Identifier et comparer la réponse de l’allométrie à différents facteurs environnementaux au sein d’une espèce cultivée

Présentation générale

Ce deuxième chapitre concerne l'étude réalisée sur le **maïs,** cultivé dans la **plateforme de phénotypage haut-débit** PhenoArch. À partir des **hybrides** de maïs, les principaux objectifs de ce deuxième chapitre sont d': i) analyser les relations allométriques au sein d'une espèce cultivée, ii) explorer la plasticité de cette contrainte biophysique sous stress hydrique et l'influence de la disponibilité lumineuse et iii) examiner le rôle de la plasticité de la surface spécifique foliaire dans la régulation de la réponse allométrique au stress hydrique.

Avec l'expérimentation 2, quatre **traits fonctionnels**, dont trois associés à la taille de la plante et un trait lié à l'utilisation des ressources foliaires (la surface spécifique foliaire), ont été étudiés chez 337 génotypes de maïs dans des conditions contrôlées avec ou non application d'un stress hydrique (voir la partie Matériel et Méthodes pour plus d'informations). Au sein du maïs, nous avons mis en évidence des **relations allométriques** entre les traits liés à la taille de la plante en conditions bien irriguées et de stress hydrique, mais avec des pentes significativement différentes. Face à un stress hydrique, la trajectoire allométrique est globalement similaire parmi les génotypes, i.e. une réduction de la taille, de la surface foliaire totale et de la croissance de la plante. Dans la plateforme de phénotypage, la présence de plantes voisines créent de l'ombrage pour la plante. La réduction de la disponibilité lumineuse ne change pas la réponse allométrique au stress hydrique, mais diminue la gamme de variations entre génotypes. Cette diminution de la **diversité génétique** peut être le résultat d'une réponse commune face à l'ombrage. La réponse allométrique au stress hydrique est liée à la plasticité de la surface spécifique foliaire. Nos résultats suggèrent le rôle important de la surface spécifique foliaire sur l'allométrie. Ce résultat est intéressant pour la prédiction de la production de biomasse des espèces cultivées, et ainsi pour les programmes de sélection variétale. Nos résultats ont mis en évidence une diversité génétique à exploiter par l'amélioration variétale pour sélectionner des génotypes de maïs selon leur trajectoire allométrique face aux fluctuations environnementales.

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

Response of plant allometric relationships to water deficit and influence of light availability: a high-throughput phenotypic evaluation using 337 Maize genotypes

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Keywords: Maize, plant allometry, high-throughput phenotyping, water deficit, light availability, specific leaf area.

Introduction

Plants alter their architecture and morphology to adapt their phenotypes to the availability of resources. Understanding plant phenotypic plasticity is pivotal in agronomy, ecology and evolution. In agriculture, understanding crop phenotypic responses to stressful conditions is crucial in the perspective of predicting and mitigating the impacts of resources scarcity on yield components (Nicotra et al., 2010). Several size-related traits, including plant biomass and plant height, are affected by abiotic stresses and resource scarcity, with direct implications for yield (Bodner et al., 2015; Osakabe et al., 2014; Prasad et al., 2008). The regulation of these phenotypic responses are however not fully resolved. One reason is the fact that the plant phenotype is an integrated network of trait covariations, which results in the dependency of size-related traits with several more-or-less integrated traits. In particular, plant allometry theories, widely discussed in ecology (Niklas, 1994; Price et al., 2007) claimed the existence of biological invariances due to the tight linkage between size-related traits M and metabolism or plant growth Y, often characterized by power laws of the form: $Y = Y_0M^b$, where b is the scaling exponent resulting from constraints of diffusion across the vascularized system and Y_0 is a normalization constant that may be characteristic of a given taxon (Price et al., 2010, 2012; West et al., 1997, 1999). Even if empirical observations considered scaling exponent as constant around ¾ across species (Enquist et al., 2007; Niklas and Enquist, 2001), recent studies have suggested variability in the scaling exponent along environmental gradients (Coomes et al., 2011; Russo et al., 2007; Vasseur et al., 2018). However the plasticity of plant allometry is still poorly explored in both cultivated and wild species.

Cross-species comparisons have been widely used in macroecology and functional ecology in the perspective of building universal laws at the origin of biological diversity (Eziz et al., 2017; Hawkins et al., 2007). Historically, this has been done at the detriment of a thorough exploration of the persistence of those laws at lower taxonomic level, notably at the species level (Albert et al., 2010; Bolnick et al., 2011). However, plant allometry results from biophysical constraints and selection pressures that act primarily within species. Cross-species studies, while meaningful due to their power of generalization, can mix different proximal and ultimate causes at the origin of plant allometry laws. This is one of the reasons for recent calls for a better recognition of intraspecific variability (and its underlying components, namely genetic diversity and phenotypic plasticity) in comparative ecology (Albert et al., 2011; Messier et al., 2017). At species level, the analysis of phenotypic diversity is necessary for the quantification of phenotypic plasticity. Analyzing the plastic response of allometric relationships to environmental factors, both in direction and amplitude, is a major step given that theoretical approaches to plant allometry have been almost exclusively developed using resource optimality criteria (Price and Weitz, 2012). However, it is known that plant-plant interactions as well as other abiotic factors can modulate scaling relationships (Okami et al., 2012; Qin et al., 2013; Vega and Sadras, 2003), which questions the claimed invariance of those relationships. In a genetic analysis of allometric relationships across natural genotypes of *A. thaliana*, Vasseur et al. (2018) showed another evidence of a control of scaling laws by the environment. Specifically, the authors reported an effect of temperature and precipitation patterns on the variability of the scaling exponent of plant growth. Further studies are needed to deepen our knowledge about possible variability of scaling exponent. Using crops to test ecological theory is growing, as illustrated by recent progress in our understanding of the underlying mechanisms of the leaf economics spectrum (Isaac et al., 2018; Martin et al., 2018a), previously described using worldwide sets of wild species (Wright et al., 2004). In that context, using several crop genotypes is a unique opportunity to explore the intraspecific variability of plant allometry (Deng et al., 2012a).

In response to water stress, stomatal closure reduces water loss by leaves but in turn limits carbon assimilation (Chaves, 1991). As a consequence, plant growth in terms of biomass accumulation or organ expansion can be limited or even stopped (Alvino et al., 1999; Fernandez et al., 1991; Newman, 1963; Prasad et al., 2008). The plasticity of allometric relationships under water stress within wild or cultivated species has been hardly investigated. With a reduction of water availability, the rare results obtained showed a stability of the scaling allometric relationships, despite changes in biomass allocation patterns (Eziz et al., 2017; Song et al., 2015; Wu et al., 2013). For example, in response to drought, biomass investment to root system increases, at the expense of aerial part, as a way to tap water from deep soil (Eziz et al., 2017). Despite those changes, the scaling exponents of the allometric relationships between stem or leaf mass with root mass did not change with the watering treatment. On the contrary, Reddy et al. (1998) showed variability of the allometric relationships between stem height and mass, between crops of the same soybean cultivars grown in different environment. Thus, the question about stability or variability into allometric relationships under water stress needs to be pursued further. The study of the allometric trajectory would help to precise crop allometric response to water stress.

In crops, the high planting densities in the field are used to maximize crop productivity within space-limited growing areas (Frick et al., 1994), but also generate strong intraspecific competition for light (Weiner and Thomas, 1986). In response to light limitation, higher plant growth enables to avoid shade by being over neighboring plants (Gommers et al., 2013). This phenotypic modification is related to an increase in carbohydrate investment in support and acquisition structures (Tardy et al., 2015), increasing both aboveground biomass and total plant leaf area. However, plant response to low light availability is constrained by physical laws (Hammond and Niklas), restraining the way biomass is allocated to aerial parts (Niklas and Hammond, 2013). In crops, the high planting density, and then light competition, limit crop growth, size and leaf area (Deng et al., 2012b, 2012a). To our knowledge, a few studies have analyzed shade impacts on plant growth and development response to an abiotic stress (Climent et al., 2006; Page et al., 2011). Under low light availability, plant allometric response to water deficit, i.e. reduction of crop size and growth, can be amplified (Climent et al., 2006), or reduced due to shade tolerance strategy (Page et al., 2011). Moreover, within crops, shade impacts can differ between genotypes, depending on their shade tolerance selected by plant breeding. In order to study and compare impacts of environmental fluctuations on plant allometry, environmental characterization through climatic factors is essential. In that context, a high-throughput phenotyping platform enables plants to grow under controlled conditions, as water deficit. Moreover, with a high number of pots, the presence of neighboring plants creates shade and light competition between plants (Cabrera-Bosquet et al., 2016). Thus, shade impacts on plant allometric response to water deficit, within maize, can be analyzed in a high throughput phenotyping platform.

