Impacts de conditions environnementales sous-optimales sur le spectre d’économie foliaire des ressources au sein d’espèces céréalières

Présentation générale

Ce troisième chapitre concerne l'ensemble des études réalisées sur le **maïs, le blé tendre, le mil et le sorgho**, cultivées dans la **plateforme de phénotypage haut-débit** PhenoDyn. À partir des **lignées** de ces quatre espèces cultivées, les principaux objectifs de ce troisième chapitre sont d': i) analyser le spectre d'économie foliaire des ressources entre et au sein de quatre espèces cultivées, ii) explorer la plasticité de ce compromis écophysiologique sous conditions sous-optimales chez les espèces cultivées et iii) examiner les relations entre le spectre d'économie foliaire et la tolérance au stress hydrique.

Avec l'expérimentation 3, quatre **traits fonctionnels**, dont deux associés au spectre d'économie foliaire et les deux autres à la tolérance au stress hydrique, ont été étudiés chez quatre espèces céréalières (maïs, mil, sorgho et blé tendre) dans des conditions optimales et de stress hydrique (voir la partie Matériels et Méthodes pour plus d'informations). À l'échelle interspécifique, il existe un compromis écophysiologique entre les deux traits fonctionnels associés au spectre d'économie foliaire. Ce compromis est maintenu dans les deux traitements hydriques et illustre **un continuum 'rapide-lent'** au sein des espèces cultivées. À l'inverse, à l'échelle intraspécifique, la relation entre les deux traits est soit faible, soit non significative. Au sein des espèces, les gammes de variation des deux traits ne permettent pas d'observer de relations significatives entre eux. Pourtant, les genotypes sont toutes présentes à **l'extrémité « acquisition des ressources »** dans l'enveloppe des contraintes illustrées chez les espèces sauvages. Ce résultat suggère d'importants facteurs sous-jacents au spectre d'économie foliaire, tels que des mécanismes physiologiques et structuraux. Ces résultats suggèrent une limite à la diversité phénotypique foliaire possible chez les espèces céréalières. Au sein de la même espèce, l'impact du stress hydrique sur la croissance foliaire varie entre les génotypes. Cette variabilité est liée à la taille de la plante, et non aux traits associés au spectre d'économie foliaire en conditions optimales. Chez les quatre espèces céréalières étudiées, la **stratégie de tolérance à la sécheresse** ne peut pas être discriminée par le compromis écophysiologique entre la conservation et l'acquisition des ressources. Nos résultats soulignent les bénéfices d'utiliser une approche à la fois écologique et agronomique pour caractériser la diversité des espèces cultivées face au stress hydrique.

Les résultats obtenus avec cette étude sont présentés sous la forme d'un article scientifique.

Does the leaf economics spectrum hold within and between crop species under both optimal and suboptimal conditions?

Agathe Roucou[1,2], Cyrille Violle[1*], Boris Parent[2], Adel Meziane[2], Alexis Bediee[2] and Denis Vile[2].

[1] Centre d'Ecologie Fonctionnelle et Evolutive (UMR 5175), CNRS, Université Paul Valéry Montpellier, EPHE, Univ Montpellier, Montpellier, France.

[2] Laboratoire d'Ecophysiologie des Plantes sous Stress Environnementaux (LEPSE), UMR759, INRA, SupAgro, Univ Montpellier, Montpellier, France.

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Introduction

Plant functional ecology has identified a set of biological laws that seem to hold for most plant species living on earth (Garnier et al., 2016). Notably, the leaf economics spectrum (LES hereafter) depicts leaf trait covariations that reflect physiological compromises at the leaf level (Wright et al., 2004). On one extreme of the spectrum, one can find species adapted to harsh environments and characterized by leaves with slow metabolism and dense tissues to cope with multiple abiotic and biotic stress (Reich, 2014). On the other end of the spectrum, species found in less stressful and more productive environments typically exhibit leaves with fast return on investment through high photosynthetic rate and thin leaves to maximize light capture (Reich, 2014). While interspecific comparisons have been very fruitful, they remain largely silent about the ecophysiological mechanisms underlying the trait-trait relationships described. First, the leaf economics spectrum is classically associated to the fast-slow continuum (Reich, 2014; Salguero-Gomez et al., 2016), but leaf growth dynamics has never been characterized in that context. Second, the response to abiotic stress is supposed to be a key driver of the LES, but the LES has been most often examined in natural conditions where environmental conditions are hardly characterized (Falcao et al., 2017; Mediavilla et al., 2018), or in experimental optimal conditions for plant growth (Baird et al., 2017; Claeys and Inze, 2013). Recently, Volaire (2018) built a bridge between ecophysiology and functional ecology in the specific context of plant response to water deficit: species/genotypes that display high return on investment should be less adapted to water deficit. Even if genotypes can buffer the negative impacts of environmental harshness on the phenotype's performance through phenotypic plasticity (Przybylo et al., 2000; Stearns, 1989; Sultan, 2000), the incorporation of this widespread and well-known mechanism in the theoretical corpus of the LES is lacking. Experimental tests are urgently needed to evaluate whether contrasting genotypes located in the LES are more or less impacted by environmental stress such as soil water deficit, or whether, on the contrary, they can modulate their response through phenotypic plasticity.

While the LES has been mostly described for wild species, recent evidence suggest its robustness in crop species, too (Donovan et al., 2014; Milla et al., 2014; Martin et al., 2017; Roucou et al., 2018). Such a test in crop species benefits to both functional ecology and crop science. On one hand, using crop species allows the use of well-known genetic material, and the subsequent intraspecific analysis of the LES. Intraspecific studies of trait-trait relationships permit to bypass the phylogenetic inertia inherent to interspecific comparisons and to go deeper into the physiological mechanisms (Albert et al., 2010; Fajardo and Siefert, 2018; Martin et al., 2017). There is indeed a living debate in functional ecology about the causes of the LES (Donovan et al., 2011), notably regarding the respective role of natural selection and biophysical constraints (Donovan et al., 2011; Messier et al., 2017; Vasseur et al., 2012). If the LES still holds within species, and in cross-genotype comparisons under both optimal and sub-optimal conditions, this would suggest a strong canalization through physiological and/or biophysical constraints (Onoda et al., 2017). The maintenance of LES relationships in both wild and crop species would also suggest that both natural and artificial selection are regulated by the same constraints. On the opposite, if the plastic response of genotypes can lead to strong shifts in LES relationships (Anderegg et al., 2018), this would suggest that the LES cannot be seen as a universal envelope of constraints for both genotypes and species, but mainly as a by-product of physiological regulation. The theoretical corpus of functional ecology can benefit to crop science, too. If the trade-offs underpinning the LES hold in crop species, the associated trait covariations need to be accounted for in plant breeding programs since, for instance, enhancing plant performance and leaf-level metabolism could be at the detriment of plant defense (Milla et al., 2015; Prieto et al., 2017).

Empirical and modelling approaches have been used to analyze the responses of crops to water deficit (WD) from the soil to the atmosphere through the study of morphological, physiological and molecular features (Farooq et al., 2009). Studies on mechanisms of drought tolerance thus span many levels of biological organization (e.g., genes, cells, organs, individual, ecosystems) (Bengough et al., 2011; He et al., 2017; de Lima Pereira et al., 2016; McDowell et al., 2008), but aboveground, and especially leaf-level, studies are predominant. Indeed, carbon and water fluxes between the plant and the atmosphere are primarily mediated by the active leaf surface (Brodribb and McAdam, 2011). Responses of the active leaf area to water stress are observable through macroscopic changes in morphological and physiological traits (Pang et al., 2011; Wellstein et al., 2017). Nevertheless, linking leaf structure and functions, related to resource fluxes in particular, in the context of plant response to water stress has been hardly done. Analyzing such a linkage through the analysis of LES relationships can reveal shifts in leaf functioning when the plant is submitted to harsh environmental conditions (Ackerly, 2004; Craine and Lee, 2003). For example, an increase in leaf mass per area can be related to a reduction of leaf elongation rate (Arredondo and Schnyder, 2003), a potential phenotypic adaptation to dehydration under water stress (Grossiord et al., 2017). As such, studying simultaneously leaf architecture, metabolism and elongation should improve our understanding of leaf strategy in response to water deficit.

Taking advantage of both ecological and ecophysiological approaches, here we experimentally investigate the effect of soil water deficit on LES traits and their covariations, using four staple crops of high economic and societal importance: maize, pearl millet, sorghum and wheat. We focused on two pivotal LES traits: leaf mass per area, i.e. the ratio of leaf dry mass to surface, and net photosynthetic rate. We also measured two size-related traits: leaf elongation rate and plant dry mass, as proxies for leaf and plant performance under both well-watered and water deficit conditions (Pang et al., 2011; Passioura and Munns, 2000; Wang et al., 2008). The change in (or robustness of) LES patterns when navigating across taxonomic scales, in particular within *versus* between species, represents a research front in functional ecology (Messier et al., 2017). To our knowledge, no study has investigating the connection between the LES and the 'fast-slow' continuum quantified at the leaf level. To develop further statements about crop response to water stress, interspecific and intraspecific comparisons are necessary. Indeed, studies with species originated from different ecological areas, with different breed histories and from temperate to tropical zones, enable to highlight the commonality and diversity of leaf response to environmental conditions (Parent and Tardieu, 2012). In that context, the normalization of leaf elongation rate by their absolute rates at 20°C help to discriminate growth response to environmental conditions among and within species (Parent et al., 2010). Benefiting from known genetic material, we used between 19 and 30 genotype lines of the four studied species in order to compare intraspecific structures of LES trait covariations, and connect them to dynamical traits for discriminating drought strategy.

