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Plasticity QTLs specifically contribute to the genotype x water availability interaction in maize

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Abstract

Key message Multi-trial genome wide association study of plasticity indices allow to detect QTLs specifically involved in the genotype x water availability interaction.

Abstract Concerns regarding high maize yield losses due to increasing occurrences of drought events are growing, and breeders are still looking for molecular markers for drought tolerance. However, the genetic determinism of traits in response to drought is highly complex and identification of causal regions is a tremendous task. Here, we exploit the phenotypic data obtained from four trials carried out on a phenotyping platform, where a diversity panel of 254 maize hybrids was grown under well-watered and water deficit conditions, to investigate the genetic bases of the drought response in maize. To dissociate drought effect from other environmental factors, we performed multi-trial genome-wide association study on well-watered and water deficit phenotypic means, and on phenotypic plasticity indices computed from measurements made for six ecophysiological traits. We identify 102 QTLs and 40 plasticity QTLs. Most of them were new compared to those obtained from a previous study on the same dataset. Our results show that plasticity QTLs cover genetic regions not identified by QTLs. Furthermore, for all ecophysiological traits, except one, plasticity QTLs are specifically involved in the genotype by water availability interaction, for which they explain between 60% and 100% of the variance. Altogether, QTLs and plasticity QTLs captured more than 75% of the genotype by water availability interaction variance, and allowed to find new genetic regions. Overall, our results demonstrate the importance of considering phenotypic plasticity to decipher the genetic architecture of trait response to stress.

Keywords: drought response, phenotypic plasticity, multi-trial GWAS, QTL, maize

Introduction

Maize, currently the leading cereal crop ahead of rice and wheat (FAOSTAT, 2022), is massively produced, traded and exported worldwide (Wu and Guclu, 2013; Erenstein et al, 2022). The major maize-producing countries are the United States, China and Brazil, with production exceeding seven hundred million tons per year since 2019 (FAOSTAT, 2022). This success is due not only to advances in agronomic practices and breeding, which have improved maize agronomic performance (Mazur et al, 1999; Balconi et al, 2007; Kelliher et al, 2019; Simmons et al, 2021), but also to the capacity of maize to adapt to a wide range of environments (Lanari, 1979; Rotili et al, 2019). Besides being the most widely produced crop in the world, maize also has a strong social and economic impact. As an easy and cheap source of calories and micro-nutrients, maize has become a staple food for many people, especially in Sub-Saharan countries. In developed countries, maize is also widely used in the starch industry and

in the production of livestock products (Shiferaw et al, 2011; Ranum et al, 2014; Ekpa et al, 2018).

One of the principal threats to maize production is drought (Zipper et al, 2016; Daryanto et al, 2016; Song et al, 2020). Despite having a C4 metabolism, which ensures good water use efficiency (Crafts-Brandner and Salvucci, 2002), maize can be severely affected by water deficits (Salehi-Lisar and Bakhshayeshan-Agdam, 2016). The defense mechanisms that decrease water losses also reduce plant growth (Tardieu et al, 2017). For instance, the water loss / CO₂ absorption trade-off associated with stomata closure leads to a decrease in photosynthetic activity and biomass production, indirectly affecting grain yield (Efeoglu et al, 2009; Wang et al, 2019; Song et al, 2019). Water deficit can also directly induce severe yield loss if it occurs during flowering and prevents silk development, an essential step for grain production (Sah et al, 2020). With climate change, drought scenarios are expected to occur more frequently in maize-producing regions (Seager et al, 2014; Cook et al, 2014; Gudmundsson and Seneviratne, 2016). Together with the increase in human population, this is a major concern for global food security (Harrison et al, 2014; Lobell et al, 2014; Meng et al, 2016). Drought tolerance is a highly integrated trait resulting from the combination of many genetically variable traits, such as water uptake, leaf growth and transpiration rate (Tardieu et al, 2014). Consequently, developing drought-tolerant maize varieties through breeding programs is a solution of choice to mitigate yield losses (Campos et al, 2004, 2006; Cooper et al, 2014). Aided by advances in genomics, genome-wide association studies (GWAS) are one of the most popular and powerful approaches for identifying genetic polymorphisms associated with inter-individual variations of traits of interest (Zhang et al, 2020; Zhao et al, 2022). These so-called quantitative trait loci (QTLs) are further used in breeding programs. However, detecting QTLs related to the genotype x water availability interaction is complex since drought tolerance strongly depends on the environmental conditions faced by the plants. When only a single trial is conducted to study drought response, it is impossible to dissociate the effect of water conditions from the effect of other environmental factors that may fluctuate between trials. To tackle these confounding effects, it is necessary to carry out multi-environment trials (METs) (Boer et al, 2007; Rodrigues, 2018).

