Journal of Experimental Botany
doi:10.1093/jxb/eru215

Journal of Experimental Botany
www.jxb.oxfordjournals.org

RESEARCH PAPER

Independent genetic control of maize (*Zea mays* L.) kernel weight determination and its phenotypic plasticity

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Received 18 November 2013; Revised 10 March 2014; Accepted 22 April 2014

Abstract

Maize kernel weight (KW) is associated with the duration of the grain-filling period (GFD) and the rate of kernel biomass accumulation (KGR). It is also related to the dynamics of water and hence is physiologically linked to the maximum kernel water content (MWC), kernel desiccation rate (KDR), and moisture concentration at physiological maturity (MCPM). This work proposed that principles of phenotypic plasticity can help to consolidated the understanding of the environmental modulation and genetic control of these traits. For that purpose, a maize population of 245 recombinant inbred lines (RILs) was grown under different environmental conditions. Trait plasticity was calculated as the ratio of the variance of each RIL to the overall phenotypic variance of the population of RILs. This work found a hierarchy of plasticities: KDR ≈ GFD > MCPM > KGR > KW > MWC. There was no phenotypic and genetic correlation between traits *per se* and trait plasticities. MWC, the trait with the lowest plasticity, was the exception because common quantitative trait loci were found for the trait and its plasticity. Independent genetic control of a trait *per se* and genetic control of its plasticity is a condition for the independent evolution of traits and their plasticities. This allows breeders potentially to select for high or low plasticity in combination with high or low values of economically relevant traits.

Key words: Grain-filling duration, kernel desiccation rate, kernel growth rate, kernel weight, maximum kernel water content, moisture concentration at physiological maturity, phenotypic plasticity, quantitative trait loci.

Introduction

Worldwide, maize is currently produced on nearly 100 million ha and, together with rice and wheat, it provides about one-third of the food calories to more than 4.5 billion people (Shiferaw *et al.*, 2011). Maize is also a major biological model (e.g. McClintock, 1950). Thus, research on the genetic control and environmental modulation of maize yield components is relevant to both global food security and the fundamental understanding of plant biology.

In common with other annual seed crops, reproductive maize plants primarily accommodate environmental variation by adjusting seed number, whereas seed weight is relatively stable (Egli, 1998; Peltonen-Sainio *et al.*, 2007). Seed size, the focus of this paper, is under stabilizing selection in nature (Smith and Fretwell, 1974) and agronomic selection further reinforces this process (Sadras, 2007). Doebley *et al.* (1994) used two F2 populations from crosses between maize and teosinte to investigate the inheritance of kernel weight. They detected six quantitative trait loci (QTL), each controlling 4–34% of the phenotypic variance in the first population, and four QTL controlling 9–31% of the phenotypic variance in their second population. This early study, however, did not present details of growing conditions and environmental sources of variation are not discussed.

More recently, Alvarez Prado *et al.* (2013a) investigated the inheritance of maize kernel weight (heritability, H^2=0.81) using 245 recombinant inbred lines (RILs) from the IBM Syn4

population (B73 × Mo17) in two environments. They also measured related traits including the rate of kernel growth (H^2=0.70), the duration of the grain-filling period (H^2=0.53), the maximum kernel water content (H^2=0.91), the rate of kernel desiccation (H^2=0.49), and the moisture concentration at physiological maturity (H^2=0.57). All these traits had large phenotypic variation and significant response to the interaction between genotype and environment. Increasingly powerful statistical models are being developed to untangle the interaction between genotype and environment (Gauch *et al.*, 2011), whereas the perspective of phenotypic plasticity is contributing to the biological interpretation of these interactions (Marguerit *et al.*, 2012; Sadras and Rebetzke, 2013).

Phenotypic plasticity, defined as 'contingent trait expression', involves the production of multiple phenotypes by a single genotype in response to environmental conditions (DeWitt and Langerhans, 2004). Bradshaw (1965) proposed that, in the evolution of processes maximizing fitness, different solutions may emerge that involve a hierarchy of plasticities (i.e. some traits are stable whereas others are more plastic). Seed number and seed size conform to this type of hierarchy (Bradshaw, 1965; Sadras, 2007; Sadras and Slafer, 2012). Whereas Bradshaw's original concept of hierarchy implied a negative correlation (e.g. high plasticity of seed number associated with low plasticity of seed size), there are also reports of positive associations between plasticities (e.g. between plasticity of phenology and plasticity of yield in annual and perennial crops; Sadras *et al.*, 2009). Thus, plasticities of different traits can be unrelated or they can be positively or negatively associated (Sadras *et al.*, 2009; Trentacoste *et al.*, 2011; Sadras and Rebetzke, 2013). Expanding on pairwise comparisons, Bonaparte and Brawn (1975) ranked the plasticity of 16 traits of four maize hybrids in response to plant population density. Grain yield per plant and yield per unit area were the most plastic traits, and the least plastic were ear height and ear row number; this plasticity ranking applied to all four hybrids. In small-grain cereals, yield components conform to the hierarchy tiller number > inflorescence number ≈ grains per inflorescence > seed size (Sadras and Slafer, 2012).