One core assumption of allometry theories, including Metabolic Scaling Theory (West *et al.* 1997, 1999; Brown *et al.* 2004), is the invariance of the structure of the leaf organs, recapitulated in leaf functional traits (Reich 1993; Wright *et al.* 2004) notably specific leaf area (SLA), with plant size (Enquist *et al.* 2007; West *et al.* 2009). Sack et al. (2002) questioned the validity of this assumption since SLA is known to vary with ontogeny within species (Poorter *et al.* 2009). Beyond SLA change with ontogeny, other studies have reported a systematic variability in SLA with plant size within species (Burns & Strauss 2012), suggesting that current theories wrongly ignored plasticity of leaf functional traits and potentially missed an important scaling law. In the ecological and ecophysiological literatures, phenotypic adjustments in response to light or water limitation have been widely studied for resource-use traits, as SLA, that have direct or indirect impacts on traits involved in scaling relationships. Lower specific leaf area reduced the transpiring leaf surface, which will reduce the water requirements under dry conditions (Poorter et al., 2009). This reduction is further associated with higher biomass allocation to foliar tissue, resulting in higher leaf density and/or thickness (Witkowski and Lamont, 1991). Specific leaf area, is also affected by the availability of incident light (Rosati et al., 2001). If the leaf is shaded, area invested per unit of mass will decrease in order to optimize carbon gain (Evans and Poorter, 2001; Gommers et al., 2013; Valladares and Niinemets, 2008). Here, we specifically examine the response of specific leaf area to water and light limitation and the consequences of possible phenotypic adjustments on crop allometry.

Responses of plant allometry to environmental fluctuations have been little studied, especially within cultivated species. Then, this lack of knowledge raises several questions: (1) Do allometric relationships vary with environmental fluctuations? (2) Does the addition of shade modify allometric response to water stress? (3) Are SLA responses to environmental conditions independent from crop allometric response? Maize is a staple crop of high economical and societal importance. A large panel of 337 maize genotypes grew up in a high-throughput phenotyping platform under controlled conditions. Each genotype was cultivated under well-watered and water deficit conditions.

Materials and methods

Plant material and experimental design

We selected 337 maize genotypes (*Zea mays*) from different European and American panels in order to have a restricted flowering window of 7 days in the field. Genotypes are F1 hybrids from the crossing between 337 dent lines and one common flint parent (*UH007*) (Prado et al., 2018).

The experiment was carried out in the phenotyping platform PhenoArch (Cabrera-Bosquet et al., 2016) hosted at the M3P, Montpellier Plant Phenotyping platform (https://www6.montpellier.inra.fr/lepse/M3P). Sowing date was May 10, 2016 and the experiment lasted 55 days in greenhouse conditions (minimal temperature = 15°C and maximal temperature = 29°C). Plants were grown in 9-L pots (0.19 m diameter and 0.4 m high) filled with a 30:70 (v/v) mixture of a clay and organic compost. Three seeds per pot were sown at 0.0255 m depth and thinned to one per pot when leaf three emerged. In the platform, plants were randomly organized in 28 columns and 60 lines (Fig. 1). Pots were separated of 20 cm on the line and 40 cm between columns, which gave a density of 18 plants per m^2.

Two levels of soil water content were maintained: well-watered (WW) and water deficit (WD) with soil water potentials of -0.05 MPa -0.6 MPa, respectively. Soil water content in pots was maintained at target values by watering each pot three times per day, using watering stations (520 U, Watson Marlow, Wilmington, MA, USA) (Prado et al., 2018). Each genotype was repeated 5 times in the experimental design, with 2 replicates under well-watered (WW) and 3 under water deficit (WD) environments.

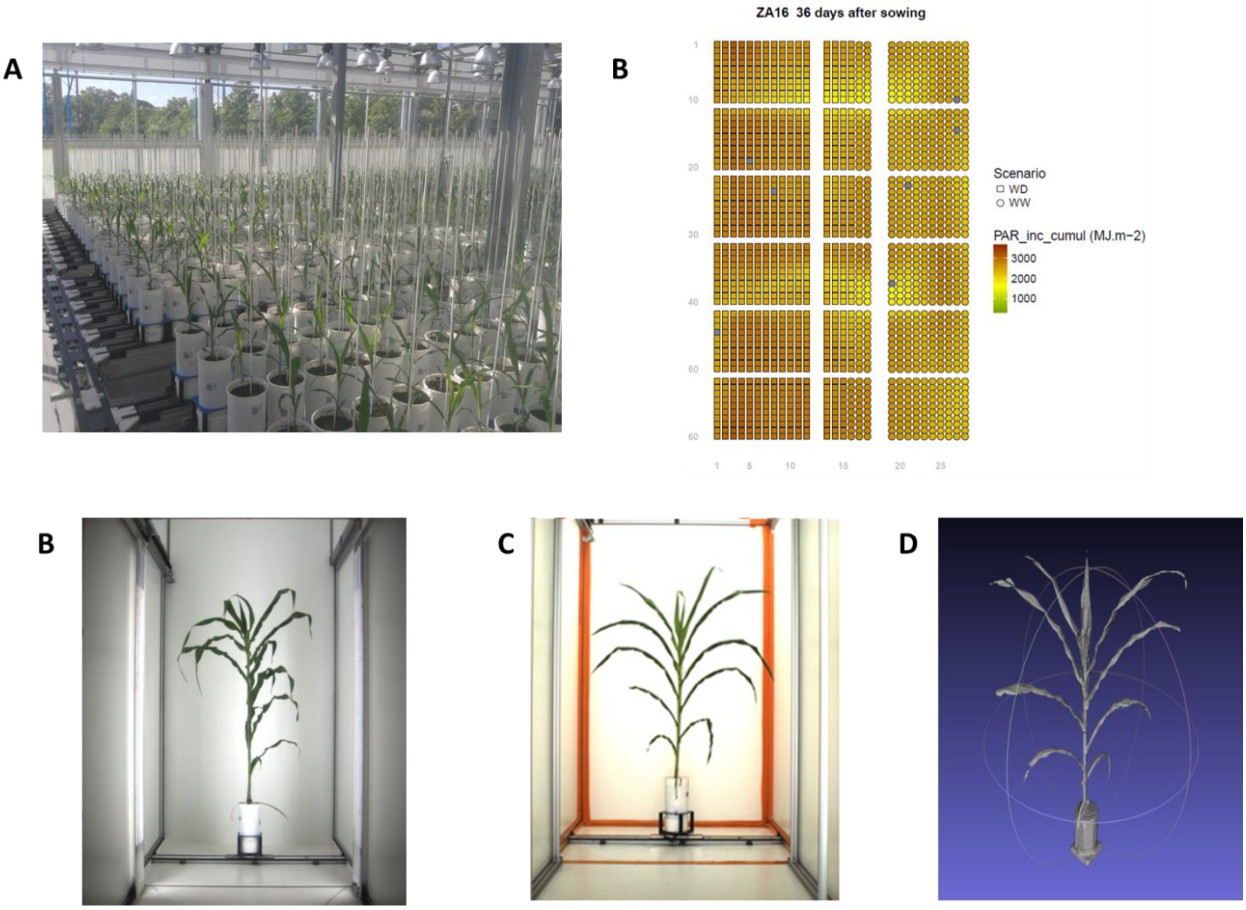

FIGURE 1 Illustrations of the experimentation. (A) Plants in the PhenoArch plaform. (B) Map of incident photosynthetically active radiation (PAR) cumulated for each plant at 36 days after germination. (C) Picture of a plant in the cabin of the imaging unit with 3D image acquisition involving top and side RGB cameras the allow daily capture of RGB colour images (2056 x 2454) from 13 views (12 side views from 30° rotational difference and one top view. (D) One example of the 13 views. (E) Representation of the 3D reconstruction of one plant. See Cabrera-Bosquet et al. 2016 for details.

