). The reverse was found under HT where no changes were detected in the biomass allocation to roots, whereas a significant positive effect was observed on reproductive allocation.

WD and HT have different effects on leaf structure

Leaves produced at HT tended to be thinner and had a higher SLA, while in WD, LDMC was increased (Fig. 4b–d; Supporting Information Fig. S2g–i). More precisely, SLA was much affected by HT in WW conditions and was significantly higher in all genotypes with little variation observed in WD, while LDMC tended to increase in response to WD, particularly at HT, and decrease under HT in WW conditions.

HT but not WD induces leaf hyponasty

In all accessions, HT induced a highly significant increase in leaf insertion angle, that is hyponasty, associated with a significant reduction in the proportion of blade compared to petiole length (Fig. 4e,f; Table 2). WD had no significant effect on hyponasty either at control or HT. By contrast, a significant increase in blade ratio was found in response to WD, resulting in significant water by temperature interaction in the ANOVA for this trait (Table 2).

WD and HT have opposite but additive effects on leaf epidermis anatomy

WD and HT had opposite effects on the cellular anatomy of leaf epidermis, but there was no water by temperature interaction as shown in the ANOVA (Table 2) indicating that the effects were globally additive. Across genotypes, cell and

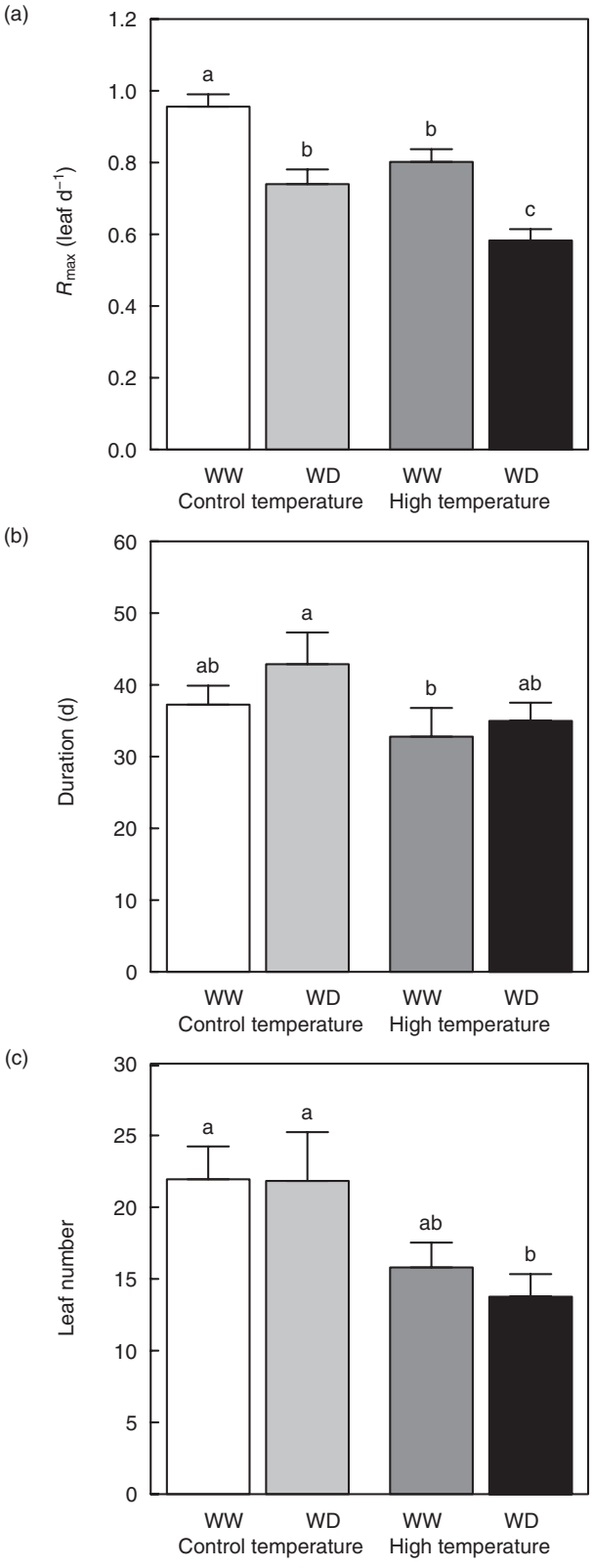

Figure 2. Dynamics of leaf production under control (CT, 20/17 °C day/night) and high temperature (HT, 30/25 °C day/night), and in well-watered (WW, 0.35 H_2O g^{-1} dry soil) and water deficit (WD, 0.20 H_2O g^{-1} dry soil) conditions. Maximum rate of leaf production (R_{max}) (a), duration of leaf production (b) and total leaf number (c). Bars are means + SE of nine accessions. Different letters indicate significant differences following Kruskal–Wallis test ($P < 0.05$).

stomata densities increased in response to WD both at control temperature and HT, whereas these traits tended to decrease in response to HT (Fig. 5). Stomatal index exhibited much less variation, but genotype and treatment effects were detected (Table 2; Supporting Information Fig. S2l–n). HT resulted in lower stomatal index (Fig. 5c). On the contrary, stomatal index tended to increase in response to WD, but the effect of this treatment was not detectable in several genotypes.