In a recent paper, D'Andrea *et al.* (2013) analysed the phenotypic plasticity of 29 physiological traits related to maize grain yield and its components in a collection of inbreds and their derived hybrids grown in contrasting nitrogen conditions. They found a large range in plasticity, from very low in traits related to crop phenology and kernel weight to very high in traits, associated with N uptake, leaf area, kernel number, and grain yield. However, they did not test the negative relationship between trait plasticity and heritability of traits *per se*, as proposed by Donovan *et al.* (2011) for leaf traits.

Bradshaw (1965) also advanced the hypothesis that plasticity is a trait on its own, with its own genetic control. Reymond *et al.* (2003) provided empirical evidence supporting Bradshaw's proposition; they showed that maximum leaf elongation rate in maize and the responsiveness of this trait to vapour pressure deficit partially map to independent QTL. An important consequence of the partial independence in the genetic control of plasticity and the trait *per se* is that plasticity can evolve independently of the trait (David *et al.*, 2004; Pigliucci, 2005; King and Roff, 2010). Breeders could, potentially, select for high or low plasticity in combination with high or low values of the trait *per se*.

This paper tested two hypotheses: (1) there is a hierarchy in the plasticity of traits related to maize kernel weight; and (2) the genetic control of the plasticity of these traits is partially independent of the genetic control of the traits *per se.* These hypotheses were tested in a 245 RIL maize population grown under different environmental conditions, where kernel weight ranged from 118 to 347 mg kernel^{-1} (Alvarez Prado *et al.* 2013a).

Materials and methods

Data set

Multitrait phenotypic data for 245 RILs from the IBM Syn4 (B73 × Mo17) maize population grown in two environments were used. Field experiments were conducted during 2009/2010 and 2010/2011 at the Campo Experimental Villarino, Facultad de Ciencias Agrarias, Universidad Nacional de Rosario, Zavalla, Argentina. Each experiment was arranged in a randomized complete block design with three replicates. Crops were sown on 15 September 2009 and 4 October 2010. Stand density was five plants m^{-2} in 2009 and seven plants m^{-2} in 2010. Thus, sowing density and weather combined to generate different growing conditions between experimental years.

The traits measured were kernel weight (KW), kernel growth rate (KGR), grain-filling duration (GFD), maximum kernel water content (MWC), moisture concentration at physiological maturity (MCPM), and kernel desiccation rate (KDR). Details of phenotyping protocols can be found in Alvarez Prado *et al.* (2013a).

Phenotypic plasticity

Phenotypic plasticity of each trait was calculated as the ratio of the variance of each RIL to the overall phenotypic variance of the population of RILs (i.e. the variance ratio as defined by Dingemanse *et al.*, 2009). This method returned: (1) 245 plasticity values for each trait; and (2) similar measures of plasticity to reaction norms which do not require assumptions on their shape (Lacaze *et al.*, 2009; Sadras and Rebetzke, 2013). To capture trait–environment relationships, the 10th, 50th, and 90th percentiles of each trait were plotted against the variance ratio for each trait and genotype, and linear regressions were adjusted for each percentile to estimate the slopes of the regressions and their significance. Percentiles were based on 245 genotypes and six data points per genotype (2 years × 3 replications). Further details of this approach are discussed elsewhere (Sadras *et al.*, 2009; Peltonen-Sainio *et al.*, 2011; Sadras and Rebetzke, 2013).

Normality of the residuals of traits *per se* and trait plasticities were verified by adjusting a Gaussian function to the data using Graph Pad Prism version 5.0. (Raduschev, 2007). D'Agostino-Pearson omnibus K2 normality test (D'Agostino, 1986) was used for testing deviations of residuals from the Gaussian distribution. Non-Gaussian distributions were transformed with the equation $y = 1 / (1 + x)$.

Correlation analyses of traits *per se* and trait plasticities were done separately. Pearson correlation coefficients and the significance of each correlation were estimated. Correlation analysis was also used to test the putative independence between traits *per se* and trait plasticities.

Heritability

Broad-sense heritability was estimated for both traits *per se* and trait plasticities using a mixed model considering genotypes, environments, blocks nested within environments, and genotype ×

environment interactions as random factors. Heritability for traits *per se* was calculated as:

$$H^2 = \sigma^2_G / \left(\sigma^2_G + \sigma^2_{GE} / \eta + \sigma^2_e / r\eta\right)$$

where σ^2_G is the genotypic variance, σ^2_{GE} is the genotype × environment variance, σ^2_e is the plot residual variance, and η and *r* are the number of environments and replicate plots, respectively (Hallauer and Miranda, 1988).