Plant and leaf trait measurements

From 7 to 36 days after sowing, pictures of the plants were daily acquired from 13 views (12 lateral views from 30° rotational difference and one top view). Cycles of plant imaging were daily made for each plant during the night. Images were converted into mm^2 by calibrating camera positions using reference objects and merged to reconstruct plant 3D-architecture. With those daily images, plant pixels were segmented from those of the background and used for estimating the daily aboveground fresh mass and whole plant leaf area (Brichet et al., 2017; Prado et al., 2018). Then, maximal fresh plant mass (PM, g) and total leaf area (PA, m^2) were estimated. Calibration curves of plant fresh mass were constructed using multiple linear regression models based on processed images taken in 13 directions against measurements of aboveground aboveground fresh mass at harvest (Brichet et al., 2017). Their time courses were expressed as a function of thermal time in equivalent days at 20°C. Aboveground fresh aboveground mass were therefore estimated at specific time by interpolation between nonsynchronous measurement points (Prado et al., 2018). With those data, maximal of absolute plant growth was calculated as an increase in aboveground plant mass during 15 days at 20°C (PG, g d^{-1}).

Before harvesting, between 36 and 42 days after sowing, 10 punches were made on both sides of the main leaf vein (area of one punch = 50.26 mm^2) on a fully expanded leaf (the eighth, ninth or tenth leaf produced). Punches were dried at 60 °C during 72 h to determine dry weight; specific leaf area (SLA, $m^2\ g^{-1}$) was calculated by the ratio of the area represented by all the punches to their dry weight.

Estimation of the shading index

At a given day, each 3D reconstructed plant was replaced in its specific position in the glasshouse to reproduce its light environment. Light interception was estimated using the CARIBU light propagation model (Chelle and Andrieu, 1998) combined with a sky radiance distribution model mixing SOC (Moon and Spencer, 1942) and clear sky (Perez et al., 2002) distributions according to the mixing ratio proposed by Mardaljevic (2000). In order to estimate light interception efficiency independently of global sky irradiance that may vary between days, normalized incident radiance was simulated on the virtual scene (1.m^{-2} of ground). The daily quantity of photosynthetically active radiation (PAR) intercepted per plant *i* for a normalized incident irradiance (m^{-2}_{ground}) in the platform was expressed as $PAR_{rel\ I}$, without considering the neighbors $PAR_{rel\ isol\ i}$.

To estimate competition for light, shading index (SI) was calculated using the following equation (*Unpublished data:* Perez et al., 2018):

$$SI_i = \int \frac{PAR_{rel\ isol\ i} - PAR_{rel\ i}}{PAR_{rel\ isol\ i}} \quad \text{(eq1)}$$

This equation was integrated during the considered leaf growth for SLA, during plant life for PA and PM and from plant germination to the end of plant growth measurements for PG.

Statistical analyses

We performed analyses of variance (ANOVA) to test genotype, water treatment, shading index and their interactions effects on the three size related traits (PA, PM and PG). Watering conditions were considered separately in a linear model with genotype as a fixed factor in order to obtain genotypic means. We then added shading index as a fixed factor to this linear model in order to analyze this specific factor. To test water stress impact on the allometric relationships, we realized standardized major axis (SMA) regressions with genotypic means. Test of common SMA slopes discriminated water deficit impact on allometric relationships. Trait responses to WD were calculated as logarithms of the response ratios as lnRR = ln ($\text{Trait}_{WD}/\text{Trait}_{WW}$). The relationships between traits and log response ratios were investigated using linear regression models. To analyse shading index influence on plant allometric response to WD, the logarithms of the response ratio of adjusted means were calculated and compared to the ones obtained with genotypic means.

All statistical analyses were performed in the R statistical environment (R Core development team, 2018) using appropriate packages. Adjusted genotypic means were extracted from the 'lsmeans' function from the *emmeans* package. We fit SMA regressions and tested for differences in slopes along main axis between watering conditions with *smatr* package. Spearman's correlation coefficients were calculated with 'cor.test' function from the *stats* package.

TABLE 1 List of leaf and plant traits studied, their abbreviations and units.

Trait	Abbreviation	Units
Leaf trait		
Specific leaf area	SLA	$m^2\ g^{-1}$
Plant traits		
Total leaf area	PA	m^2
Aboveground fresh mass	PM	g
Maximal growth rate	PG	$g\ d^{-1}$
Light variable		
Shading index	SI	

Results

Response of plant allometry to water deficit

Highly significant variation in plant size-related traits was found across the 337 maize genotypes. Under WW, aboveground fresh mass (PM) varied 3 times (151-445 g), whole-plant growth rate (PG) 5.7 times (from 9.4 to 53.3 g d^{-1}), and total leaf area (PA) 2 times (0.23-0.48 m^2) across genotypes (Fig. 2). The soil WD applied resulted in a significant reduction of plant size in almost (286 out of 337) all maize genotypes (Table 2; Supporting Fig. S1 and S2). Under WD, the range of values was narrower for the three traits, especially for higher values (PM = [167; 331] g; PG

= [8.77; 20.12] g d^{-1}; PA = [0.21; 0.41] m^2). This resulted from the significant negative relationship between plant size and plant size response to WD (see Fig. 3 for PM), i.e. bigger plants exhibited higher size reduction than smaller ones. However, weak correlations between values of plant size related traits under WW and WD ($r_{spearman}$ = 0.3, 0.32 and 0.38 with $P < 0.001$, respectively for PM, PG and PA) indicated significant variability in plant response to WD across the 337 maize genotypes (Supporting Fig. S2). Across genotypes, broad-sense heritability (H^2) was similarly strong for PM (0.62), PG (0.61) and PA (0.56) under WW, whereas it was a bit weaker under WD where H^2 was equal to 0.54 for and 0.49 for PM, and 0.55 for PA (Table 2). Across genotypes, highly significant allometric relationships were found between PM, PG and PA both under WW and WD treatments (Fig. 2). The SMA slopes of the log-linear relationship between PM and PA significantly differed between WW and WD treatments ($P < 0.001$ with slope equal to 0.68 and 0.8 under WW and WD, respectively) (Fig. 2A). They also significantly differed between treatments for the relationships with PG (PG vs. PM, $P < 0.001$ with slope equal to 1.3 under WW; PG vs. PA, $P < 0.001$ with slope equal to 1.94) (Fig. 2B and C).

TABLE 2 Estimation of broad-sense heritability of the studied leaf, plant and growth-relative traits by separating watering conditions.

	Trait			
Scenario	**SLA**	**PM**	**PG**	**PA**
WW	0.56	0.62	0.61	0.56
WD	0.53	0.49	0.54	0.55