Materials and methods

Plant material

We selected 30 genotypic lines from maize (*Zea mays*), 23 from pearl millet (*Pearl millet*), 19 from sorghum (*Sorghum bicolor*) and 25 from wheat (*Triticum turgidum*). First, those genotypes were chosen to maximize genetic diversity of species. Then, this panel was restricted to some genotypic lines, to maximize the phenotypic diversity of drought response.

For maize, drought response was studied through response of leaf elongation to soil water content. Fifteen temperate and fifteen tropical lines were selected. For pearl millet and sorghum, drought response was studied through water use efficiency. For wheat, 25 genotypic lines were chosen for their diversity of geographical localizations drought resistance.

Experimental design

We set up the experiment at the INRA-SupAgro campus (France, 43° 37' 02''N, 3° 183 51' 18'' E) in the phenotyping PhenoDyn platform (https://www6.montpellier.inra.fr/lepse/M3P) for a duration of 35 days in controlled conditions (minimal temperature = 19°C and maximal temperature = 26°C). Plants were grown in 9-L pots (17.5 cm diameter and 38.5 cm depth) filled with a soil

30/70% (v/v) of clay and organic compost. Plants were maintained at a soil water potential higher than -0.05 MPa and lower than -0.1 MPa under well-watered conditions, and between -0.3 and -0.4 MPa under water deficit environment.

Leaf trait measurements

We determined maximal photosynthetic rate (A_{max}, nmol CO_2 s^{-1}) at vegetative stage on a fully expanded leaf (the fifth, sixth or seventh leaf produced) by using a portable photosynthesis system with a leaf assimilation chamber (CIRAS, PP systems and LI-6400XT, LI-COR). Measurements were performed at constant relative humidity (80%), ambient temperature (25°C), constant $[CO2]_{atm}$ (400 ppm), and at saturating light (PAR = 1500 µmol s^{-1} m^{-2}) on 30, 8, 10 and 25 genotypes from respectively maize, pearl millet, sorghum and wheat.

Just before harvest, the same leaves were collected and rehydrated at 4°C during 24 hours. Then, we scanned them (150 dpi) and leaf area was determined by image analysis using the ImageJ software (U.S. National Institutes of Health, Bethesda, MD, USA) on 23, 19 and 25 genotypes from pearl millet, sorghum and wheat. Then, each leaf was dried at 60°C during 72 h to determine leaf dry weight. For pearl millet, sorghum and wheat genotypes, we calculated leaf mass per area (LMA, g m^{-2}) as the ratio of leaf dry weight to leaf area. For maize genotypes, after leaf width and length measurements, 10 punches were made on both sides of the main leaf vein (area of one punch = 50.26 cm^2). Those punches were dried at 60°C during 72 h to determine their total dry weight; leaf mass per area (LMA, g m^{-2}) was calculated by the ratio of their dry weight to the area represented by all the punches. Then, for all species, we used LMA to calculate the maximal photosynthetic rate per unit of leaf dry mass as A_{mass} (nmol CO_2 g_{-1} s_{-1}). Plant dry mass (PDM, g) was measured by aboveground dry weight at harvest.

For each sowing, leaf elongation (mm) was measured daily on the fifth leaf for wheat genotypes and the sixth for the others every 15 minutes with rotating displacement transducers (RDTs 60-1045 Full Smart Position Sensor; Spectrol Electronics, Ltd, Wiltshire, England) from 9 AM to 00 PM during 5-12 days - depending on leaf maturation - following the protocol detailed in Sadok *et al*. (2007). For each day, we calculated mean and standard deviation of leaf elongation. The means which were inferior to 1.5 of standard deviation were considered as measurement errors and were removed. The leaf elongation rate (LER, mm d^{-1}) was calculated by averaging the means of the first three days.

Statistical analyses

Within each species, we computed genotypic means of each trait under both watering conditions using a linear fixed-effect model with genotypes, watering conditions and their interaction as fixed factors. Variance partition between genotype, watering condition and their interaction was performed using ANOVA for each trait. Statistically significant differences were determined using pairwise comparisons based on Tukey-adjusted least-squares means.

Across and within species, for studying correlations between leaf (LMA and A_{mass}) and size-related traits (PDM and LER), we calculated bivariate Spearman's correlation coefficients between traits. Trait-trait relationships were characterized using standardized major axis (SMA) regressions on log-transformed trait values per watering condition. For size-related traits, we calculated the logarithm of the response ratios of plant aboveground dry biomass and leaf elongation rate as lnRR = $Trait_{WD}/Trait_{WW}$ in order to compare plant responses to WD between genotypes.

All statistical analyses were performed in the R statistical environment (R Core development team, 2017) using appropriate packages. Genotypic means were extracted using the 'lsmeans' function from the *emmeans* package. We fit SMA regressions and tested for differences in slopes and shift along main axis between watering conditions with the *smatr* package.

TABLE 1 List of morphological and physiological studied traits, their abbreviations and units.

Trait	Abbreviation	Units
Leaf traits		
Maximum net photosynthetic rate	A_{mass}	nmol g^{-1} s^{-1}
Leaf mass per area	LMA	g m^{-2}
Leaf elongation rate	LER	mm d^{-1}
Plant dry mass	PDM	g

Results

At the interspecific level, A_{mass} and LMA covaried in a pattern that is consistent with the LES under both WW ($r_{Spearman} = -0.68$, $P < 0.001$) and WD ($r_{Spearman} = -0.70$, $P < 0.001$) conditions (Fig. 1). In our greenhouse conditions, A_{mass} and LMA mean values of the four species were located in the upper part of the negative A_{mass}-LMA relationship quantified for wild species worldwide (Supporting Fig. S2). Maize and sorghum had significantly higher A_{mass} and LMA values than pearl millet and wheat (Table 2).

LER values discriminated genotypes from maize to wheat along the LMA-A_{mass} relationship under WW but not under WD (Fig.1), which indicates that the LES reflects a fast-slow continuum at the leaf level in optimal conditions for plant growth. More precisely, under WW, LER values were significantly positively correlated to A_{mass} ($r = 0.51$ with $P < 0.001$) and negatively to LMA ($r = -0.48$ with $P < 0.001$) across species (Fig. 2B and D).

TABLE 2 Changes in leaf traits of the four studied species (maize, pearl millet, sorghum and wheat) in response to water deficit. Values are probabilities associated with genotype, watering treatment and their interaction effects in linear fixed-effect models. The number ***n*** of genotypes studied in each species is reported. For abbreviations, see Table 1.

Species		A_{mass}	LMA	LER	PDM
Zea mays	**Genotype**	0.03	< 0.0001	0.03	0.004
(Maize, n = 30)	**Watering**	< 0.001	< 0.001	< 0.001	< 0.001
	Genotype * Watering	0.71	< 0.001	0.05	0.25
Pearl millet	**Genotype**	0.08	0.0002	0.04	0.0003
(Millet, n= 23)	**Watering**	0.32	< 0.001	< 0.001	< 0.001
	Genotype * Watering	0.48	0.16	0.81	0.01
Sorghum bicolor	**Genotype**	0.08	0.0002	0.04	0.0003
(Sorghum, n = 19)	**Watering**	0.32	< 0.001	< 0.001	< 0.001
	Genotype * Watering	0.48	0.16	0.81	0.01
Triticum aestivum	**Genotype**	0.11	< 0.001	< 0.001	0.0003
(Wheat, n = 25)	**Watering**	0.03	< 0.001	< 0.001	< 0.001
	Genotype * Watering	0.53	0.05	0.46	0.82