METs are experiments where a trait of interest is measured in several trials. For example, in Millet et al (2016), yield was measured in 29 different fields representing multiple trials with contrasting conditions. More complex METs can include paired conditions in each trial. For example, in Alvarez Prado et al (2017), stomatal conductance in maize was measured in four trials, each with two different watering conditions (well-watered and water deficit). To detect QTLs of interest from METs, GWAS can be carried out for each trial separately. Then, among all identified QTLs, some are selected for their contribution to the genetic effects using a modeling approach (Diouf et al, 2020) or because they are present in a specific set of trials (Millet et al, 2016; Alvarez Prado et al, 2017; Touzy et al, 2019; Hu et al, 2021). Another approach in METs with paired conditions is based on phenotypic plasticity, *i.e.* the variation in phenotype for a given genotype in response to different conditions (Bradshaw, 1965). Plasticity indices can be computed for each trait with regression models as proposed by Finlay and Wilkinson (1963) or by computing the relative difference or ratio between two studied conditions (Peleg et al, 2009; Zhai et al, 2014; dos Santos Silva et al, 2021). Performing GWAS on plasticity indices then allows the detection of condition-responsive QTLs, hereafter called plasticity QTLs (Wang et al, 2013; Zhai et al, 2014; Ye et al, 2019). Recent studies in rice and wheat have shown that plasticity QTLs tend to be positioned near stress-responsive genes (Mai et al, 2021; Fatiukha et al, 2021) and that many of them do not overlap with the QTLs detected separately in each studied condition (Kusmec et al, 2017; Diouf et al, 2020).

In this study, we investigated the genetic bases of the drought response in maize using a MET approach. To this end, we analyzed previously published phenotypic data acquired for six drought-related ecophysiological traits and 254 maize genotypes grown under two watering conditions repeated in four independent trials (Alvarez Prado et al, 2017). Because the four trials were conducted over three years and two seasons, high variations in vapor pressure deficit and light were observed (Alvarez Prado et al, 2017) and were considered as trial effects. We first assessed the effect of the trial on QTL detection by performing multi-trial GWAS on well-watered and water deficit phenotypic means. Then, considering plasticity indices as different traits, as postulated by Bradshaw (1965), we investigated the relative contribution of QTLs and plasticity QTLs to the genotype by water availability interaction for the six studied ecophysiological traits.

Methods

Description of the phenotypic and genomic data used

The phenotypic dataset used in this study was previously published by Alvarez Prado et al (2017). This dataset consisted of six ecophysiological traits, namely biomass (Biom), leaf area (LA), transpiration rate (Transp), stomatal conductance (gs_max), water uptake (WU) and water use efficiency (WUE), measured on a diversity panel of maize hybrids obtained by crossing 254 dent lines selected for their restricted flowering window with a standard flint line (UH007). Three replicates of each hybrid were grown under two watering conditions (well-watered, WW, and water deficit, WD). This experimental design was replicated in four different trials during three different years and two different seasons: spring 2012, spring 2013, spring 2016 and winter 2013. Plants were grown at the INRAE PhenoArch phenotyping platform located in France at Montpellier (Cabrera-Bosquet et al, 2016; Alvarez Prado et al, 2017), with applied soil water potentials equal to -0.05 MPa for the WW condition and ranging from -0.3 to -0.6 MPa for the WD condition. For each trait, watering condition and trial, the average of the three replicates (hereafter called the phenotypic mean) was adjusted by taking into account the greenhouse spatial effect, as described in Alvarez Prado et al (2017).

The genomic dataset contained 977,459 SNPs obtained using a combination of a 50K Infinium HD Illumina array (Ganal et al, 2011), a 600K Axiom Affymetrix array (Unterseer et al, 2014) and 500K markers obtained by genotyping by sequencing (Negro et al, 2019). SNPs with a minor allele frequency (MAF) below 0.05 or a heterozygosity rate above 0.15 were filtered. Missing values were imputed by Beagles 3.1 (Browning and Browning, 2007). SNPs were mapped on the Zm00001d.2 gene models annotation of the B73 reference assembly (ZmB73_RefGen.v4) of the maize genome obtained from MaizeGDB (<https://www.maizegdb.org/assembly#downloads>)

Calculation of plasticity indices

Plasticity indices were calculated as ecophysiological trait-related stability indices (Bousslama and Schapaugh Jr., 1984). They are defined as ratios between the phenotypic mean of a trait for a genotype under water deficit to the phenotypic mean of the same trait for the same genotype under the well-watered condition. These plasticity indices were computed for each ecophysiological trait in each trial.

Multi-trial GWAS

Multi-trial GWAS was performed by adding a fixed effect of the trial in the single locus mixed model of Yu et al (2006) :

$$Y_{gt} = \mu + T_t + \alpha.X_g + G_g + \varepsilon_{gt} \quad (1)$$

where Y_{gt} is a plasticity index or a phenotypic mean under the WW or the WD condition of genotype g in the trial t ; μ is the overall mean; T_t is the fixed effect of trial t ; α is the fixed effect of the SNP allelic dose X_g (coded as 0,1 and 2) for the genotype g ; $G_g \sim \mathcal{N}(0, \sigma_g^2.K)$ is the random effect of genotype g , with K the kinship matrix; $\varepsilon_{gt} \sim \mathcal{N}(0, \sigma^2.I_n)$ is the residual error. The kinship matrix K was computed with the whole set of SNPs except those located on the same chromosome as the tested SNP (Rincent et al, 2014), following the approach published by Astle and Balding (2009) and implemented in the R package *statgenGWAS*. Model (1) was run using the function *GWAS* of the R package *rrBLUP* (Endelman, 2011).

SNPs were considered to be significantly associated if their p-values were below 10^{-5} . SNPs less than 0.1 cM apart were clumped into QTLs identified by the ecophysiological trait to which they are related and the most significant SNP.

QTLs associated with plasticity indices were defined as plasticity QTLs while those associated with phenotypic means each of the two watering conditions were defined as QTLs.