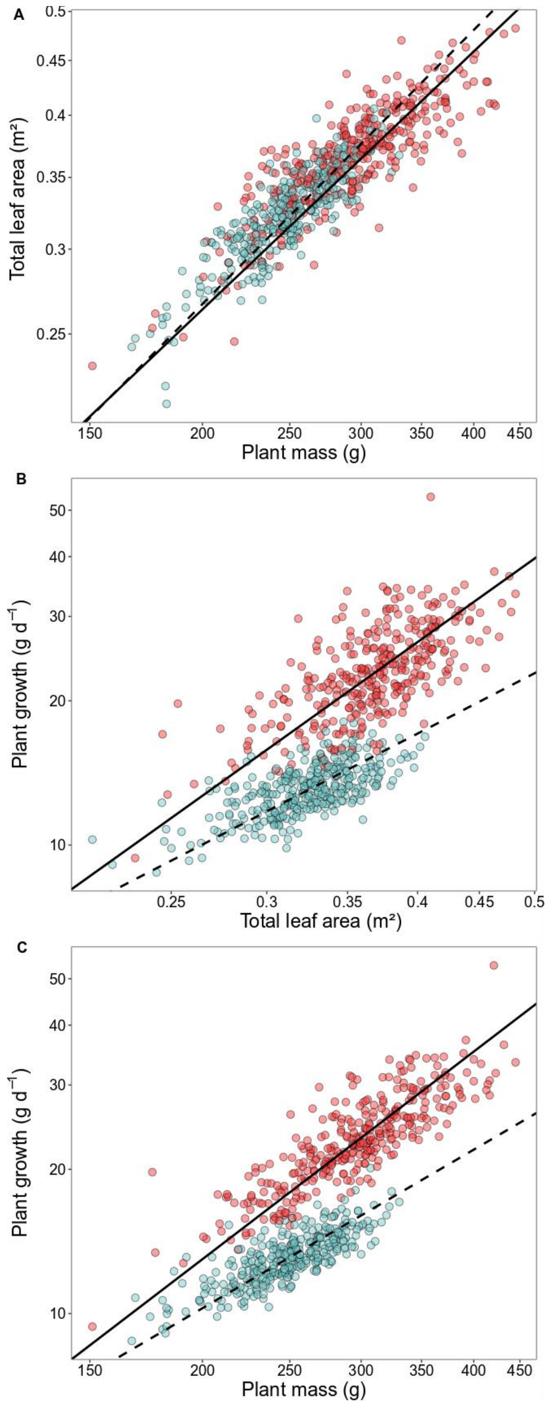

FIGURE 2 Allometric relationships of aboveground fresh mass (PM), total leaf area (PA) and plant growth rate (PG) in 337 maize genotypes under well-watered (WW) and water deficit (WD) conditions. Each point represents the genotypic mean value of each maize genotype under WW (blue) and WD (red). Solid and dashed lines represent the statistically significant SMA regressions under WW and WD, respectively. (A) For of PA to PM the relationships were $\log PA_{WW} = -4.88 + 0.68 (\log PM_{WW})$; $R^2 = 0.71$, $P < 0.001$ and $\log PA_{WD} = -5.51 + 0.8 (\log PM_{WD})$; $R^2 = 0.74$, $P < 0.001$; both n = 337, under WW and WD, respectively. (B) For of PG to PA the relationships were $\log PG_{WW} = -2.61 + 0.51 (\log PA_{WW})$; $R^2 = 0.46$, $P < 0.001$ and $\log PG_{WD} = -3.1 + 0.77 (\log PA_{WD})$; $R^2 = 0.45$, $P < 0.001$; both n = 337, under WW and WD, respectively. (C) For of PG to PM the relationships were $\log PG_{WW} = -4.37 + 1.32 (\log PM_{WW})$; $R^2 = 0.72$, $P < 0.001$ and $\log PG_{WD} = -3.12 + 1.03 (\log PM_{WD})$; $R^2 = 0.63$, $P < 0.001$; both n = 337, under WW and WD, respectively.

Influence of light availability on plant allometric response to WD

Under low light availability, i.e. increasing shading index values (SI), plant size (PM, PA) and plant growth rate (PG) significantly decreased under both watering treatments (Supporting Fig. S3, Table 3). Linear relationships of PM, PG and PA with higher SI values were stronger under WW ($R^2 = 0.38$, 0.34 and 0.23; all $P < 0.001$, for PM, PG and PA, respectively) than under WD ($R^2 = 0.15$, 0.03 and 0.09; all $P < 0.001$ for PM, PG and PA respectively) (Supporting Fig. S3, Table 3). Taking light availability into account by adding SI as a covariate did not significantly change the negative relationship between plant mass and its response to WD (Fig. 3). However, the range of values of PM response to WD was narrower with SI ([-0.7; 0.29]) than without SI as a covariate ([-0.67; 0.41]). Adding SI to the model changed the lnRR of PM, PG and PA (Fig. 4). The slopes of the relationships of lnRR with SI taken into account to lnRR without SI taken into account were slightly and significantly lower than 1 (slopes equal to 0.8, 0.88 and 0.87 respectively for PM, PG and PA), therefore lnRR was slightly but not significantly overestimated when SI was not taken into account (test of student to compare means of lnRR with $P > 0.05$ for the three traits) (Fig. 4).

TABLE 3 Changes in the three plant traits of maize genotypes in response to water deficit. Values are probabilities associated with genotype, scenario, shading index and their interaction effects model. 337 genotypes were studied. For abbreviations, see Table 1.

	Traits	**Genotype**	**Scenario**	**SI**	**G*S**	**G*SI**	**S*SI**	**G*S*SI**
Traits	**PM**	< 0.001	< 0.001	< 0.001	0.98	0.58	< 0.001	0.9
	PG	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	PA	< 0.001	< 0.001	< 0.001	0.9	0.64	< 0.001	0.74

Note. G: Genotype, S: Scenario and SI: Shading Index.

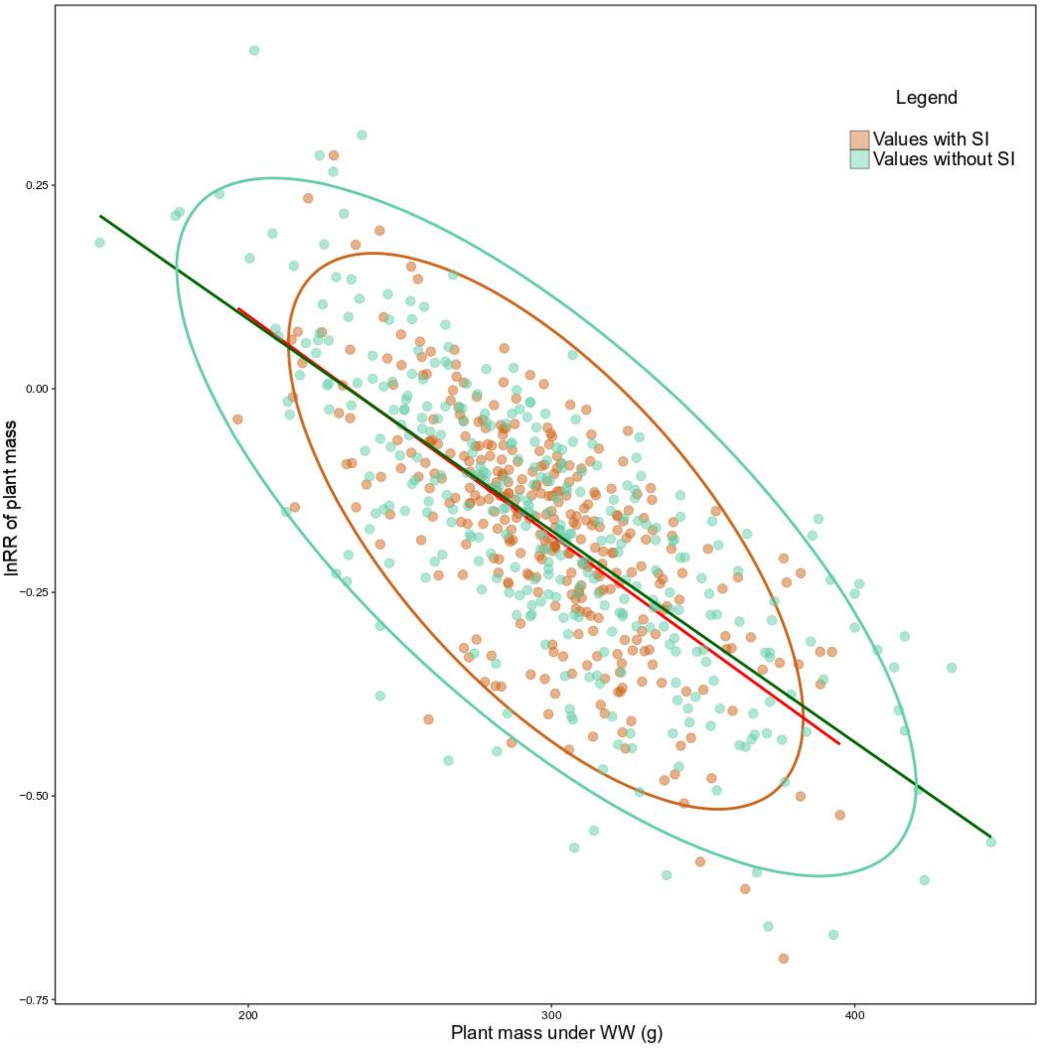

FIGURE 3 Relationship between plant mass (PM) response to water deficit (WD) and PM values under well-watered (WW) conditions with and without considering the shading index (SI). Points represent genotypic means (green) and adjusted means with the shading index as fixed effect (brown) (n = 337), respectively. Corresponding ellipses represent 95% confidence intervals. Lines are significant linear regression lines for genotypic means (green; $R^2 = 0.54$ with $P < 0.001$) and adjusted means for SI (brown; $R^2 = 0.45$ with $P < 0.001$). Slopes of the two regressions were not significantly different ($P = 0.6$).