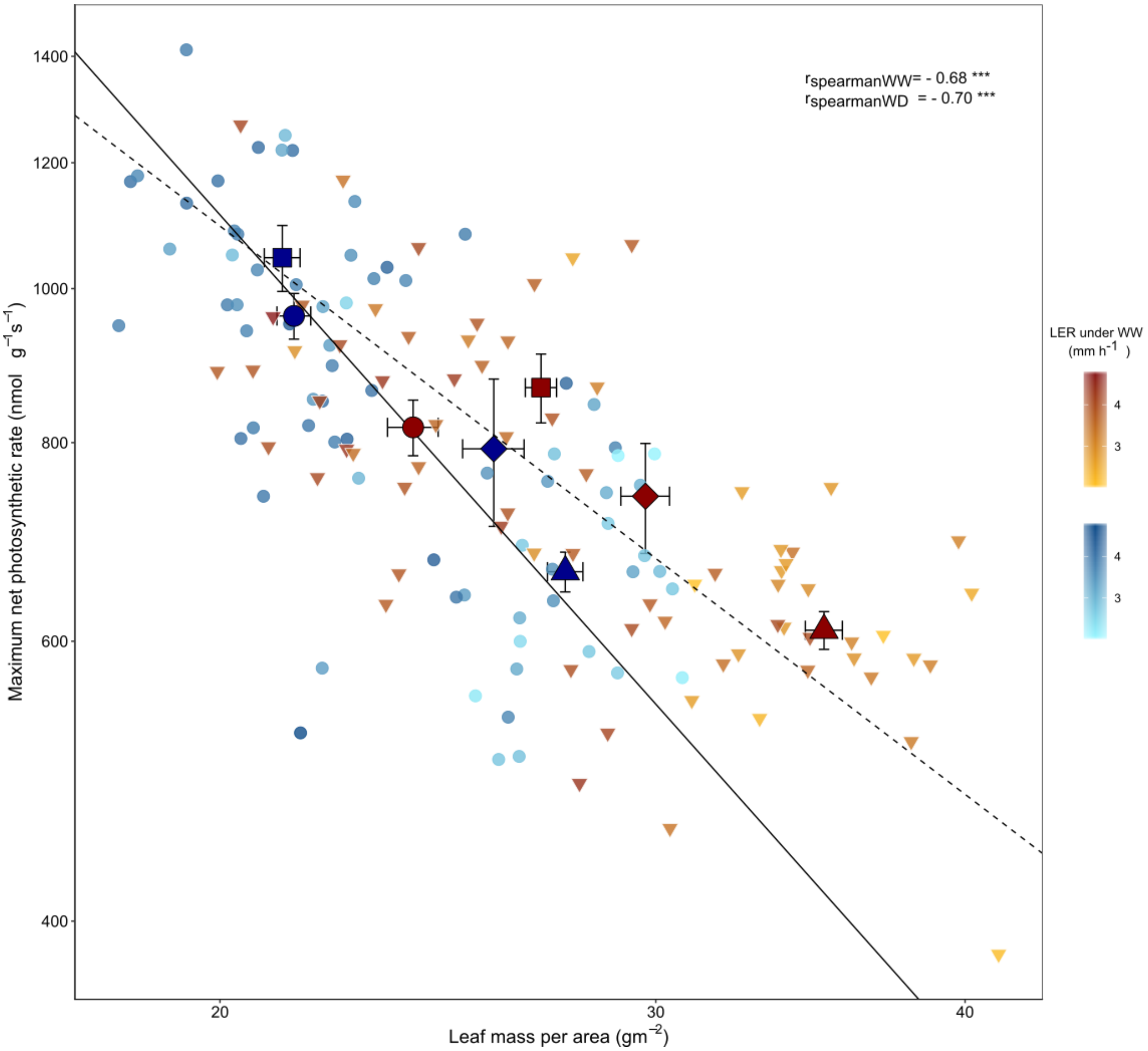


FIGURE 1 Relationship between leaf mass per area (LMA) and maximum net photosynthetic rate (A_{mass}) among and within four crop species cultivated under well-watered (WW) and water deficit (WD) conditions. Small symbols represent mean values of genotypes within species cultivated under well-watered (WW; blue symbols; n = 73) and water deficit (WD; red symbols; n = 73) regimes. Large symbols represent mean ± s.d. values of maize (circles), pearl millet (diamonds), sorghum (squares) and wheat (triangles). Fill color gradient scales with leaf elongation rate (LER) under WW from lighter blue for low to darker blue for high LER values. Solid and dashed lines represent SMA regression lines under WW ($R^2 = 0.45$ with $P < 0.001$) and WD ($R^2 = 0.47$ with $P < 0.001$), respectively. Spearman's rank coefficients ($r_{spearman}$) are shown between species (n = 4) under both watering regimes, and within maize (n = 30) ($r_{spearmanWW} = -0.43$ * and $r_{spearmanWD} = -0.66$ ***), pearl millet (n = 8) ($r_{spearmanWW} = -0.38$ ns and $r_{spearmanWD} = -0.74$*), sorghum (n=10) ($r_{spearmanWW} = -0.4$ ns and $r_{spearmanWD} = -0.6$ ns) and wheat (n = 25) ($r_{spearmanWW} = 0.3$ ns and $r_{spearmanWD} = -0.06$ ns) (***: P < 0.001 ; **: P < 0.01 ; *: P < 0.05 ; ns : not significant).

Response of LES traits to water deficit

No significant species-by-watering condition interaction was found for A_{mass} which significantly decreased in response to WD in all species but pearl millet ($P = 0.67$; Table 2; Supporting Figure S1). Conversely, LMA significantly increased under WD in all species with different effect sizes between species (Supporting Figure S1). Across all genotypes, a significant negative log-linear relationship between the two leaf traits was observed both under WW ($R^2 = 0.45$ with $P < 0.001$) and WD ($R^2 = 0.49$ with $P < 0.001$) but the slopes differed significantly between watering conditions (test of common SMA slopes: $P = 0.0025$; Fig.1). Within species, A_{mass} was discriminated between genotypes only for maize, while there were significant LMA differences between genotypes in all species but sorghum (Table 2). A negative log-linear relationship between A_{mass} and LMA was only marginally significant for maize ($R^2 = 0.13$ with $P = 0.053$) under WW and for maize and sorghum under WD ($R^2 = 0.52$ and 0.45 with $P < 0.001$ and $P = 0.033$, respectively; Fig.1).

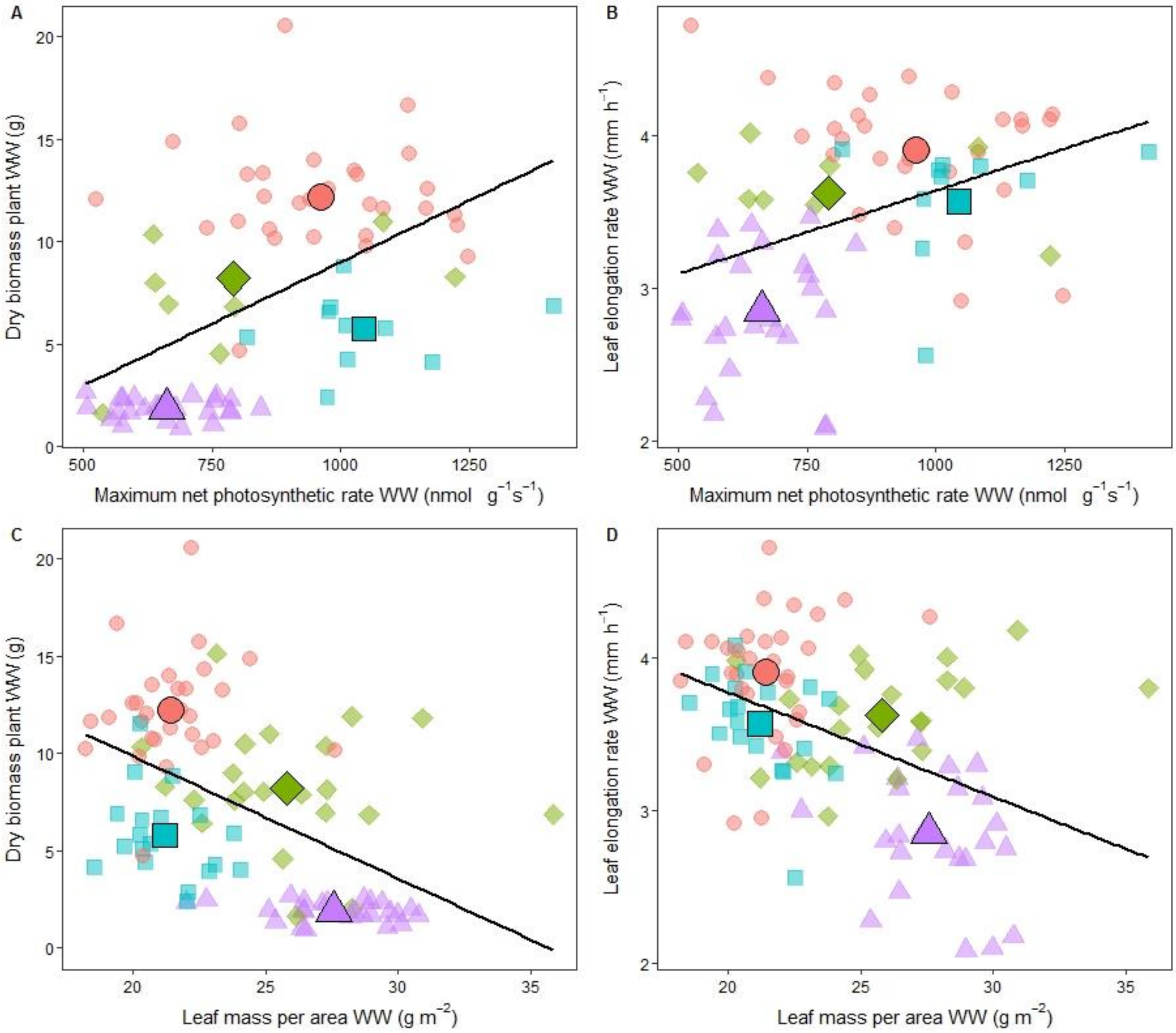


FIGURE 2 Projection of the four studied crops in the growth related– leaf trait values under well-watered (WW) conditions relationships. The two growth related traits were represented with two other leaf trait values under WW: (A) the plant dry mass (g) – maximum net photosynthetic rate (nmol g^{-1} s^{-1}) under WW relationship; (B) the leaf elongation rate (mm h^{-1}) – maximum net photosynthetic rate (nmol g^{-1} s^{-1}) under WW relationship; (C) the plant dry mass (g) – leaf mass per area under WW (g m^{-2}) relationship and (D) the leaf elongation rate (mm h^{-1}) – leaf mass per area under WW (g m^{-2}) relationship. For the four relationships, red circles, green diamonds, blue square, blue diamonds and purple triangles represent mean values of genotypes within maize, pearl millet, sorghum and wheat species measured within WW (n = 73 for A and B, and n=97 for C and D). The bigger circle, diamond, square and triangle represent the species mean under WW. Solid lines represent the statistically significant linear regression between the two traits for A ($R^2 = 0.14$ with $P < 0.001$), B ($R^2 = 0.26$ with $P < 0.001$), C ($R^2 = 0.2$ with $P < 0.001$) and D ($R^2 = 0.23$ with $P < 0.001$).