QTL colocalization is defined by the overlap of the linkage disequilibrium (LD) windows of QTLs as described in Negro et al (2019). Genes associated with QTLs, *i.e.* genes located in QTL LD windows, were retrieved from the Zm00001d.2 gene models annotation .gff3 of the B73 reference assembly (<https://www.maizegdb.org/assembly#downloads>)

Estimation of the relevance of QTLs and plasticity QTLs

To assess the biological relevance of the detected QTLs and plasticity QTLs, we followed the approach previously described by [van Eeuwijk et al \(2010\)](#). A multi-environment mixed model with random effects for genotype (G), genotype by water availability interaction ($G \times W$), genotype by trial ($G \times T$) was first fitted following [Alvarez Prado et al \(2017\)](#) in order to estimate the variance components of random effects by the restricted maximum likelihood (REML):

$$Y_{gwt} = \mu + E_{wt} + PC_g + (PC \times E)_{gwt} + \underline{G}_g + \underline{(G \times W)}_{gw} + \underline{(G \times T)}_{gt} + \underline{\varepsilon}_{gwt} \quad (2)$$

where Y_{gwt} is the phenotypic mean of genotype g in the watering condition w and the trial t ; μ is the overall mean; E_{wt} is the fixed effect of the environment defined as the combination between the watering condition w and the trial t ; PC_g are coordinates of genotype g projected onto principal component analysis axes built with the kinship matrix K . The number of axes used was chosen following the Kaiser criterion; $(PC \times E)_{gwt}$ are the fixed interaction effects between the genetic structure PC_g and the environment defined as the combination between the watering condition w and the trial t ; $\underline{\varepsilon}_{gwt}$ is the residual of the model.

For each ecophysiological trait, the significance of \underline{G} , $\underline{G \times W}$, $\underline{G \times T}$ random effects were tested by comparing the model defined Equation (2) and the same model without the tested random effect. These random effects were considered to be significant if their p-values were below 0.05.

Then, a multi-locus multi-environment mixed model ([van Eeuwijk et al, 2010](#); [Alvarez Prado et al, 2017](#)) was fitted by adding the fixed effects of the QTLs and QTLs by environment interaction in (2):

$$Y_{gwt} = \mu + E_{wt} + PC_g + PCQ_g + (PC \times E)_{gwt} + (PCQ \times E)_{gwt} + \underline{G}_g + \underline{(G \times W)}_{gw} + \underline{(G \times T)}_{gt} + \underline{\varepsilon}_{gwt} \quad (3)$$

where PCQ_g is the fixed effect of a given set of QTLs or plasticity QTLs. PCQ_g are coordinates of the genotype g projected onto principal component analysis axes built with a kinship matrix computed with a set of significant SNPs that describe the QTLs or plasticity QTLs. The number of axes used was chosen following the Kaiser criterion.

Let r be one of the three random effects : G , $G \times T$, and $G \times W$. The proportion of variance γ_{qr} explained by a given set of SNPs q for the random effect r is defined by:

$$\gamma_{qr} = \frac{\Gamma_r - \Gamma_r^*}{\Gamma_r} \quad (4)$$

where: Γ_r is the variance component of the random effect r in (2) and Γ_r^* is the variance component of the random effect r in (3).

Results

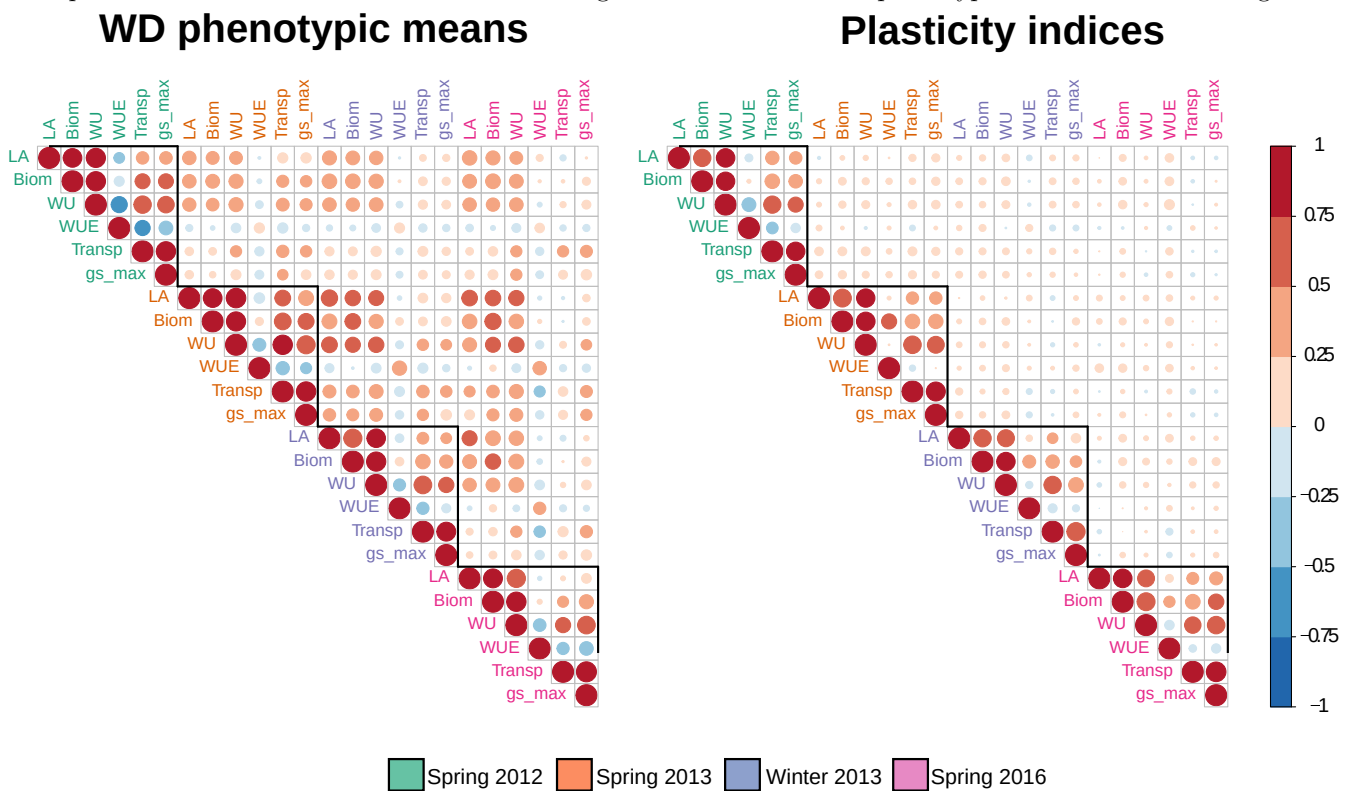
GxE interactions are driven more by trial effects than by water availability