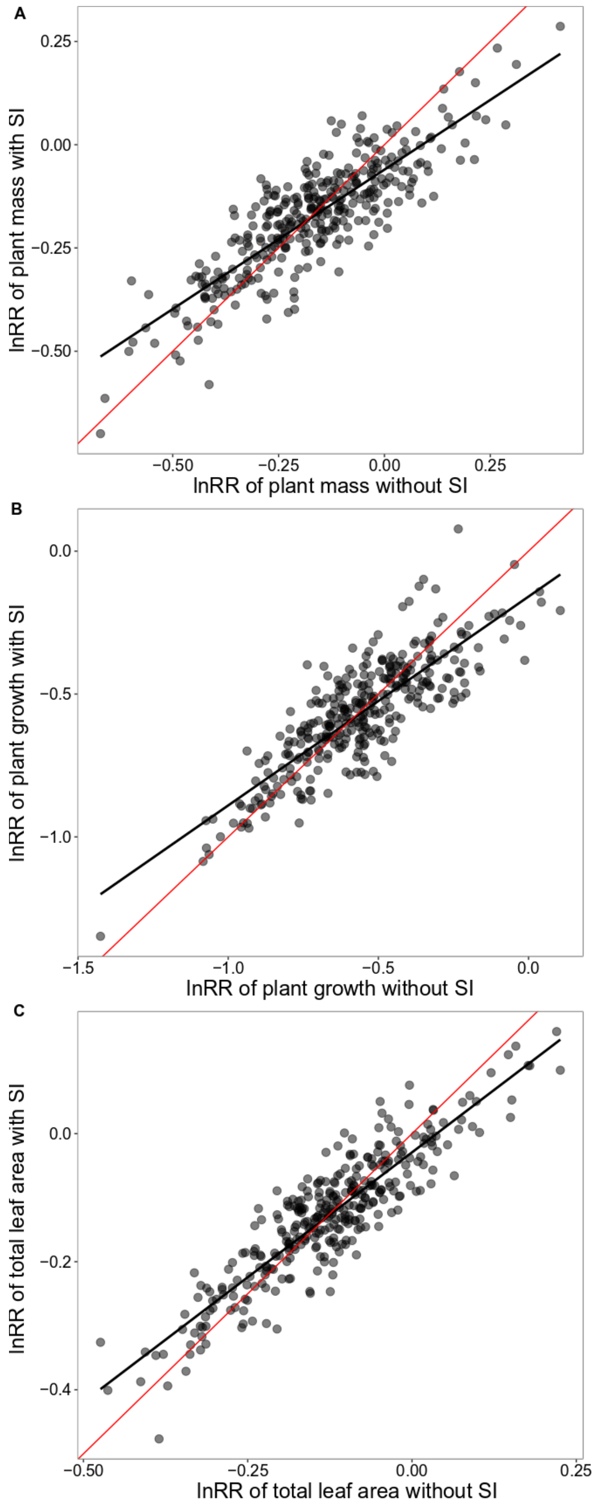

FIGURE 4 Log response ratios (lnRR) to water deficit (WD) with and without taking the shading index (SI) into account in the calculation of genotypic trait means. Red lines are y = x lines. Black lines represent statistically significant linear regressions (all n = 337). (A) LnRR of plant mass (R^2 = 0.80; $P < 0.001$) with slope equal to 0.79 (Confidence interval = [0.75-0.84]). (B) LnRR of plant growth rate (R^2 = 0.72; $P < 0.001$) with slope equal to 0.88 (Confidence interval = [0.83 – 0.93]). (C) LnRR of total leaf area (R^2 = 0.69; $P <$ 0.001) with slope equal to 0.87 (Confidence interval = [0.83 – 0.91]).

Plasticity in SLA modulates plant allometric response to WD

Significantly lower SLA values were observed under WW than under WD conditions (Supporting Fig. S1D; Table 3; Supporting Fig. S4). Broad sense heritability of SLA was similar under both conditions (H^2 = 0.56 and 0.53, under WW and WD, respectively) (Table 2). SLA significantly increased in response to low light availability (high SI) under both watering treatments (R^2 = 0.26 and 0.12; both $P < 0.001$, under WW and WD, respectively; Supporting Fig. S4A). Across genotypes, the addition of SI in the model reduced the estimate of lnRR of SLA to WD (slope equal to 0.76) (Supporting Fig. S4B).

SLA was significantly negatively related to PM, PG and PA (Fig. 5). Significantly different slopes were detected between WW and WD for the relationships of PM and PG to SLA (both $P <$ 0.001), but nor for the relationship of PA to SLA ($P = 0.5$). The response of the three size-related traits to WD, i.e. reductions of PM, PG and PA, significantly increase with higher SLA values under optimal conditions (Fig. 6).

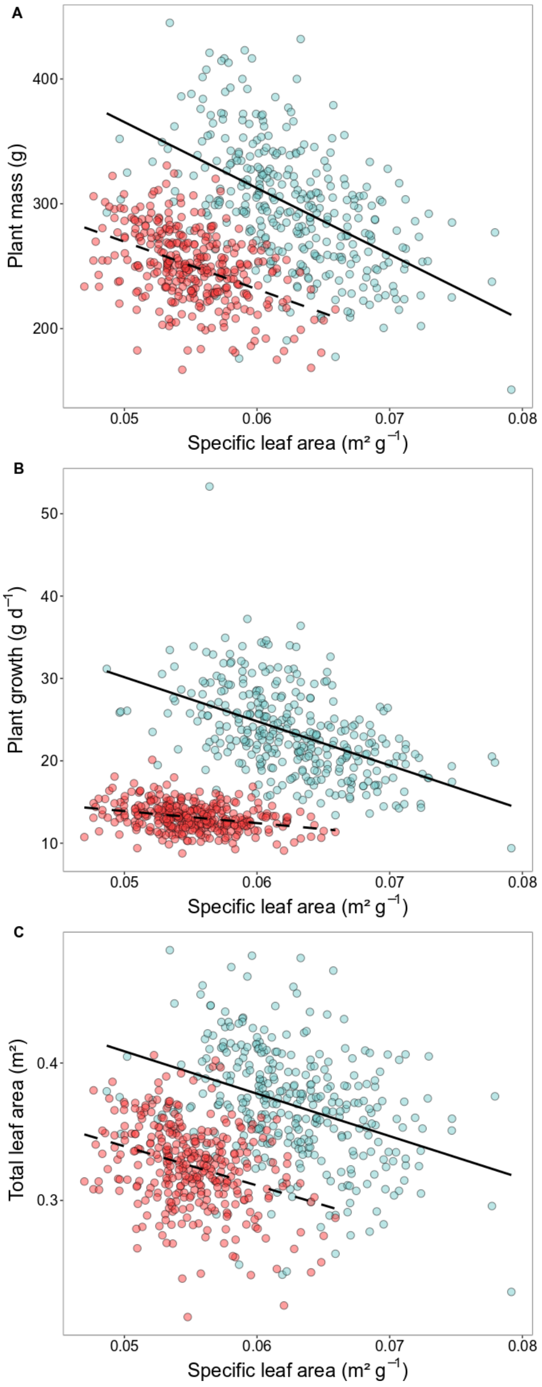

FIGURE 5 Relationships of aboveground fresh mass (PM), total leaf area (PA) and plant growth rate (PG) to specific leaf area (SLA) under well-watered (WW) and water deficit (WD) conditions. Each point represents the genotypic mean value without SI of each maize genotype under WW (blue) and WD (red). Solid and dashed lines represent the statistically significant SMA regressions under WW and WD, respectively. (A) For PM the relationships were $\log PM_{WW} = 2.5 – 1.13 * (\log SLA_{WW})$; $R^2 = 0.27$, $P < 0.001$ and $\log PM_{WD} = 3 - 0.86 * (\log SLA_{WD})$; $R^2 = 0.2$, $P < 0.001$; both n = 337, under WW and WD, respectively. (B) For PG the relationships were $\log PG_{WW} = -0.95 – 1.5 * (\log SLA_{WW})$; $R^2 = 0.28$, $P < 0.001$ and $\log PG_{WD} = 0.8 – 0.61 * (\log SLA_{WD})$; $R^2 = 0.09$, $P < 0.001$; both n = 337, under WW and WD, respectively. (A) For PA the relationships were $\log PA_{WW} = -2.5 – 0.53 * (\log SLA_{WW})$; $R^2 = 0.13$, $P < 0.001$ and $\log PM_{WD} = -2.6 – 0.5 * (\log SLA_{WD})$; $R^2 = 0.10$, $P < 0.001$; both n = 337, under WW and WD, respectively.