Response of plant and leaf growth to water deficit

Whole-plant as well as leaf-level growth decreased in response to the WD applied, as indicated by the significant decrease in PDM and LER across the four species (Table 2; Supporting Fig. S1). On average, pearl millet was the most tolerant species (Fig. 3B; Supporting Fig. S3B). Within species, significant differences between genotypes were identified for PDM and LER, except LER for sorghum (Table 2). Growth responses to WD were highly variable across genotypes within species (Fig.3 and Supporting Fig. S3). LER relative reduction due to WD ranged from -64.8% to -8.3% in maize, -52.6 to 2.8% in pearl millet, -48.9% to -0.9% in sorghum and -48.9% to -0.6% in wheat (Fig.3 and Supporting Fig.S4), whereas PDM reductions due to WD ranged from -80.6% to -46.5% in maize, -76.9 to 14.4% in pearl millet, -76.9% to 2.2% in sorghum and -68.6% to -29.6% in wheat for the less and most drought tolerant genotypes, respectively (Supporting Fig. S3).

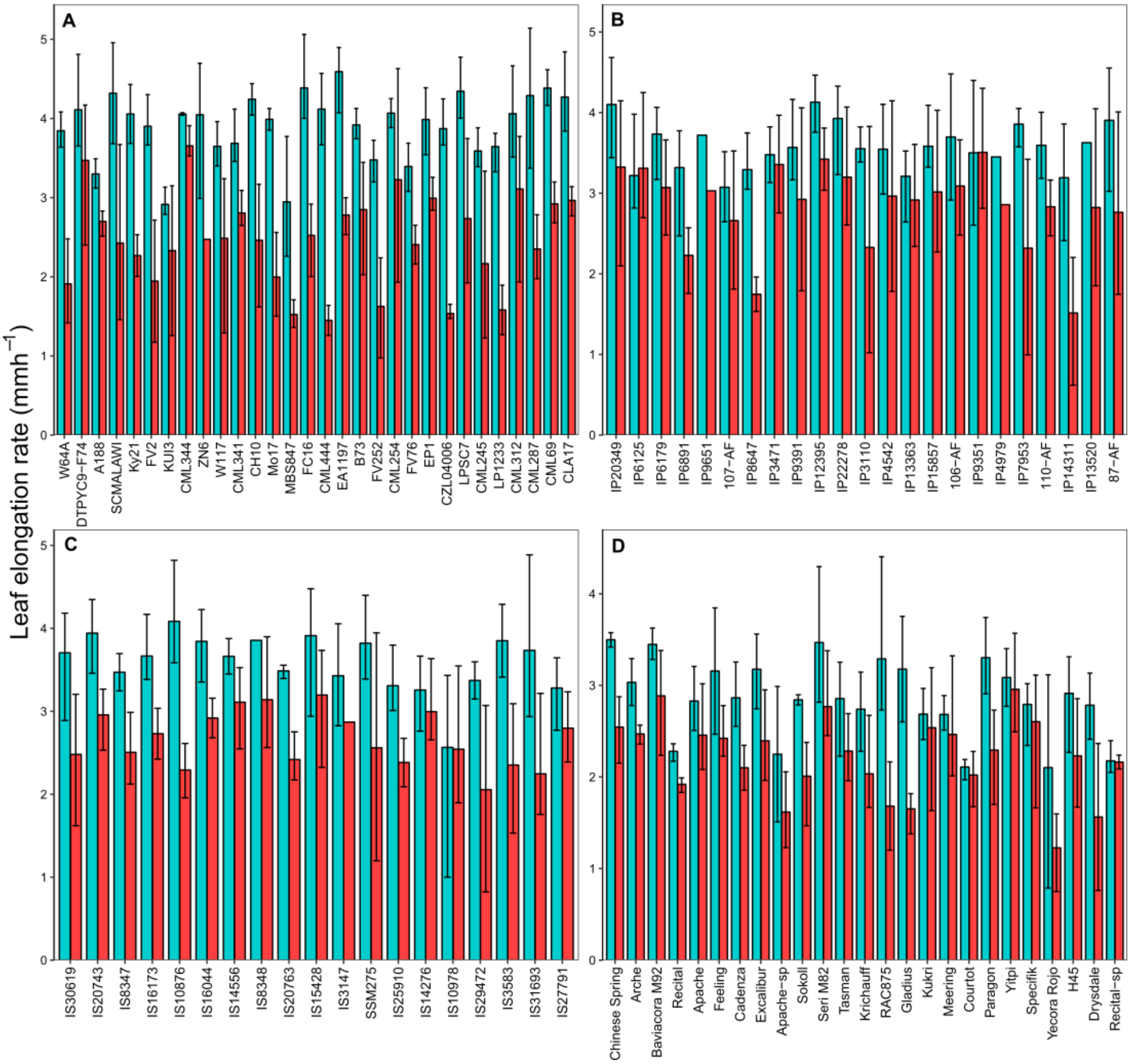


FIGURE 3 Water deficit effect on the leaf elongation rate among genotypes within the four species. Each species are represented separately: (A) maize genotypes (n = 30); (B) pearl millet genotypes (n = 23); (C) sorghum genotypes (n = 19) and (D) wheat genotypes (n = 25). For each histogram, blue and red colors represent respectively plant dry mass under well-watered and water deficit conditions. Genotypes are ordered by their ascending order for LMA values under well-watered. Error bars represent the confidence interval of 95 % for each genotype within the two treatments. They were measured with a nonparametric bootstrap which enables to obtain confidence limits for the population mean without assuming normality.

Across species, significant negative relationships between LER and PDM values under WW and their log response ratios were observed (r = -0.56 and r = -0.35 with both $P < 0.001$, respectively) (Fig.4). A similar trend was found within species but the negative correlations were not all significant (Table 3).

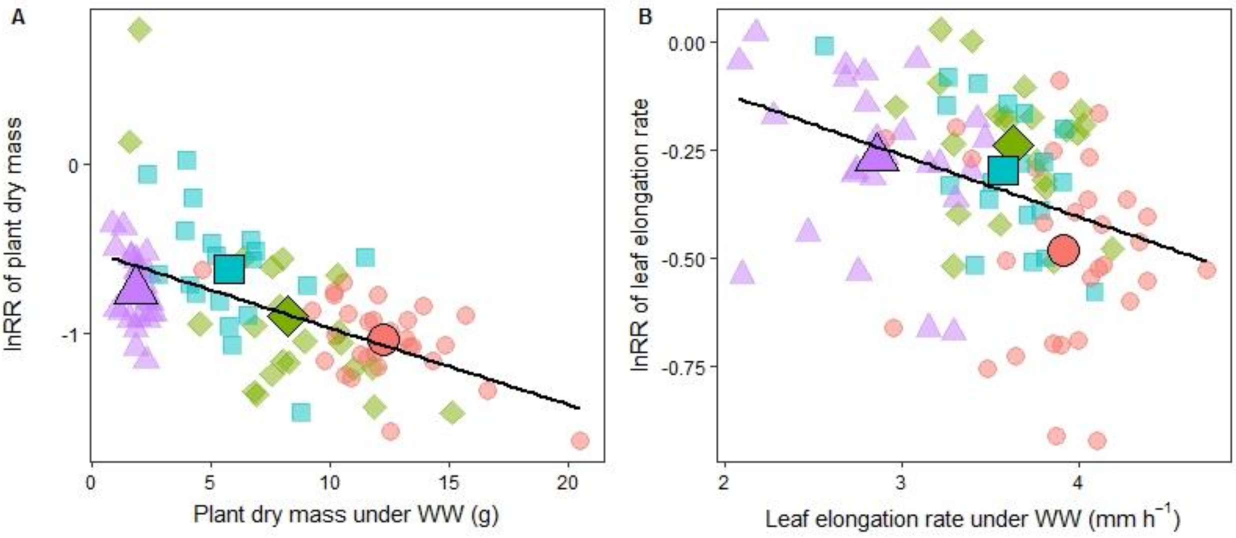


FIGURE 4 Projection of the four studied crops in the log response ratios (lnRR) of trait–trait values under well-watered (WW) conditions relationships. The two relationships were represented with growth related and leaf trait values: (A) the lnRR of plant dry mass – plant dry mass under WW (g) relationship; (B) the lnRR of leaf elongation rate - leaf elongation rate under WW (mm h^{-1}) relationship. For the two relationships, red circles, green diamonds, blue square and purple triangles represent mean values of genotypes within maize (n = 30), pearl millet (n = 23), sorghum (n = 19) and wheat (n = 25) species measured within WW. The bigger circle, diamond, square and triangle represent the species mean under WW. Solid lines represent the statistically significant linear regression between the two traits for A ($R^2 = 0.31$ with $P < 0.001$) and B ($R^2 = 0.12$ with $P < 0.001$). For the two relationships, the coefficients of spearman were calculated with all species and within each one, for results see Table 3.