To evaluate the trial effects, we calculated the Pearson correlation coefficients between the four trials for each phenotypic mean and plasticity index (Fig. 1). This showed that the phenotypic means or plasticity indices computed for a same ecophysiological trait in two different trials are more distant from each other than the phenotypic means or plasticity indices computed for different ecophysiological traits in the same trial. This is even more apparent for plasticity indices (Fig. 1B) than for phenotypic means (Fig. 1A and Fig. S1). This result highlights the importance of the effects of the trials on the phenotypes and response phenotypes of drought-related ecophysiological traits.

Table 1: Variance components of each random effect calculated from (2) and their contribution in percentage to the total ecophysiological trait variance. The p-values in brackets indicate the significance of the associated effect.

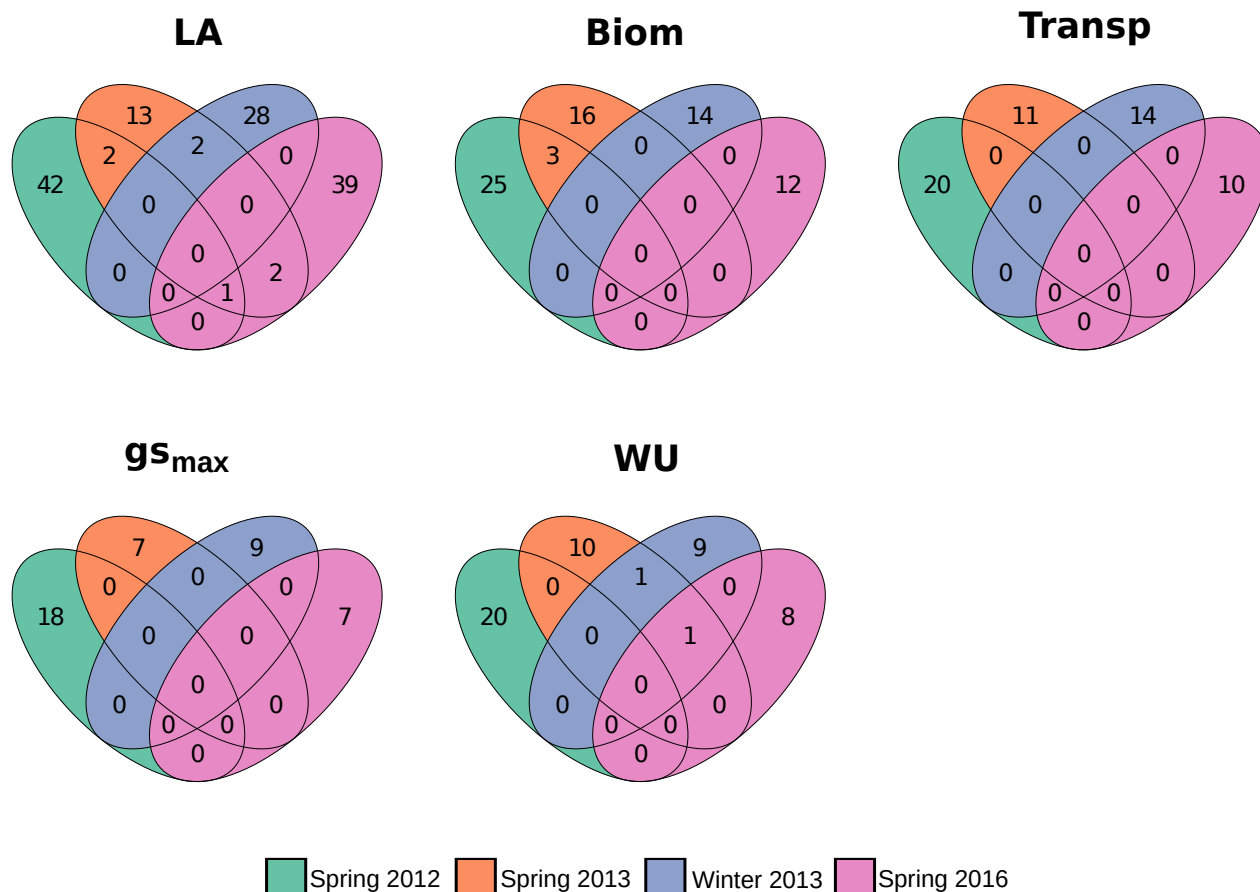
Trait	G		$G \times W$		$G \times T$		ϵ	
	Γ_G	%	$\Gamma_{G \times W}$	%	$\Gamma_{G \times T}$	%	Γ_ϵ	%
LA	$5e^{-4}$ ($<1e^{-16}$)	44	$2,7e^{-5}$ (0,051)	3	$1e^{-4}$ ($6,7e^{-7}$)	9	$5e^{-4}$	44
Biom	484 ($<1e^{-16}$)	40	104,2 ($3e^{-8}$)	9	31,9 (0,15)	3	582	48
Transp	0,23 ($<1e^{-16}$)	28	0,007 (0,6)	1	0,05 (0,005)	6	0,54	65
gs_max	9,02 ($<1e^{-16}$)	22	0,61 (0,4)	1	2,8 (0,015)	7	28,9	70
WU	0,08 ($<1e^{-16}$)	39	0,009 (0,001)	4	0,017 ($5,5e^{-5}$)	8	0,1	49
WUE	6,5 ($1e^{-13}$)	19	1,66 (0,007)	5	4,96 ($2,6e^{-7}$)	14	21,6	62