Photosynthesis is reduced at HT and ABA content increases under WD and HT

In WW conditions, net photosynthetic rate was significantly reduced by HT from 3.95 ± 0.73 at 20 °C to 3.30 ± 0.56 μmol CO_2 s^{-1} m^{-2} at 30 °C (Fig. 6a; Table 2). No significant genotype by temperature interaction was detected ($P = 0.29$; Table 2). Across all genotypes, leaf ABA content was significantly increased under WD and HT, and it was even more increased in response to the combination of the two stresses WD and HT (Fig. 6b).

Do responses to HT and WD relate to accessions climatic origin?

Beyond mean responses to single or combined treatments, the accessions studied herein displayed a range of sensitivities for their different traits. We explored whether any part of the responses of the accessions was related to the climatic conditions at geographical origin of the populations in which they were collected. The data from the PCA were used in order to reduce the number of comparisons and therefore the risk of type I error.

For each treatment, no trend was observed between accessions coordinates on PC1 from the PCA on trait values and mean monthly temperature at geographical origin of the populations. However, for plants grown under HT in WW conditions, a positive trend was found between coordinates on PC2 and temperature of origin (Fig. 7a). Inspection of Fig. 7 revealed that the accession from Cape Verde Island (Cvi-0) had a contrasted response compared to the other accessions. When excluding Cvi-0 from the analysis, the correlation was high and significant ($r = 0.80$; $P < 0.01$; Fig. 7a). The collection site of this accession presents the higher temperature, although it was reported that Cvi-0 has been collected at 1200 m asl (Tonsor *et al*. 2008), thus possibly encountering lower temperatures. As seen earlier, PC2 was negatively correlated to stomatal and cell density and biomass allocation to roots. Therefore, the accessions that originate from sites with higher temperature tend to have less stomata per unit leaf surface, and to allocate less biomass to the roots than accessions from colder sites when cultivated under HT (Fig. 7b,c).