Considering that plasticity variance is composed by the environmental and genotype × environment variances and that the heritable component of this plasticity variation is the genotype × environment variance (Scheiner and Goodnight, 1984), heritability for plasticity of traits was calculated as:

$$H^2 = \sigma^2_{GE} / \sigma^2_P$$

where σ^2_{GE} is the genotype × environment variance and σ^2_P is the phenotypic variance, similarly to equation 1 (Scheiner and Lyman, 1989).

QTL analysis

Phenotypic plasticity calculated as the variance ratio of each trait and RIL was used for QTL analysis as in Lacaze *et al.* (2009). The genetic map extracted from Alvarez Prado *et al.* (2013a) was used. It consisted on 641 molecular markers from the Maize GDB website distributed along the entire genome. Each trait was analysed separately using composite interval mapping with model six of the Zmapqtl procedure of the Win QTL Cartographer version 2.5 (Wang *et al.*, 2006). A threshold for logarithm of odds (LOD) scores of two was used to identify QTL. Given the population size, this threshold was chosen to balance the chances of missing small-effect QTL with false-positive QTL declarations because trait plasticities show low heritability estimates, denoting a reduced power to detect QTL (Beavis, 1994). Therefore, this work considered the mean chromosome length of the genetic map and a probability of 92–95% (van Ooijen, 1999). Several markers were selected as cofactors to control genetic variation of possible linked or unlinked QTL and to improve the power to detect minor QTL. Stepwise regression analysis was performed for cofactor selection with a scanning interval of 1 cM and a window size of 10 cM. The statistical model for QTL detection was:

$$y_i = \mu + bx_j + \sum_k b_k x_{jk} + e_j$$

where y_i is the observed phenotype of RIL *i*, μ is the general mean, *b* is the effect of the putative QTL, x_j refers to the genotype of RIL *i* (1 or –1 for AA or aa, respectively), *k* refers to the markers selected as cofactors, b_k refers to the effect of cofactors, x_{jk} refers to the genotype of RIL *i* for those markers selected for genetic background control, and $e_j \sim N(0, \sigma^2)$.

QTL positions were assigned to the points of maximum LOD scores. For establishing the final QTL model, all detected significant QTL in the composite interval mapping scan were included in an initial model. QTL with nonsignificant effects inside the model were removed. Additionally, new QTL and epistatic interactions were tested using the Bayesian information criteria (BIC) for accepting or rejecting each model refinement. Final multi-QTL model was refined by optimizing QTL positions. Multiple interval mapping model used for each plasticity trait was:

$$y_i = \mu + \sum_{r=1}^{m} a_r x_{ir} + \sum_{r<s}^{t} \beta_{rs} x_{ir} x_{is} + e_i$$

where y_i is the trait value of RIL *i*, μ is the general mean of the model, *m* refers to the number of significant markers detected, α_r is the QTL effect and x_{ir} and x_{is} are the genotypes of RIL *i* in positions *r* and *s*, β_{rs} is the epistatic effect between putative QTL r and s, *t* is the number of pairwise epistatic effects, and e_i is the random residual effect. The final QTL model for trait plasticities was compared with the final multi-QTL model for traits *per se* reported by Alvarez Prado *et al.* (2013a).

Results

Phenotypic plasticity and heritability of kernel traits

The phenotypic plasticity of kernel traits was negatively correlated with the heritability of traits *per se* (Fig. 1). Statistically, this is the result of both plasticity and heritability being derived from components of the phenotypic variances and their ratios. Biologically, a negative association is expected because for traits where the environment overrides genetic control of the phenotype, the heritability is low and the plasticity correspondingly high, and, reciprocally, for traits under strong genetic control and low environmental responsiveness, the heritability is high and the plasticity low (Donovan *et al.*, 2011). This negative relationship was not associated with trait experimental error because coefficients of variation from evaluated traits (reported in Alvarez Prado *et al.*, 2013a) were not associated with heritability or plasticity values. A hierarchy for the plasticity of kernel traits was thus determined: KDR ≈ GFD > MCPM > KGR > KW > MWC.

Relationships between plasticity of kernel traits and environmental conditions

A statistical approach was used to relate the plasticity of a given trait with the phenotype under poor (10th percentile), intermediate (50th percentile), and favourable (90th percentile) conditions. No attempt was made to link the phenotype to particular features of the environment.