TABLE 3 Spearman coefficients ($r_{spearman}$) and their *pvalue* (*P*) for the 6 correlations between leaf and size-related traits with log response ratios (lnRR) of the size-related traits within the four studied species (maize, pearl millet, sorghum and wheat). The leaf and size-related traits used for the correlation were values under well-watered regime. For each correlation, five $r_{spearman}$ were calculated with the four species (interspecies), and within maize, pearl millet, sorghum and wheat. For abbreviations, see Table 1.

Variables		**Interspecies**		**Maize**		**Pearl millet**		**Sorghum**		**Wheat**	
Y	**X**	$r_{spearman}$	*P*	$r_{spearman}$	*P*	$r_{spearman}$	*P*	$r_{spearman}$	*P*	$r_{spearman}$	*P*
PDM	LMA	-0.61	< 0.001	0.18	0.33	-0.17	0.44	-0.38	0.1	-0.24	0.24
LER	LMA	-0.46	< 0.001	0.24	0.21	0.29	0.17	-0.43	0.07	-0.37	0.07
PDM	A_{mass}	0.57	< 0.001	-0.13	0.5	0.32	0.41	0.14	0.71	-0.03	0.9
LER	A_{mass}	0.44	< 0.001	-0.27	0.14	-0.2	0.6	0.27	0.45	0.13	0.5
lnRR_PDM	LMA	0.06	0.58	0.1	0.61	-0.13	0.56	0.25	0.29	-0.21	0.32
lnRR_LER	LMA	0.2	0.05	-0.11	0.56	-0.22	0.33	0.05	0.83	0.07	0.7
lnRR_PDM	A_{mass}	-0.36	0.002	-0.11	0.56	-0.57	0.15	0.006	1	-0.16	0.44
lnRR_LER	A_{mass}	-0.26	0.02	-0.01	0.96	0.12	0.8	-0.42	0.23	-0.14	0.49
lnRR_PDM	PDM	-0.58	< 0.001	-0.36	0.05	-0.53	0.01	-0.45	0.05	-0.35	0.08
lnRR_LER	LER	-0.4	< 0.001	-0.08	0.65	-0.35	0.11	-0.5	0.03	-0.25	0.22

Relationships between plant size responses to WD and LES traits

We found no significant relationship between leaf trait values under WW and responses of growth-related traits to WD at the interspecific level (all $P > 0.05$) (Fig.5). While A_{mass} was negatively correlated to the log response ratios of LER ($r_{Spearman} = -0.36$ with $P = 0.002$) and PDM ($r_{Spearman} = -0.26$ with $P = 0.02$), LMA was only positively significantly correlated with the log response ratio of LER ($r_{spearman} = 0.20$ with $P = 0.05$) (Table 3). Within species, leaf traits were not significantly correlated to the responses of size-related trait to WD (all $P > 0.05$) (Table 3).

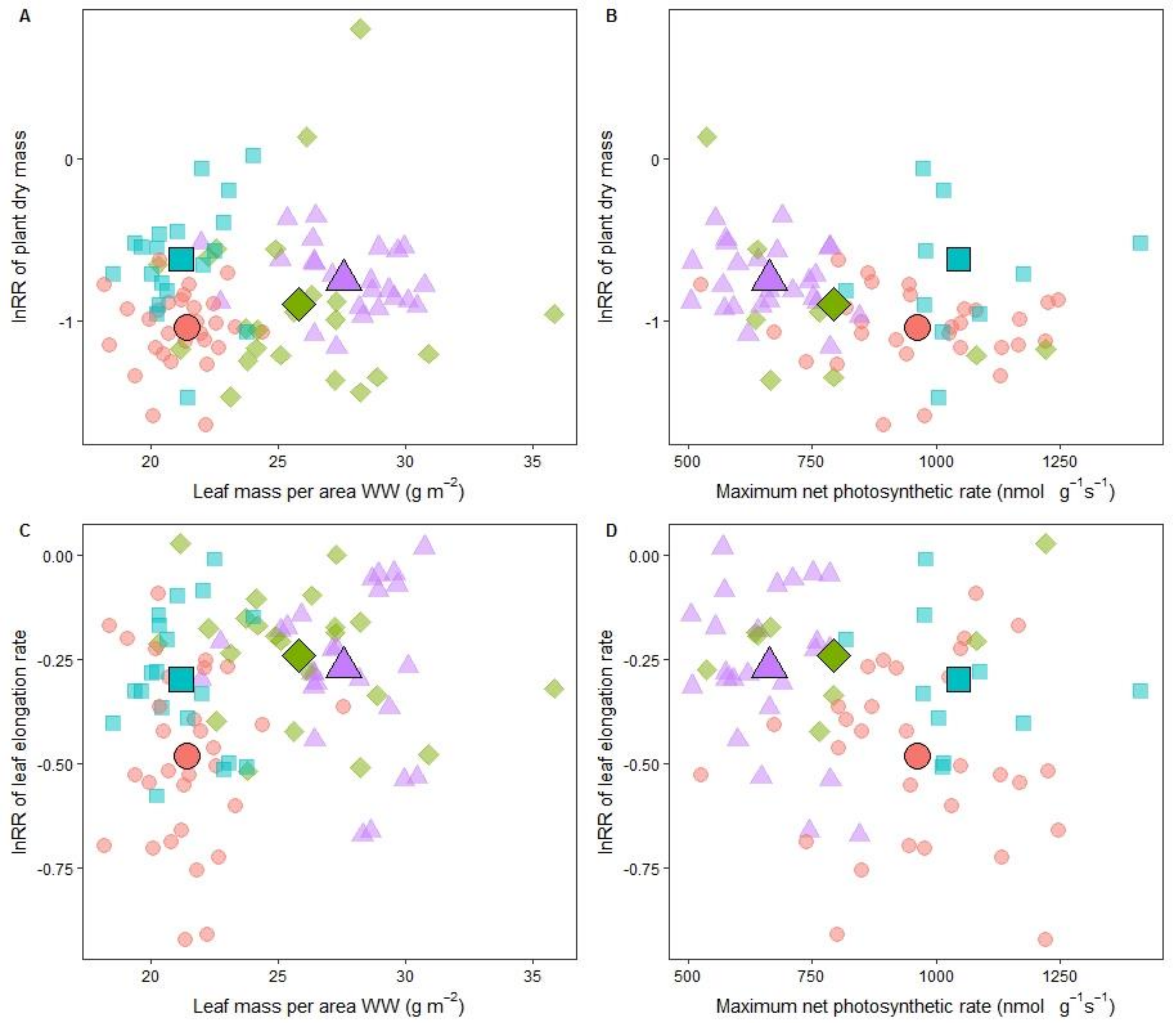


FIGURE 5 Projection of the four studied crops in the log response ratios (lnRR) of growth related– leaf trait values under well-watered (WW) conditions relationships. The lnRR of two growth related traits were represented with two other leaf trait values under WW: (A) the lnRR of plant dry mass – leaf mass per area under WW (g m^{-2}) relationship; (B) the lnRR of plant dry mass –maximum net photosynthetic rate (nmol g^{-1} s^{-1}) under WW relationship; (C) the lnRR of leaf elongation rate (mm h^{-1}) – leaf mass per area under WW (g m^{-2}) relationship and (D) the lnRR of leaf elongation rate (mm h^{-1}) – maximum net photosynthetic rate (nmol g^{-1} s^{-1}) under WW relationship. For the four relationships, red circles, green diamonds, blue square, and purple triangles represent mean values of genotypes within maize, pearl millet, sorghum and wheat species measured within WW (n = 97 for A and C, and n = 73 for B and D). The bigger circle, diamond, square and triangle represent the species mean under WW. For the four relationships, the coefficients of spearman were calculated with all species and within each one, for results see Table 3.