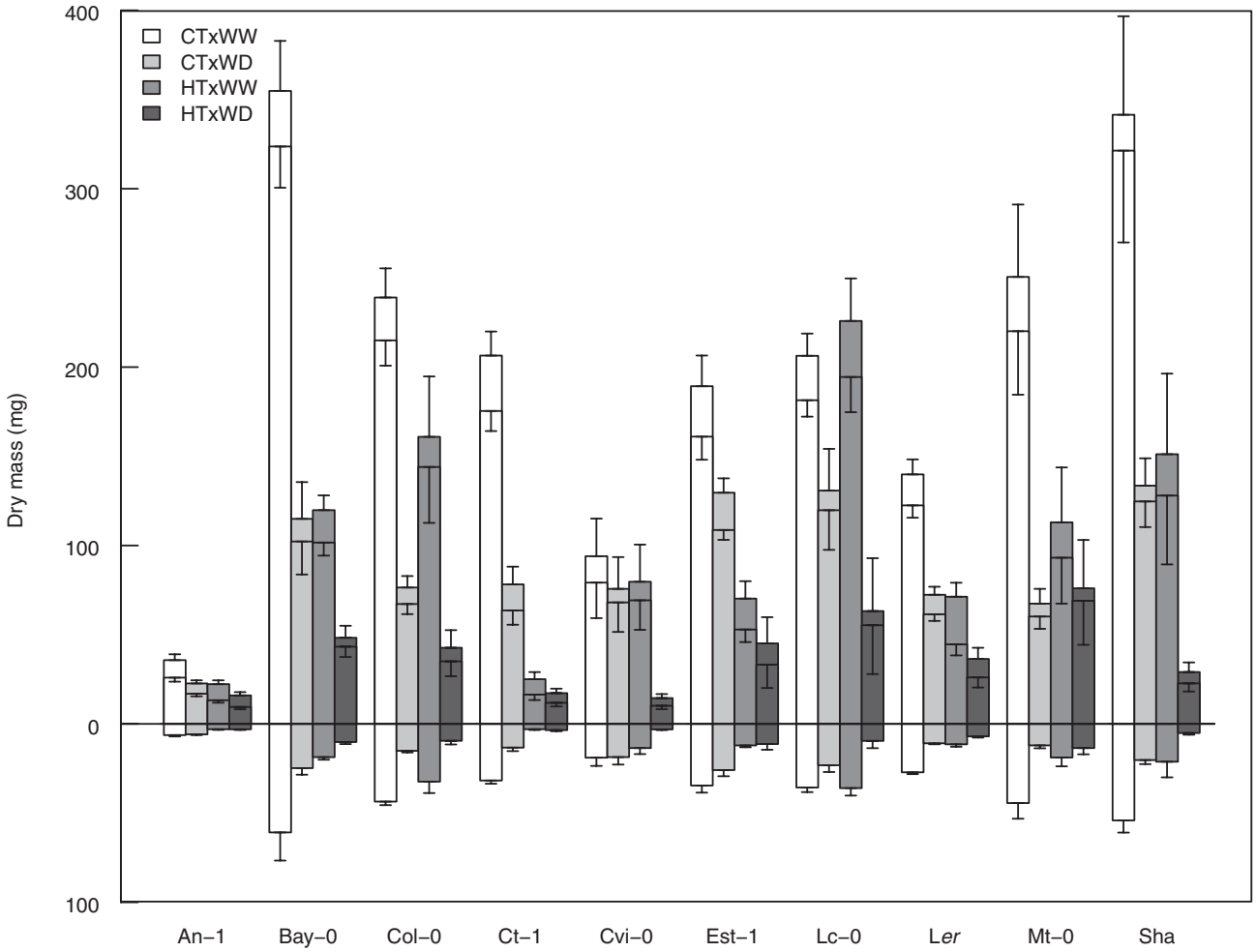

Figure 3. Plant dry mass under control (CT, 20/17 °C day/night) and high temperature (HT, 30/25 °C day/night), and in well-watered (WW, 0.35 g H_2O g^{-1} dry soil) and water deficit (WD, 0.20 g H_2O g^{-1} dry soil) conditions. Bars are means ± SE ($n = 4$ to 9) for the roots (below), vegetative leaves (intermediate) and reproductive stems (top) of 10 *Arabidopsis* accessions.

Positive trends were also found between the coordinates on PC2 from the PCA on trait values and mean monthly precipitation from September to May in all treatments ($r = 0.40$ to 0.73). While not statistically significant, this corresponded to a stronger reduction in stomatal density under WD, HT or both for accessions originating from sites with high precipitations ($r = –0.36, –0.51$ and $–0.56$, respectively).

Relationships between plant traits and tolerance to HT and WD

We explored the relationships between plant traits as measured in controlled conditions and accessions response to HT and WD. A negative correlation was found between absolute plant size in controlled conditions and the response ratio of plant size to the treatments. This trend was significant in response to WD ($r = –0.73$; $P = 0.03$; Fig. 8a) but not to HT ($r = –0.27$; $P = 0.48$) or the combination of HT and WD ($r = –0.50$; $P = 0.17$). Thus, stunted accessions (e.g. An-1) tend to be more tolerant to WD. Furthermore, the root-to-shoot ratio in controlled conditions was positively correlated with the response ratio of plant size to WD ($r = 0.68$; $P = 0.04$; Fig. 8b) and with the response ratio of leaf production rate under combined HT × WD ($r = 0.72$; $P = 0.04$). Thus, accessions with bigger root compartment relative to shoot tended to better maintain growth under WD, and to keep producing leaves at the same rate as control under combined stresses.

DISCUSSION

WD and HT: independent or interacting responses?

Complex interactive responses can occur in plants experiencing multiple environmental stresses (Mittler 2006). Here, we report the single or combined effects of soil WD and HT on a large set of plant traits from the cellular to the whole-plant levels in a collection of accessions of the model plant *A. thaliana*. Plant growth was significantly reduced under HT and WD, and their combination was more