Kernel weight was normally distributed (P=0.35) and ranged from 118 to 347 mg kernel^{-1} across RILs and environments (Fig. 2A). KW plasticity was also normally distributed (P=0.18); the variation in plasticity (Fig. 2A, X-axis) was

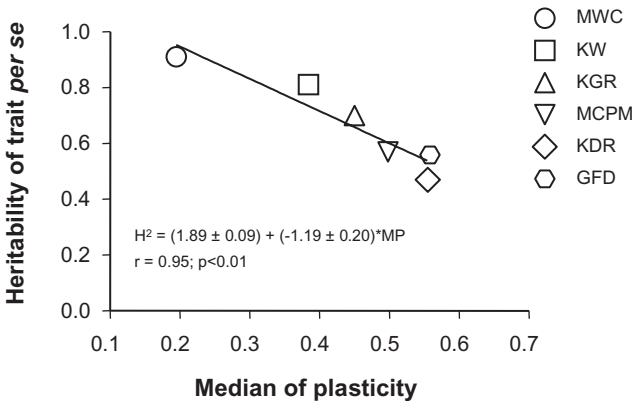

Fig. 1. Relationship between broad-sense heritability and plasticity of kernel-related traits in maize. Both heritability and plasticity are unitless. GFD, grain-filling duration; KDR, kernel desiccation rate; KGR, kernel growth rate; KW, kernel weight; LOD, logarithm of odds; MCPM, moisture concentration at physiological maturity; MWC, maximum kernel water content.