Discussion

The Leaf Economics Spectrum (LES) that recapitulates leaf-level physiological trade-offs at play for resource acquisition and use across plant species, has been the most described pattern in functional ecology for the last decade. Despite this success, several primary underlying hypotheses of the LES remain untested. First, the LES should reflect a fast-slow continuum at both organ and whole-plant levels (Reich, 2014), but the quantification of leaf elongation rate is absent from most studies in functional ecology. Second, ecological strategies at both ends of the LES are expected to be selected in different environmental conditions: acquisitive strategies in optimal conditions for plant growth *vs.* conservative strategies in stressful conditions. Even if this is what we can observe in interspecific comparisons (Wright et al., 2005), the lack of intraspecific studies testing this idea impedes a deeper exploration of this hypothesis (Vasseur et al., 2012). Indeed, one genotype at the acquisitive (resp. conservative) part of the spectrum should be more (resp. less) impacted by an experimental stress, if and only if genotype-by-environment interactions are negligible. The latter condition seems largely improbable; this is however an implicit postulate of several theories in functional ecology. Here, using a unique crop material, we applied both interspecific and intraspecific comparisons to investigate LES relationships under both optimal and suboptimal conditions, and how LES traits can be used as proxies for growth strategies and response to water deficit.

First demonstrated in a set of 2,500 wild species (Wright et al., 2004), recent studies reported some evidence that the LES of domesticated species stands within this spectrum. This is for instance the case for the bivariate relationship between leaf mass per area (LMA) and leaf nitrogen content for several panels of crop genotypes (Milla et al., 2014, 2015), as well as along domestication series (Roucou et al., 2018). Here, LMA and mass-based photosynthetic rate (A_{mass}) values of 73 genotypes from the four cereal species under study stand at the acquisitive extreme of the major axis of variation, and the relationship between them mirrors the negative relationship described by the interspecific worldwide LES. This suggests that similar ecophysiological tradeoffs shape leaf trait covariations in artificially selected cultivated lines, although both trait values were respectively lower and higher than the values of wild species. Martin et al. (2018) found a similar trend for maize and wheat at the interspecific level, but lack of paired LMA and photosynthetic rate values within species impaired inspection of intraspecific relationships. Importantly, we also highlighted that the LES line covaries with variation in leaf elongation rate. To our knowledge, this is the first demonstration that the LES reflects a fast-slow continuum at the leaf level, as often suggested but never demonstrated at the organ level (Reich, 2014a). Next, when investigating the LES within species, we found a weak but significant covariation between LMA and A_{mass} across maize lines, whereas the relationship was not significant in wheat, pearl millet and sorghum. This contrasts with several recent studies who found consistent LES relationships within species (Blonder et al., 2013; Hu et al., 2015; Niinemets, 2015; Vasseur et al., 2012). A general explanation advanced that seems to apply here is that the range of trait values is not enough to let a significant relationship emerge (Funk and Cornwell, 2013). Beyond mathematical consideration, this can prevent selection for leaf traits in crop elites, even if further studies using larger panels could tone down this assertion.

The response of LES to environmental changes is largely unknown in both cultivated and crop species (Keenan and Niinemets, 2017; Wright and Sutton-Grier, 2012). Notably, to our knowledge, our study is the first to analyze the impact of water stress on LES within crop species. LMA is expected to increase (Baird et al., 2017; Poorter et al., 2009; Wellstein et al., 2017) and net photosynthetic rate to decrease (Chaves, 1991, 2002; Farooq et al., 2009), in response to drought. Here, accordingly, we found thicker and/or denser leaves and a reduction of net photosynthetic rate across the four cereals. Beside plasticity of both LMA and A_{mass} under water deficit, we highlighted that the general LMA-A_{mass} relationship was stable in response to WD across the four cereals. This result suggests existence of physiological and/or structural constraints limiting the independent evolution of these two traits (Onoda et al., 2017). Obviously, differences in the experimental conditions and treatment procedures may exert contrasted changes in LMA and/or A_{mass} that would challenge this result. Across species, plastic response of LES traits to stressful conditions would be thus constrained by physiological regulations. As a consequence, some leaf phenotypic combinations remain impossible, even under stressful conditions. Such limits need to be considered in plant breeding in the targeted selection of traits.

Volaire (2018) proposed a translational approach in which functional ecology theories could be used in ecophysiology to predict the response of species or genotypes to environmental changes. In particular, plant response to water deficit could be related to the tradeoff between resources acquisition and conservation (Volaire, 2018). In other words, soft traits associated to the LES measured in optimal conditions could be used as proxies for plant performance in suboptimal conditions. Genotypes with high return on investment (resources acquisition) are expected to be less adapted to water deficit (Volaire, 2018). On the opposite, genotypes or species with a more conserved strategy is expected to be more drought tolerant (Volaire, 2018). Here, we highlighted that crop genotypes with faster growth (higher leaf elongation rate and plant biomass) under control condition tended to exhibit stronger reduction of growth in response to water deficit. But, neither leaf growth response to WD nor aboveground biomass accumulation under WD was related to the position of the crop genotype in LMA-A_{mass} values under WW conditions. Drought tolerance strategy between genotypes could not be discriminated by leaf functional traits related to LES under optimal conditions. Yet, drought strategy was reported to be related to resource-use strategy (Reich, 2014). But, across species, each genotype adjusts their phenotype to stressful conditions (Freschet et al., 2018). In that context, plasticity of both A_{mass} and LMA to water deficit depends on genotypic adaptations to stressful conditions. Using their values under optimal conditions does not predict genotypic response under other environmental conditions. This result suggests the important role of intraspecific variability when assessing plant response to water stress.

Face to the challenge of climate change, the number of water limited environments for crops will increase (Pereira, 2017). Understanding crop strategy in response to water stress enable to provide an appropriate framework for targeting selection of adapted pre-breeding material (Nakhforoosh et al., 2016). Indeed, highlighting attractive behaviors would help to target interesting traits and then their genetic determinisms. Recently, a translational ecological research emerged (Martin and Isaac, 2015): it advocates mutual benefits from ecological approaches and crop science in order to better characterize phenotypic diversity of plant species and further improve our understanding of natural and cultivated ecosystems under a changing environment (Milla et al., 2015). In that context, the comparative approach used in this study experimentally examined the response of four main crop species to soil water deficit (WD) through the lens of functional ecology theory. Our study highlighted an ecophysiological compromise not “easily modifiable” under stressful conditions. Plasticity of the leaf phenotypic *space of possibles* was determined in response to water deficit according to drought tolerance. Exploring and comparing this ‘leaf phenotypic space’ across species and under stressful conditions enable to better understand the existing leaf phenotypic plasticity, and then to target the appropriate trait combinations for improving drought tolerance.

Supporting Information

The following Supporting Information is available for this article:

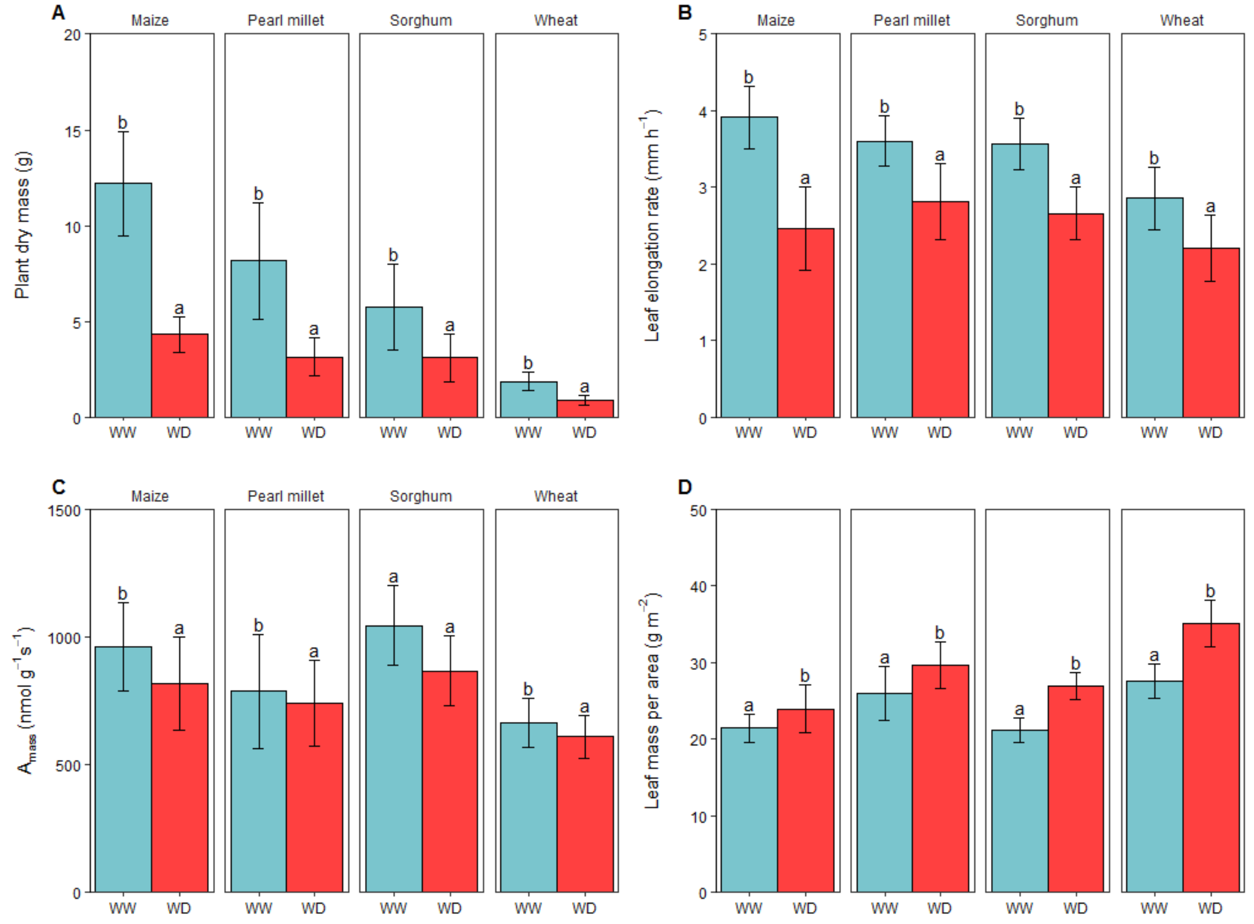
Supporting Fig. S1 Variation of leaf and plant traits in four crop species grown under two contrasted watering conditions.