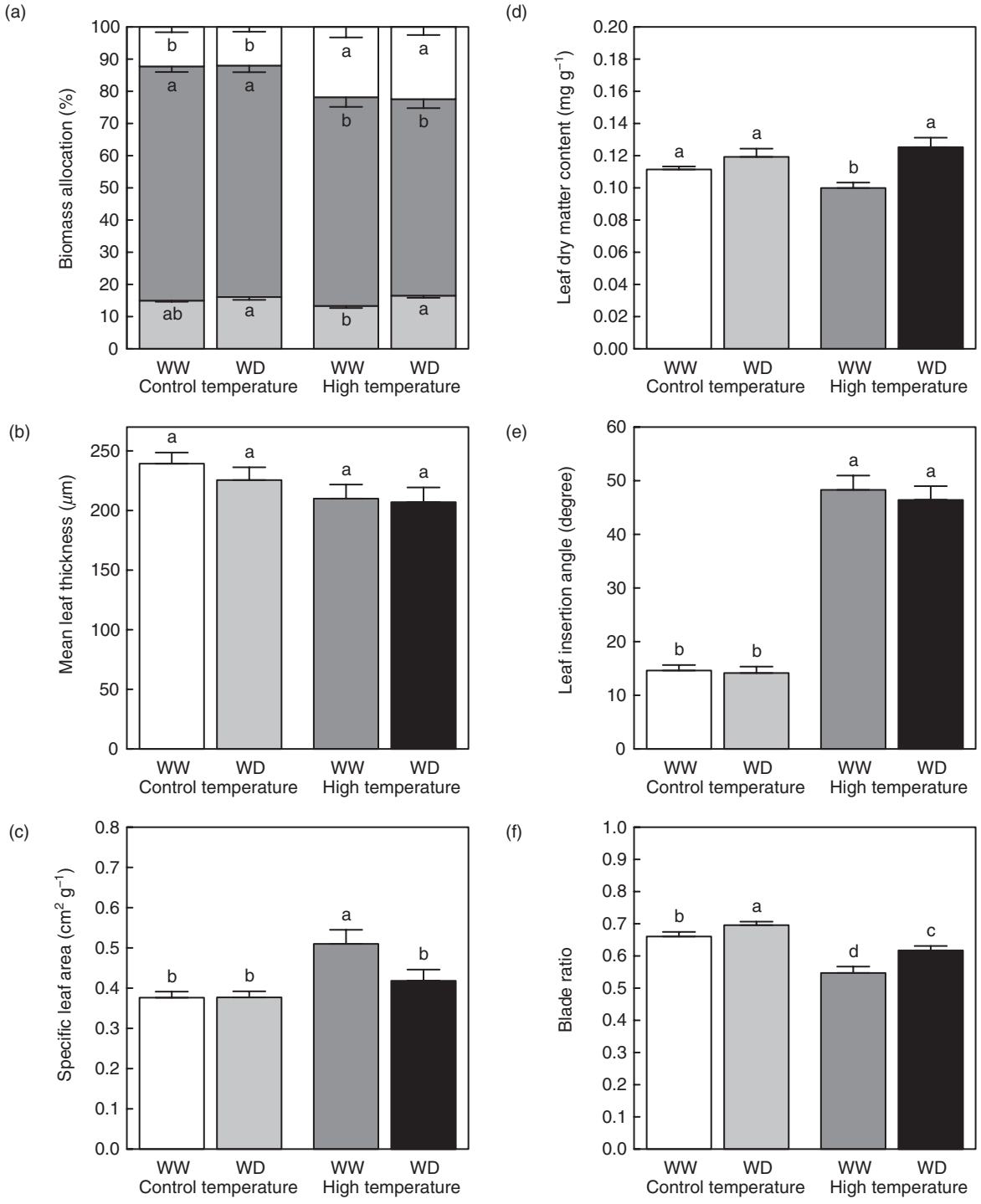

Figure 4. Biomass allocation and leaf morphology under control (CT, 20/17 °C day/night) and high temperature (HT, 30/25 °C day/night), and in well-watered (WW, 0.35 g H_2O g^{-1} dry soil) and water deficit (WD, 0.20 g H_2O g^{-1} dry soil) conditions. Dry mass allocation to the roots (below), vegetative leaves (intermediate) and reproductive stems (top) (a), leaf thickness (b), specific leaf area (c), leaf dry matter content (d), leaf insertion angle (e) and blade ratio (f). Bars are means ± SE of nine accessions. Different letters indicate significant differences following Kruskal–Wallis test ($P < 0.05$).

detrimental to plant performance as also described in previous studies (Xu & Zhou 2006; Prasad *et al*. 2008). Interestingly, single trait as well as multiple traits analyses revealed that the combined effects of these two stresses were globally additive. This held true for traits responding in the same (e.g. plant mass) or reverse (e.g. stomatal density) directions to the two stresses and suggests a certain degree of independency between the mechanisms involved

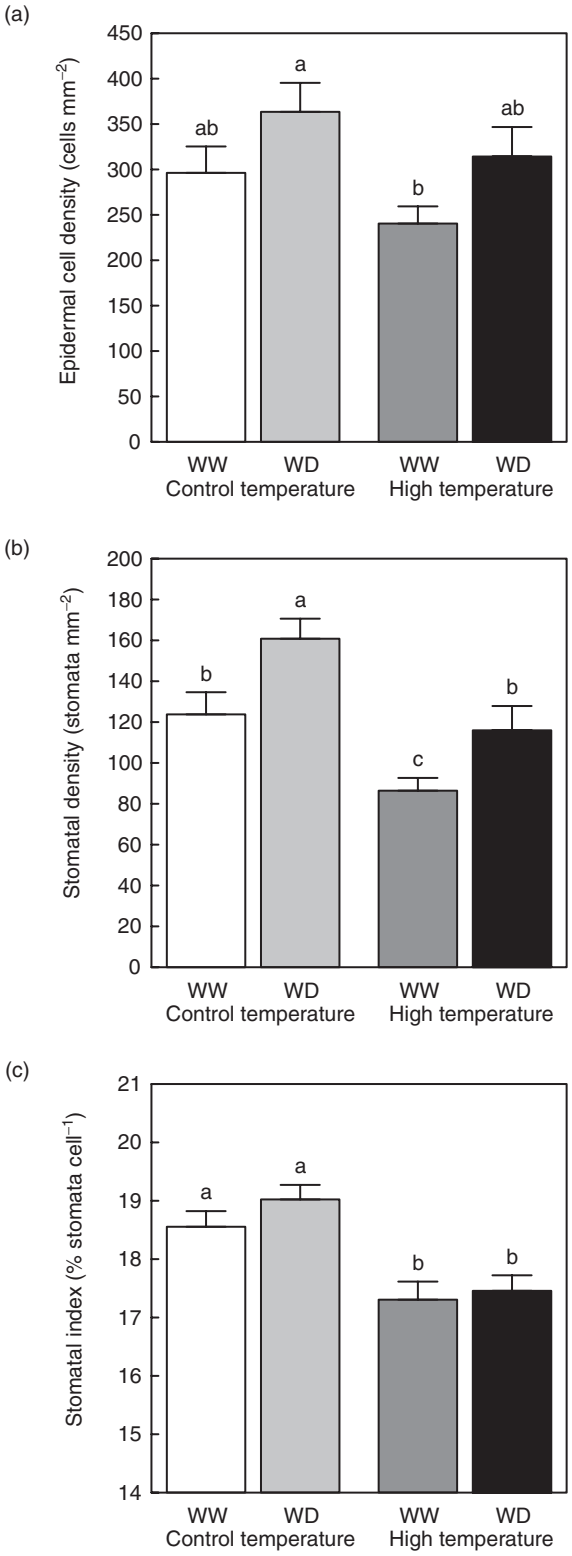

Figure 5. Leaf epidermal anatomy under control (CT, 20/17 °C day/night) and high temperature (HT, 30/25 °C day/night), and in well-watered (WW, 0.35 g H_2O g^{-1} dry soil) and water deficit (WD, 0.20 g H_2O g^{-1} dry soil) conditions. Cell density (a), stomatal density (b) and stomatal index (c). Bars are means + SE of nine accessions. Different letters indicate significant differences following Kruskal–Wallis test ($P < 0.05$).