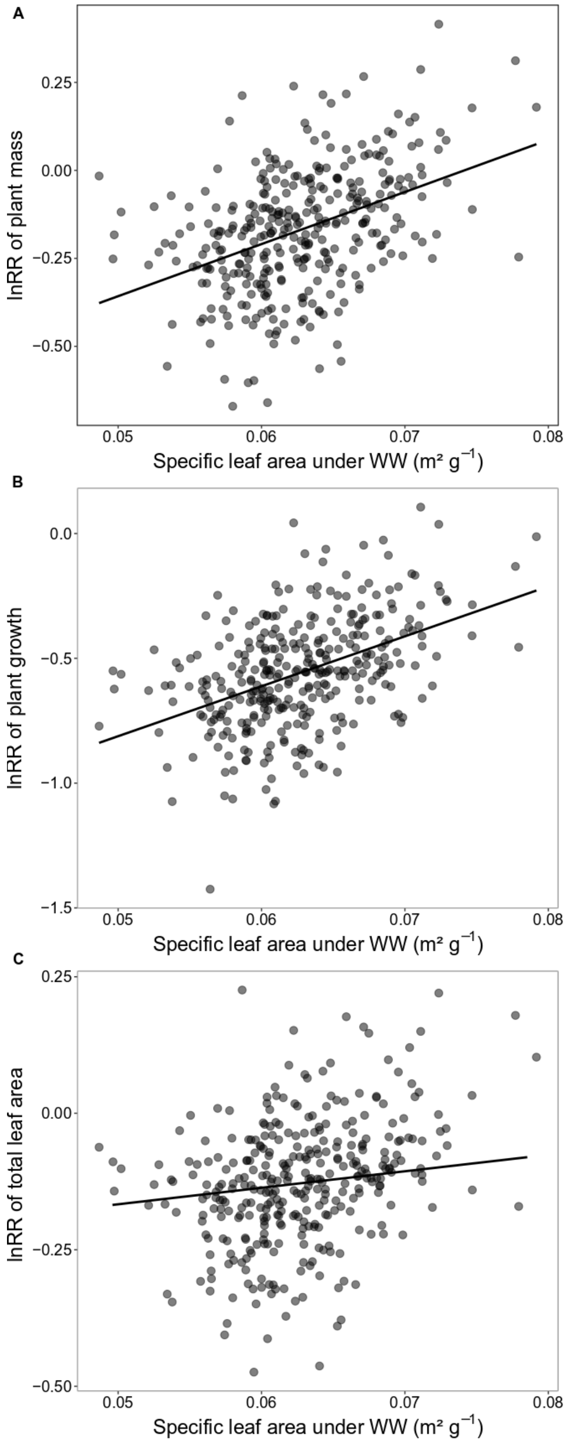

FIGURE 6 Linear relationships between the log response ratios (lnRR) of plant growth, size and total leaf area with specific leaf area under WW. The three relationships were between: A, lnRR of plant growth and specific leaf area under WW (m^2 g^{-1}); B, lnRR of plant mass and specific leaf area under WW (m^2 g^{-1}) and C, lnRR of total leaf area and specific leaf area under WW (m^2 g^{-1}). Black points represent genotypic means (n = 337). Black lines represent the statistically significant linear regressions between lnRR of plant mass, growth and total leaf area with specific leaf area under WW ($R^2 = 0.17$, 0.20 and 0.11 with $P < 0.001$, respectively).

Discussion

Here we used a unique panel of 337 maize genotypes to explore water deficit impacts on plant allometry. Not surprisingly, we highlighted highly significant plasticity of aboveground plant size and plant growth rate (PG) in response to WD. Aboveground fresh mass (PM) and total leaf area (PA) were indeed significantly reduced by WD and both traits were highly correlated both under WW and WD conditions with a slight but significant change in slopes. This is indicative of significant changes in biomass partitioning in response to WD. In addition to the global reduction of plant organ size, it has commonly been observed that the proportion of biomass allocated to the different plant organs change in response to WD (Poorter et al., 2015; Eziz et al. 2017). For instance, increased allocation to root biomass is a general response to WD in many species (Eziz et al., 2017; Vile et al., 2012). Here we specifically investigated the aboveground mass to total leaf area allometric relationship. Plant growth rate was also reduced by WD and this stress significantly affected the slopes of the allometric relationships of aboveground fresh mass and plant area to aboveground growth rate.

Within maize, allometric slopes were significantly different from the expected ¾ (Niklas and Enquist, 2001). Few studies get interested into possible variability around plant allometric slope (Vasseur et al., 2012), while several animal studies have questioned the valor of the slope between body size and metabolism (Bolstad et al., 2015; Bonduriansky and Day, 2003; Ray et al., 2016). Allometric exponent is often considered stable due to structural constraints related to body size (Voje and Hansen, 2013). But, hypothesis suggested that allometric exponent resulted from natural selection, and then could differ from the "expected valor" according to environmental conditions (Bonduriansky and Day, 2003; Gould, 1966). By restricting plant growth, experimental conditions could cause variability in allometric slopes. For example, maize plants were cultivated into pots, which can constrain plant size compared to natural conditions (Dambreville et al., 2017; Poorter et al., 2012). Then, within crops, plants grew up until planting density became critical for their size. In that context, Deng et al. (2012b) showed that there was a scaling relationship between plant mass (M) and critical planting density (N_{crit}), as $N_{crit} = M^{-3/4}$. According to Deng et al. (2012b), with the plant biomass mean obtained on 337 maize genotypes, critical density would be around 14 plants per m², while we have 18 plants per m² in the phenotyping platform (Deng et al., 2012b). Plant size would be limited by a high planting density. As a consequence, allometric slope would differ from the expected valor as a way to adapt plant phenotype to the phenotyping platform conditions.

Our findings highlighted significant water stress effect on allometric relationships between total leaf area, aboveground mass and growth. To our knowledge, few studies have shown possible variations of plant allometric relationships in response to environmental conditions. This result suggests possible variations in biomass allocation pattern to aerial part under abiotic stress (Poorter et al., 2015). Within *A. thaliana*, Vasseur et al. (2018) explained this variation as advantageous for stress resistance, despite their cost for seed production. Here, variability of crop allometry could result from phenotypic adaptations to water stress (Pan et al., 2013; Vasseur et al., 2018). Despite this variability in response to water deficit, the allometric relationships between plant growth, total leaf area and plant mass were conserved. This result confirms that allometric combinations are “hardly modifiable”, even under abiotic stresses (Voje et al., 2014). One hypothesis is the existence of developmental and structural constraints selection (Climent et al., 2006; Egset et al.; Voje et al., 2014). In that context, plasticity of plant allometry would be possible only if the phenotype obtained is viable under natural conditions (Bolstad et al., 2015). Even if more studies are needed, our results are interesting for Maize breeding. Better knowledge about plant allometric response to abiotic stresses enable to highlight changes in biomass allocation patterns (Poorter et al., 2015; Weiner, 2004), and then, inform about structures and functions reflecting plant strategy.