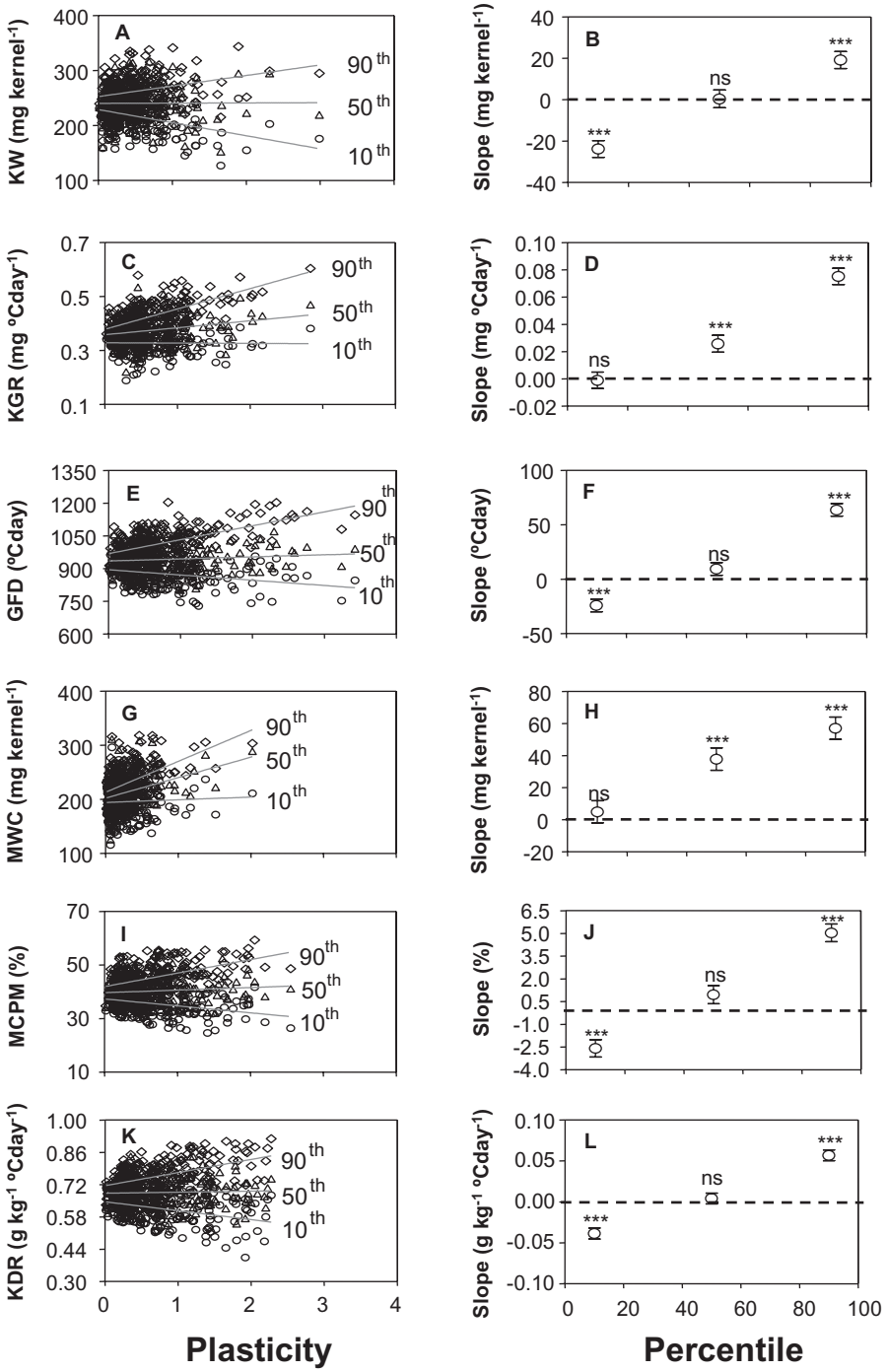

Fig. 2. Relationship between kernel-related traits and their plasticities under favourable (90th percentile, open diamonds), median (50th percentile, open triangles) and unfavourable (10th percentile, open circles) conditions. (A, B) kernel weight (KW), (C, D) kernel growth rate (KGR), (E. F) grain-filling duration (GFD), (G, H) maximum kernel water content (MWC), (I, K) moisture concentration at physiological maturity (MCPM), (K, L) kernel desiccation rate (KDR). Each data set includes a total of 735 data points (245 genotypes × 2 years × 3 replications); percentiles were derived from six data points for each of the 245 genotypes (2 years × 3 replications). In B, D, F, H, J, and L, dotted lines indicate y = 0; mean slope of regressions±SE are also indicated. Asterisks indicate $P< 0.0001$. ns, not significant ($P>0.05$).

almost 11-fold greater than the variation in the trait *per se* (Fig. 2A, Y-axis). The regressions between KW percentiles and KW plasticity of each line (Fig. 2A) returned a 2-fold range of slopes, from –23.8 mg kernel^{-1} for the 10th percentile to 19.2 mg kernel^{-1} for the 90th percentile, with a flat relationship (slope ≈ 0) for the 50th percentile (Fig. 2B). Thus, for this combination of RILs and environments, KW showed a symmetrical capacity to capture favourable and unfavourable environmental conditions.

Kernel growth rate was normally distributed (P=0.84) and highly variable and ranged from 0.171 to 0.790 mg °Cday^{-1}. KGR plasticity was also normally distributed (P=0.30). The relationship between KGR and KGR plasticity (Fig. 2C) showed moderate capacity to capture intermediate and high

capacity to capture favourable conditions while no response was observed under less favourable conditions (Fig. 2D).

Grain-filling duration had a normal distribution (P=0.95) and ranged from 683 to 1399 °Cday across RILs and environments. GFD plasticity showed a non-Gaussian distribution (P=0.03) associated with a heavy right tail, reflecting a high frequency of RILs with high plasticity (Fig. 2E). Differences among lines in their capacity to capture favourable conditions were larger than differences in their capacity to capture unfavourable environments (P<0.0001; Fig. 2F).

Maximum kernel water content was normally distributed (P=0.67) and ranged from 115 to 321 mg water kernel^{-1}. MWC plasticity was normally distributed (P=0.09) showing the lowest variation of all traits under investigation (Fig. 1). The regression slope of MWC *per se* and MWC plasticity increased from ~0 mg water kernel^{-1} for the 10th percentile to approximately 60 mg water kernel^{-1} for the 90th percentile (Fig. 2G). Thus, for this combination of RILs and environments, the plasticity of this trait was fully accounted for by the capacity to capture favourable conditions and was independent of variation under unfavourable conditions (Fig. 2H).

Moisture concentration at physiological maturity was normally distributed (P=0.64) and ranged from 19 to 60% across RILs and environments. MCPM plasticity was normally distributed (P=0.16) and showed an asymmetric response to environmental conditions (Fig. 2I). The regression slope increased 3-fold, from –2.5% under unfavourable conditions to 5% under favourable conditions (Fig. 2J).

Kernel desiccation rate was normally distributed (P=0.48) and ranged from 0.296 to 1.171 g kg^{-1} °Cday^{-1} across RILs and environments (Fig. 2K). It had the highest plasticity of the evaluated traits (Fig. 1). KDR plasticity was normally distributed (P=0.33) and the environmental response of this trait was symmetric, increasing from approximately –0.05 g kg^{-1} °Cday^{-1} for unfavourable environmental conditions to approximately 0.05 g kg^{-1} °Cday^{-1} for favourable conditions (Fig. 2L).

Correlations between traits and between plasticities

Trait plasticities were poorly correlated between each other whereas significant correlations were found between traits *per se* (Table 1). KW was positively correlated with KGR (r=0.79, P<0.001) and to a lesser extent with GFD (r=0.32, P<0.001). Regarding water-related traits, while KGR was positively correlated with MWC (r=0.86, P<0.001), GFD was negatively and weakly associated with MCPM (r=–0.45, P<0.001) and KDR (r=–0.25, P<0.001). In addition to the low correlation coefficients between plasticities, there was no pattern between the trait *per se* correlation coefficients and the correlation of trait plasticities. However, there was a stronger relationship among traits with low plasticity than those with high plasticity.