Supporting Fig. S2 Projection of the four species in the net photosynthetic rate-leaf mass per area trade-off from the Leaf Economics Spectrum (LES) with a zoom on cultivated genotypes.

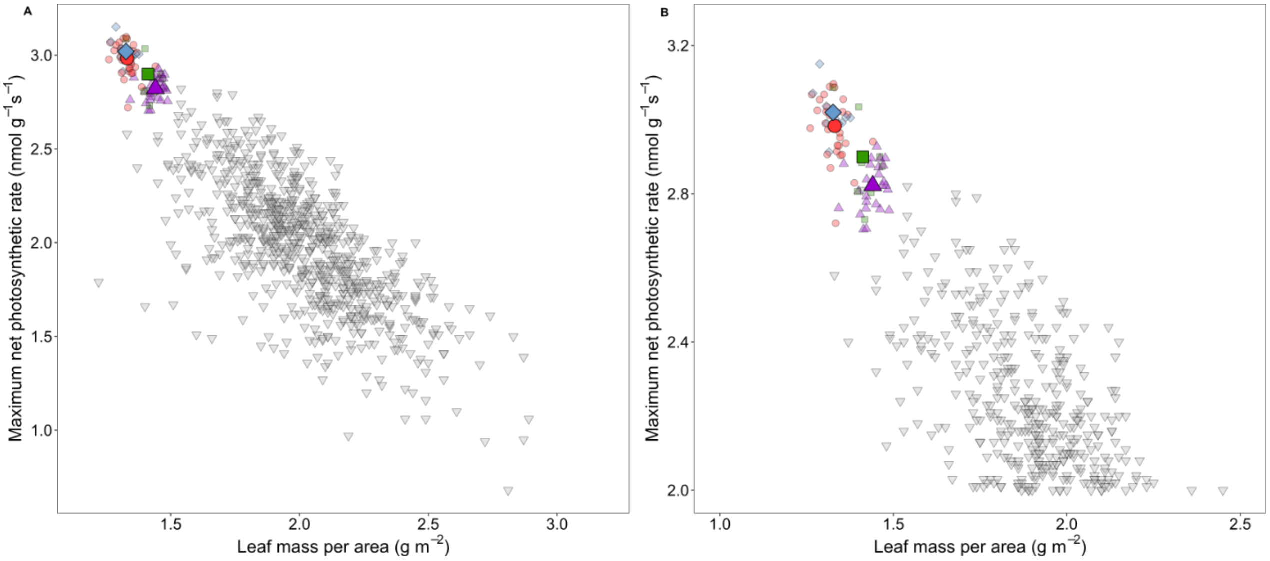
Supporting Fig. S3 Water deficit effect on the aboveground dry weight among genotypes within the four species.

Supporting Fig. S4 Variations in the relative change of leaf expansion for studied genotypes within the four species.

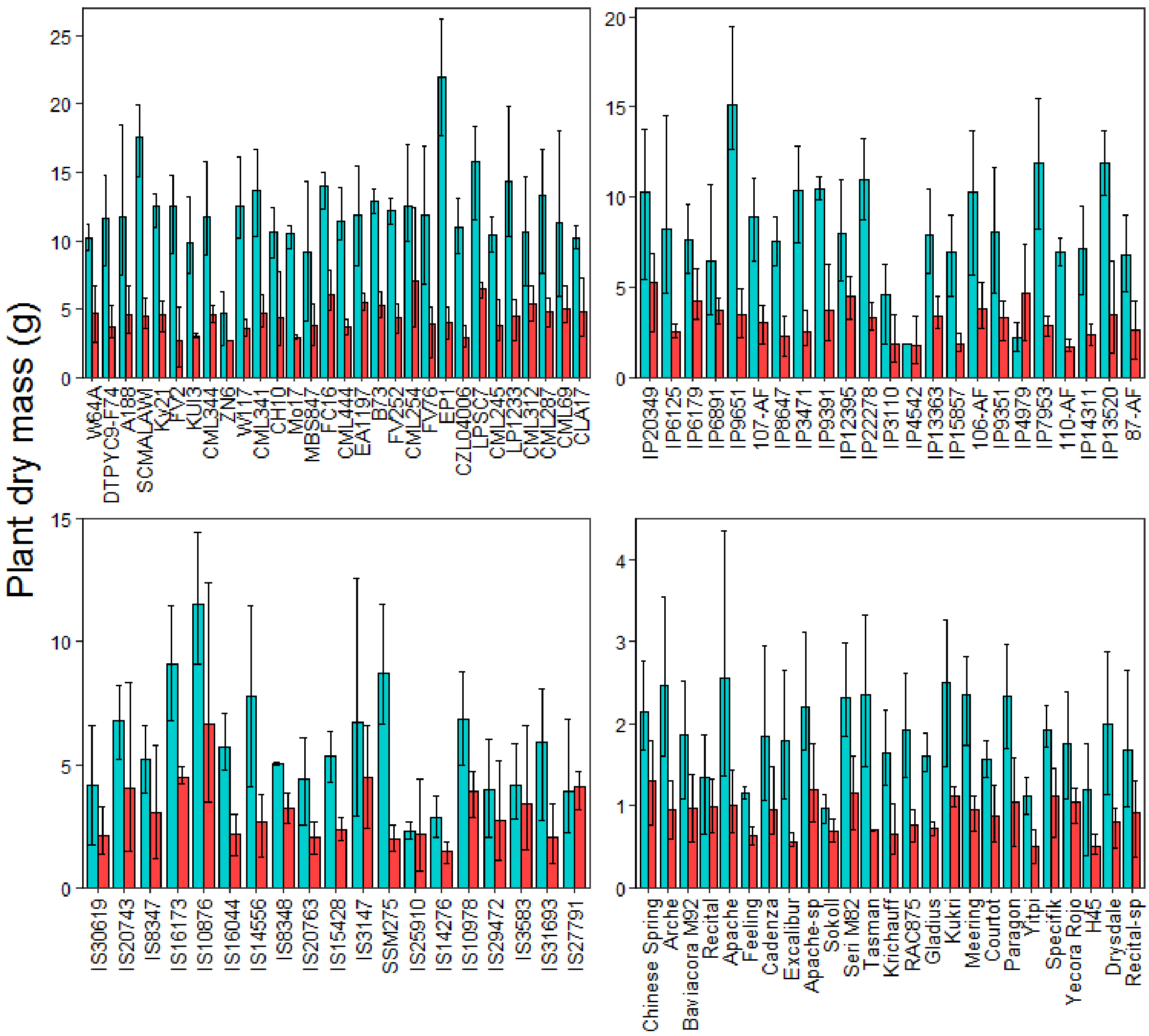
Supporting Fig. S5 Variations in the log response ratio (lnRR) of plant dry mass for studied genotypes within the four species.