Lower biomass accumulation to aboveground compartment is an usual way for plants to reduce biomass production (Eziz et al., 2017; Poorter and Nagel, 2000). This phenotypic response often occurs as drought tolerance strategy (Erice et al., 2010; Villagra and Cavagnaro, 2006; Yin et al., 2005). Here, we observed a common reduction of plant size, total leaf area and growth under water deficit. In the high-throughput phenotyping platform, plants were surrounded by other plants. When shade created by neighboring plants increased, our study showed reduction in the development of aerial parts (total leaf area, aboveground mass and growth) necessary to develop light interception. Across maize genotypes, shade mitigates water deficit impact on plant size, total leaf area and growth. Those results confirmed shade influence on water stress tolerance within Maize (Page et al., 2011). In face to shade, genetic diversity of plant size response to water deficit was less important between genotypes. This result suggests similar phenotypic adaptations for limiting size-related trait reductions under shade. Increasing plant size and growth enable to be above neighboring plants to have more light, and then avoid shade (Gommers et al., 2013). Level of shade is not important enough, compare to water deficit, to observe stronger differences. But our results suggest that this mitigation of size-related trait reductions would result from a similar plant strategy for avoiding shade within maize. In an agricultural field, it is unless that only one stress occurs, better knowledge of size-related trait sensitivities to cumulative stresses would enable to better understand and select crop allometric trajectory.

In our study, we showed that plant size and growth reductions under water stress can be related to specific leaf area adjustments. In response to stress, plants may exhibit phenotypic plasticity in key functional traits allowing short-term adjustment to environmental conditions (Hamann et al., 2018). With higher SLA, plants will capture more light per unit of leaf weight, and would be expected to exhibit stronger competitive ability (Funk et al., 2016). For example, in a light-limiting environment due to high planting density, shade-leaves have higher SLA and therefore are more efficient in capturing light on a unit per mass (Chen and Klinka, 1998). Under drought, specific leaf area decreases as a way to enhance water use efficiency (Wellstein et al., 2017). Those phenotypic adjustments are associated with changes in leaf structure and/or anatomy (Poorter et al., 2009; Wilson et al., 1999; Witkowski and Lamont, 1991). Thus, plasticity in SLA is advantageous as it allows plants to adjust light interception and stress tolerance to environmental conditions (Hamann et al., 2018; Scheepens et al., 2010; Wright et al., 2004). Our findings highlight the fundamental role of SLA in the maximization of total leaf area and growth rate. The strong within-maize relationship found between SLA and aboveground mass is of great interest for the predictions of biomass production of crop species and for the programs of plant breeding. Moreover, intraspecific plasticity of both biomass allocation patterns and specific leaf area response highlight genetic diversity. As heritability of the studied traits was high, this variability between genotypes could be used by plant breeding to select Maize strategy for both biomass allocation and drought tolerance.

Authors' Contributions

All the authors designed the study; A.R. compiled and analysed the data collected by the co-authors; all the authors wrote the manuscript. All the authors gave their final approval for publication.

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Supporting Information:

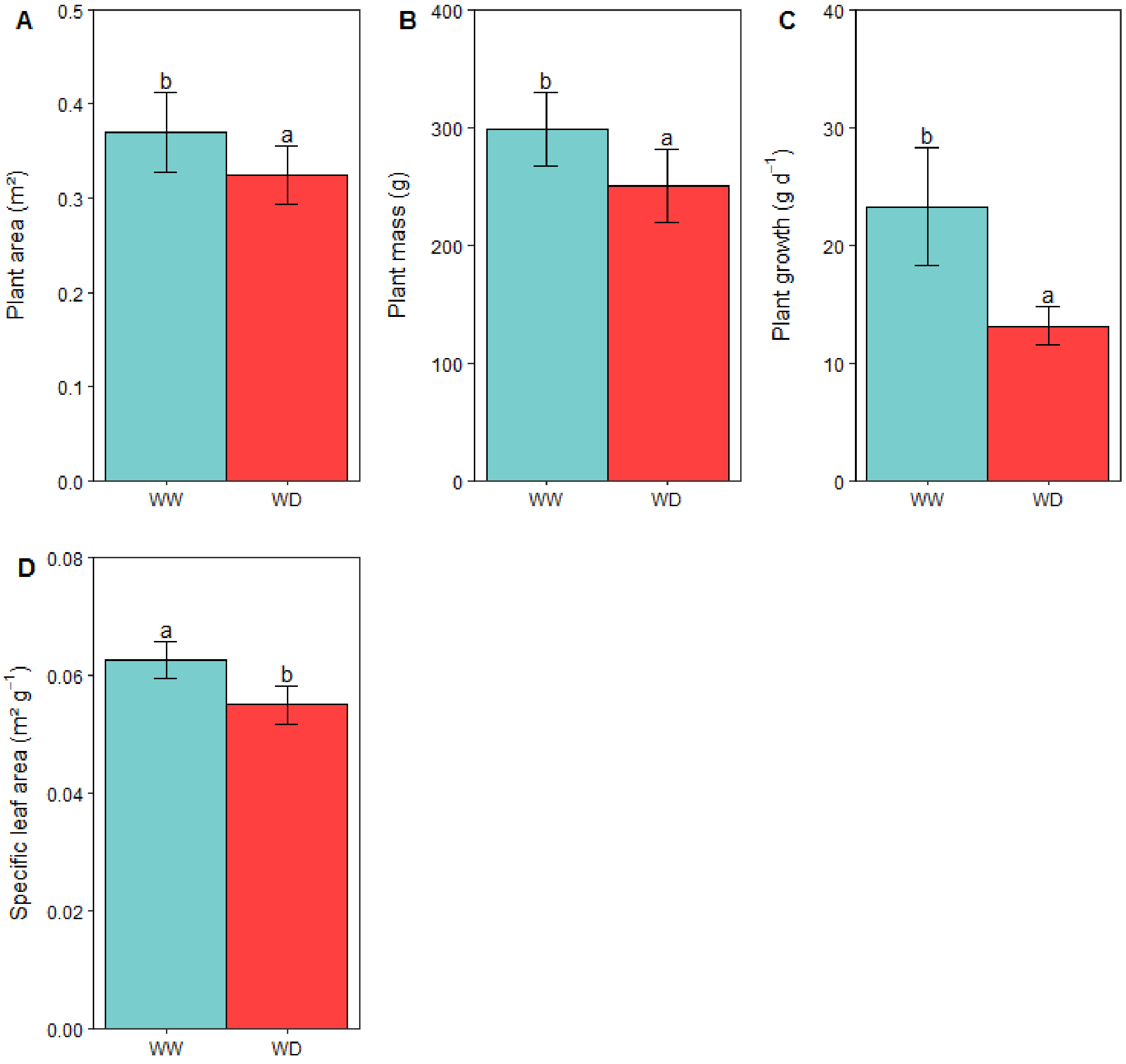
The following Supporting Information is available for this article:

Supporting Fig. S1 Variation of lead and plant genotypic means in 337 maize genotypes grown under well-watered (WW) and water deficit (WD) environments.

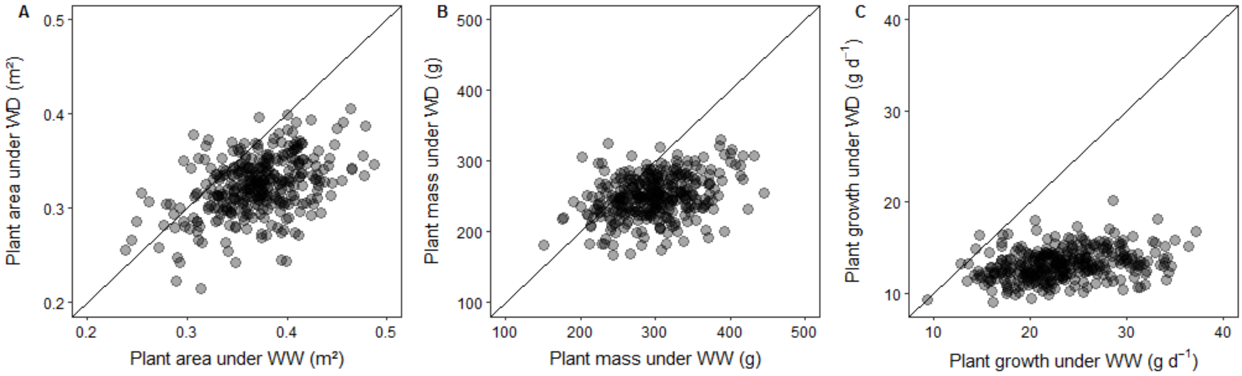
Supporting Fig. S2 Relationships of genotypic mean trait values under water deficit (WD) versus well-watered (WW) conditions.