A second analysis was done correlating traits *per se* with their plasticities in order to test their putative independence. Correlation coefficients were generally low (Table 2). The highest correlation coefficients were observed for KGR (r=0.35) and MWC (r=0.27), while the lowest correlation coefficients were observed for KW (r=0.01) and KDR (r=–0.05).

QTL of kernel trait plasticities

The plasticities of kernel traits showed low-to-medium heritability: 0.09 for MWC, 0.16 for KGR, 0.25 for KW, 0.34 for GFD, 0.37 for KDR, and 0.48 for MCPM. Low heritability values denoted a low heritable portion of the variation.

Owing to the weak correlations between plasticities of kernel traits (Table 1), a QTL analysis was carried out for each trait plasticity independently. A total of 28 significant QTL were detected for all traits. Three to seven QTL for the

Table 1. *Pearson correlation coefficients and significance for the combination of traits* per se *and trait plasticities*

GFD, grain-filling duration; KDR, kernel desiccation rate; KGR, kernel growth rate; KW, kernel weight; MCPM, moisture concentration at physiological maturity; MWC, maximum kernel water content; ns, not significant.

Trait	Trait	Trait *per se*		Trait plasticity	
		r	*P*-value	*r*	*P*-value
KW	KGR	0.79	<0.0001	0.10	ns
	GFD	0.32	<0.0001	0.19	<0.01
	MWC	0.81	<0.0001	0.32	<0.0001
	MCPM	–0.17	<0.0001	0.31	<0.0001
	KDR	0.26	<0.0001	0.05	ns
KGR	GFD	–0.24	<0.0001	0.37	<0.0001
	MWC	0.86	<0.0001	0.25	<0.0001
	MCPM	0.21	<0.0001	0.08	ns
	KDR	0.12	<0.01	0.20	<0.01
GFD	MWC	0.04	ns	0.18	<0.01
	MCPM	–0.45	<0.0001	0.40	<0.0001
	KDR	–0.25	<0.0001	0.01	ns
MWC	MCPM	0.33	<0.0001	0.09	ns
	KDR	0.34	<0.0001	0.09	ns
MCPM	KDR	0.23	<0.0001	–0.05	ns

Table 2. *Pearson correlation coefficients and significance of traits* per se *and trait plasticities*

GFD, grain-filling duration; KDR, kernel desiccation rate; KGR, kernel growth rate; KW, kernel weight; MCPM, moisture concentration at physiological maturity; MWC, maximum kernel water content; ns, not significant.

Trait	Correlation between traits *per se* and plasticity	
	R	***P*-value**
KW	0.01	ns
KGR	0.35	<0.0001
GFD	0.19	<0.01
MWC	0.27	<0.001
MCPM	0.12	ns
KDR	–0.05	ns

plasticity of each individual trait were identified, accounting for 1.5–6.4% of phenotypic plasticity variance (Table 3).

The final QTL model for KW plasticity showed seven additive QTL, located on chromosomes 1, 2, 3, 8, and 9 (Table 3). Epistatic interactions were detected between QTL located on chromosomes 2 and 5 and QTL located on chromosomes 5 and 7. All but two QTL, located in chromosomes 2 and 8, showed negative additive effects, meaning that the presence of alleles from the high-KW parental inbred line Mo17 reduced KW plasticity. The final QTL model for KW plasticity explained almost 30% of the phenotypic plasticity variance (Table 3).

The final QTL model for KGR plasticity returned three QTL and accounted for 8% of the phenotypic plasticity variance. Presence of Mo17 alleles increased KGR plasticity (Table 3). No colocalizations between KGR and KW plasticity QTL were observed.

For GFD plasticity, six QTL were significant and accounted for approximately 26% of the phenotypic variance (Table 3). Two out of six QTL reduced GFD plasticity when Mo17 alleles were present, while the remainder increased it.

The final QTL models for plasticity of kernel water-related traits accounted for 14–20% of the phenotypic variance, more for MWC plasticity than for plasticity of MCPM and KDR (Table 3). In all three models, four QTL were detected where half of the QTL increased plasticity when Mo17 alleles were present (Table 3). For MWC plasticity, an epistatic interaction between QTL located on chromosomes 2 and 4 was significant ($P<0.05$). No colocalization was observed between QTL of different trait plasticities (Fig. 3).

When comparing the QTL models detected for trait plasticities in this study and the previously described multitrait *per se* QTL for grain-filling traits detected by Alvarez Prado *et al.* (2013a), two QTL associated with MWC plasticity colocalized with multitrait QTL on chromosomes 1 and 7 (Fig. 3). Those multitrait QTL showed consistent effects across environments where the presence of Mo17 alleles increased the trait mean. In the case of MWC plasticity, QTL showed opposite effects, where the presence of Mo17 alleles increased the plasticity in chromosome 7 and decreased it in chromosome 1 (Table 3). In chromosome 10, one QTL associated with MCPM plasticity colocalized with one multitrait QTL from Alvarez Prado *et al.* (2013a). However, that specific multitrait QTL was not significant for MCPM (Table 3).