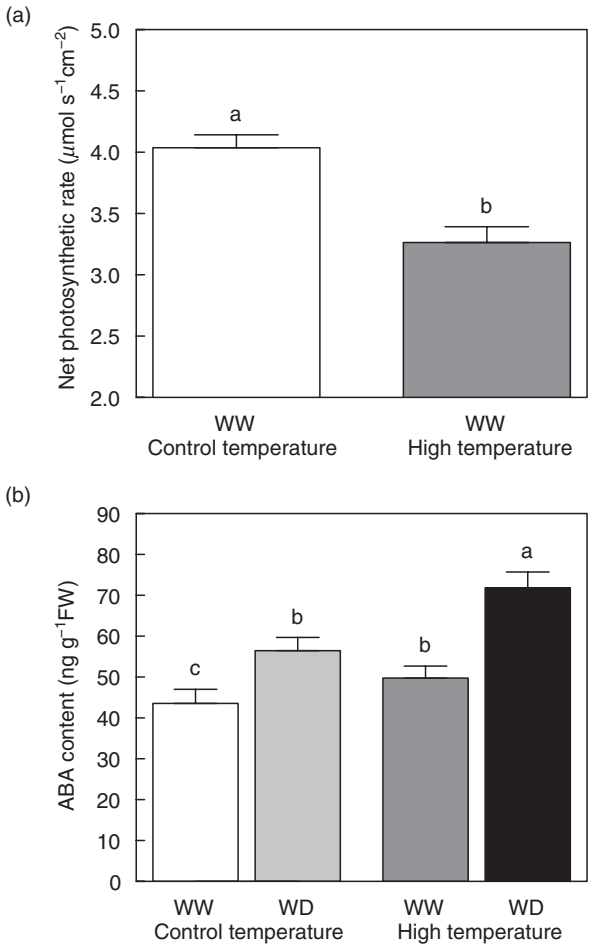

Figure 6. Net photosynthetic rate (a) and abscisic acid (ABA) content (b) under control (CT, 20/17 °C day/night) and high temperature (HT, 30/25 °C day/night), and in well-watered (WW, 0.35 g H_2O g^{-1} dry soil) and water deficit (WD, 0.20 g H_2O g^{-1} dry soil) conditions. Bars represent means + SE of nine accessions. Different letters indicate significant differences following Kruskal–Wallis test ($P < 0.05$). Net photosynthetic rate was not measured in WD conditions. FW, fresh weight.

in the responses to WD and HT applied herein. Some traits were specific of the response to either WD or HT. This was the case for biomass allocation to roots which increased in response to WD, and conversely for reproductive allocation, leaf insertion angle and SLA which significantly increased in response to HT (Xu & Zhou 2006). However, among the large number of traits investigated, no single trait was affected only by the combination of HT and WD. The impact of the combined stresses has been rarely studied. In wheat and sorghum, Machado & Paulsen (2001) found that plant water status in response to HT was highly dependent on soil water availability. The work by Rizhsky and collaborators showed that some molecular responses were specific to the combination of heat and drought compared to either stress alone (Rizhsky *et al*. 2002, 2004). Yet our study is, to our knowledge, the first addressing this issue in different ecotypes and using a broad range of growth, developmental and physiological traits, and the lack of