Supporting Fig. S3 Projection of the studied maize individual plants in the three studied traits with the shading index relationships by separating well-watered (WW) from water deficit (WD) conditions.

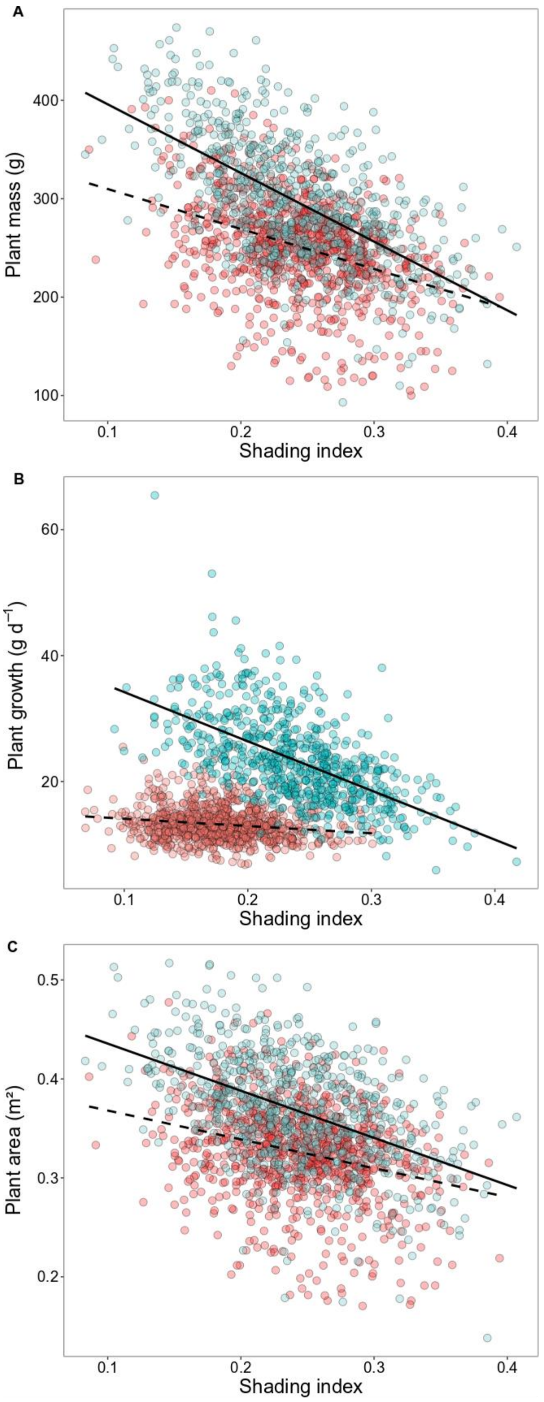
Supporting Fig. S4 Impact of the shading index (SI) on specific leaf area response to water deficit (WD).



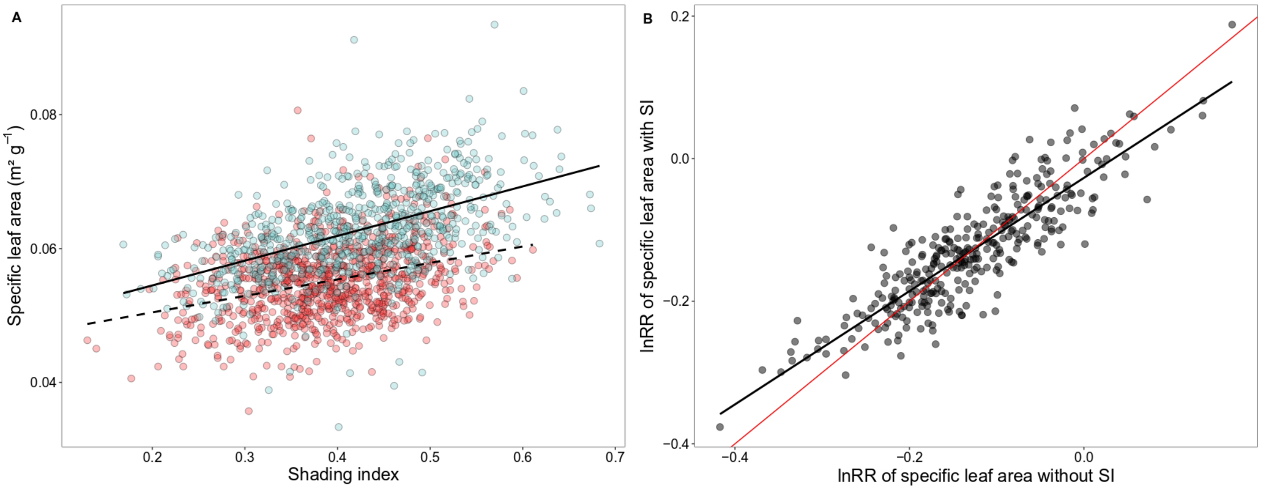
SUPPORTING FIG. S1 Variation of leaf and plant genotypic means in 337 maize hybrid genotypes grown under well-watered (WW) and water deficit (WD) environments. The five traits represented: (A) total leaf area (m^2); (B) fresh aboveground mass (g); (C) plant growth (g d^{-1}) and (D) specific leaf area (m^2 g^{-1}) were determined on plants grown under well-watered (WW) and water deficit (WD) conditions. Bars and error bars represent means ± SEM (n = 337). Different letters above bars indicate significant differences at $P < 0.05$ using Tukey’s multiple comparison tests.



SUPPORTING FIG. S2 Relationships of genotypic mean trait values under water deficit (WD) versus well-watered (WW) conditions. The three figures represent the relationships between the size-related traits: (A) the relationship between values of total leaf area (m²) under WW - under WD ($r_{spearman}$ = 0.38 with $P < 0.001$); (B) the relationship between values of fresh aboveground plant mass (g) under WW - under WD ($r_{spearman}$ = 0.3 with $P < 0.001$) and (C) the relationship between values of plant growth (g d^{-1}) under WW - under WD ($r_{spearman}$ = 0.32 with $P < 0.001$). Black points represent genotypic means (n = 337). Solid lines represent the linear regressions with intercepts and slopes equal to 0 and 1, respectively.



SUPPORTING FIG. S3 Projection of the studied maize individual plants in the three studied traits with the shading index relationships by separating well-watered (WW) from water deficit (WD) conditions. Blue and red points represent values of individual maize plants within well-watered (WW) (n = 705) and water deficit (WD) (n=930) regimes. (A) : the fresh aboveground plant mass – shading index relationship with the solid and dashed lines represent the statistically significant linear regression under WW ($R^2 = 0.38$ with $P < 0.001$) and under WD ($R^2 = 0.15$ with $P < 0.001$); (B) : the plant growth – shading index relationship with the solid and dashed lines represent the statistically significant linear regression under WW ($R^2 = 0.34$ with $P < 0.001$) and under WD ($R^2 = 0.03$ with $P < 0.001$) and (C) : the total leaf area – shading index relationship with the solid and dashed lines represent the statistically significant linear regression under WW ($R^2 = 0.23$ with $P < 0.001$) and under WD ($R^2 = 0.09$ with $P < 0.001$).



SUPPORTING FIG. S4 Relationship between shading index (SI) and specific leaf area (SLA) under well-watered (WW) and water deficit (WD) conditions. (A) Projection of the studied maize individual plants in the SLA with SI by separating well-watered (WW) from WD conditions. Blue and red points represent values of individual maize plants within WW (n = 705) and WD (n = 930) regimes. The specific leaf area – shading index relationship with the solid and dashed lines represent the statistically significant linear regression under WW ($R^2 = 0.26$ with $P < 0.001$) and under WD ($R^2 = 0.12$ with $P < 0.001$). (B) The relationship between log response ratio (lnRR) of specific leaf area with and without SI. Black circles represent maize genotypes (n = 337). The black line represents significant linear regressions ($R^2 = 0.76$ with $P < 0.001$) and the red line the 0:1 line.