Discussion

Hypothesis 1: hierarchy of plasticities of traits related to maize kernel weight

Bradshaw (1965) first proposed a hierarchy (i.e. negative association) between plasticity of seed number and size, which was later extended to other components of yield in maize (Bonaparte and Brawn, 1975; D'Andrea *et al.*, 2013) and in small-grain cereals (Sadras and Slafer, 2012). The current work studied the hierarchy of plasticities of maize kernel traits. A close, negative correlation between plasticity and heritability was expected from statistical and biological principles (Donovan *et al.*, 2011; Sadras and Slafer, 2012), and heritabilities commensurate with independent reports in maize (Alvarez Prado *et al.*, 2013b) and sorghum (Gambín and Borrás, 2011) reinforce the reliability of the current estimates. A ranking of plasticities was thus identified: KDR ≈ GFD > MCPM > KGR > KW > MWC.

The hierarchy of plasticities between MWC, the trait with the lowest plasticity, and KDR, the trait with highest plasticity, is consistent with physiological principles. The low response of MWC to the environment is associated with the upper limit for kernel size established before flowering, as reflected in correlations between ovary size and final seed mass (Egli, 1990; Calderini *et al.*, 1999; Gambín *et al.*, 2006; Yang *et al.*, 2009). In soybean, disruption of the seed testa allowed maintenance of seed filling in comparison to seeds with intact testa (Egli, 1990), reinforcing the proposition that MWC plasticity is both likely limited by maternal tissue and under strong genetic control (Sadras and Denison, 2009). In contrast, KDR correlates with temperature (Hallauer and Russell, 1961). Of interest, MWC was unresponsive to unfavourable conditions (Fig. 2G,H) whereas KDR was responsive to both favourable and unfavourable conditions (Fig. 2K,L), further explaining the differential plasticities of these traits.

The hierarchy between duration and rate of grain filling, GFD > KGR, is also consistent with physiological observations. Maize KGR is established during early grain filling (Jones *et al.*, 1996) and tends to remain stable regardless of environmental conditions during grain filling (Egharevba *et al.*, 1976; Jones and Simmons, 1983). In contrast, environmental stress (i.e. reductions in source through defoliation) during this period shortened GFD with an associated increase in MCPM (Sala *et al.*, 2007) and/or KDR. This is reflected in the symmetry in environmental responses of GFD (Figs. 2E,F) in contrast to the response of growth rate that was restricted to the most favourable conditions (Figs. 2C,D).

Hypothesis 2: genetic control of plasticity of kernel traits is partially independent of genetic control of traits per se

Bradshaw (1965) advanced the notion that plasticity is a trait itself with its own genetic control. Reymond *et al.* (2003)

Table 3. *Location, additive effect, and* R^2 *of QTL from final multi-QTL model for trait plasticities*

Final multi-QTL models include main additive effect QTL and epistatic interactions between detected QTL (additive × additive interaction). LOD scores refer to multi-QTL models for each trait. GFD, grain-filling duration; KDR, kernel desiccation rate; KGR, kernel growth rate; KW, kernel weight; LOD, logarithm of odds; MCPM, moisture concentration at physiological maturity; MWC, maximum kernel water content; QTL, quantitative trait loci.

Trait	LOD	QTL	Chromosome	Location	Additive effect	R^2
KW	21.3	1	1	141.6	–0.078	2.6
		2	1	220.9	–0.094	3.6
		3	2	116.1	0.060	1.5
		4	2	142.0	–0.099	4.9
		5	3	16.0	–0.102	4.5
		6	8	28.8	0.071	2.2
		7	9	121.6	–0.089	3.7
		2×5	–	–	0.069	2.6
		5×7	–	–	0.084	3.6
		Total				29.2
KGR	5.4	1	3	79.7	0.077	2.6
		2	4	156.0	0.081	3.0
		3	7	21.2	0.076	2.4
		Total				8.0
GFD	12.9	1	1	32.0	–0.044	6.4
		2	1	210.7	0.025	2.0
		3	8	76.5	–0.028	2.7
		4	9	86.9	0.046	6.8
		5	10	44.2	0.036	4.5
		6	10	86.7	0.032	3.7
		Total				26.3
MWC	12.9	1	1	186.0	–0.046	2.7
		2	4	148.6	0.052	3.6
		3	5	65.6	–0.054	4.5
		4	7	25.6	0.069	5.7
		2×4	–	–	0.050	3.2
		Total				19.7
MCPM	7.4	1	1	75.2	–0.089	3.6
		2	4	64.4	0.098	4.1
		3	4	167.4	0.101	4.7
		4	10	78.4	–0.112	5.2
		Total				17.6
KDR	7.6	1	2	25.2	–0.112	4.2
		2	2	113.0	0.101	3.6
		3	7	46.6	–0.111	4.2
		4	9	30.1	0.085	2.3
		Total				14.3

provided empirical evidence supporting this hypothesis in studies with a focus on maize leaf expansion. Marguerit *et al.* (2012) dissected the genetic architecture of the control of transpiration and its acclimation to water deficit in grapevine (*Vitis vinifera*), detecting different QTL for transpiration rate *per se* and transpiration rate acclimation to water deficit, again supporting the independent genetic control of the trait and its plasticity. The current work provides further support to this hypothesis in maize kernel traits, which are important to understand and potentially manipulate these yield-related traits.