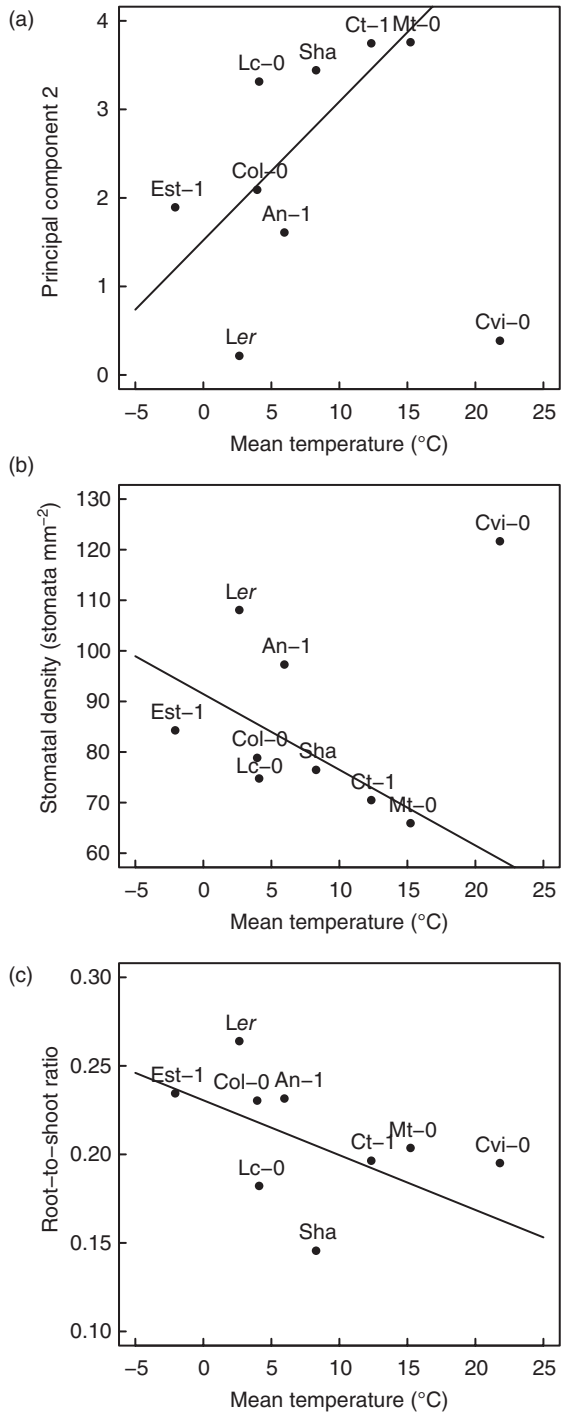

Figure 7. Relationships between mean temperature at the collection sites of nine *Arabidopsis* accessions and (a) PC2 coordinates (Fig. 1), (b) stomatal density and (c) root-to-shoot ratio under high temperature (30 °C) but well-watered (0.35 g H_2O g^{-1} dry soil) conditions.

HT × WD interaction is the rule for most of them, at least for the moderate levels of stresses applied during the whole plant cycle.

As generally found, plant growth dynamics (leaf production and leaf expansion) were significantly impaired in response to HT (Loveys *et al*. 2002) and WD (Granier *et al*. 2006; Hummel *et al*. 2010), leading to reduced plant size at reproductive stage and therefore reduced seed production (Aarssen & Clauss 1992). However, the two stresses had contrasting effects onto the timing of reproduction. As commonly found in natural and crop species (McMaster *et al*. 2009), WD delayed reproduction, but contrasted effects on final leaf number were found across accessions. By contrast, under HT, fewer leaves were produced when early reproduction occurred. Early reproduction following a moderate increase in temperature has been previously reported in *A. thaliana* (Balasubramanian *et al*. 2006) and other species (Barnabas, Jäger & Fehér 2008). However, very sparse data are available on the combined effects of HT and WD on reproductive phenology in natural species (but see Barnabas *et al*. 2008 for a review in cereals). Here, we found that the effects were globally additive in such a way that WD also delayed flowering under HT.

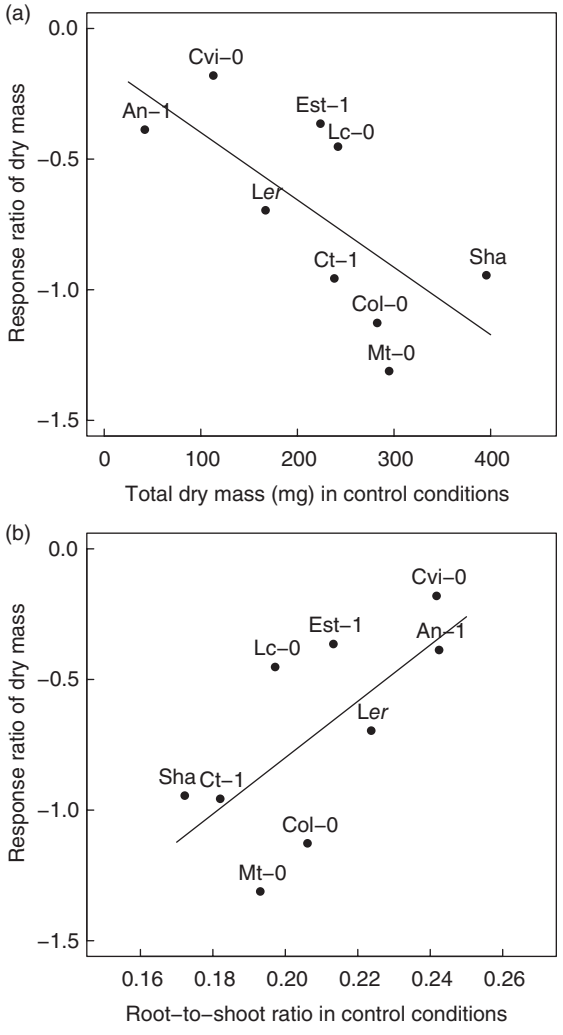

Figure 8. Relationships between the response ratio of total dry mass to water deficit (0.20 g H_2O g^{-1} dry soil/20 °C soil water content/air temperature) of nine *Arabidopsis* accessions and (a) the total dry mass and (b) root-to-shoot ratio in control conditions (0.35 g H_2O g^{-1} dry soil/20 °C).

Although the majority of plants reached the flowering stage and a significant increase in biomass allocation to the reproductive stem was found under HT, flower abortions were clearly visible on later reproductive stages and very few pods reached maturity (not shown). The fecundity of the plants was particularly impaired under combined stresses. This was not surprising since reproductive structures are particularly sensitive to heat stress (Zinn, Tunc-Ozdemir & Harper 2010) and even more to combinations of heat and drought (Barnabas *et al*. 2008). Notably, HTs (31–33 °C) very close to that experienced here (30 °C) have been shown to be sufficient to impair anthers development in non-acclimated plants of *A. thaliana* (Sakata *et al*. 2010). Apparently, vegetative acclimation to long-lasting treatments as experienced here did not change this response.