Fig. 1: Correlogram of Pearson's correlations obtained for WD phenotypic means and plasticity indices between each pair of trial x trait combinations. The correlogram obtained for WW phenotypic means is shown in Fig. S1.



To further quantify the contribution of the trials to genetic variance, we computed, for each ecophysiological trait, the significance of the G, GxT and GxW effects and their variance components (Table 1). We observed that G effect was significant for all ecophysiological traits, and it was the most important effect explaining 19-44% of the trait variances. Concerning the GxW effect, the contributions in the trait variance was low (1 - 9%) and this effect was not significant for LA, Transp, and gs_max. The contribution on the GxT effect on the trait variance was 2 to 7 times higher than the variance explained by GxW, and the GxT was significant except for Biom. These results indicate that by conducting single-trial GWAS, the probability of detecting a trial-responsive QTL is higher than that of detecting a water availability-responsive QTL. This result is supported by the fact that there is almost no overlap between the sets of QTLs detected by Alvarez Prado et al (2017) in the four trials (Fig. 2).

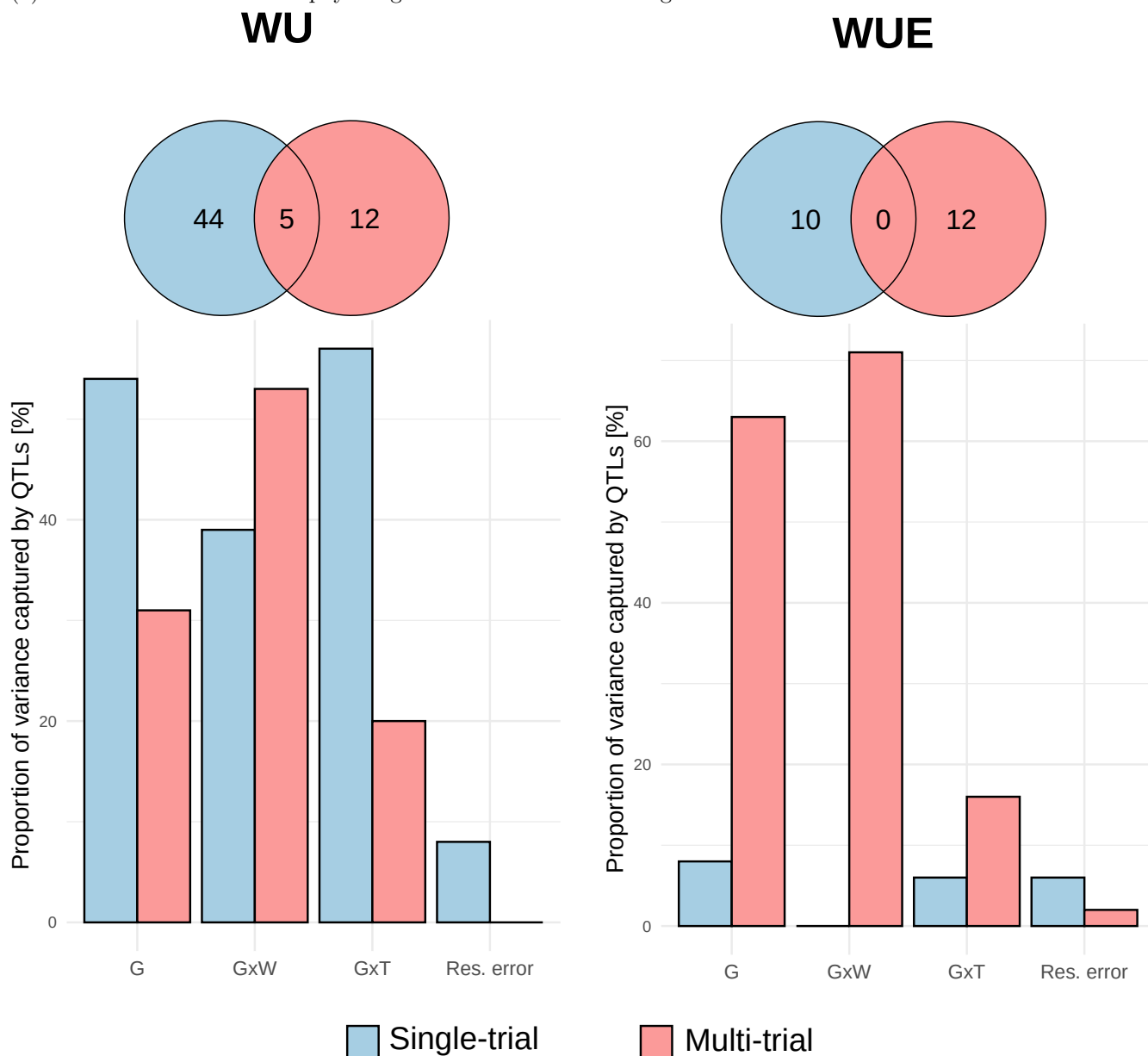
Fig. 2: Venn diagrams showing the overlap between the QTL sets detected by Alvarez Prado et al (2017) in four trials by using single-trial GWAS.