The genetic control of KW and its physiological components were independent of the genetic control of the plasticities of these traits, as no colocalization of QTL were observed in five out of six traits. The only exception was MWC, which was the trait with the lowest plasticity and hence the highest heritability. For this trait, there was colocalization of QTL for MWC and MWC plasticity. The extent to which trait *per se* and its plasticity are independent might, therefore, depend on the actual degree of plasticity.

A major difficulty in the detection of QTL for different traits subjected to different environmental conditions is the lack of repeatability across experiments. For example, Liu *et al.* (2011) detected 12 QTL when studying grain-filling rate in maize, but only three were common to the four environments, and Guo *et al.* (2011) identified seven QTL for maize KW, but only two were common across experiments. Such instability is not surprising because all traits are influenced

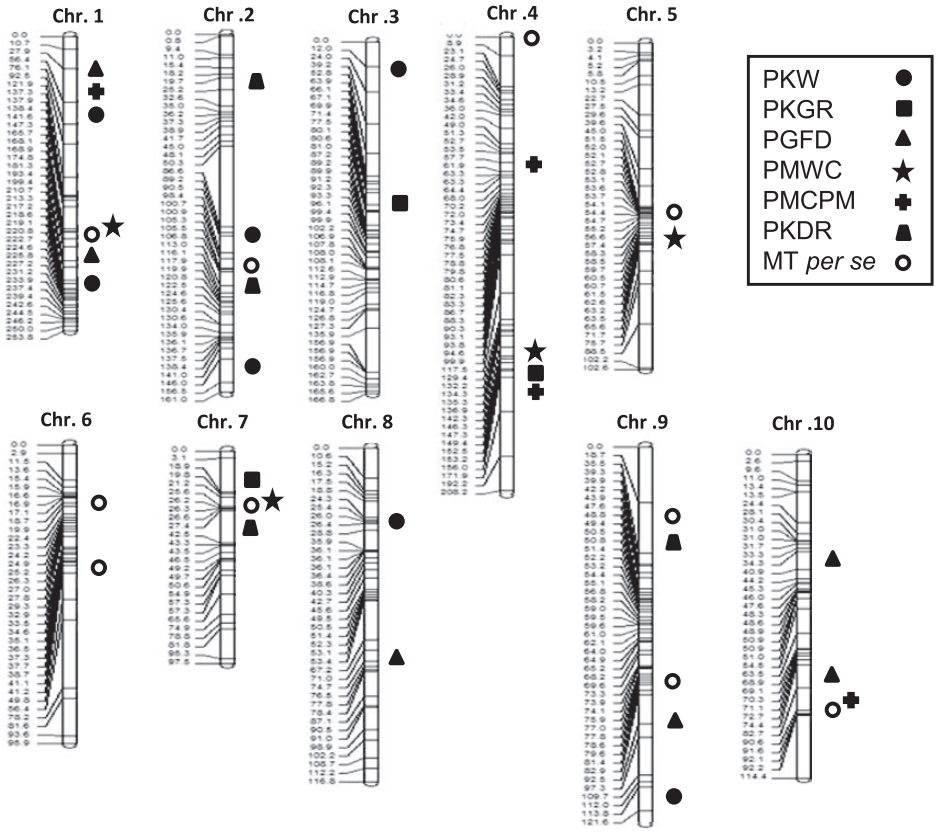

Fig. 3. Chromosomal locations of detected QTL for plasticity of kernel weight (PKW), kernel growth rate (PKGR), grain-filling duration (PGFD), maximum kernel water content (PMWC), kernel desiccation rate (PKDR), and moisture concentration at physiological maturity (PMCPM) and for multitrait QTL detected in Alvarez Prado *et al.* (2013a) (MT *per se*).

by environmental conditions. Most QTL did not colocalize, suggesting that these independent traits were regulated by different genes. KW depends upon KGR and GFD, and the QTL behind these two traits are independent (Alvarez Prado *et al.*, 2013a). The current results agree with previous findings on vegetative traits (Reymond *et al.*, 2003; Marguerit *et al.*, 2012) and for the first time expand these principles to reproductive traits in maize that are relevant to crop production.

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