Is genetic variability of responses related to the climate of origin?

In our study, except the young seedling stage (before the emergence of the firsts true leaves), plants developed entirely under HT, WD or both. This may have led to acclimation processes possibly reinforcing plant tolerance to these stresses. Applying steady-state contrasted temperatures would also have produced different responses than those identified in the case of acute increase of temperature applied at a particular developmental stage as it is largely found in the literature. Nevertheless, a high genotypic variability in traits values was observed in the different growing conditions, and a significant genotype by environment interaction was found. This is not surprising given that the chosen accessions originated from a wide range of environments with varying temperature and drought constraints. A high variability of traits related to growth and phenology has been identified in natural populations of *A. thaliana* (Montesinos-Navarro *et al*. 2011). And genotypic variability among natural accessions has previously been identified for traits related to adaptation to WD (McKay, Richards & Mitchell-Olds 2003) and temperature (Tonsor *et al*. 2008). Here, we applied a HT level within the physiological range of *A. thaliana* and close to the basal thermotolerance of the accession Col-0 (Ludwig-Muller *et al*. 2000). Unfortunately, as far as we know, basal thermotolerance has not been consistently evaluated for other accessions than Col-0. Therefore, we cannot exclude that the variability of responses to HT observed here between the accessions was related to contrasted basal thermotolerance, which could also depend on the environment encountered in their habitat of origin. Few relationships between plant tolerance to HT and the climatic environment at the collection site of the accessions were found in this study. This is in accordance with Loveys *et al*. (2002) who found no relationship between thermal origin of the accessions and the production of dry matter in response to increasing temperature at the interspecific level. However, a lack of association could arise from the small number of accessions considered in our study. In a more geographically restricted study but including a large set of *Arabidopsis* natural populations, Montesinos-Navarro *et al*. (2011) showed that the variation of traits exhibited in controlled conditions was consistent with the temperature and water constraints encountered at the collection sites along an altitudinal gradient, pointing towards a likely adaptive differentiation of the populations to the environmental conditions. Here, we found that accessions that originate from sites with higher mean temperature during the vegetative growth tend to have less stomata per unit leaf surface, and to allocate less biomass to the roots than accessions from colder sites when grown under HT.

Stomatal density and plant response to HT and WD

Despite the prevailing opinion that stomatal density would increase in response to HT (Wahid *et al*. 2007), data from the literature are not unanimous (see Luomala *et al*. 2005). Indeed, it is most likely that stomatal density depends on tight interactions between plant water balance (water status and transpiration) and the environmental conditions, particularly relative humidity and VPD (VPD_{air}) encountered by the plant during leaf growth (Lake & Woodward 2008). Assuming that conditions favouring expansion dilute stomata at the leaf surface, increases in humidity in the vicinity of the plant are expected to reduce stomatal density. In this study, the possible effects of VPD_{air} on stomatal density at HT were excluded since VPD_{air} was maintained equal between the control (20 °C) and the HT (30 °C) treatment. In order to fulfil this condition of constant VPD_{air}, air relative humidity was maintained higher under HT (85%) than under control (65%) conditions, possibly favouring the development of leaves with lower stomatal density at HT compared to control temperature. This was observed despite the significantly higher transpiration rate under HT compared to control temperature (Supporting Information Fig. S4). In addition, our results unequivocally show that soil WD led to increases in stomatal densities either at control or HT, thus counteracting the effects of HT. The same trend of decreasing and increasing stomatal density in response to HT and WD, respectively, was found in almost all genotypes. Despite the fact that VPD_{air} was maintained equal between the two temperature treatments, accelerated depletion of soil water or lower leaf water potential may have interfered with plant responses at HT due to higher rates of transpiration (Machado & Paulsen 2001; Supporting Information Fig. S4). Interestingly, relationships were found between stomatal density and meteorological conditions at the collection sites. Stomatal density was lower in accessions collected in warmer sites and/or sites with higher amount of precipitations, particularly when considering the responses to HT and WD.

Contrary to what was suggested by Lake & Woodward (2008), we found no relationship between ABA content in the rosette leaves and stomatal density. We cannot exclude a differential response of abaxial versus adaxial leaf epidermis in our experiments (see Luomala *et al*. 2005); however,

we observed that stomatal densities of both sides of the leaves are correlated either under WW or WD conditions (Vile & Pervent, unpublished results).

Similarities between responses to HT and low light

It is noteworthy that some of the specific responses to HT were also characteristic of responses to low light intensity. For instance, it is well known that SLA increases and LT decreases in response to low light (Poorter *et al*. 2009), and that shade leaves have higher SLA and are thinner than leaves exposed to direct sun light (McMillen & McClendon 1983). Chabot & Chabot (1977) reported that decreasing light and moderately elevated temperature had similar effects on thickness. In *Arabidopsis*, a clear similarity between the responses to light and HT resides also in hyponastic growth, that is the increase in leaf insertion angle (Van Zanten *et al*. 2009). These authors reported very similar trends of variation in leaf angle in response to HT and low light, and we have recently shown that the hyponastic response to HT can be reversed by increasing light intensity (Vasseur, Pantin & Vile 2011). Taken together, these results suggest that part of the responses to a moderate heat stress could be associated to a defect in carbon acquisition through photosynthesis, which is impaired under HT, and/or an increased competition for carbon use due to enhanced physicochemical processes and increased protection mechanisms (notably heat shock proteins; Heckathorn *et al*. 1996). Accordingly, tolerance to warm temperatures is increased at high CO_2 concentration in C3 plants (Huxman *et al*. 1998; Taub, Seemann & Coleman 2000), and decreased at low nitrogen supply due to a limited production of nitrogen-costly heat shock proteins (Heckathorn *et al*. 1996). The interactive effects of HT and light on plant functioning were analysed here under lower light than encountered in natural conditions. To test whether our results would hold under higher light conditions as found in the nature, especially at HT, experiments should be performed at higher light intensities. Interactions between WD, HT and light also remain to be investigated (Vasseur *et al*. 2011).