Multi-trial GWAS enhances the detection of water availability-responsive QTLs

To assess the effect of the trials on QTL detection, we performed multi-trial GWAS on phenotypic means. This allowed us to detect 102 QTLs, (60 in the WD condition and 42 in the WW condition) (Table S1), spanning 395 genes in total (Table S4). Compared to the QTLs previously obtained by single-trial GWAS with a p-value threshold of 10^{-5} (Alvarez Prado et al, 2017), QTLs obtained by multi-trial GWAS were less numerous (2.9 to 7.9 times less for all traits except WUE) and were mainly new QTLs (see the Venn diagrams in Fig. 3, Fig. S2). We then compared the contribution of all QTLs detected in the four single-trial GWAS *vs* those detected by multi-trial GWAS to the variations due to G, GxT and GxW (see bar plots in Fig. 3, Fig. S2, Table S2). For three traits, (Biom, gs_{max} and Transp), the QTLs detected only by multi-trial GWAS contributed less to the variations due to G, GxT and GxW than the QTLs detected only by single-trial GWAS. For gs_{max} and Transp, this may be explained by the fact that the number of multi-trial QTLs considered was very low compared to that of single-trial GWAS (2 and 5 *vs* 45 and 53, respectively). For the two traits LA and WU, the QTLs detected only by multi-trial GWAS contributed less to the variations due to G and GxT but much more to the variations due to GxW. Finally, for WUE, the QTLs detected only by multi-trial GWAS contributed more to the variations due to G, GxT and GxW. Overall, these results show that for half of the traits considered, multi-trial GWAS allowed to decrease the noise from the trials and detect new QTLs that were more responsive to water availability than to the trials.

Fig. 3: Comparison between QTLs detected by single-trial and multi-trial GWAS for WU and WUE. The Venn diagrams show the overlap between the two QTL sets. The barplots show the proportions of variance of the G, GxT interaction and GxW interaction effects that were captured by a given QTL set, as computed from (2) and (3). Results for the others ecophysiological traits are shown in Fig. S2.



Plasticity QTLs specifically contribute to the GxW interaction

Using multi-trial GWAS, we identified 40 plasticity QTLs (Table S1), none of which overlapped with the 102 QTLs (see the Venn diagrams in Fig. 4 and Fig.S3). These plasticity QTLs are highlighted on the karyoplot shown in Fig. 5, and cover 240 genes (Table S4).

Fig. 4: Comparison between QTLs and plasticity QTLs detected from multi-trial GWAS for WU and WUE. The barplots show the proportions of variance of the G, GxT interaction and GxW interaction effects that were captured by a given QTL set, as computed from (2) and (3). For each trait, 1000 control sets were constituted with the QTLs and n SNPs randomly selected among the total number of SNPs available (977,459) in order to control over-fitting. With n the number of plasticity QTLs detected for the trait. Results for the others traits are shown in Fig.S3.