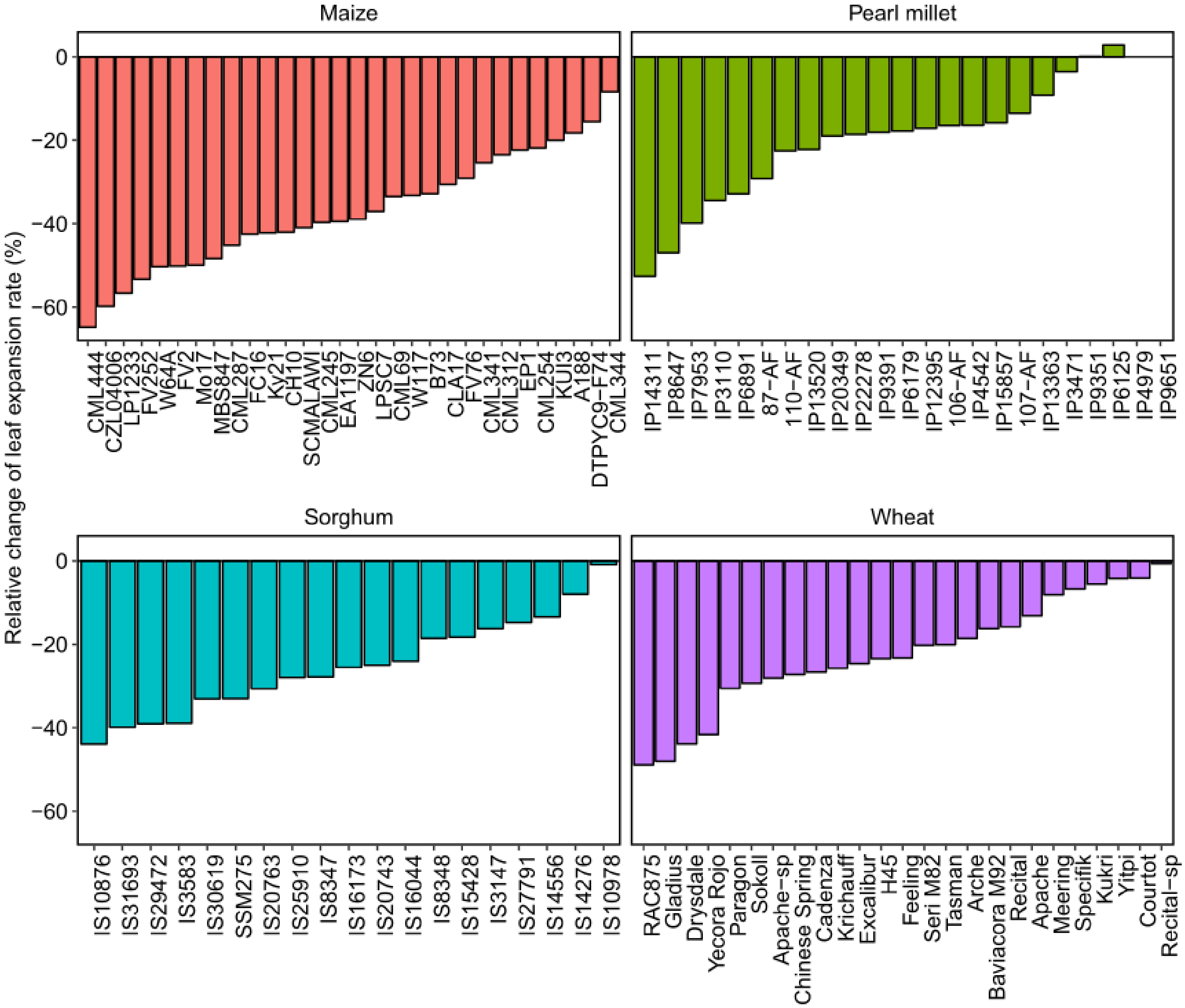
SUPPORTING FIG. S1 Variation of leaf and plant traits in four crop species grown under two contrasted watering conditions. (A) Plant dry mass; (B) leaf elongation rate; (C) maximal photosynthetic capacity and (D) leaf mass per area were determined on plants grown under well-watered (WW) and water deficit (WD) conditions. Bars and error bars represent means ± SEM (n = 30 genotypes for maize, n = 25 genotypes for wheat, n = 19 genotypes for sorghum except for maximal photosynthetic capacity (n=10), n = 23 genotypes for pearl millet except maximal photosynthetic capacity (n=8)). Different letters above bars indicate significant differences at $P < 0.05$ using Tukey's multiple comparison tests.



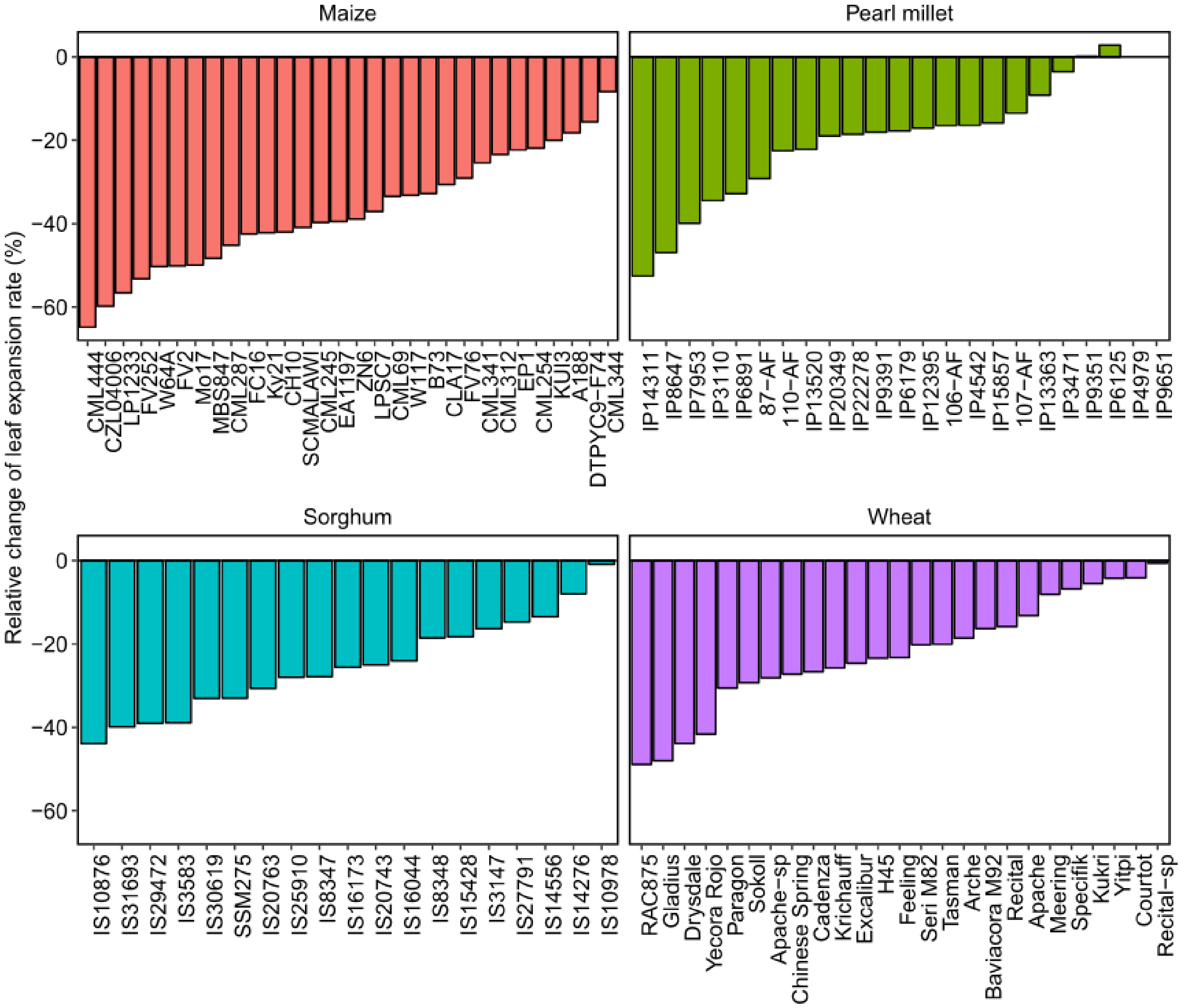
SUPPORTING FIG. S2 Projection of the four species in the net photosynthetic rate-leaf mass per area trade-off from the Leaf Economics Spectrum (LES) with a zoom on cultivated genotypes. Grey triangles represent mean trait values for individual wild species (data from Wright et al., 2004; n = 2548) and red circles, blue diamonds, green squares and purple triangles represent the mean trait values of the studied maize genotypes (n=30), pearl millet genotypes (n=8), sorghum genotypes (n=10) and wheat genotypes (n=25).



SUPPORTING FIG. S3 Water deficit effect on the aboveground dry weight among genotypes within the four species. Each species are represented separately: A, maize genotypes (n=30); B, pearl millet genotypes (n=23); C, sorghum genotypes (n=19) and D, wheat genotypes (n=25). For each histogram, blue and red colors represent respectively plant dry mass under well-watered and water deficit conditions. Genotypes are ordered by their ascending order for LMA values under well-watered conditions. Error bars represent the confidence interval of 95 % for each genotype within the two treatments. They were measured with a nonparametric bootstrap which enables to obtain confidence limits for the population mean without assuming normality.



SUPPORTING FIG. S4 Variations in the relative change of leaf expansion for studied genotypes. Each species are represented separately by different colours: maize genotypes (n=30) (red); pearl millet genotypes (n=23) (green); sorghum genotypes (n=19) (blue) and wheat genotypes (n=25) (purple). Genotypes are ordered in decreasing order of relative change of leaf expansion rate.



SUPPORTING FIG. S5 Variations in the log response ratio (lnRR) of plant dry mass for studied genotypes. Each species are represented separately by different colours: maize genotypes (n=30) (red); pearl millet genotypes (n=23) (green); sorghum genotypes (n=19) (blue) and wheat genotypes (n=25) (purple). Genotypes are ordered in decreasing order of log response ratios of plant dry mass. Error bars represent the confidence interval of 95 % for leaf elongation rate values for each genotype within the two treatments. They were measured with a nonparametric bootstrap which enables to obtain confidence limits for the population mean without assuming normality.