Inherent trait variation and plant tolerance to HT and WD

Ecological research has engaged major efforts to identify plant traits, as measured in controlled or natural conditions, that could be good predictors of plant responses to changes in their environment (Grime 2001; Vile, Shipley & Garnier 2006; Violle *et al*. 2007). Here, we found a trade-off between plant size in control conditions and tolerance to WD. A similar negative relationship between plant size and plant tolerance to WD was found in an analysis of 20 accessions capturing much of the genetic variation of *A. thaliana* worldwide (Clark *et al*. 2007) and a new collection of 88 accessions from Europe and Asia (Bouteillé *et al*., unpublished results; $r = -0.54$ and -0.25; $P = 0.013$ and 0.022, respectively). A re-analysis of the data from Bouchabke *et al*. (2008) also showed a significant negative relationship between total leaf area in WW conditions and its response to a mild WD applied for 10 d ($r = -0.49$; $P = 0.014$). Interestingly, we found a similar ranking of responses to WD for the six common accessions (but Sha to a lesser extent) between Bouchabke *et al*. (2008) and our study. Such a trade-off between plant size and the response ratio to WD was also found in a re-analysis of the data of a recent study on stress-related specific mutants of *Arabidopsis* (Skirycz *et al*. 2011), although plant size variation between lines was weak ($r = -0.43$; $P = 0.014$). These authors report that growth reduction caused by stress was independent of plant size under control conditions, but they used the relative response of mutants compared to the wild type, not the response ratio for each line. A first explanation for this trade-off would reside in the fact that large plants consume more water and therefore experience greater water shortage. However, the experimental procedure used in the present study as well as in Bouchabke *et al*. (2008) and in Skirycz *et al*. (2011), that is a daily irrigation to adjust the soil water content, is unlikely to have favoured small plants that consume less water. A trade-off between plant size and plant tolerance to WD is in accordance with the results of He *et al*. (2010) that populations of *Centaurea stoebe* with inherently bigger plant size are more susceptible to stressing (water and nutrient) conditions. In contrast to these authors, who did not observe any relationship with other traits than plant size, here, we found a positive relationship between the root-to-shoot ratio and plant tolerance to WD which could give a proportionate advantage under inherent water shortage.

On the other hand, the negative trend between plant size and *Arabidopsis* tolerance to HT was weaker and not significant. No single trait was identified as a good predictor of plant response to HT. Some elements suggest that changes in leaf inclination could participate to thermotolerance adjustments by reducing intercepted light and hence tissue temperature (Salvucci & Crafts-Brandner 2004). Although leaf insertion angle increased in response to HT and this response varied between accessions, in our data, hyponasty was not related to thermotolerance. Furthermore, in contrast to the results of Van Zanten *et al*. (2009), no relationship was observed between the change in leaf angle in response to HT and the diurnal temperature range at the geographical origin of the accessions. This discrepancy could in part be explained by the higher but shorter temperature treatment experienced in Van Zanten *et al*. (38 °C during 7 h) compared to our study (30 °C during *c.* 15 d).

Finally, plant tolerance to WD under HT, in terms of plant size reduction, was also related to plant size in WW and control temperature conditions albeit the relationship was weaker than for WD under control temperature. Thus, inherent plant size would participate in soil–water–plant relationships to a larger extent than to the response to increasing temperature.

CONCLUSION

Despite the likely interactive processes involved in plant response to HT and WD, here, we showed that at least moderate levels of these two stresses have additive effects on a large set of plant traits related to growth and development in the model species *A. thaliana*. This would have important consequences for modelling plant growth under combined stresses. Some traits were affected only by one or the other stress, highlighting the specific sensitivity of some processes such as reproduction in response to HT and resources allocation for a better water acquisition in response to water deprivation. In natural environments, variation in temperature and water availability can act together or independently on co-varying traits and on the distribution of plant species. It was therefore not surprising to find a significant natural variation in *Arabidopsis* tolerance to HT and WD applied separately or in combination. Genetic variability in the responses of several traits to the different stresses accompanied this natural range of tolerances and was in good correspondence with some characteristics of the climatic origin of the natural populations. This opens several avenues to explore the underlying physiological processes shaping the distribution of this and other species.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Leaf production dynamics in *A. thaliana* Col-0.
Figure S2. Mean trait values by genotypes and treatments.
Figure S3. Correlation matrix of response ratios for total dry mass.
Figure S4. Night and day transpiration rates of Col-0 and L*er* accessions.
Table S1. Loadings of the variables included in the PCA on mean trait values.
Table S2. Correlations between genotypes coordinates on first and second principal components from the PCA performed on trait values.

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