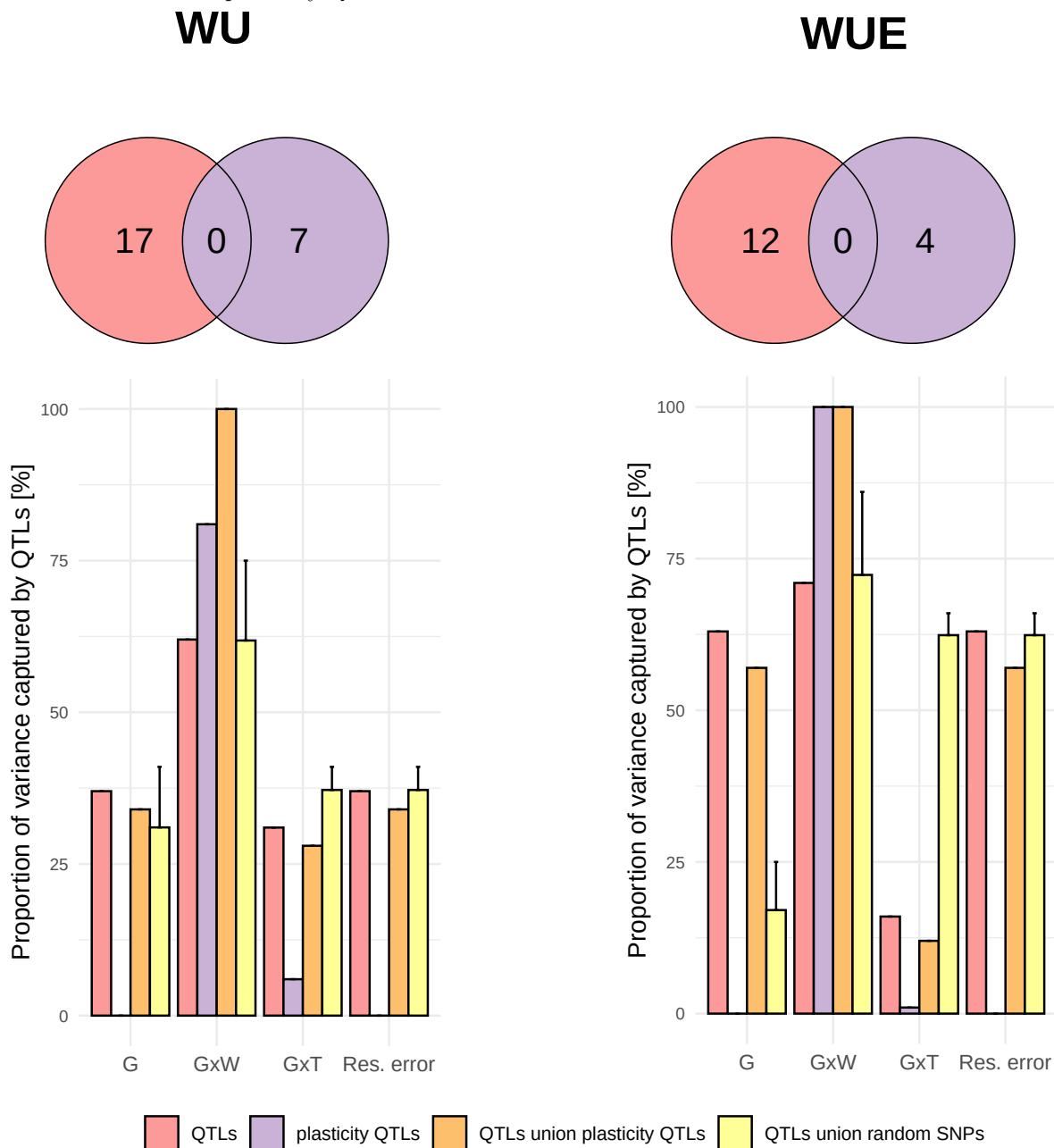
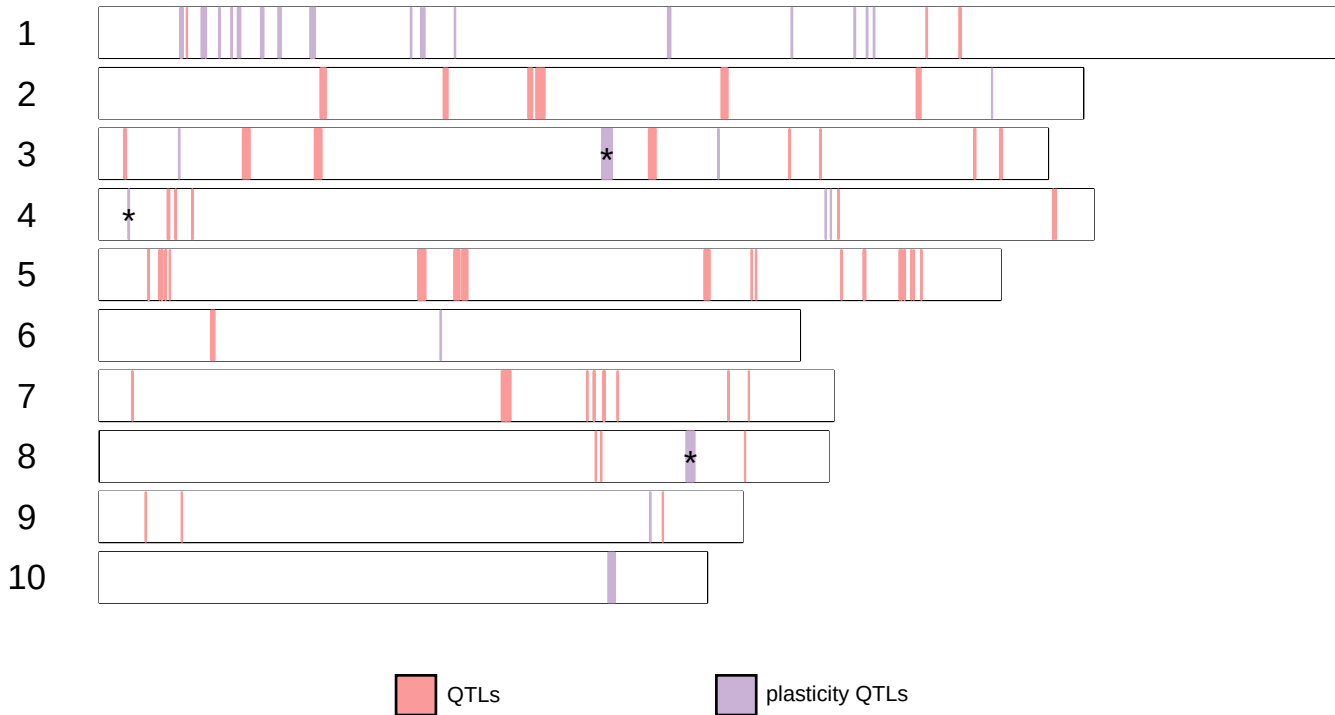


Fig. 5: Karyoplot showing the results of the multi-trial GWAS performed on phenotypic means and plasticity indices. The QTLs identified on phenotypic means are depicted in red and those identified on plasticity indices in purple. Asterisks correspond to a colocalization between QTLs and plasticity QTLs.



Plasticity QTLs were 2 to 7 times less numerous than QTLs and contributed weakly to the variance due to G and GxT effects (Fig. 4, Fig.S3 and Table S3). However, excepted for *gs_max*, plasticity QTLs strongly contributed to the variance due to GxW effects (60 - 100%). QTLs mainly contributed to the variance due to GxW effects in four traits (LA, *gs_max*, WU and WUE), as well as to the effects of G and GxT. Plasticity QTLs contributed more to the GxW effect than QTLs. To evaluate to what extent plasticity QTLs were complementary to QTLs, we considered jointly plasticity QTLs and QTLs and compared their biological relevance to a control set comprising QTLs and randomly selected SNPs (Fig. 4 and Fig.S3). Excepted for *gs_max*, QTLs and plasticity QTLs jointly contributed more to the GxW effect than the union of the QTLs and randomly selected SNPs. Altogether, these results show that plasticity QTLs are good candidates for understanding the genetic architecture of the GxW interaction.

Discussion

Plants being sessile, they are continuously exposed to variable and potentially harsh environmental conditions that can cause biotic or abiotic stress. In crops, the ability to resist or tolerate such stresses is of great importance to maintain productivity without resorting to inputs. Here, we investigated the genetic bases of the drought response in maize using a MET approach. The objectives of our study were i) to assess the effect of the trials on QTL detection, and ii) to estimate the extent to which plasticity QTLs contribute to the GxW effect on drought-related ecophysiological traits in maize compared to QTLs.

Even though the trials were carried out in a greenhouse with well-controlled watering conditions, the meteorological conditions outside the greenhouse were different from one trial to the other. We took these different meteorological conditions into account, which allowed us to decompose the GxE interaction into GxW and GxT. We showed that the GxE interactions observed in the data were driven more by trial effects than by water availability. This may explain the low overlap between the sets of QTLs identified from each trial by Alvarez Prado et al (2017).

Compared to single-trial GWAS, multi-trial GWAS allowed to better fit the ecophysiological traits related to drought response: residual errors and GxT interactions were both smaller in multi-trial GWAS than in single-trial GWAS. In addition, the newly detected QTLs captured a larger part of the GxW variability for LA, WU and WUE. For the three other traits (Biom, Transp and *gs_max*), results are more mitigated: the number of newly detected QTLs was small compared to the several dozens of QTLs detected by the single-trial GWAS, which already explained a large part of the GxW interaction. Overall, by performing multi-trial GWAS, we multiplied

individual observations and thereby increased the power to detect QTLs across trials (Cantor et al, 2010; Thomas, 2010). These results are consistent with those of Benaouda et al (2022), who showed that four multi-environment QTLs explained 20.6% of the heading time variance in wheat compared to 9.5% for the six single-environment QTLs detected by Langer et al (2014).

In this study, we also explored the gain provided by performing GWAS on six plasticity indices for dissecting the genetic architecture of ecophysiological trait response to drought. We identified 40 plasticity QTLs and highlighted 38 genetic regions that differed from those associated with the QTLs. The results obtained with the multi-locus multi-environment model showed that plasticity QTLs specifically captured the variance of the GxW interaction. By comparison, QTLs not only captured the variance of the GxW interaction, but also a large part of the G variance and, to a lesser extent, part of the variance of the GxT interaction. These results indicate that, for the ecophysiological traits studied, the genetic control of phenotypic plasticity in response to drought does not completely overlap with that of the genetic control of the phenotypic mean. Similar results were previously observed in maize (Kusmec et al, 2017), tomato (Diouf et al, 2020) and cassava (dos Santos Silva et al, 2021).

All together, our results help dissecting the genetic basis of response to water deficit. Indeed, three mutually non exclusive genetic models have been proposed to explain phenotypic plasticity (Scheiner, 1993; Via et al, 1995). First, the over-dominance model assumes that phenotypic plasticity is related to the number of heterozygous loci (Gillespie and Turelli, 1989). Second, the allelic-sensitivity model considers that the environment affects the allelic effect of the genetic factors that determine a trait. Third, the gene-regulatory model assumes that phenotypic plasticity results from epistatic interactions between structural and regulatory alleles. Our results favor this last model, in agreement with the hypothesis of Bradshaw (1965) stating that the plasticity of a trait is an independent property of that trait and is under its own specific genetic control.

In conclusion, considering phenotypic means and plasticity as different traits and taking trial effects into account allow us to gain a more precise understanding of how ecophysiological traits respond to water availability. In the short term, our perspective is to go deeper into the functional annotation of the genes associated with the QTLs and plasticity QTLs. By comparing the two gene lists against gene regulatory databases, a strong result in favor of the gene-regulatory model would be to find that genes associated with the plasticity QTLs are regulators of genes associated with the QTLs. These findings may shed further light on the genetic regulatory system underlying the response of plants to stress.

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Declarations

Competing interests. The authors have no relevant financial or non-financial interests to disclose.

Consent to participate. Not applicable

Consent for publication. Not applicable

Data availability. The genotyping datasets analyzed during the current study are published in Negro et al (2019) The phenomics dataset analyzed during the current study is published in Alvarez Prado et al (2017)

Code availability. <https://forgemia.inra.fr/gqe-base/djabali-drought-plasticity-qtls-article1>

Authors' contributions. MBN and MLM defined the research project. YD carried out the analyses. RR, MBN, and MLM supervised the work. YD wrote the manuscript and MNB, RR, and MLM revised and approved it.

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